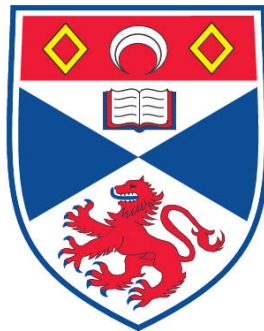


**PUTTING POLLINATION QUALITY INTO ANALYSES OF FLORAL
ECOLOGY: TESTING SYNDROMES THROUGH POLLINATOR
PERFORMANCE**

Caroline King

**A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews**



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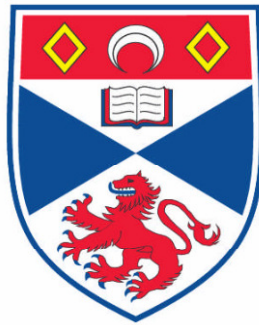
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Putting Pollination Quality into Analyses of Floral Ecology: Testing Syndromes through Pollinator Performance

Caroline King



This thesis is submitted in partial fulfilment for the degree of PhD
at the
University of St Andrews

Date of Submission: 05/09/2011

This thesis is dedicated to my parents, Kevin and Fiona King, who have put up with a lot over the years, but never stopped supporting me and pretending to understand what I was studying. I wouldn't have got this far without them.

"In the long history of humankind (and animal kind, too) those who learned to collaborate and improvise most effectively have prevailed." *Charles Darwin*

"No power in the 'verse can stop me." *River Tam, Firefly*

"Live now; make *now* always the most precious time. Now will never come again"
Jean-Luc Picard, Star Trek Next Generation

I, Caroline King hereby certify that this thesis, which is approximately 85,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in June, 2007 and as a candidate for the degree of PhD in September 2011; the higher study for which this is a record was carried out in the University of St Andrews between 2007 and 2011.

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of PhD in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

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This thesis would not have been completed without the assistance of coffee, chocolate and (the occasional) Jack Daniels. It would probably have been completed sooner without the "assistance" of my cats Grissom and Horatio. I would like to clarify that any further typos or spelling mistakes are likely the result of them sprawling across the laptop whenever the opportunity arose.

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Abstract

Over recent years, the extent of specialised and generalised plant-pollinator relationships, and the predictive powers of floral traits (often grouped into “pollination syndromes”) as indicators of the most effective pollinators of plant species, have been questioned. Such studies, however, have used proxies such as visitation frequency rather than direct measurements of pollinator effectiveness (PE). The main objective of this thesis was to test the predictive powers of various pollination syndromes using a specific measure of PE: single-visit stigmatic pollen deposition (SVSPD).

Six different classical pollination syndromes were tested, using 13 different plant species from tropical and temperate habitats, and in the case of flowers typical of the hummingbird, hoverfly, bee, oil flower and long-tongued insect syndromes, the expected pollinators were the most effective at a single-visit scale. For generalist pollination syndrome flowers, not all observed visitors were significant pollinators, and the species studied were not as broadly generalised as their visitor assemblages would suggest.

In all 13 plant species, pollinator performance could appear consistent within functional visitor groups but was variable between visitor species, and in almost all cases not all of the observed visitors were effective pollinators. The pollinator performance proxies of visit duration and feeding behaviour were neither significantly, nor consistently, related to PE. Visit duration was not an accurate indicator of pollinator performance on its own, though it was useful when combined with SVSPD to define pollinator performance at a given time scale, for example per hour, per day or per season. My findings suggest that the results of recent “pollination” networks and webs, based on visitors but not necessarily pollinators, should be treated with caution.

SVSPD therefore proved to be an effective and relatively simple direct measure of PE, confirming the predictive powers of pollination syndromes, and giving further insight into the extent of specialisation and generalisation.

Chapter 1: Introduction

“Among plants, the nuptials cannot be celebrated without the intervention of a third party to act as a marriage priest” (Rothrock, 1867)

The Importance of Animal Pollination

Pollination by Animals

Pollination represents a key animal-plant relationship vital to both wild flower communities (Ashman *et al.*, 2004; Aguiler *et al.*, 2006) and agricultural productivity (Klein *et al.*, 2007; Ricketts *et al.*, 2008), playing an important functional role in most terrestrial ecosystems. Angiosperms, or flowering plants, account for approximately one sixth of all the described species on earth (Willmer, 2011) and it is estimated that 90% or more of angiosperm species benefit from animal pollination (Linder, 1998; Renner, 1998).

Flowers are usually hermaphroditic, with both male and female parts. For effective fertilisation, the male gametophyte (pollen) produced from the male structure of the flower (the anther) must come into contact with the female structure (the stigma) and the genetic material must then be transported to the female gametophyte (the ovule). While this process can be effected by abiotic pollination methods such as wind or water, biotic pollination, or animal pollination, is a much more common strategy (Linder, 1998; Renner, 1998; Willmer, 2011).

The majority of plant-pollinator interactions can be considered mutualistic. The plant is fertilised by pollen (which must be conspecific but preferably non-self), effecting reproductive success. The animal receives a benefit of some kind; either food for the individual in the form of pollen, nectar or tissues; pollen or fatty oil to feed their brood; a site in which to lay eggs, usually a developing seed or surrounding structures that provide larval hatching and/or feeding sites; nest building material; liquid fragrances for use in mating

displays; or a site for shelter, warmth or rendezvous (Renner, 2006). Over millions of years, many features of flowering plants have evolved in relation to the effective dispersal of gametes by animals, and the animals involved have also evolved special traits adapting them towards obtaining food or other resources efficiently from the plants they visit, and in particular the flowers. For example, it has long been known that the blooming periods of certain flowers have evolved to coincide with the flight period of effective pollinators (Robertson, 1895). As they visit these flowers, the animals pick up pollen, which can be transferred to subsequent flowers visited. This effects the dispersal of gametes from the plant, promoting outcrossing and leading to greater genetic diversity.

Pollination by animals can be extremely beneficial to plant reproductive success, especially to plants in isolated habitats, such as islands, where pollinator limitation can be an important selective force (Spears, 1987). An effective pollinator must have the ability to passively pick up pollen as it touches the anthers of a flower that it visits, and then be able to carry that pollen to the stigma of another flower. The animal should be a good physical fit to the flower, in terms of size and shape, so that some part of its body reliably touches the anther during flower visitation. In addition, appropriate surface structures on the body of the visitor, such as feathers, fur, hair or scales to which pollen adheres readily, will aid in the pickup and transfer of pollen, while shiny or waxy surfaces are not conducive to effective pollen transfer, and in some species surface secretions may even damage pollen (Willmer, 2011).

Animal visitor-flower interactions can also be more one-sided however, and not all insects observed to visit or feed from a plant will necessarily pollinate it effectively. Nectar-robbing, for example, is where the nectar of the flower is removed by a visitor without pollination occurring, usually by perforation of the corolla (Inouye, 1980a). In this case the relationship is generally antagonistic, as the insect often causes harm to the flower. Less harmful forms of nectar thievery can also occur, as in the case of honeybees inserting their tongues between the sepals and bases of the petals of *Brassica* (Free and Williams, 1973)

in order to steal nectar; while this does not cause any specific damage or benefit to the plant, it does still result in a loss of resources. There are also examples of plants benefiting at the expense of (or lack of benefit to) an animal. One such example is deceptive flowers where rewards are “faked”, as in food deception in the orchid *Orchis boryi* (Gumbert and Kunze, 2001) or brood site imitation in the genus *Asarum* (Vogel, 1978a and 1978b), where animals are “tricked” into pollinating the plant. While, in most of these cases, no specific harm is caused to the flower visitor, foraging time wasted on rewardless flowers could be considered a negative effect.

Animal Flower-Visitors

At least 130,000 animal species, possibly up to 300,000, make regular visits to flowers, and are therefore potential pollinators (Buchmann and Nabhan, 1996; Kearns *et al.*, 1998). While the most well-known species of pollinators generally come from the orders Hymenoptera, Lepidoptera, Diptera and Coleoptera these are by no means the only families that participate (Kevan and Baker, 1983). Dating far back to the beginnings of pollinator studies, various comprehensive visitation records of flowers (Müller, 1883; Willis and Burkill, 1895-1908; Burkill, 1897; Knuth, 1906-1909; Hagerup, 1950; Pigott, 1958; Porsch, 1957; Popham, 1961; Corbet, 1970; Proctor and Yeo, 1973; Kevan and Baker, 1983) have indicated how widespread flower visitor species are amongst the insect groups, not to mention examples of bird and mammal flower visitation.

Insects within the subclass Apterygota (including the Collembola, or springtails) have been observed feeding on pollen from flowers (Proctor and Yeo, 1973; Berg *et al.*, 2004). From the superorder Exopterygota, members of the orders Dermaptera, Dictyoptera, Plecoptera, Neuroptera, Mecoptera, Trichoptera and Hemiptera have all been observed feeding on nectar, pollen, or sap on flowers (Proctor and Yeo, 1973; Kevan and Baker, 1983; Dupont and Olesen, 2009). As well as the above, some species of Thysanoptera, or thrips,

are pollinators of certain flowers species (Kevan and Baker, 1983; Yi-Bo and Zhen-Yu, 1999; Sakai, 2001; Kitching *et al.*, 2007).

There are also many known pollinators among the birds, both hummingbirds and passerines (e.g. Stiles, 1981; Bruneau, 1997; Ollerton *et al.*, 2008; Hoffman *et al.*, 2010), from the families Trochilidae, Thraupidae, Nectariniidae, Zosteropidae, Promeropidae, Meliphagidae, Dicaeidae, Fringillidae (in particular the sub-family Drepanidinae), Icteridae and Psittacidae (sub-families Loriinae and Loriculinae). To be added to this are the fruit-eating and flower-feeding bats of the order previously known as Chiroptera, and now more commonly divided into the two separately evolved orders Megachiroptera and Microchiroptera, which are known to be pollinators of many plants, for example *Eucalyptus* and *Melaleuca* (Beardsell *et al.*, 1993). Furthermore several species of non-flying mammals are known to pollinate flowers in Australia, South Africa and tropical America (Lumer and Schoer, 1980; Steiner, 1981; Janson *et al.* 1981; Gribel, 1988; Goldingay *et al.*, 1991; Carthew and Goldingay, 1997; Yumoto *et al.*, 1999), and there are occasional records of lizard pollinator species (Elvers, 1977; Eifler, 1995; Perez-Mellado and Casas, 1997; Traveset and Sáez, 1997; Nyhagen *et al.*, 2001; Olesen and Valido, 2003; Hansen *et al.*, 2006; 2007; Sazima *et al.*, 2009). Thus we can see that the list of potential animal pollinator species is quite extensive.

The Importance of Animal Pollination in Natural Communities

In his iconic book, *The Origin of Species* (1859), Darwin emphasised that “plants and animals, most remote in the scale of nature, are bound together by a web of complex relations”. The vast majority of terrestrial organisms exist in trophic systems based on plants, and as we climb the trophic ladder species richness increases by orders of magnitude. A given plant species, for example birch, oak or willow, may be host to 200-300 insect herbivore species, and each herbivorous insect may be utilised by 10-20 carnivores, either predators or parasites (Herrera and Pellmyr, 2002). Given the quantity and variety of

organisms associated with plants, and the dependence of many of their plants upon animal pollinators, pollinator diversity is an important factor in maintaining ecosystem diversity.

Around 80% of wild flower species are directly dependent on animal flower visitors for seed and fruit set (Potts *et al.*, 2010). Almost three quarters of these species show some degree of pollen limitation (Larson and Barrett, 2000; Ashman *et al.*, 2004; Aguilar *et al.*, 2006; Klein *et al.*, 2007). Insufficient pollen delivery to stigmas can result in angiosperms producing fewer mature fruits and seeds than the maximum suggested by the number of flowers and ovules they produce (Larson and Barrett, 2000). In addition to pollination by animals being more common, and usually more effective, than abiotic methods of pollination as mentioned above (Renner, 1998), it is frequently also associated with more rapid speciation of plants (Dodd *et al.*, 1999; Kay *et al.*, 2006).

The Importance of Animal Pollination in Agriculture

Animal pollination is not only of importance to natural floral communities; mankind relies on a variety of pollinators for the fertilisation of some 90 commercial crops worldwide (Benjamin and McCallum, 2008) as well as many non-commercially-grown fruits and nuts. As agricultural practices have changed, becoming more intensively managed and tending towards monocultures, methods of anthropogenic management of crop pollination have changed in order to keep up with demand. The art of keeping bees began with the ancient Egyptians, for whom beekeeping and the transportation of bees for honey production was well established by 2,400BC (Crane, 1999), and the practice has spread across cultures ever since.

The honey bee, or most often the Western honey bee *Apis mellifera*, was found to be well suited to intensively-managed rearing, and commercial bee breeding became a lucrative business. In America in particular the practice is extremely widespread, with 2.44 million colonies being kept there in 2008, and transported across the country to pollinate valuable crops (Benjamin and McCallum, 2008). It is asserted that a third of all food consumed in the

US requires bee pollination for its production, and the value of honey bee pollination in the US is estimated at an annual \$15 billion (Johnson, 2007). *Apis mellifera* is capable of increasing yield in 96% of animal-pollinated crops (Klein *et al.*, 2007), and provides a pollination service to many wild plants, though its quality as a pollinator is not always well supported by evidence, and the contributions of other pollinators may be more substantial (Klein *et al.*, 2007).

Worldwide, an estimated 75% of all food crops used directly for human food rely on pollination by animals, and by bees in particular (Potts *et al.*, 2010). Although wind-pollinated crops such as wheat and rice are among the highest in volume (Ghazoul, 2005a), a large proportion of fruit crops such as apples, melons and berries are reliant on animal pollinators, as are many nuts. Cultivation of pollinator-dependent crops has shown an increase between 1961 and 2006. Aizen *et al.* (2008) used FAO data to examine temporal trends in yield, total production and cultivation of crops in relation to pollinator dependency over the five decades between 1961 and 2006, finding a disproportionate increase in the area cultivated by pollinator-dependent crops in comparison to pollinator declines and suggesting that the continuation of this trend will lead to an decrease in crop yields as the demand for pollinator services exceeds those available due to declines.

Many studies have attempted to put a financial value on worldwide pollination services. In 1997, taking into account the benefits to the environment as well as to agriculture, the value was estimated at \$117 billion per year (approximately £72 billion, Costanza *et al.*, 1997). A more recent study (Gallai *et al.*, 2009) put this figure at €153 billion (approximately £136 billion), 9.5% of the total economic value of the agricultural output of crops used directly for human food consumption.

Without animal pollinators, the cost of manually pollinating crops would be extortionate. In Southern Sichuan in China, for example, uncontrolled pesticide use wiped out the natural pollinator community in the 1980s. Since then, the pollination of the area's

pear trees must be carried out by hand, using bamboo sticks with chicken feathers on the end. Farmers scrape off pollen from the anthers of the trees, allow it to dry for two days and then use the feathers to apply the pollen to the stigma of the flower. Although an effective method, it is much more costly and time-consuming than if performed by insect pollinators, as well as leading to other problems such as over-pollination of trees, adding to labour costs through the need for fruit thinning, and a loss of genetic diversity from the repeated pollination of target pears by the same pollen sources. While there are a few beekeepers available in the area, they refuse to place their colonies in the fields of pear trees due to the continued extensive pesticide use to control pear lice, where pear trees are sprayed 12 times before harvesting (Ya *et al.*, 2005). If such a process were to be implemented in the USA alone it would cost an estimated \$90 billion, or £55 billion (Benjamin and McCallum, 2008).

Pollinator Declines

The importance of pollinators for environmental and agricultural purposes has made recent well-publicised declines a worldwide concern. Honey bee colonies in the USA fell by 59% between 1947 and 2005 (van Engelsdorp *et al.*, 2008), and central European colonies suffered a decline of 25% between 1985 and 2005 (Potts *et al.*, 2010) with several factors receiving the blame. One of these, the ectoparasite mite *Varroa destructor*, an invasive species from Asia (Sammataro *et al.*, 2000), a significant vector for diseases, has wiped out the majority of wild and feral honey bee colonies in Europe and the USA, as well as a significant proportion of the colonies of beekeepers (Kraus and Page, 1995; Moritz *et al.*, 2007; Jaffée *et al.*, 2010).

Moving beyond the honeybees, a widespread pattern of declines in pollinator abundance and diversity as a result of habitat loss and agricultural intensification has been suggested (Ricketts *et al.*, 2008; Winfree *et al.*, 2009). Bumblebee populations across Belgium and the UK have shown declines in species diversity (Rasmont and Mersch, 1988;

Goulson *et al.*, 2008; Williams and Osborne, 2009). Worldwide nearly 200 species of vertebrate pollinators (Nabhan and Buchmann, 1997) and an untold number of invertebrate pollinators (Matheson *et al.*, 1996) may be on the brink of extinction. Pollinator extinctions are thought to be biased towards pollinators with specialised dietary or habitat requirements (Biesmeijer *et al.*, 2006), for example bumblebee species with narrow pollen specialisation (Kleijn and Raemakers, 2008). Biased extinctions such as these will have important implications for the resilience of pollination services across species, time and space via the loss of important functional roles such as long-distance pollen dispersal (Larsen *et al.*, 2005).

Factors Driving Pollinator Declines

Pollinator abundance and diversity are under threat from a range of anthropogenic factors (Kearns *et al.*, 1998), as recognised by the UN Sao Paulo declaration (1998-1999), and the International Pollinator Initiative (IPI) was founded in 2000 in order to coordinate worldwide investigations of the subject. Such declines are unlikely to be caused by a single factor acting in isolation; rather, declines in both wild and managed pollinator populations are more likely to be caused by a variety of factors interacting with one another, with one sub-lethal factor increasing the severity of another (Oldroyd, 2007; Le Conte and Navajas, 2008; Settele *et al.*, 2008).

The majority of studies have analysed possible factors in isolation. One of the most important factors thought to be driving bee declines is that of habitat fragmentation (Brown and Paxton, 2009). Pollinator diversity and abundance decline with habitat fragmentation (Rathcke and Jules, 1993; Steffan-Dewenter and Tscharntke, 2000; Ricketts *et al.*, 2008; Winfree *et al.*, 2009). Conversely however, several studies have demonstrated a positive effect of particular kinds of habitat conversion on certain bee guilds, such as cavity-nesters within urban areas (Cane *et al.*, 2006; Carré *et al.*, 2009), or on general bee abundance and diversity (Winfree *et al.*, 2007). This could be due to intermediate levels of disturbance which will promote availability of resources for pollinators across multiple partial habitats (Cane *et*

al., 2006; Winfree *et al.*, 2008), or the introduction of novel foraging and/or nesting resources or micro-habitats (Cane *et al.*, 2006; Winfree *et al.*, 2007). In addition, certain bee species are able to tolerate or even benefit from a moderate level of disturbance (Carré *et al.*, 2009), including habitat loss (Winfree *et al.*, 2009), due to the highly mobile nature of bees and their ability to adapt to using patchy resources.

Habitat fragmentation may also have a negative effect on wild pollinator populations; however few studies have investigated the effects this has on pollination itself (Steffan-Dewenter *et al.*, 2006; Brosi *et al.*, 2008; Winfree *et al.*, 2009). Fragmentation produces declines in species richness and abundance of bees, in particular solitary or parasitic species, and those with narrow ranges of pollen hosts (Steffan-Dewenter *et al.*, 2006); and also in butterflies, particularly those which are monophagous (Tscharntke *et al.*, 2002).

Changes in climate have been a growing concern for many years, and higher temperatures alter the flowering times of plants (Sparks *et al.*, 2000) as well as bringing the earlier onset of spring (Sparks and Menzel, 2003) which will affect the behaviour of pollinators, who in seasonal habitats must time their emergence after winter with the blooming period of their host plants. Mismatches of temporal (Hegland *et al.*, 2009) and spatial (Schweiger *et al.*, 2008) co-occurrence of animal-visited flowers and pollinators can potentially disrupt their relationships (Memmot *et al.*, 2007). Butterfly distributions have been affected by recent climate change (Hickling *et al.*, 2006), and future, more severe changes in climate are expected to have even greater impacts (Settele *et al.*, 2008). A relationship between narrow climatic niches and vulnerability to declines in British bumblebees has also been shown (Williams *et al.*, 2007). Impacts of climate change are evident at various levels: *individual level*, for example changes in the temporal activity of bees (Stone and Willmer, 1989); *species level*, in changes in phenology (Hegland *et al.*, 2009), such as local or regional extinction of butterfly species (Parmesan *et al.*, 1999; Thomas *et al.*, 2001); *population level*, such as evolutionary change leading to an increase in the variety of habitats colonised by two butterfly species in England (Thomas *et al.*, 2001) and *community*

level, for example in changing composition and functioning of pollinator communities (Memmot *et al.*, 2007).

Other suggested causes of pollinator declines include changes in land use, modern agricultural practices, the use of pesticides and herbicides which are known to have a devastating effect on abundances of non-target organisms as well as the pests they are used against, and invasions of non-native plants and animals (Kearns *et al.*, 1998; Potts *et al.*, 2010), as well as diseases and parasites, such as tracheal and *Varroa* mites in honey bee colonies brought into previously clean countries by illegal bee importations (Ball and Allen, 1988; Watanabe, 1994; Hung *et al.*, 1995; 1996; Shimanuki and Knox, 1997; Bowen-Walker *et al.*, 1999; Brodsgaard *et al.*, 2000; Sammartaro *et al.*, 2000; Shen *et al.*, 2005).

Concerns over the impacts these declines will have in areas of interest such as crop production have led to a number of suggestions as to the direction of future research. Allen-Wardell *et al.* (1998) suggested that priorities for research and conservation of pollinators should include:

- Increased attention to invertebrate systematics, monitoring and reintroduction as part of critical habitat management and restoration plans
- Multi-year assessments of lethal and sub-lethal effects of pesticides
- Assessments of herbicides and habitat fragmentation on wild pollinator populations in and around cropland
- Inclusion of the monitoring of seed and fruit set and floral visitation rates in endangered plant management and recovery plans
- Inclusion of habitat needs for critically important pollinators in the critical habitat designations for endangered plants
- Identification and protection of floral reserves near roost sites along the “nectar corridors” (feeding sites) of threatened migratory pollinators
- Investment in the restoration and management of species diversity of pollinators and their habitats adjacent to croplands in order to stabilize or improve crop yields

Research into these areas has increased over the past decade, but the results are worrying. The realisation has dawned that the loss or decline of plant-pollinator relationships could lead to the depletion of flowering plants, both wild and cultivated varieties, as well as having an adverse effect on crop production and thus on commodity markets (Kevan and Phillips, 2001; Potts *et al.*, 2010).

Pollinator Syndromes and Specialisation

Pollinator Syndromes

There are many different areas of research within the field of plant-animal interactions, several of which will be investigated and tested further through this thesis, the first being the concept of pollinator syndromes.

Flowers show remarkable adaptive radiation, however certain features and forms are often found to have evolved convergently in many different families. Broad floral types such as bowl-shaped or tubular flowers, or bright red colouring or scent types and other recurring features, form the basis of the theory of pollinator syndromes. Convergent evolution of certain morphologies or reward structures has occurred because flowers are exploiting the preferences and abilities of certain types of flower-visitor, and the recurrence of such traits indicates pollination by similar visitor species (Faegri and Van der Pijl, 1979; Thomson and Wilson, 2008; Willmer, 2011).

Classification of flowers has been tackled by several different authors beginning with Sprengel's (1793) class system based upon number and arrangement of stamens, combined with the reward for pollinators and sex expression at the individual flower and whole plant level, and Delpino's (1868-1875) proposition of two general schemes for classification of flowers. Following from these studies, the concept that floral-trait combinations are related to pollinator type has been put forward by many authors since (Müller, 1883; Delpino, 1868–1875; Müller and Delpino, 1869; Knuth 1906, 1908; Baker, 1963; Grant and Grant, 1965; Faegri and van der Pijl, 1966; 1971; 1979; Stebbins, 1970; Johnson and Steiner, 2000;

Fenster *et al.*, 2004; Willmer, 2011). Stebbins (1970) expanded upon this by formulating the Most Effective Pollinator Principle (MEPP), stating that a given plant will evolve specialisations that are suited to the most effective pollinator species of that plant.

A pollination syndrome is defined as a suite of floral traits, a combination of morphology, colour, scent, and other phenotypic traits representing adaptations towards pollinators, including rewards that are associated with the attraction and utilisation of a specific group of animals as pollinators (Fenster *et al.*, 2004). There is much evidence to suggest that the evolution of certain floral traits is mediated by pollinator selection (Galen and Newport, 1988; Nilsson, 1988; Campbell, 1989; Galen, 1989; Schemske and Horvitz, 1989; Robertson and Wyatt, 1990; Herrera, 1993; Andersson and Widén, 1993). Fenster *et al.* (2004) stressed the importance of grouping pollinators into functional groups according to presumed similarities in the selection pressures they exert, and stated that different functional groups varied in their effectiveness as pollinators for different plant species, as well as varying in the selection pressures they exert. Several of the various pollination syndromes defined by the above studies are described in further detail in later chapters.

Specialisation and Generalisation

A fundamental aspect of pollinator syndromes is the degree to which the interaction between the plant and its pollinators is either specialised or generalised. If pollination syndromes truly exist, then flowers within each syndrome must show specialisation towards a particular pollinator species or type. As the floral trait adaptations mentioned above distinguish angiosperm species, specialisation to a particular pollinator has been considered critical to plant speciation and evolutionary radiation (Grant and Grant, 1965; Stebbins, 1970; Crepet, 1983) and a general evolutionary trend towards specialisation has been suggested (Stebbins, 1970; Crepet, 1983; 1984).

Examples of pollinator-mediated specialisation in nature are not difficult to find. Among the most extreme examples of obligate specialisation are the frequently-studied fig

wasps and their relationship with fig trees. Due to the highly coupled relationship between the figs and their pollinators, outcrossing levels in figs are high, even when the density of flowering conspecifics is low. As this is an obligate mutualism, the figs have no other pollinators (Wiebes, 1979; Berg, 1989) and the fig wasps visit no other flowers. Fig wasps from the family Agaonidae pollinate at least 750 species of *Ficus*, and each wasp species pollinates a particular species of fig, or occasionally two closely related species (e.g. Bronstein, 1992; Cook and Rasplus, 2003; Cook *et al.*, 2004; Jusselin *et al.*, 2008). The relationship between figs and their wasps was thought to be almost exclusively 1:1, however, more recently, exploitation of the interaction in the form of the occurrence of wasps of species other than the known pollinator species has been found in up to 50% of fig species studied. Most of these wasp species may be participating in pollination, however many are known to be parasitic and non-pollinating (Cook and Rasplus, 2003; Molbo *et al.*, 2003).

Even with these exceptions, figs and fig wasps show an extreme example of obligate specialisation which, though found in several other species [for example yuccas and yucca moths (Powell, 1992; Pellmyr *et al.*, 1996), senita cacti and senita moths (Fleming and Holland, 1998; Holland and Fleming, 1999; 2002) and *Glochidium* trees and *Epicephala* moths (Kato *et al.*, 2003)], is by no means a common feature in natural communities, and both participants will have an inevitable selective pressure to exploit one another as a result of evolutionary conflicts (Pellmyr, 1997).

Less extreme examples of pollinator-mediated specialisation can also be found, and these should be considered both from the perspective of the plant and the perspective of the animal. From the point of view of the visitor, some species of solitary bee in particular are extremely specialised in their flower visiting behaviour (Wcislo and Cane, 1996). *Andrena hattorfiana* is specialised towards flowers of the family Dipsacaceae, which restricts it to flowers of the genus *Knautia* in Europe (Larsson and Franzen, 2007), while *Andrena vaga* is specialised towards flowers of the genus *Salix*, also known as willows. European species of

Melitta are also rather specialist towards certain legumes and campanulas (Kwak and Bekker, 2007). Specialisation is frequently found in those solitary bee species which visit oil-producing flowers. *Macropis* sp. offer a good example of this, being observed collecting pollen and oils almost exclusively from flowers of yellow loosestrife (*Lysimachia*; Kwak and Bekker, 2006), and *Rediviva* bees are specialists on oil-producing *Diascia* species in Southern Africa (Steiner and Whitehead, 1990). Analysis of the gut pollen spectra of hoverflies has also shown that some species, in particular *Melanostoma*, *Cheilosia*, *Rhingia* and *Volucella*, are rather specialised in their flower feeding (Gilbert, 1981; 1985; Haslett, 1989a; Hickman *et al.*, 1995; Gilbert and Jervis, 1998; Willmer, 2011). Examples of floral specialisation are also apparent in checkerspot butterflies of the genus *Euphydryas* (Murphy, 1984) and white pierid butterflies of the genus *Pieris* (Lewis, 1986; 1989; Goulson and Cory, 1993; Kandori and Ohsaki, 1996); as well as examples from the beetles, such as *Cetonia* sp., which show specialisation towards *Viburnum opulus* flowers (Englund, 1993), and *Byturus tomentosus* which visits raspberry flowers almost exclusively (Willmer *et al.*, 1996). Some degree of specialisation in terms of flower visiting can also be found, at least at a local level if not always at a species level, in many different visitor species, including migratory species such as hummingbirds and long-lived vertebrates (Willmer, 2011).

The terms monolectic, oligolectic and polylectic offer a means of describing pollen-feeding behaviour, referring to species which collect pollen from only one, or a few, or many different plant species respectively. The vast majority of pollen-feeders are polylectic (Cane and Sipes, 2006). However, many bees show narrow oligolecty in their flower visiting behaviour, either over a whole day, or at the very least in a single foraging bout (Westerkamp, 1996), but some show extreme “fussiness” in pollen choice, ceasing nesting or failing to develop when only non-host pollen is offered (Praz *et al.*, 2008a,b). Minckly and Roulston (2006) reviewed the “lecty” terms, offering clarification of their limits and pointing out that the majority of bees which are described as oligolectic (Cane and Stipes, 2006) visit plants with good rewards that are also visited by a variety of different species and should

therefore be considered generalists; whereas more generalist, or polylectic, bees tend to visit flowers with few flower visitors and low rewards, leading to asymmetry in the plant-pollinator relationship (Jordano *et al.*, 2006; Vásquez and Aizen, 2004; 2006).

Examples of specialisation from the point of view of the plant can also be readily found, for example the pollination of the violet *Viola cazorlensis* solely by the hawkmoth *Macroglossum stellatarum* (Herrera, 1993), and the various species of *Passiflora* visited by only one or a few bat, hummingbird or *Xylocopa* bee species (e.g. Amela Garcia and Hoc, 1998; Kay, 2001; Varassin *et al.*, 2001; Storti, 2002; Holland and Lanza, 2008). In addition, many species of deceptive orchids are only pollinated by a single (or narrow range of) visitor species (e.g. Boyden, 1980; Bierzychudek, 1981; Dafni and Ivri, 1981; Nilsson, 1983; Peter and Johnson, 2008), and many oil-collecting bees have rather specialised relationships with particular plants (e.g. Steiner, 1989; Bittrich and Amaral, 1996; Steiner and Whitehead, 1996; 2002, see chapter 4).

Morphological features of flowers have often been used as a measure of their level of specialisation or generalisation, and in particular the “openness” of a flower is often seen as a determinant of its specialisation. According to the definitions of Faegri and van der Pijl (1979), open flowers include those with dish (also known as bowl), bell and brush shapes, while closed flowers include gullets, flags and tubes (see fig. 1). Using these groupings, Olesen *et al.* (2007) assigned over 1400 flower species to an appropriate category, and calculated a “flower visitor generalisation level” (L), the number of visitor species at a given site, and a relative value (L/A), the proportion of total visitor animal species that visited a given flower species. The level of flower openness was not well-correlated with either L or L/A, although 6 of the 10 most generalised flowers in terms of flower visitors were within the dish or bowl shaped category. On similar lines flag and gullet shaped flowers have been found to be more specialised in terms of number of visitor species, being visited by only a few different types (Ramírez, 2003).

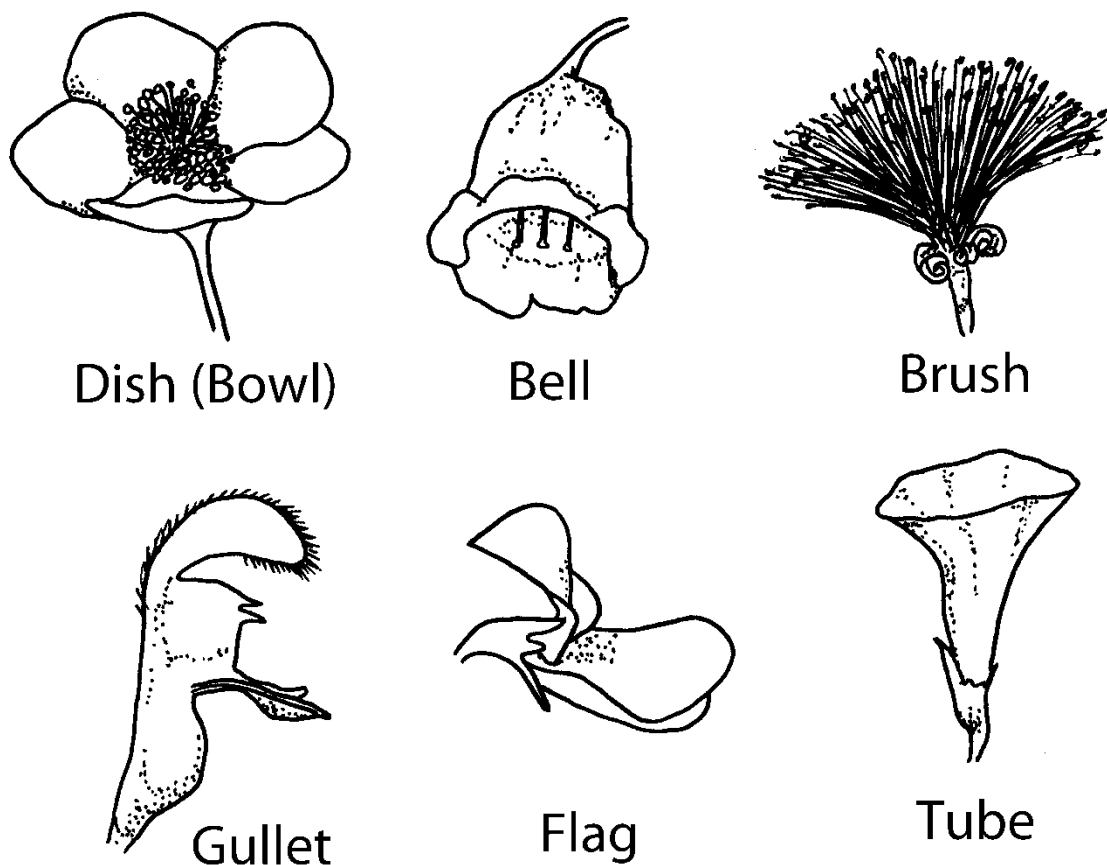


Fig. 1: The six basic blossom types.

From the perspective of the visitor, generalisation in flower visiting, and gathering a reward from all plants that it meets regardless of species, may be considered the more effective strategy (Gómez and Zamora, 2006). Contrasting with this, however, specialisation and floral constancy in visitors, thereby allowing transference of pollen to the stigmas of conspecifics, is the most effective strategy from the point of view of the plant. With such a mismatch between the most effective strategies for both participants, it is perhaps surprising that there are widespread examples of specialisation and generalisation, rather than a compromise interaction somewhere in the middle of the two extremes.

It has been suggested however that generalisation can itself become an adaptive strategy for plants, where pollinators are strong agents of selection, but have similar levels of effectiveness, flower preference etc., and therefore act together to generate floral

adaptations (Gómez and Zamora, 2006; Willmer, 2011). Two different phenomena should be distinguished here: non-adaptive generalisation and adaptive generalisation. Non-adaptive generalisation is where spatiotemporal variability may mean that extrinsic factors override the selective pressures of pollinators, or that selective regimes may fluctuate over time and space. Adaptive generalisation is where different types of visitor impose similar selective effects on floral traits (Gómez and Zamora, 2006). For example, flowers of *Erysimum* are visited by more than 100 species of insect, however this degree of generalisation varies between local and regional populations. Plants with an intermediate level of generalisation have the highest seed set, suggesting that there is an optimum level of generalisation for any given generalised plant species (Gomez *et al.*, 2007).

However, taking into consideration that, from the point of view of the plant, selection should be expected to favour traits that lead to increased visitor efficiency, visitor fidelity and specialisation, and avoid visitation from poor pollinators or parasites, we would expect that there would be at least some level of specialisation and floral constancy, or effective pollination of plants would not occur due to a loss of viable pollen when pollinators do not show floral constancy. Theoretically, there are certain conditions in which specialisation to a particular pollinator may be most effective: where pollinator availability, abundance and behaviour are reliable; when plants are long-lived, and/or capable of vegetative reproduction if pollination fails; and when the plants are rare or sparsely distributed, and therefore specialisation to visitors with high floral constancy will avoid the clogging up of stigmas with heterospecific pollen grains and increase the chances of effective pollination by conspecific pollen grains (e.g. Charlesworth, 1989; Wesselingh *et al.*, 2000; Gardner and Macnair, 2000). Therefore, in environments where pollinators are unpredictable from year to year, and where annuals and weedy plants are attempting to colonise unpredictable habitats with unknown pollinator abundance and availability, generalisation would be expected to be the more effective strategy (Gómez and Zamora, 2006).

Specialisation and Generalisation: Problems with Terminology

The terms specialisation and generalisation suggest a dichotomy, whereas in reality obligate specialisation and extreme generalisation are at opposite ends of a continuum in resource use and niche breadth (Waser *et al.*, 1996; Waser and Ollerton, 2006). Furthermore, there is much confusion over terminology in the literature concerning specialisation and generalisation, and the terms specialised and generalised can be used to describe both the partners in the interaction, as well as the nature of the interaction between them.

Several authors have raised concerns over this confusion in terminology (Renner 1998; 2006; Armbruster *et al.*, 2000; Vázquez and Simberloff, 2003; Minckley and Roulston, 2006; Ollerton *et al.*, 2007; reviewed in Willmer, 2011), and the main issues that have arisen are described below.

The factors involved in determining the extent of specialisation or generalisation often involve a measure of the resource items used by the organism that is being classified; however in a mutualism such as pollination, this can be viewed from the perspective of either participant, in this case the plant or the animal. A bee that visits many different flowers will be termed a generalist, whereas a plant that is visited by only that particular bee species would be termed a specialist.

As well as referring to the participants, the terms specialised and generalised can be applied to the interaction itself. Therefore a bee visitor to a flower may be termed a specialist or a generalist from observations on its foraging behaviour, and the interaction between it and the flower it visits may also be termed specialised or generalised. These two approaches have been termed “evolutionary specialisation or generalisation”, a process, and “ecological specialisation or generalisation”, a state (Armbruster *et al.*, 2000). The term “functional specialisation or generalisation” has also been used by some authors in the similar role as the latter term (Dalsgaard *et al.*, 2008). Ecological specialisation or

generalisation may be easier to measure in principle, while evolutionary specialisation or generalisation is more difficult to approach (Armbruster *et al.*, 2000).

As a further problem, the relationship between animal species and plants is not static, and can vary across both space and time. A visitor species that has a specialised relationship with a plant species in one habitat, or at one time of year, may have a more generalised relationship involving other plant species in a different habitat or at a different time. In addition to this, a certain bee species may visit only one plant species in a single foraging bout, and therefore be termed a specialist; however over the course of its lifetime an individual bee may visit a variety of different species, and be considered a generalist. Also, in social bees in particular, individuals may be specialised in their flower visiting behaviour, whereas the colony as a whole may be considered generalised if individuals visit different plant species.

Flowers can also be termed specialised or generalised on the basis of morphological features without any regard for actual visitors. Following the pollinator syndromes approach, features such as flower shape, colour and scent may be used to define the specialisation of a species, without the confirmation of visitor species observed. This is described as phenotypic specialisation or generalisation (Ollerton *et al.*, 2006), and is distinct from the cases of evolutionary and ecological specialisation or generalisation described above (Armbruster *et al.*, 2000).

Specialisation or generalisation may also be measured by the number of different species visiting a plant. When relative abundances of these species are included in analyses, a conclusion of generalisation may turn out to be inaccurate (Herrera, 2005). It is therefore important when investigating plant-pollinator interactions to include not only a record of species, but their abundance and their quality of visit (see below).

We also must consider the problem of absolute and relative specialisation or generalisation. For example, a plant in a small or isolated habitat may be classified as

specialised purely because of the low abundance of pollinators in an area, or it could be classified as generalised because it is utilising a high proportion of all the available pollinators in the area.

Finally, there is the issue of fundamental specialisation or generalisation and realised specialisation or generalisation, in the same way that the niche breadth of a given species may be fundamental or realised. Fundamental specialisation refers to all of the possible positive interactions for a species in all possible ecological interactions (Vázquez and Aizen, 2006), whereas realised specialisation is the actual level of specialisation recorded in a given environment and its conditions. In practice, the latter is used more frequently in studies, and is easier to measure.

Considering the problems outlined above, the argument has been made that the use of the terms specialist and generalist should not be used to describe the interaction, but rather to refer solely to the plant or the animal visitor. In particular, a specialised flower species should refer to a species that is effectively pollinated by one or a few animal visitor species (Renner and Feil, 1993; Armbruster and Baldwin, 1998; Armbruster *et al.*, 2000; Fleming *et al.*, 2001), and a specialised visitor should refer to an animal species that collects resources from a narrow range of flower species, for example a monolectic or oligolectic bee species (Cane and Sipes, 2006).

Using this approach, a generalised interaction could involve a specialised visitor or a specialised plant species, or even both, and it is not possible to extrapolate from the degree of specialisation of a plant species to the degree of specialisation of its animal visitors (Renner, 1998; Armbruster *et al.*, 2000; Waser and Ollerton, 2006).

Questioning the Pollination Syndromes Concept

While pollinator syndromes have long been used as a basis for determining the pollination interactions of a given plant species, more recently some authors have

questioned the validity of this concept. Many ecologists have claimed that pollinator syndromes are over-simplified, and that the majority of plant species are generalised in their pollination, rather than specialised.

On this view, while the evolution of floral diversity seems to be based upon specialised relationships with pollinators, the vast majority of angiosperms are considered generalists in their pollinator requirements, being serviced by a spectrum of taxonomically diverse animals (Waser, 1983; Renner and Feil, 1983; Waser *et al.*, 1996; Ollerton, 1996). In addition, the range of pollinators may vary over the course of an individual plant's flowering period (Gross and Werner, 1983; Ashman and Stanton, 1991), between seasons (Pettersson, 1991; Fishbein and Venable, 1996) and over the lifetime of a plant (Herrera, 1995) due to fluctuations in pollinator assemblages over these time periods. Ollerton (1996) described this mismatch between phenotypically specialised yet ecologically generalised plants as a "paradox".

Herrera (1996) argued that, while evidence for pollinator-mediated selection on some floral traits was available (e.g. Galen and Newport, 1988; Nilsson, 1988; Campbell, 1989; Galen, 1989; Schemske and Horvitz, 1989; Robertson and Wyatt, 1990; C.M. Herrera, 1993; Anderson and Widén, 1993 (and more has accumulated since, e.g. Sandring and Ågren, 2009; Sletvold *et al.*, 2010)), this does not necessarily mean that the phenomenon occurs universally, or on all floral traits. The indiscriminate application of pollinator syndromes to plants is suggested to have exaggerated the degree of adaptations of plants to pollinators (Baker, 1963; Macior, 1971; Waser, 1983; Herrera, 1996). Herrera suggested that, in practice, the floral traits that characterise pollinator syndromes, such as colour, flower shape and nectar production, are poor predictors of the pollinators of a given plant species, and there is evidence that syndromes are of little value in explaining interspecific variation in pollinator composition. Flowers of *Delphinium nelsonii*, possessing blue flowers classic to the bee-pollination syndrome, are pollinated by bees as expected in the Rocky Mountains of North America, but also visited by hummingbirds in Western Colorado, with both species

found to deliver pollen of equal quality (in terms of outcrossing distance), though bees deposit more pollen per visit (Waser and Price, 1990). Herrera (1988) found variation in pollinators of *Lavandula latifolia* both spatially and temporally, and McCall and Primack (1992) found that preferences in flower colour within insect species varied between communities, suggesting that pollinator syndromes may be community specific.

Contrasting with the few examples of specialised plant-pollinator interactions, the flowers of many plant species are visited by a diverse assemblage of pollinators; for example the neotropical herb *Calathea ovandensis* (Horvitz and Schemske, 1990), the shrub *Hormathophylla spinosa* (Gómez and Zamora, 1999), plants of the genus *Calochortus* (Dilley *et al.*, 2000) and the shrub *Jasminum fruticans* (Thompson, 2001), all of which are visited by a diverse range of animals from various taxa (see Chapter 7). Thompson (1983) noted the rarity of obligate specialist interactions in pollination biology, and pointed out that pollination webs existed, as in food webs, and were likely to be complex and cross-connected. Since then, several authors have proposed that, while many flowers show adaptations indicating specialisation to particular pollinator types, observations often detect multiple types of visitors to flowers (Herrera, 1988; 1996; Waser *et al.*, 1996; Waser, 1998). This concept was formalised by Ollerton (1996) as a “paradox”, where flowers may appear to be phenotypically specialised but ecologically generalised. The long-standing view that plant-pollinator evolution moved towards increased specialisation is now being challenged, most notably by Waser and Ollerton (2006; and authors within), and is seen by some as far from universal. Floral generalisation is often predicted as the best strategy for a flower visitor when abundances of preferred species are low, or fluctuating in time and space (Waser *et al.*, 1996), and specialisation and floral constancy are not to be expected, or to be viewed as indicators of an advanced pollinator (Waser, 2001; 2006). It has been pointed out that, where abundances of a key pollinator become low, a specialised plant would become vulnerable; similarly, a visitor adapted towards a particular plant species would suffer should abundances of that plant decline (Buchmann and Nabhan, 1996). A high reciprocal

specialisation between plant and pollinator would also be risky where abundances or quality of interactions varies over time (Waser *et al.*, 1996; Renner, 1998; Vásquez and Simberloff, 2002; Memmot *et al.*, 2004), and the risks would be even greater in an obligate one-to-one relationship.

Plants may be effectively pollinated even if the floral traits involved have not evolved in relation to their present pollinators, therefore some floral traits can be considered exaptations rather than strict adaptations (Herrera, 1996). There are concerns over the effective pollination of imported crops and plant species, illustrated by the often quoted examples of alfalfa, pollinated by the solitary ground-nesting alkali bee *Nomia melanderi*, and the leaf-cutting bee *Megachile rotundata*, which are commercially managed for pollination of the crop (Cane, 2002); and red clover, which is dependent on bumblebees for effective pollination, shown by a dramatic increase in yield following the importation of bumblebees when the crop was introduced to New Zealand, despite adequate native populations of honeybees (Plath, 1925; Fussell, 1992; Rao and Stephen, 2007). Many plants which are introduced into foreign continents, however, are able to be successfully pollinated by completely new pollinator assemblages (Rick, 1950; Milton and Moll, 1982; Podoler *et al.*, 1984; Kohn and Barrett, 1992). Examples such as these can also be seen in a more natural context, without the influence of anthropological introductions, such as in the many species of typical “bird-flowers” found on the Canary Islands, which are pollinated effectively by opportunistic sylviid warblers as no true “flower birds” exist on these islands. It is thought that the current pollinators of these flower species are not the original pollinators which may have caused the original selective pressure towards bird-flower traits, but they are still able to effect efficient pollination (Vogel *et al.*, 1984; Olesen, 1985).

Following from the many authors who have questioned the validity of pollinator syndromes (e.g. Waser *et al.*, 1996; Ollerton, 1998); Ollerton and Watts (2000) tested the predictive powers of 11 classic pollination syndromes. They found that the close proximity of these syndromes in phenotypic space, and their response to a random trait-deletion

sensitivity analysis to test the robustness of their results, led to difficulty in distinguishing between them on the basis of flower traits (see fig. 2). They proposed that the 11 syndromes resolve into four distinct groups in phenotypic space: the mammal and bat group; the fly, carrion fly, beetle and wasp group; the bee and butterfly group; and the moth and hawkmoth group, (with bird pollination as an intermediate between the bee and butterfly group and the moth and hawkmoth group). They suggested that syndromes may provide a useful first indication of a plant's pollination ecology, but should not be used to draw conclusions in the absence of field data.

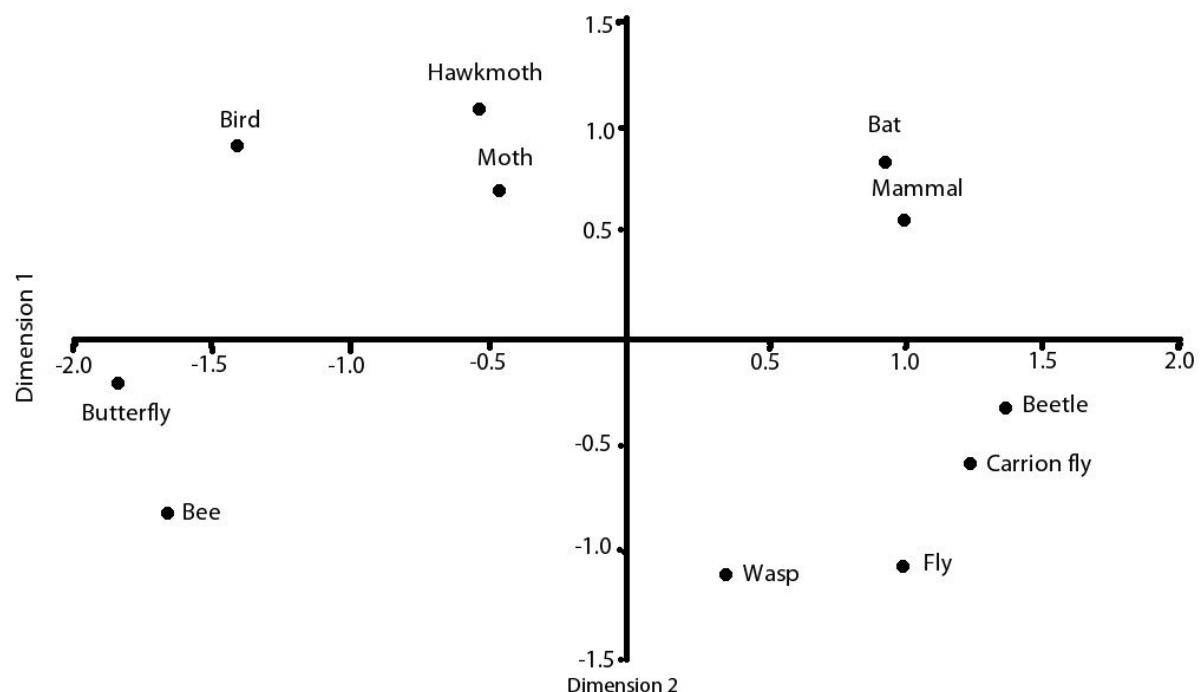


Fig. 2: Multidimensional scaling analysis of classical pollination syndromes. Reproduced from Ollerton and Watts (2000).

Later, Ollerton *et al.* (2009) again tested the predictive powers of flowers in six communities in three continents. They found that that majority of species studied did not fall within the discrete pollinator syndromes, and in approximately two thirds of the plant species the most common pollinator could not be successfully predicted by placing the species in the syndrome closest to it in phenotypic space.

Questioning Specialisation

The concept of pollinator syndromes suggests that floral trait evolution tends towards specialisation (e.g. Schluter, 2000). A more recent stance, however, is that generalisation in pollinator syndromes is the “norm”. Many mutualisms, pollination included, evolve from relationships which are essentially exploitative or parasitic in their nature, and it has previously been assumed that co-evolution should lead to increasing specialisation and stability where the aims of both participants are realised. It has become clear however that the “goals” of the plant and the visitor are different and often conflicting, therefore the outcome would be expected to be a more generalised compromise (Waser and Ollerton, 2006).

According to many authors, generalisation may be widespread among natural pollination systems (e.g. Herrera, 1996; Waser *et al.*, 1996; Armbruster *et al.*, 2000; Oleson, 2000), likewise, these authors propose that extreme specialisation may only be found in plants that provide neither nectar nor pollen as rewards, but rather unusual rewards such as seeds, resins, non-volatile oils, or fragrances (Buchman, 1987; Armbruster, 1997; Pellmyr, 1997; Fleming and Holland, 1998, Steiner and Whitehead, 2002), or those where no reward is offered at all and pollination is by deceit (Dafni and Bernhardt, 1990). In such systems, a reduction in common rewards, such as pollen and nectar, allows for the evolution of specialisation by discouraging the visits of other, non-target pollinators (Fenster and Dudash, 2001).

Contrasting null-model analyses (the generation of randomized data sets in the absence of a hypothesized mechanism) with patterns of specialisation in five plant-pollinator interaction webs, Vázquez and Aizen (2003) suggested that generalist relationships are much more common than was previously thought. Following on from this, Vázquez *et al.* (2005) used mathematical models to show that the more generalist an animal visitor is, or the more frequently it participates in interactions, the more it contributes to plant

reproduction, regardless of its effectiveness on a per-interaction basis, a concept covered in further detail below. In fact, some researchers believe that specialisation is rare (Jordano, 1987; Waser *et al.*, 1996; Memmot, 1999; Oleson and Jordano, 2002), and that generalist relationships may be a profitable evolutionary outcome under certain circumstances. Specialist relationships between mutualistic partners are thought to be more susceptible to extinction caused by anthropogenic factors such as habitat fragmentation (Rathcke and Jules, 1993; Waser and Ollerton, 2006), with generalist relationships being able to “bounce back” more effectively when exposed to stresses. If the abundance of a key pollinator of a specialised plant was to decline, the plant would become vulnerable, as would a visitor adapted for a single type of plant if faced with a decline of that species (Buchmann and Nabhan, 1996). A high level of specialisation between a plant and a visitor is particularly risky where the abundance or quality of interactions varies over time (Waser *et al.*, 1996; Renner, 1998; Vázquez and Simberloff, 2002; Memmott *et al.*, 2004).

Plant-pollinator interactions are essentially a type of network, and parallels have been drawn between them and studies of networks from outside the field of biology. Tests of distributed communication systems have found that complex networks are more tolerant of random extinctions than more simple networks, although this comes at the cost of attack survivability when a component with many links is ‘attacked’ or removed (Albert *et al.*, 2000). This concept was shown to apply to pollinator networks of plants and animal visitors by Memmot *et al.* (2004), who found that those interactions with the most links between plant species and pollinators were more robust to losses from the network. It has also been shown that mutualistic networks are highly nested, leading to asymmetry in the organisation of the community, and assortative mixing, where specialists interact, not with other specialists, but with more stable generalists (Newman, 2002; Bascompte *et al.*, 2003). Perhaps this low observation of specialisation is partly due to our own impact on the environment, forcing plants and pollinators to “hedge their bets” with more generalised interactions, to increase their survival rate should one partner be removed.

Visitation Webs

A recent innovation in pollination studies is the construction of what are termed “Pollination Webs”, but which may be more accurately described as “Visitation Webs”, as described below, and they will be referred to as such for the remainder of this thesis. Visitation webs are based upon ecological studies of food webs, and involve the recording of all visitors to all plants in a given community, which are then mapped out as an interacting web structure which can be analysed statistically.

One of the first visitation webs was created by Jordano (1987), who investigated all the possible interactions of animals and plants in a community, calculating the connectance value, or the proportion of realised interactions, between them, as well as the magnitude of the interactions. The conclusion of this study was that visitation webs, or networks as they have been more recently termed, are more generalised than was previously assumed.

Memmot (1999) calculated several visitation webs for British meadow communities, finding that, although there were some specialised insects within the webs, their preferred plant species were also visited by a number of more generalist species which also visited many other plant species, therefore leading to high levels of connectance within the web. Following this, a visitation record set collected by Robertson (1928, previously used by Waser *et al.*, 1996 to argue for generalisation) was used to calculate a visitation web of Colorado communities, which showed varying levels of generalisation between visitor groups, with flies being the most generalised in their flower visiting, followed by bees, and moths being more specialised in their visiting behaviour (Memmot and Waser, 2002, see fig. 3).

Generally, a high connectance value in a web suggests a high level of generalisation (Dunne *et al.*, 2002), however the use of connectance values as a means of comparison between webs is limited as the calculated values of connectance rely heavily on web size and sampling effort (Olesen and Jordano, 2002; Medan *et al.*, 2006; Petanidou and Potts,

2006; Willmer 2011), therefore an effective web requires an extremely large sampling effort covering the whole flowering season, and over several seasons, as flower visitation may vary greatly between years (Herrera, 1988; Williams *et al.*, 2001).

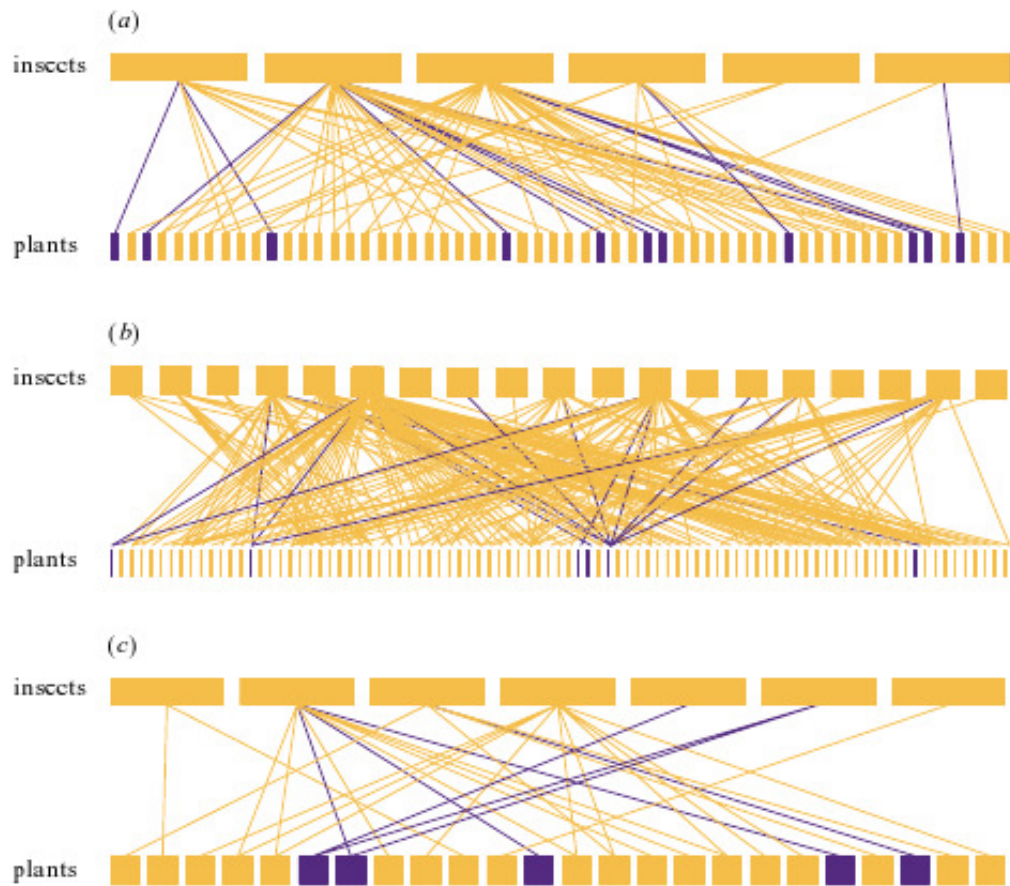


Fig. 3: Example of a “Pollinator” web for a community in Central USA showing the interactions between insect visitors and native and alien plant species. Alien plant species are highlighted in blue. Interactions shown are involving: (a) Anthophoridae (*sensu stricto*, Hymenoptera); (b) Sarcophagidae (Diptera); and (c) Sphingidae (Lepidoptera); (from Memmot and Waser, 2002).

High values of connectance, and therefore levels of generalisation, are not universal amongst visitation webs however. It has been shown that connectance values are lower in communities at high altitudes, on islands or within the tropics, and higher connectance values are more common in high latitudes and in the lowlands (Olesen and Jordano, 2002).

An alternative method to calculating connectance values is the calculation of the linkage of a web, in basic terms the number of taxa with which a given species interacts. This term is similar to the measures of “phily” for plants or “trophy” for animals used in earlier literature (cf. Petanidou and Potts, 2006). Linkage increases with network size, or the sum of all plant and visitor species within the community studied, therefore linkage values for small island communities are low (Lundgren and Olesen, 2005). Using this method in the study of visitation webs means that the general strengths and importance of links within a network can be measured, and the link strength can also be factored into analysis, for example in Blüthgen *et al.* (2007), where interaction frequency was used as a measure of link strength.

Arguments for Pollinator Syndromes, and the Extent of Specialisation

While the authors above have questioned the predictive powers of pollination syndromes and the extent to which the degree of specialisation is prevalent, the studies from which these arguments have been drawn may be flawed in some aspects of their design. In addition to the confusion surrounding the terminology of specialisation and generalisation described above, there are several factors influencing pollination interactions which are often overlooked in pollinator studies.

As with all field experiments a variety of outside factors can have an influence on the experiment, from temperature, to precipitation, to the interactions of other species. The influences of these outside factors, if not appropriately accounted for, may influence the outcome of studies and lead to unreliable or misleading results. In the case of pollination

biology, studies into the effects of herbivory on plant-pollinator interactions have surfaced in the past few years where the pollinator visitation rate was positively correlated with plant fecundity in the absence of, but not in the presence of, herbivory; for example ungulate herbivory on *Erysimum mediohispanicum* (Gómez, 2003 and 2005). These studies concluded that factors which act upon the same fitness components as pollinators can prevent a plant becoming adapted and specialised towards a particular pollinator by obscuring or counteracting the phenotypic selection exerted by them. It has also been shown (Herrera, 1988; Petanidou and Ellis, 1993; Williams *et al*, 2001) that there can be a significant difference in both flora and fauna and therefore plant-pollinator interactions between different years, and so studies that are only one year or shorter in length should be treated with caution. The small scale of some pollinator studies creates another problem. Studies at anything less than a community level will not account for important influences such as inter- and intra-specific competition. Densities of other pollinators in the area must also be recorded, as it has been shown that levels of generalisation can vary with forager density (Fontaine *et al*, 2008). In line with Optimal Foraging Theory predictions, that study found that a higher density of *Bombus terrestris* led to a broader diet, while lower densities allowed the feeding behaviour of the bees to become more specialised. Failure to account for the above variables and outside factors in pollination studies may influence the outcome of such studies and obscure the true nature of the plant-pollinator relationships within them.

Perhaps the most glaring error in pollinator studies, however, is the confusion of flower visitation with actual pollination. In order to be an effective pollinator, a flower visitor must be able to pick up pollen as its body moves past the anthers of a flower, and then deposit a sufficient amount of this pollen, while it is still viable, on the stigma of the next (con-specific) flower it visits. Usually, to facilitate this, the animal visitor will be a good **physical fit** to the flower in terms of size and shape, so that when it lands on the flower, or inserts its mouthparts to feed, a particular part of the animal's body will come into contact with the anthers of the flower. A good example is the strong match between flower tube

length of *Dianthus carthusianorum* and proboscis length of the two butterfly species *Inachis io* and *Melanargia galathea* (Bloch, 2009). Conversely, ill-fitting flower visitors who do not come into contact with the reproductive parts of the flower will not effectively pollinate that flower, regardless of how frequent their visitation is, as for *Dianthus carthusianorum* mentioned above, where a mismatch between floral tube length and proboscis length was shown to decrease pollinator efficiency (Bloch, 2009).

Appropriate **surface structures** such as hair, feathers, scales or fur will also allow pollen to adhere to the body more easily, whereas pollen will not adhere so well to shiny or waxy surfaces, and may even be damaged by surface secretions, for example the antimicrobial secretions of ants (Beattie *et al.*, 1984; 1985; Hull and Beattie, 1988; Peakall *et al.*, 1990).

In terms of **behaviour**, an effective pollinator will visit after dehiscence has begun, but before pollen depletion has occurred, otherwise its visit will be of little value in fulfilling the male role of the flower. The visitor should also visit flowers when the stigma is receptive to incoming pollen in order to fulfil the female role of the plant. Visitors' movements on and within the flower during feeding, known as flower handling, should also allow for pollen to be picked up on an appropriate part of the body which can subsequently deposit the pollen on the stigma of the next flower. The **handling time** of an insect will affect how many flowers it can visit in a given period of time, and therefore its pollination effectiveness. The directionality and speed of movement between plants will also affect pollen dispersal. Effective grooming or eating of pollen by visitors will also lower pollinator effectiveness.

Most importantly, the **floral constancy** of a given visitor species, in other words the likelihood that it will go on to visit another flower of the same species, plays a huge part in the effectiveness of a given visitor. A high level of floral constancy, or specialisation, from a visitor is highly beneficial to the reproductive success of the flower (Willmer, 2011). Floral constancy implies that a flower visitor moves sequentially and reliably through conspecific

flowers, either for pollen, nectar or unusual rewards such as oils or scents. Floral constancy is beneficial to the visitor as well as to the plant, as constancy to a flower species, and/or a site, can minimize travel distances, handling times and overall foraging times, therefore increasing the foraging efficiency of the visitor. In terms of benefits to the plant, high floral constancy will ensure reproductive isolation and maintain species differences. Floral constancy can be divided into “passive constancy” (Thompson, 1982), where flowering plant species are closely aggregated, or where only one plant species is flowering, effectively enforcing intraspecific movement; and “active constancy”, where several plant species are flowering but only one is visited. Floral constancy can be used to refer to successive trips, or even trips on successive days (e.g. Free, 1970), but is more commonly used to refer to behaviour within a single trip.

Finally, **physiological** aspects of a pollinator, such as its ability to regulate heat or water balance, will influence its effectiveness at pollinating. Such factors will affect the times of day at which a visitor can be effective, as well as the length of its foraging bouts. In addition to the more sophisticated physiology of bats and birds, some insects (including many bees, a few hoverflies and some moths and beetles) show some degree of endothermy, notably the ability to generate heat to initiate flight when ambient temperatures are low (e.g. Willmer, 1983; Willmer and Stone, 2004).

Pollinator studies have often concentrated on visitor frequency as a measure of the effectiveness of a given pollinator, assuming that all visitors are equal in their pollinating performance. Given the above, it is clear that not all animals which feed upon or land on a flower are effectively pollinating it. Illegitimate visitors may have several ways of gaining rewards from flowers without effecting pollination. Some may be poor physiological fits; others may have low floral constancy and “lose” large amounts of pollen on non-conspecific flowers; and others still may be considered cheats in their flower visiting, collecting rewards without offering any reproductive benefit to the flowers. Inouye (1980a) offered a useful clarification of the terminology of floral larceny, both of pollen and nectar. Nectar can be

removed via primary or secondary robbery, the former involving the making and use of a hole, usually in the corolla, and the latter the use of an existing hole made by others, both of which result in nectar depletion and flower damage, and usually only a slight chance of direct or indirect pollination. Nectar can also be removed by theft, where flowers are entered in the usual manner but a morphological mismatch precludes pollination, or by baseworking, where visitors gain basal entry to corollas between the petals or sepals; both of which result in nectar depletion, though no damage, and a slight chance of direct or indirect pollination. Pollen may be removed by robbery, where pollen is gathered and tissues of the flower are damaged; or theft, where pollen is removed without damage to the floral tissues. Both these methods of larceny result in pollen depletion, the former also resulting in damage to floral tissues, and no effective pollination.

What is needed, therefore, is good data on “visitors” versus “pollinators”. “Pollinator webs” (e.g. Jordano, 1987; Memmot, 1999; Waser *et al.*, 1996; Memmot and Waser, 2002) and other studies questioning the prevalence of specialisation in nature (e.g. Waser and Ollerton, 2006) have recorded the visitor species and frequency of visitation to each flower species as a base for their analyses. The study of specialisation and generalisation in natural communities by Waser *et al.* (1996) for example, used visitation surveys to conclude that generalisation was the more common strategy. The study, however, while referring to these surveys as “pollinator surveys”, made no distinction between mere visitation and actual, effective pollination of flowers, only taking the frequency of visits into account.

Tests of the effectiveness of pollinator syndromes as predictors of intended pollinators have usually relied on visitor frequency as an indicator of the “intended” pollinator species, matching the pollinator indicated by floral traits, or, in some cases, highlighting a mismatch. The apparently sapromyophilic *Tacca chantrieri* for example, found to be largely self-pollinating (Zhang *et al.*, 2005), or the Neotropical palm *Astrocaryum vulgare*, showing traits of both beetle and wind-pollination but only being effectively pollinated by beetles (Consiglio and Bourne, 2001). Ollerton *et al.*, (2009) determined that pollinator syndromes

were ineffective at determining the most frequent flower visitor in many species studied; however there is no mention of the effectiveness of visitors, and the most frequent visitor is not necessarily the intended or most effective. In addition, studies purporting to defend the effectiveness of pollinator syndromes at predicting pollinators have also relied on visitor frequency as an indication of the intended pollinator (e.g. Wilson *et al.*, 2004; 2006; Streisfeld and Kohn, 2007).

Relying on visitation frequency values, or quantity of visitors, rather than quality, will lead to inaccurate conclusions on the effectiveness of pollinator syndromes as a determinant of intended pollinators and the nature of plant-pollinator interactions, and in many cases the nature of the relationship between the two will be over-generalised. While visitor frequency does play a part in the effectiveness of a visitor (in that an infrequent visitor, either through pollinator rarity or low flower constancy, is likely to provide insufficient pollination), the quality of a pollinator is also important as a visitor may visit frequently yet provide an inferior pollination service. To gain an accurate understanding of the predictive powers of pollinator syndromes and the true level of specialisation and generalisation in natural communities it is therefore necessary to include a measure of pollinator quality, or pollinator effectiveness, in pollination studies.

Pollinator Performance

Past Studies of Pollinator Effectiveness and Efficiency

As can be seen above, the ability to assess the performance of flower visitors in order to determine their ability to effectively pollinate a flower, and their relative performance in comparison to other flower visitors, is extremely important if we hope to achieve accurate and meaningful results from pollinator studies. Including a measure of pollinator performance can improve studies in a variety of areas, such as comparing performance to flower morphology, habitat type, temporal or spatial patterns of plant distribution (both at an individual and population level), and changes in the environment (e.g. Potts, Dafni and

Ne'eman, 2001). Pollinator performance is also of importance to agronomists and plant breeders, who are interested in improving seed or fruit set in crops (e.g. Morison *et al.*, 2000); conservation biologists interested in the effects of extinction of particular pollinators on the reproduction of rare plants (Bond, 1994; Kearns *et al.*, 1998; Biesmeijer *et al.*, 2006); and evolutionary biologists testing the predictive powers of pollinator syndromes, who are interested in whether floral traits are adaptations to specific pollinator species, types or assemblages (Waser *et al.*, 1996; Johnson and Steiner, 2000; Fenster *et al.*, 2004).

As described above, plants within a certain pollination syndrome are thought to have evolved in response to a suite of traits of the pollinator with the best “performance” which contributes the greatest to plant reproductive success (Stebbins, 1970; Grant, 1971; Wilson and Thompson, 1991; Olsen, 1997), and these pollinators are thought to have shaped the evolution of both floral characteristics (Campbell, 1989; Wilson, 1995; Wilson and Thomson, 1996; Schemske and Bradshaw, 1999) and of plant lineages (Stebbins, 1970; Crepet, 1983; Grimaldi, 1991). Stebbins (1970) proposed the “Most Effective Pollinator Principle”, stating that “the characteristics of flowers will be moulded by those pollinators that visit most frequently and effectively”. Most recent tests of pollinator syndromes, however, and the resultant specialisation and generalisation of plant-pollinator interactions have ignored the first part of this principle and relied upon visitation frequency only as a measure of a pollinator’s importance. Clearly, given the immense variation in characters such as size, shape, floral constancy and behaviour, not all flower visitors will pollinate a flower equally, and it cannot be assumed that the most frequent visitor is also the most effective.

Although the concept of assessing pollinator performance has been around for over 30 years, until the recent review of Ne'eman *et al.* (2010; described below) there was no general consensus on the definitions of terminology within the subject, nor was there a commonly accepted conceptual framework or methodology. The terms effectiveness, efficiency, efficacy and importance have all been used, often interchangeably, to refer to a variety of aspects pertaining to pollinator performance, leading to great confusion within the

literature. Without defined terminology and methodology, comparisons between multi-site and multi-year investigations, required for international, long-term assessments of pollinator status and trends, are almost impossible.

Basing their study on a comprehensive review of the literature, and focusing on 70 representative studies carried out between 1975 and 2007, Ne'eman *et al.* produced a summary of the various terminology used across the years, and the methods of assessing pollinator performance each term referred to, a simplified version of which is shown below (see table 1).

Term Used	What is measured/estimated	Source
Direct measure of pollen deposition success (as pollen deposition on stigma)		
Pollination Intensity	The number of pollen grains deposited on a virgin stigma after a single visit by a specific pollinator	Primack and Silander 1975; Rodet <i>et al.</i> , 1998; Falque <i>et al.</i> , 1996; Mitchell, 1997
Pollination efficiency	The number of pollen grains deposited on a virgin stigma after a single visit by a specific pollinator	Waser and Price, 1990; Ashman and Stanton, 1991; Pettersson, 1991; Willmott and Burquez, 1996; Cane and Schiffhauer, 2001; Hiei and Suzuki, 2001; Bloch <i>et al.</i> , 2006
Pollination efficacy	The number of pollen grains deposited on a virgin stigma after a single visit by a specific pollinator	Cane and Schiffhauer, 2003
Per visit effectiveness	The number of pollen grains deposited on a virgin stigma after a single visit by a specific pollinator	Mayfield <i>et al.</i> , 2001
Pollinator effectiveness	The number of pollen grains deposited on a virgin stigma after a single visit by a specific pollinator	Dieringer, 1992; Osorio-Beristain <i>et al.</i> , 1997
Stigma pollen load per visit	The number of pollen grains deposited on a virgin stigma after a single visit by a specific pollinator	Kearns and Inouye, 1993; Inouye <i>et al.</i> , 1994
Pollination effectiveness	The number of pollen grains deposited on a virgin stigma after a single visit by a specific pollinator	Motten <i>et al.</i> , 1981; Suzuki <i>et al.</i> , 2002
Pollination efficiency	Cumulative pollen deposition on stigma during the flower's lifespan	Arroyo and Dafni, 1995; Tandon <i>et al.</i> , 2001
Pollination intensity	Cumulative pollen deposition on stigma during the flower's lifespan	Vaissière, 1991; Mitchell, 1997; Falque <i>et al.</i> , 1996

Pollination level	Cumulative pollen deposition on stigma during the flower's lifespan	Morandin <i>et al.</i> , 2001
Estimating pollen deposition success with pollinator behaviour parameters		
Pollination effectiveness	Number of pollen grains removed from anthers per single visit	Suzuki <i>et al.</i> , 2002
Pollination efficiency	Percentage of flower visits with stigma touch in a given foraging bout	Dafni <i>et al.</i> , 1987
Pollinator efficiency	Visit frequency in a given flower	Calzoni and Speranza, 1998
Pollinator efficiency	Time it takes for a flower visitor to visit a given number (10 or 50) of flowers	Richards, 1987
Combining pollen deposition success with pollinator behaviour parameters		
Pollination efficiency	Fraction of the pollen load of the vector that was deposited on the stigma in a single visit	Kearns and Inouye, 1993; Lau and Galloway, 2004
Vector pollinating efficiency	The relative pollen load contributed by a specific pollinator	Inouye <i>et al.</i> , 1994
Absolute pollination efficiency	Probability of the removed pollen reaching the target stigma	Galen and Stanton, 1989
Index of pollen transfer effectiveness	Mean flower number visited per unit time, multiplied by mean number of pollen grains deposited on the stigma in a single visit	Herrera, 1990
Pollinator importance	Mean flower number visited per unit time, multiplied by mean number of pollen grains deposited on the stigma in a single visit	Bloch <i>et al.</i> , 2006

Pollinator effectiveness	Proportion of visited flowers that receive pollen	Herrera, 1987
Pollinator importance	The product of a species' pollination effectiveness and its relative abundance, where relative abundance is calculated as the number of visits made by the species divided by the total number of insect visits observed during the period of study	Olsen, 1997
Combining pollen deposition success with plant parameters		
Pollination efficiency	Proportion of the conspecific pollen load on stigma in relation to number of ovules	Richards, 1996
Stigmatic fertilisation success	Proportion of the conspecific pollen load on stigma in relation to number of ovules	Kearns and Inouye, 1993
Pollination intensity	Proportion of the conspecific pollen load on stigma in relation to number of ovules	Beatie, 1976
Pollen deposition efficiency	Proportion of the conspecific pollen load on stigma in relation to number of ovules	Gómez and Zamora, 1999
Pollination effectiveness	The number of pollen grains of the right morphotype deposited in a single visit on reproductive parts of the flower	Muchhala, 2003
Pollinator effectiveness	Proportion of deposited pollen in single visits that develops pollen tubes reaching the ovules	Motten, 1986
Pollination efficiency	Presence or absence of germinated pollen grains	Guo <i>et al.</i> , 1990
Germination number	Presence or absence of germinated pollen grains	Inouye <i>et al.</i> , 1994
Pollination efficiency	Average number of conspecific pollen grains on the stigma during the activity period of a given pollinator population	Vaissière, 1991

Pollination efficiency	Fraction of the produced pollen that reaches the stigma	Richards, 1986
Pollination efficiency	Number of pollen grains deposited per stigma in relation to the total pollen production of the flower	Cruden <i>et al.</i> , 1990
Total source efficiency	Number of pollen grains deposited per stigma in relation to the total pollen production of the flower	Inouye <i>et al.</i> , 1994
Combining pollen deposition success with pollinator behaviour parameters and plant parameters		
Pollinator efficiency	Proportion of removed pollen that actually fertilises and ovule	LeBuhn and Holsinger, 1998
Pollination intensity	Number of functional (compatible) pollen grains per one visit “converted” into chances of siring seeds, multiplied by visitation rate	Galen and Newport, 1987
Pollination efficiency	The pollination efficiency was assessed in four ways: (i) pollen deposition (stained pollen grains with a pollen tube were counted), (ii) pollen removal, (iii) visit frequency, (iv) response to nectar production pattern	Canto-Aguilar and Parra-Tabla, 2000
Index of pollination effectiveness	Stigmatic pollen load related to a given pollinator while stigma is receptive	Potts <i>et al.</i> , 2001
Combining pollinator behaviour parameters with plant parameters		
Pollinator efficiency	Amount of pollen a given visitor carries and proportion which is conspecific	Schlindwein and Wittman, 1995
Pollination relative efficiency	Amount and the identity of pollen grains on the visitor's body	Ashman and Stanton, 1991
Pollinator efficacy	Relative potential of a flower visitor species as a successful pollen vector for a given proportion of plants	Sugden, 1986

Pollinator efficiency	Yield of germinated pollen grains at end of anthesis in relation to maximum possible transferred pollen grain number during flower anthesis (= mean pollen load on the vector multiplied by number of flower visits a female flower receives during anthesis)	Nepi and Pacini, 1993
Direct measure of plant female reproductive success (seed set, fruit set)		
Pollination Effectiveness	Fruit set as a result of intermorph pollinations with stuffed hummingbirds	Ornelas <i>et al.</i> , 2004
Pollination effectiveness	Seed set per flower as a result of individual visits of different pollinators (in an enclosure)	Waser and Price, 1983
Pollination effectiveness	Percentage of receptive florets in an inflorescence setting seed following one visit by a given species	Olsen, 1997
Pollinator effectiveness	Seed yield as a result of single visits to a virgin flower	Motten <i>et al.</i> , 1981
Pollination effectiveness	Seed yield as a result of single visits to a virgin flower	Vaissière <i>et al.</i> , 1996; Mayfield <i>et al.</i> , 2001
Pollination efficiency	Seed yield as a result of single visits to a virgin flower	Suzuki and Akazome, 2000; Kandori, 2002
Seed set per visit	Seed yield as a result of single visits to a virgin flower	Inouye <i>et al.</i> , 1994
Pollination efficiency	Seed yield per single visit per flower head of a sunflower	Parker, 1981
Seed set per visit	Seed yield per single visit per flower head of a sunflower	Inouye <i>et al.</i> , 1994
Pollen transfer efficiency	Percent fruit set as a result of one visit	Klein <i>et al.</i> , 2003
Pollination efficiency	Percent fruit set as a result of one visit	Sampson and Cane, 2000

Female pollination efficiency	Seed set per flower	Andersson, 1996
Pollination efficiency	Fruit production per unit time of pollinator activity in the inflorescence	Keys <i>et al.</i> , 1995
Pollination efficiency	Percentage of fruit set attributed to a specific activity period of different pollinators	Dafni <i>et al.</i> , 1987
Pollination efficiency	Seed and fruit yield as a result of the pollinator activity	Guo <i>et al.</i> , 1990; Cauich <i>et al.</i> , 2004
Pollinating efficiency	Seed and fruit yield as a result of the pollinator activity	Vicens and Bosch, 2000
Combining plant female reproductive success with pollinator behaviour parameters		
Pollination efficiency	Number of pollinator visits needed for 100% seed production	Spears, 1983; Titze, 2000
Pollination effectiveness	The relative contribution to seed set of pollinators active at different times of the day (measured as seed production and pollen movement distance)	Young, 2002
Pollination efficiency	Fruit production per unit distance the vector travels in the inflorescence	Keys <i>et al.</i> , 1995
Pollination efficiency	Correlation between the forager visitation frequency and seed set (seed set plotted against visit frequency per flower for each pollinator for an individual plant)	Waser and Price, 1990
Pollinator effectiveness	The relative pollinator effectiveness was assessed in three ways: (i) observation of visitor behaviour, (ii) effect of visitors on seed production in experiments, (iii) correlation between seed and fruit set and flower visitation	Montalvo and Ackerman, 1986
Combining pollen deposition success with plant female reproductive success		
Pollination effectiveness	Percentage of fruits resulting from pollinated flowers	Gudin and Arene, 1991
Fertilisation efficiency	Relative contribution on a given pollinator to set seed in relation to the pollen deposited by a given pollinator	Vaissière, 1991

Stigmatic seed set success	Proportion of deposited pollen that produces seeds	Kearns and Inouye, 1993
Combining plant reproductive success with plant parameters		
Pollination efficiency	Percentage of flowers that develop into fruit	Schneider <i>et al.</i> , 2002; Van Praagh and Hauschildt, 1991
Pollination efficiency	Percentage of ovules that developed into seeds in an inflorescence	Tamura and Kudo, 2000
Pollination effectiveness	Percentage of flowers that develop into fruit	Mesquida and Renard, 1981; Motten <i>et al.</i> , 1981; Donovan and Read, 1991; Meisles and Chiasson, 1997
Pollination effectiveness	The proportion of unrestrained seed set caused by a single visit of a species corrected by the amount of seed set when no visitation occurs.	Spears, 1983
Fruit set per 100 flowers	Percentage of flowers that develop into fruit	Inouye <i>et al.</i> , 1994
Plant Parameters		
Pollination efficiency	Reciprocal of pollen-ovule ratio	Richards, 1996

Table 1: Summary of the various terms used to describe pollinator performance, and the variables used to quantify them. Reproduced from Ne'eman *et al.*, 2010.

As can be seen from table 1, there is at present a great deal of ambiguity in the literature with more than 30 different definitions for the terms “pollination efficiency” or “pollinator efficiency”, something which led Inouye *et al.*, (1994) to exclude the terms “efficiency” and “effectiveness” from the lexicon of their review, instead proposing a number of much more specific terms. They stated that they “will consider this paper successful if it eliminates the confusion about the concepts and definitions of some of these terms”; however, despite their formation of a consistent terminology for the pollination process, the terms “efficiency” and “effectiveness” were still widely used after this point, and no consensus as to an effective means of evaluating pollinator performance had been reached.

Assessment Concepts for the Comparison of Pollinator Performances

Generally, there are two main types of assessment concepts for the comparison of pollinator performances (Gross, 2005; Ne’eman *et al.*, 2010). The first is pollination success, defined as the contribution to stigmatic pollen deposition; and the second is the consequent “female reproductive success”, or contribution to seed set, of the pollinated plants. Theoretically, both approaches can be analysed from the perspective of either the male or female fitness of the plant; however, given the logical constraints of following the fate of pollen grains, the focus is usually on the female perspective, such as pollen deposition on receptive stigmas, fertilised ovules or seeds produced per plant.

Studies that do assess male fitness include pollen removal and pollen loss or wastage in pollinator performance measures (Harder and Wilson, 1994; LeBuhn and Holsinger, 1998; Thompson *et al.*, 2000; Thompson, 2006), therefore enabling a measurement of “pollen transfer efficiency” (Thompson, 2006) which can be categorised as a combination of high or low pollen removal combined with high or low pollen deposition (Thompson *et al.*, 2000). In this way a good pollinator may become a pollen thief in the presence of better pollinators if they are wasting pollen that the better pollinator could be depositing. At present however, measuring reproductive success from the perspective of

male fitness requires expensive, complex laboratory techniques which would not be suitable for large-scale comparative pollination field studies, therefore studies from the female perspective of reproductive success are more common and simpler to perform.

In assessing pollination success as measured by stigmatic pollen deposition, the performance of the pollinator ends after its visit to the flower, once it has deposited pollen on the stigma. A direct measureable indicator of pollinator performance, therefore, is the number of pollen grains which are deposited on a receptive stigma. This direct measure has often been modified or substituted with parameters such as pollinator behaviour within the flower, duration of visit or by visitor frequency or abundance (see table 1), which are assumed to be correlated with pollination success, though evidence for this is not forthcoming (see Chapter 9).

The second assessment concept, female reproductive success, is usually measured in terms of seed set, and characteristics of the plant are included, as well as a number of stages of the pollination process leading to seed set. The essential question of this approach is how much of the pollen deposited on the stigma has the ability to fertilise ovules and sire seeds.

Pollinator behaviour in or on flowers and frequency of visits have often been used as proxies for both approaches to pollinator performance; pollen deposition success and female reproductive success. Stebbins (1970) identified visitation frequency as an integral component of pollinator performance, and in their revised methodology Ne'eman *et al.* (2010), while agreeing that visitation rate is not necessarily a proxy for pollen deposition, used this parameter to scale up either temporally or spatially from a single visit to a single flower to a rate of pollen deposition.

The term “pollinator importance” is frequently used for the product of two parameters: (i) the probability of a visit, measured for example by visit frequency or relative abundance (Armbruster, 1988) and (ii) the pollinator performance per visit or per unit time, such as the

mean number of seeds produced, percent fruit set, or the number of pollen grains deposited in a single visit (Waser and Price, 1983; Schemske and Horvitz, 1984; Inouye *et al.*, 1994; Olsen, 1997). Sahli and Conner (2006) concluded from a review of 17 plant species that visitation rate was the primary indicator of pollinator importance, rather than differences in performance per visit; however it is argued that due to the limited number of species in their analysis they could not determine whether performance per visit is important for plants with specialised pollen removal and deposition mechanisms, as was indicated by the two species of *Asclepias* that were included in the study.

Visitation rate itself is comprised of two components: the visit activity of each individual pollinator per unit time, and the number of visitors per flower per unit time or per patch of flowers. This is important when analysing pollen deposition patterns, as 10 flower visitors which each make one visit in a given unit of time will result in the deposition of pollen of different quality or quantity than a single flower visitor which makes 10 flower visits in a given unit of time. In addition, not all flowers in a patch have equal probabilities of being visited. Preferential visits to flowers due to differential nectar reward availability are well-documented (Andersson, 1988), and there may also be an unbalanced ratio of male to female flowers, or male to female stages of flowers (Harder and Wilson, 1998; Thomson, 2001). In practice, preferential visitation is extremely difficult to observe or measure, therefore more field studies are required in order to identify the underlying distribution patterns of visits which accurately represent the real situation in natural plant populations.

As mentioned above, pollen deposition on stigmas is often used as a measure of pollinator performance, but may be substituted with visit frequency, visit duration, stigma contact and pollen load on the pollinator's body in cases where it is not feasible to directly measure stigmatic pollen deposition. In the case of some Brassicaceae and Asteraceae, for example *Scalesia affinis* (Asteraceae), a full pollen load is deposited on stigmas before any visitors arrive, making it impossible to determine the pollen deposition of a single visitor (Nielsen *et al.*, 2003). By including a measure of pollinator visitation rates, single visit pollen

deposition can be scaled up temporally or spatially to a rate of pollen deposition. In combination with other parameters, pollen deposition success can be used to measure the potential, context independent, performance of a given pollinator species, regardless of other factors such as comparison to other species, which may influence the final plant reproductive success. It can also be used to infer the actual, context dependent, performance of a given pollinator. Potential pollinator performances are important in many agricultural and conservation studies, and relatively simple to implement. Measures of actual pollinator performance, more important for evolutionary questions, require the inclusion of factors such as time of pollen deposition; for example if visitor species A is only the best pollinator in the absence of visitor B, which deposits the pollen earlier in the day and pollen competition for ovules plays a role.

Seed and fruit set, either as the result of single, sequential or unrestricted visits of several pollinators, are often used as a measure of female reproductive success, in some cases being related to plant input such as ovule or flower production, pollinator input such as pollen deposition, or to pollinator behaviour such as visit frequency. The drawback to using seed set as a measure of pollinator performance is that post-pollination processes can reduce actual relative to potential fruit or seed set (Cane and Schiffhauer, 2003), therefore a developing fruit may abort despite adequate pollination if limited maternal resources are usurped by neighbouring fruits (Stephenson, 1981; Corbet, 1998), which would lead to an underestimation of pollinator performance. In addition, sources of error in pollinator performance studies can come from the assumption that single visits relate to a monotonic, incremental increase in seed set for successive visits of the same flower, which is rarely true (Motten *et al.*, 1981; Olsen, 1997). Measuring pollinator performance using single visits may also not be a viable method in species with numerous ovules per flower as these flowers may require a minimum threshold number of pollen grains to be deposited before fruit is able to develop and seeds are produced (Vaughton and Ramsey, 2000; Cane and Schiffhauer, 2003).

As such factors can influence the measures of seed set from a given pollinator and these measures are often impractical, several indirect methods have been substituted in order to estimate pollinator performance based on female reproductive success. Commonly used measures include pollinator activity and abundance, such as visitation frequency and visit duration, in addition to pollen deposition per flower and per unit time, which are also used as proxies for pollen deposition success.

Some studies have shown that pollinator activity can be directly related to seed production or fruit set with or without data on pollen deposition on the stigma, for example an increased visitation frequency of a visitor may increase the chances of pollen delivery (Motten *et al.*, 1981; Schemske and Horvitz, 1984). Female reproductive success, however, is not always correlated with either pollen deposition success or other variables such as the abundance of flower visitors or their visit frequency (Crome and Irvine, 1986; Sahli and Conner, 2006).

Another variable which must be considered when measuring pollinator performance is stigma receptivity, both pollen-capture ability and stigma selectivity (Lord and Russell, 2002), an important concept as only visits to receptive stigmas can be regarded as successful. The quality of deposited pollen is also an important factor when measuring pollinator importance. From the viewpoint of the plant, pollen quality is related to a number of factors, such as conspecific pollen grains in a pollen load (Rathke, 1983; Wilcock and Neiland, 2002), pollen viability (Dafni and Firmage, 2000), pollen compatibility (De Jong *et al.*, 1992; Ramsey and Vaughton, 2000), the genetic identity and the number of conspecific pollen donors represented in the pollen load (Bertin, 1986; Price and Waser, 1979), pollen allelopathy (Morison *et al.*, 2000) and pollen clogging (Ashman *et al.*, 1993). Pollen quality can be influenced by a single pollinator's behaviour because it affects the composition of the pollen load brought to the stigma, however the quality of this pollen load can only be assessed in relation to the specific features of a given plant. The number of pollen donors contributing to the stigmatic pollen load may also influence the quality of the offspring (Bertin, 1986).

Pollinator foraging behaviour will influence pollen quality by improper pollen transfer (Rathke, 1983) and by geitonogamous pollination. A pollinator which typically has a long flight distance may improve the quality of the deposited pollen on the stigma by increasing the probability of cross pollination (Herrera, 1987), although outcrossing depression may also occur (Banyard and James 1979; Ritland and Ganders 1987; Dudash 1990; Fenster 1991; Waser 1993; Waser and Price 1995; Trame *et al.*, 1995). Finally, the number of pollen grains, ovules and seeds must also be considered. Combining pollinator behaviour parameters with plant parameters such as number of pollen grains and ovules produced follows an economics approach, judging pollinator performance in relation to the used, or wasted, resources. In an evolutionary sense, the pollen to ovule ratio can be interpreted as an indicator of the effectiveness of the pollination (Cruden, 1977), and the reciprocal term ovule to pollen ratio has been termed the pollination efficiency (Richards, 1996). The minimum number of pollen grains that have the ability to fertilise ovules, and therefore have a high enough pollen quality, and are required to be deposited on the stigma for maximal seed production is an important factor affecting pollination efficiency, although this have rarely been investigated. Although such information is lacking, the value is expected to vary between species, though it is often assumed in the absence of this information that the minimal number of good pollen grains required for the maximal number of seeds produced per flower is equal to the maximal number of seeds produced under natural conditions. A more accurate solution to this problem is to analyse the dose-response relationships between the number of pollen grains in stigmatic loads and the consequent seed set (Bosch and Waser, 2001; Cane and Schiffhauer, 2003; Hoffman, 2006); however the creation of a pollen saturation curve like this is labour intensive, and a more practical approach is to ignore correction for threshold number of pollen grains needed for seed set per ovule.

A Revised Lexicon and Model for Pollinator Performance Studies

In their review, Ne'eman *et al.*, (2010) set out to define the terminology and methodology of assessing pollinator performance. Analysis of the most meaningful and practical parameters from the literature led them to suggest a modular approach, based upon the two main assessment concepts for comparing pollinator performance. They identified the two most important questions when investigating pollinator performance as:

- (i) What is the contribution by a flower-visiting species to the pollen deposited on a given plant species?
- (ii) What is the contribution to the plant's female reproductive success in terms of seed set?

To avoid further confusion, given the variation in meanings as illustrated in Table 1, the terms pollination efficiency and pollinator effectiveness were avoided, however the terms effectiveness and efficiency were used to signify different aspects of performance and, despite their interchangeable use in the English language, were given more precise definitions. Effectiveness was defined as “the potential to bring about an effect that is the capability of, or success in, achieving a given goal”; while efficiency was defined as “an effect in relation to the resources spent or the input or output of a system”.

Pollen deposition effectiveness was therefore defined as the pollinator's contribution to pollen deposition alone, as the essence of effectiveness as defined in this approach is the achievement of the goal as such and is not related to the resources that are available or spent. This measure does not take into account any variables which are involved in the plant's female reproductive success, such as pollen quality, pollen or ovule production, or consequent seed set.

Pollen deposition efficiency was defined as a measure of whether a given pollinator deposits sufficient pollen to achieve full seed set per flower. As the term efficiency includes a

consideration of how well the goal is achieved given the available resources, the amount a given pollinator contributes to female reproductive success must be measured. This is measured by seed set, and depends on plant variables such as the quality of pollen and the availability of ovules to receive the pollen, therefore to determine whether a flower visitor is a “good” pollinator in terms of seed set we must relate the pollinator’s contribution to the available maximal seed set of the flower under the given constraints of the plant. Pollen deposition efficiency can range from 0, or no contribution, to 1, maximum contribution or full seed set per flower.

Using these definitions of effectiveness and efficiency means that not every effective flower visitor, considered “good” in terms of pollen deposition, will necessarily also be an efficient pollinator, considered “good” in terms of seed production. These connotations can be further extended to investigate indices for other components of the pollination process, for example the term pollen transfer efficiency is an appropriate derived index relating to the efficiency of the transfer process in terms of the pollen removed that gets wasted.

The modular approach suggested by Ne’eman *et al.* (2010) is based upon the key basic unit of the number of pollen grains deposited on the stigma in a single visit. Both pollen deposition effectiveness and pollen deposition efficiency can be scaled up to the next level, which is based on temporal and spatial scaling up by incorporating visit frequency, or the number of visits per flower per hour, when observing a flower patch. From this second level it is then possible to develop higher order indices by adding more parameters or summarising to higher temporal or spatial scales such as day or seasonal levels, or whole pollinator assemblages. These higher order derived indices can then be reduced back to their basic units, permitting comparisons across different temporal and spatial scales to facilitate comparisons between different investigations.

Notably, measures of pollen removal rates are excluded from the proposed modular approach of Ne’eman *et al.* (2010). The use of pollen removal data in the assessment of

pollinator performance is debatable, as their accuracy is affected by the numerous opportunities for pollen loss. This is illustrated in the flow diagram below (fig. 4), created by Inouye *et al.* (1994) to show the many paths that pollen may follow after production, including its possible fate after removal by vectors. It shows a variety of ways in which pollen can be lost from a pollination system, such as consumption by vectors, or deposition of pollen on different species of flower, and emphasises the point that pollen removal and pollen loads are not effective methods for measuring pollinator performance. This point is agreed upon by the majority of authors, even though many still include this as a component of pollinator effectiveness, probably due to the relative ease with which it can be measured. Freitas and Paxton (1998) for example, allowed single bee visits to marked flowers of the cashew *Anacardium occidentale* and measured pollen removal and deposition rates in order to compare the effectiveness of the introduced honey bee *Apis mellifera* and the indigenous bee *Centris tarsata*. Ivey *et al.* (2003), in line with earlier studies (Herrera, 1987; Utelli and Roy, 2000), defined pollinator effectiveness as “any characteristic of a pollinator or pollinator’s behaviour that contributes to its ability to affect plant fitness, including components of both quality and quantity”, and measured pollen load and both removal and deposition of pollen, as well as flower handling time and the potential for geitonogamy to determine the “pollination effectiveness” of different visitors to a population of swampy milkweed.

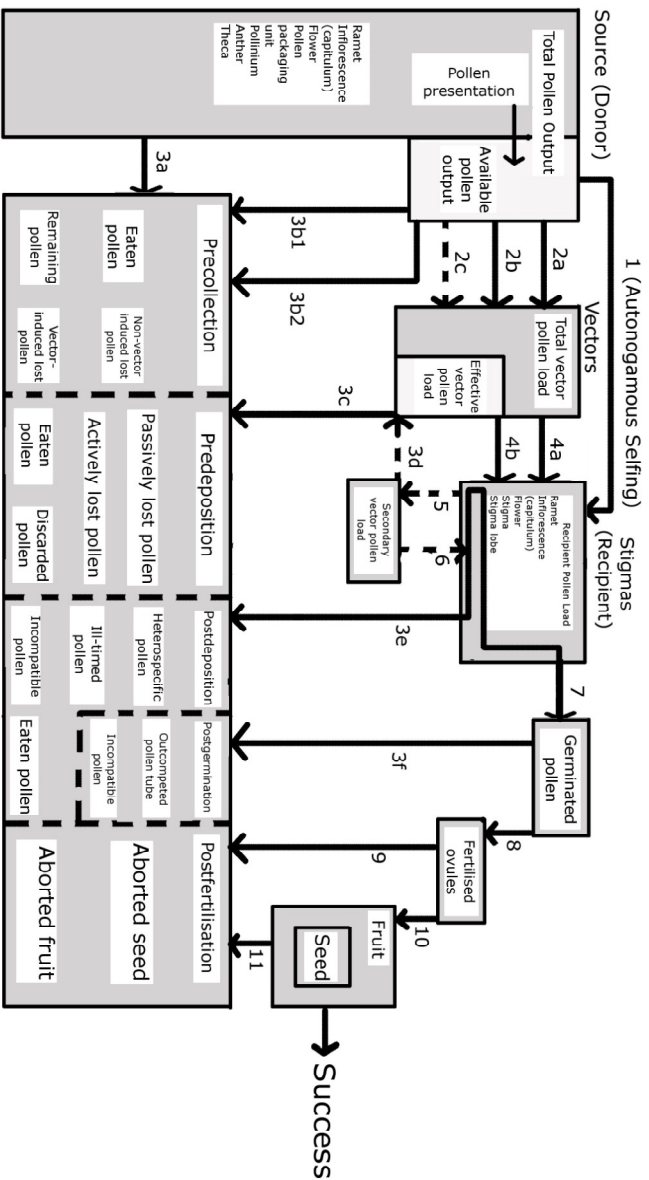


Fig. 1. 4: Flow chart for biotic describing the various paths pollen can take after production (reproduced from Inouye *et al.*, 1994). Dotted lines indicate secondary transfers of pollen that may involve minimal quantities of pollen.

Terms suggested for pathways:

1: Autogamous selfing

2: Transfer of pollen from source to vector

2a: Pollen placement (placed on the vector by the flower, either passive or active)

2b: Pollen collection (gathered by the vector, either passive or active)

2c: Secondary pollen transfer

3a: Prerepresentation pollinivory (pollen never made available for dispersal)

3b1: Postrepresentation lack of collection/postrepresentation pollinivory

3b2: Non-vector induced pollen loss/vector induced pollen loss

3c: Passive vectorial pollen loss, via gravity or shear forces/predposition pollinivory (eaten directly or groomed off and given to larva)

3d: Passive vectorial pollen loss, via gravity or shear forces/predposition pollinivory (of secondary pollen)/ pollen discarding, by the vector (grooming of secondary pollen)

3e: Heterospecific pollen deposition (primary or secondary pollen)/ unrecipive pollen deposition (primary or secondary pollen)/ incompatible pollen deposition (primary or secondary pollen)(stigmatic incompatibility)/ postdeposition pollinivory (primary or secondary pollen)

3f: Pollen tube competition/incompatible pollen deposition (styler incompatibility)

4: Transfer of pollen from vector to stigma

4a: Pollen acquisition (from the plant's perspective); active or passive

4b: Pollen deposition (from the vector's perspective); active or passive

5: Secondary pollen placement, or pollen collection (stigma pollen load to secondary vector pollen load)

6: Secondary pollen acquisition, or pollen deposition (secondary vector pollen load to secondary stigma pollen load).

7: Germination

8: Fertilisation

9: Seed development; seed set/ fruit development; fruit set

10: Seed abortion/ fruit abortion

Equations for Assessing Pollinator Performance

Pollen Deposition Effectiveness

Ne'eman *et al.* proposed several formulas for calculating the various indices of their modular approach to pollinator performance. The first refers to the measuring of pollen deposition effectiveness, D_v at the single visit level, which is equal to d , the number of pollen grains delivered by a given pollinator to the stigma of a given flower in a single visit (see equation 1). This has also been referred to as the stigmatic pollen load (Inouye *et al.*, 1994). It is suggested that, in practice, an investigator waits in front of an unvisited flower with a receptive stigma, or a patch of unvisited flowers until a pollinator visits, then counts the pollen grains deposited on the stigma in this visit.

$$D_v = d \quad \text{Equation 1}$$

It is important to scale up from this single-visit level as pollen deposition may be improved if the flower receives more visits over time; therefore the rate of successive depositions is a critical component of the pollination process. A pollinator species with a low single-visit pollen deposition effectiveness may increase its overall deposition either by the activity of individuals, by making more frequent visits, or by the abundance of individuals, by having many individuals visit the same flower. Pollen deposition effectiveness at the per hour level, or D_t was therefore defined as the pollen deposition effectiveness per single visit multiplied by the visit frequency f (see equation 2).

$$D_t = D_v f r \quad \text{Equation 2}$$

Here, r refers to the proportion of flowers in the observed patch which possess receptive stigmas at the given time. Ideally this measure would be the proportion of the pollinator visits to flowers with receptive stigmas out of the total number of flowers visited by the given pollinator. For practical reasons, and under the assumption of a random visitation pattern to all flowers, the proportion of visits to receptive flowers can be inferred from the

percentage of flowers with receptive stigmata in the patch during flower visiting observations. Measuring receptivity of stigmas can be done using a stain specific to esterase activity, which is concomitant with stigma receptivity in some species (Berlyn and Miksche, 1976; Mattsson *et al.*, 1974), however this is a difficult and time-consuming method to implement, and it is difficult to determine receptivity by eye in many species (Tangmitcharoan and Owens, 1997). In addition, there is variation in visitation due to intraspecific variation in factors such as inflorescence size, corolla size, flower colour, scent and nectar volume (e.g. Waser, 1983; Galen and Newport, 1987; Thompson, 1988; Galen, 1989; Campbell *et al.*, 1991; Eckhart, 1991; Kearns and Inouye, 1993; Connor and Rush, 1995). In the case where flowers are marked with chemical scents following visits (Eltz, 2006; Saleh and Chittka, 2006, and references therein), it must be considered that visits may not be at random, but may depend on the half life of the chemical marking. Pollen deposition effectiveness can be calculated for each period separately, and the daily effectiveness then calculated as a weighted average of the specific visitation rates during the day. However, for all the reasons given the value of r is in practice rather difficult to produce with accuracy.

Pollen Deposition Efficiency

Pollen deposition efficiency, P , is defined as a measure of how much a pollinator can contribute to maximal seed set per flower via its pollen deposition. P therefore is an indication of whether sufficient pollen has been deposited to produce full seed set, and is related to the quality of the pollen and the maximum female reproductive potential of the flower considering resource constraints, but without pollen limitation. Due to the practical difficulties of measuring seed set after a single visit in order to assess pollinator performance, Ne'eman *et al.* (2010) proposed that the number of pollen grains deposited on a stigma be used as a proxy for the potential seed set that a given pollinator can contribute to a given plant.

In contrast to pollen deposition effectiveness, which only requires recording of the number of deposited pollen grains, assessing pollen deposition efficiency requires that the quality of deposited pollen be evaluated. Pollen quantity, q , was defined as the number of pollen grains deposited that are able to fertilise ovules and sire seeds. The quality of pollen is determined by three factors which are independent of each other. Firstly, pollen must be conspecific (k), as well as viable (m), and compatible (n). To determine a value of pollen quality, it is necessary to determine how much of the deposited pollen (d) meets all three of these conditions at the same time. In mathematical terms, this is equivalent to the subset of deposited pollen grains that represent the intersection of all three conditions, as shown below (see equation 3).

$$q = |k \cap m \cap n| \quad \text{Equation 3}$$

The viability and conspecificity of pollen can be determined using enzymatic techniques and microscopy (Dafni *et al.*, 2005), however determining the compatibility of pollen is a more complex task which depends on the breeding system of the plant, the degree of self-compatibility and the previous activity of the pollen vector, and so far no quick and simple method for assessing the proportion of compatible pollen in a pollen load is available. Rather, time-consuming investigations of the breeding system, prior pollinator behaviour and pollen deposition patterns are required in order to estimate the proportion of compatible pollen that has been deposited. Using genetic markers can enable direct measurements of outcrossed and selfed pollen (Sage *et al.*, 2005), and in self-compatible plants compatible pollen (n) can be assumed to equal 1, however in the case of self-incompatible plants which have a high level of pollinator-mediated geitonogamy, pollen compatibility remains problematic and cannot be ignored (De Jong *et al.*, 1993; Snow *et al.*, 1996; Sage *et al.*, 2005).

The next step in an “ideal” investigation of a pollinator’s contribution to seed set is to relate the amount of pollen deposited to how much is required for maximum seed set under

given environmental conditions, and without pollen limitations. Pollen limitation is where less pollen is received by the plant than is needed to produce a full seed set (Burd, 1994) as explained above. In contrast to this however, is pollen surplus, where more pollen than is required for maximum seed set is received. This concept has received much less attention in the literature, although in the assessment of pollen deposition efficiency it is of great importance as there would seem to be no advantage to the plant if a pollinator deposits surplus pollen in comparison to one that deposits the correct amount, unless a threshold of excess pollen is needed or additional pollen has an effect on the seed quality through pollen competition (e.g. Burd, 1994; Ashman, *et al.*, 2004). The number of quality pollen grains (q) should therefore not exceed the number needed, and should be capped at the maximal potential seed set per flower by subtracting the surplus pollen delivered, therefore giving a capped value for quality pollen (c).

In order to calculate this value, it is necessary to first estimate the maximal seed set capacity of a given flower under field conditions, and without pollen limitation. The maximum potential seed set (s) is estimated by artificially supplementing pollen on flowers which are exposed to natural pollination given current resource constraints. If the amount of quality pollen deposited in a single visit (q) is larger than the maximum potential seed set (s) then q is capped at the upper limit of s to give c , however if q is less than that of the upper limit of s , then c is equal to the value of q .

It is important to calculate the final value of c in the correct sequence. Capping to the limit of s should not be performed on the overall average of q for a given pollinator; rather the truncation must be performed separately for every value of q at the single visit level before averaging over all replicates. This sets s as the upper limit for each single visit to a flower; therefore no extremely high value of q will skew the results of c .

Once the two preceding steps have been completed, pollen deposition efficiency at the single-visit level can be calculated. Pollen deposition efficiency (P_v , see equation 4) is

the capped quality pollen (c) divided by the maximal seed set potential for the flower (s). As the value for capped quality pollen can never exceed the maximum number of potential seed set, this ratio will range from 0 to 1.

$$P_v = c/s \quad \text{Equation 4}$$

The pollen deposition efficiency per hour (P_t , see equation 5) can be calculated using a method similar to that of pollinator deposition effectiveness per hour (see equation 2) using visit frequency (f) and the proportion of flowers with receptive stigma (r), as well as by applying capping for pollen surplus in a similar manner for pollen deposition efficiency per hour, setting the upper limit of P_t at 1.

$$P_t = P_v fr \quad \text{Equation 5}$$

Plant-Pollinator Overlap: Duration and Timing

One of the issues in making generalisations concerning pollinator performance is the conversion from event-related measurements such as single visits, or time related measurements of pollinator activity, such as per hour, to longer period such as the lifetime of the flower. Different pollinators may have different lengths and timings of activity during the day, for example a certain bee species may be active only during the morning while another is active over the whole day (Herrera, 1990; Willmer and Stone, 2004; Hoffman, 2006). In order for the results of the time-related indices at the per hour level to reflect the different overlap times, observation units must be either randomly, or evenly, distributed over the entire lifetime of the flower at the appropriate resolution for capturing these differences. In addition, the chronological time of flower visitor activity must be noted, for example if a pollinator starts early in the morning it may have already deposited sufficient pollen for producing full seed set, then all subsequent flower visitors will not contribute to the actual seed set, even if it is a good pollinator with a potential for inducing high seed set in the given

plant species, for example in the absence of other pollinators (Herrera, 1990; Thomson *et al.*, 2000).

If it is not possible to capture the differences in overlap with a practical sampling regime, a measure of the duration and timing of the overlap between stigma receptivity of the flower and the pollinator's activity can be devised and incorporated. This modification of the basic equations above has potential to be scaled up to higher levels such as inflorescence, plant or population or over longer durations such as an entire season. The development of higher order indices will prove valuable for comparing pollinators' performance at the level of seed yield for the flowering season of plant populations.

The points above are the main focus of this thesis, however Ne'eman *et al.* (2010) also offered clarifications and equations for calculating a variety of other components relating to pollinator performance which are not explored further here. These include stigmatic pollen deposition over the lifetime of a flower; pollen deposition efficiency over flower lifetime; pollen deposition effectiveness and pollen deposition efficiency over an entire pollinator assemblage; and autonomous selfing efficiency and open pollination efficiency.

Other Problems with Past Pollinator Performance Studies

Another issue with previous studies of pollinator performance which was not addressed by Ne'eman *et al.* (2010) is that of the taxonomic or functional scale at which pollinator performances are assessed. In many studies visitor species have been pooled into larger functional groups rather than being assessed by individual species, for example by the nine functional groups originally suggested by Robertson (1928): Long-tongued bees; short-tongued bees; other Hymenoptera; Diptera; Coleoptera; Lepidoptera; Hemiptera; Neuroptera; and birds, (though in more recent studies Neuroptera are rarely included).

Herrera (1987), for example, considered the frequency of pollen transfer, the number of pollen grains deposited on a stigma, selection of floral sexual stage (the flowers studied

were distinctly protandrous), and patterns of flight distance between flowers to determine the relative effectiveness of 34 floral visitors to *Lavandula latifolia* flowers in southern Spain, defining flower visitors as three functional groups, Hymenoptera, Lepidoptera and Diptera. The study found that species of Hymenoptera deposited more pollen and more often than did species of Lepidoptera and Diptera and there was no significant difference in pollen deposition between the latter two. Variation in “pollinator effectiveness” was found within the categories, and the author acknowledged that, while the grouping together of individual species into broader categories such as order or family makes statistical sense, it would almost certainly underestimate the actual range of pollinator performances encountered by the plant.

Despite this admission in early pollinator performance studies, the grouping of flower visitors into large, broad categories (e.g. Fishbein and Venable, 1996), or the investigation of only one or a few species from within the visitor assemblage (e.g. Freitas and Paxton, 1998; Fumero-Cabán and Meléndez-Ackerman, 2007; Madjidian *et al.*, 2008), are still common practices. Given the wide variation within these functional groups of features which will have an effect on pollination performance, such as size, hairiness, body shape and behaviour, pollinator performance would be expected to vary greatly within these groups. To accurately assess the performance of all members of a pollinator assemblage, and more accurately identify the most effective pollinators of a given species, it is necessary to assess pollinator performance at the individual species level rather than at the family, order or other large functional group level.

Using the Modular Approach of Ne’eman *et al.*

The modular approach above is complex, and in places approaches the impractical, but it illustrates the scale at which effective pollinator performance studies must ideally be carried out. This thesis tests the viability of some of the components described above. Chapters 3 through 8 utilise single visit pollen deposition of a variety of visitors to determine

their pollen deposition effectiveness, and therefore the most effective pollinators, of flower species showing traits indicative of various pollinator syndromes, and identifying those flower visitors which are not effectively pollinating flowers. It is hoped that being able to exclude non-pollinating visitors from pollinator assemblages will clarify the true level of specialisation or generalisation within these plant-pollinator interactions, and offer a more accurate test of the pollinator syndromes approach. Chapter 9 uses records of flower visitor frequencies to further analyse the performance of a pollinator assemblage using the methods described above, scaling up the single visit pollen deposition effectiveness to an hourly rate.

To accurately determine the performance of all members of the visitor assemblage, and avoid over or under-estimation of visitor performances, visitor pollination performance will be assessed at the individual species level rather than at a larger, broader functional group level. The predictive powers of other measures of performance such as visitation frequency, duration of visit and feeding mechanism and the accuracy of such measures at indicating the most effective pollinators of a given species will also be investigated in Chapter 10.

This thesis aims to provide a test of the viability of some of the pollinator performance protocols proposed by Ne'eman *et al.* (2010). These performance assessments will then be used to accurately test the predictive powers of the pollinator syndrome approach, and determine the true level of specialisation and generalisation in some natural pollination systems.

Chapter 2: Assessing Single Visit Pollen Deposition

Introduction

One of the aims of this thesis is to test the validity of pollination syndromes as predictors of the effective pollinators of a given plant species. Chapters 3 through 7 investigate a variety of different plant species showing traits indicative of different pollinator syndromes, and test the predictive powers of these syndromes at identifying the most effective pollinators within the visitor assemblage of each. In order to do so, a measure of the effectiveness of each visitor of the given plant species must be determined. In line with recent consensus views (Inouye *et al.*, 1994; Ne'eman *et al.*, 2010; Willmer, 2011, see Chapter 1), and the methodologies of earlier pollinator performance studies (e.g. Primack and Silander 1975; Motten *et al.*, 1981; Dieringer, 1992; Kearns and Inouye, 1993; Inouye *et al.*, 1994; Falque *et al.*, 1996; Mitchell, 1997; Osorio-Beristain *et al.*, 1997; Rodet *et al.*, 1998; Mayfield *et al.*, 2001; Suzuki *et al.*, 2002; Cane and Schiffhauer, 2003), single visit pollen deposition was used as a determinant of pollinator performance.

Chapter 8 uses the equations defined by Ne'eman *et al.* (2010) to scale up single visit pollen deposition of the visitor assemblage of *Agrimonia eupatoria*, a plant species with traits indicative of a hoverfly pollination syndrome (see Chapter 6) by means of a visitation survey detailing visitation frequency over the course of a day for the visitor assemblage of this species.

To ensure the consistency of results, the following protocol was applied to each of the different pollinator syndromes in Chapters 3 through 7. Any deviations from the methods below noted are in each particular chapter.

Materials and Methods

Sampling Periods

Sampling, where possible, occurred throughout the day to allow for variations in visitor assemblage in different time periods. Field research occurred throughout the summer months of 2008, 2009 and 2010, and observations were restricted to days with weather conditions optimal to pollinator activity, namely dry, calm and preferably sunny days. Temperature and humidity readings were taken from a shaded area of a given study site using a HM34 Vaisala Pocket Size Relative Humidity Meter every half hour during each sampling session. Sampling occurred at three sites: West Quarry Braes, Fife, Scotland; Loch Tay, Perth and Kinross, Scotland; and Parque Nacional Santa Rosa, Guanacaste Province, Costa Rica. Details of each study site, period and timing of observations are described in the relevant chapter.

Pollinator Effectiveness

To investigate pollinator effectiveness, flowers of each study species were selected as buds and covered in netting with a 2mm wide mesh to exclude flower visitors, but allow for air and water to pass through. Once flowers had fully opened they were uncovered and observed until a single visitor had landed. The visitor species was either photographed or captured for identification using keys from Fogden and Fogden (2006; for birds), Michener (2000; Hymenoptera), Prŷs-Jones (1987; bumblebees), Stubbs and Falk (2002; hoverflies) and Chinery (2005; general invertebrates).

The visit duration was timed using a stopwatch, with records of whether the visitor was feeding on nectar, pollen or both by observing behaviour such as tongue extension and collection of pollen with the legs or mouth. The stigma of the flower was then carefully removed with forceps and placed into an individual, numbered dry well of a NUNC

Polypropylene 384 well plate which was covered and sealed. The number of pollen grains present was counted on the same day where possible (or following preservation in a freezer where not possible) using a Meiji EMZ 5 dissecting microscope at between 10x and 100x eyepiece magnification depending on the size of the stigma and the pollen grains of each given species.

For each species a number of unvisited control flowers were covered with netting for the same period of time as the experimental flowers and the number of pollen grains present on the stigmas was once again counted and recorded. This was to account for self-pollen transfer by wind or by the handling of flowers during the study.

Statistical Analyses

Statistical analyses were carried out using SPSS 17 and followed the advice given in Barnard, Gilbert and McGregor (2011), and following personal conversations with Will Cresswell and Jane Wishart (University of St Andrews). Raw pollen counts were tested for normality and homogeneity of variance and transforms were applied as required. Where normal distribution and homogeneity of variance could be achieved with transformation, variance between groups was tested using ANOVA and post hoc Least Significant Differences (LSD) tests were applied. Where data had normal distribution, but homogeneity of variance could not be achieved by transforming the data a more robust comparison of means, Welch's Robust test for Equality of Means, was used to test for variation between groups and Tamhane's Multiple Comparison's post hoc test, a more robust post hoc test, was applied (Hochberg and Tamhane, 1987). Where data had non-normal distribution which could not be resolved by transformation, comparisons of pollen depositions to those of controls were carried out by multiple Mann-Whitney U tests, corrected for Type I errors using the Bonferroni method (Bland and Altman, 1995).

Using the above, a value of mean pollen deposition per stigma (MPS) was determined for each flower visitor for which sufficient or appropriate data were available. The MPS of each visitor species was then compared to the MPS of the relevant control stigmas. A pollinator was defined as **a species which deposited a significantly greater MPS in comparison to control stigmas**, whereas those species which did not deposit a significant MPS were identified as either inefficient pollinators, or nectar or pollen thieves, and were excluded from further analysis. The species which deposited the highest significant MPS was identified as the most effective pollinator species for that particular plant species.

The results of each of the pollinator syndrome tests are described further below in Chapters 3 through 7, followed by a calculation of the daily pollen deposition of visitors to *Agrimonia eupatoria* (see Chapter 8) and a test of other frequently used proxies of pollinator effectiveness as described by Ne'eman et al. (2010; see Chapter 9).

Notations

The following notations are used in the graphs of later chapters to represent the significance of MPS in comparison to control stigmas, in this case the P-value of the ANOVA (or non-parametric test) performed:

. =< 0.5

* = < 0.05

** = < 0.005

*** =<0.001

Chapter 3: Testing Pollination Syndromes

The Hummingbird Pollination Syndrome

Introduction

Bird pollination, ornithophily, is a common pollinator syndrome in the USA, as far north as Alaska, as well as the neotropics, the eastern Mediterranean, Middle East (although not northern Asia), most of Australasia and Africa. The syndrome is absent in Europe, aside from reports of occasional nectar feeding by some passeriforms (Kay, 1985; Proctor *et al.*, 1996; Schwilch *et al.*, 2001; Merino and Nogueras, 2003, Willmer, 2011), and one native bird-pollinated plant in Spain, *Anagyris foetida* L. (Leguminosae), pollinated by three species of warblers (Ortega-Olivencia *et al.*, 2005).

About 500 genera of angiosperms are known to be pollinated, if not exclusively, at least partly, by birds (Renner and Ricklefs, 1995). Flower-visiting is a widespread phenomenon amongst the birds, found in at least 50 families (Proctor and Yeo, 1973; Proctor *et al.*, 1996; Renner, 1996), having evolved separately in the ancestors of at least seven different bird families (Cronk and Ojeda, 2008). Of these visitors, there are perhaps 8 separate groups that have developed this behaviour to the point of being adequate plant pollinators. The main groups are the Trochilidae (hummingbirds), Nectariniidae (sunbirds) and Meliphagidae (honey-eaters), but the Icteridae (American orioles), Thraupidae (honeycreepers), Fringillidae (Hawaiian honeycreepers), Zosteropidae (white-eyes), Promeropidae (South African sugar-birds), Parulidae (New World Warblers), and Coeribinae (bananaquits) are also important as nectar feeders (Lein, 1972; Cronk and Ojeda, 2008). This is equivalent to about 10% of all bird species.

Birds have several different attributes that aid their effectiveness as pollinators. Their long flight distances and high visual acuity can be especially valuable especially during inclement weather conditions when other pollinators, such as bees, are inactive. Birds can therefore be important pollinators in environments such as high altitude ecosystems, arid environments, and isolated islands where insects have low population densities (Van der Pijl and Dodson, 1966; Stiles, 1978; Dupont *et al.*, 2004; Micheneau *et al.*, 2006) and for winter flowering plants when insects are rare (Kunitake *et al.*, 2004).

Nectar-feeding bird species tend to be small in size in comparison to other bird species, though they are among the largest pollinators in terms of body size (Brown *et al.*, 1978). For this reason they require more energy than do insect pollinators, therefore bird-pollinated plants tend to put more resources into nectar production and often produce larger flowers which can accommodate avian visitors. They may also need to deploy more resources in floral structures that protect against nectar thieves (Stiles, 1978). Environments with low photosynthetic rates such as tropical forest understories, cold, hyper-arid and nutrient poor environments have few bird pollinated plants, perhaps because they suffer from low plant productivity, which may be limiting for nectar production and the bird-pollination syndrome in general (Stiles, 1978).

Floral Traits of General Bird Pollination

Flowers can have several different traits that encourage effective visits by nectar-feeding birds. Aspects of flower size, shape, colour, scent, anther and stigma placement as well as nectar volume and composition all serve to attract birds to flowers and increase their effectiveness as pollinators. These traits are summarised in Table 1.

Flowering time	Day-flowering, flowers open early morning
Dehiscence	Early morning
Nectar secretion	Early morning, often refilled
Nectar composition	Low concentration, high volume (typically between 10 and 50µl in volume and 15-25% in concentration)
Colour	Vivid, red and orange dominant
Nectar Guides	Absent
Flower Shape	Tubular, often pendant or nodding
Lower Lip	Absent or folded back
Corolla	Long, walls thickened or protected basally by sepals and bracts
Anther and Stigmas	Filaments stiff, anthers and stigma protruding
Ovary	Protected, usually located low down in the flower structure
Odour	Absent or very faint
Arrangement of Flower Parts	Large separation between reproductive parts and nectar

Table 1: Summary of the typical characteristics of bird-pollinated flowers according to Faegri and van der Pijl (1979) and reviewed by Willmer (2011).

Perching Bird Pollination

Bird pollination can be split into two categories: perching bird pollination and hovering bird pollination. As the name suggests, perching birds cannot adequately hover to feed on flowers and must therefore perch to feed. The most common examples of pollinating perching birds occur mainly in the Old World tropics, such as the sunbirds of Africa and Southern Asia and the Australasian honeyeaters and wattlebirds, although there are rare examples from the New World such as the bananaquits and honeycreepers.

Pollination by perching-birds tends to be more widespread and involves fewer specialist adaptations to flower feeding than is required for hovering pollination (Cronk and Ojeda, 2008). Pollination of both Old and New World flowers can be facilitated by passerine

birds (Steiner, 1979). Passerines tend to forage and travel in groups and can be much more effective at cross-pollinating even large trees (Stiles, 1981). In general, passerines perch on flowers or branches in order to feed, although hovering is known to occur in some species (Pyke, 1980; Stiles, 1981; Westerkamp, 1990; Dreisig, 1997; Cheke & Mann, 2001). On average, sunbirds weigh more than twice as much as hummingbirds, and honeyeaters more than five times as much (Fleming and Muchhala, 2008). These differences in hovering ability and mass have important implications for the evolution of flower or inflorescence size and nectar content (Cruden et al., 1983; Opler, 1983; Pellmyr, 2002). Flower size and nectar volume have been shown to be positively correlated with pollinator size (Opler, 1983), and flowers pollinated by passerine birds tend to possess a perch, or sturdy inflorescences on which the bird can land (Westerkamp, 1990). Low flowering herbaceous plants can also be pollinated by passerine birds that land on the ground, and usually orient their flowers vertically erect, for example *Lotus berthelotii* Masf. and its relatives in the Canary Islands (Oleson, 1985).

Hovering Bird Pollination

Pollination by hovering birds involves the hummingbirds, family Trochilidae. They evolved in South America, spreading to North America around the late Tertiary (Grant, 1994). Some migratory species are even found as far north as Canada in summer (Grant and Grant, 1968). Hummingbirds are most abundant in the North and West of South America, but have been more extensively studied in California and Costa Rica. They tend to be very small birds, ranging from 3-10g in body weight (Brown *et al.*, 1978), in comparison to passerine birds, which range from about 9-55g (Kendeigh, 1970 and references within). As they do not require a perch or landing platform, the flowers of hummingbird-pollinated plants are often hanging or pendant (Cronk and Ojeda, 2008).

Endotherms have metabolic rates up to 10 times that of ectotherms, therefore requiring much higher calorific inputs. In the case of endothermic flower visitors such as

birds, bats and mammals, the nectar rewards gained from flowers must be high to account for daily energy expenditures. In bats and sunbirds, the daily turnover of calories is amongst the highest recorded for any animals; about 65% of the total caloric content of the body (Willmer, 2011).

Size also has a significant effect on the energy requirements of birds, with larger species of birds having higher energy requirements than smaller species. Metabolic rate (H_m) of resting birds increases with body mass (m_b) according to a fractional exponent ($H_m = km_b^{0.75}$, where k represents ambient temperature in kelvins) (reviewed in Calder and King, 1974 and, more recently by Heinemann, 1992). The daily energy expenditure and resting metabolic rate of birds is found to correlate positively with body mass (Furness and Speakman, 2008). Smaller hummingbird species perform better than larger species when collecting nectar from flowers as, although they collect nectar at the same volumetric rate, they expend less energy in doing so (Mendonça & Dos Anjos, 2006).

If not stressed by energy constraints from scarcity of food or low temperatures, all birds maintain relatively constant body temperatures of about 40°C (Calder and King, 1974). Reaching and maintaining temperatures above the ambient requires a balance between heat production and heat loss from the body surface. The range of ambient temperatures at which metabolic rate is constant and body temperature is maintained at approximately 40°C is termed the thermoneutral range. When environmental temperature is below the thermoneutral range, metabolic rates become elevated above resting levels in order to raise body temperature. Metabolic rates also become raised during periods of activity. Although standard metabolic rate, rate of heat loss and daily energy cost decrease with body size, standard metabolic rate and heat loss scale with fractional exponents, whereas energy reserves should scale linearly in relation to body size. Therefore small birds must feed more frequently and have a lesser ability to withstand periods of food scarcity or long, harsh winters than large birds (Calder and King, 1974; Brown *et al.*, 1978; Pyke and Waser, 1981; Bednekoff *et al.* 1994).

The cost of flight in small birds is substantial, up to 5-7 times the standard metabolic rate for hovering in hummingbirds and linear flight in other birds (Lasiewski, 1963; Tucker, 1970; Bernstein *et al.*, 1973; Greenwalt, 1975; Heinemann, 1992). As they tend to fly continuously while foraging, hummingbirds in particular may expend a large amount of energy on flight. Field estimates of energy expenditure for territorial, nectar-feeding hummingbirds suggest that flight, both foraging flight and non-foraging flight, may account for up to 50% or more of daily energy expenditure (Wolf and Hainsworth, 1971; Carpenter and MacMillen, 1976; Heinemann, 1992) and energy expenditure for hummingbirds during foraging reaches the upper limits recorded for vertebrates (Suarez, 1998; Chai and Dudley, 1999).

Animals that rely on energetically demanding foraging methods such as hovering flight are likely to experience energetic bottlenecks (Prinzinger *et al.*, 1992; Dawson and Whittow, 2000). Because of these energy constraints, most hummingbirds have the ability to enter into and arouse spontaneously from a state of torpor. Torpor in hummingbirds is usually triggered at night, when temperatures are lowest, foraging is impossible, and predation risk is at its lowest (Brown *et al.*, 1978; Dawson and Whittow, 2000). Torpor is an adaptive mechanism allowing for energy conservation during periods of food scarcity, when birds have difficulty maintaining a positive energy balance (Calder, 1974; Calder and Booser, 1973; Carpenter, 1976; Hainsworth *et al.*, 1977; Brown *et al.*, 1978; Geiser, 2004). In general, torpor is more common in smaller birds with a body mass of less than 100g (Schleucher, 2004). Smaller birds are able to rapidly cool body temperature (T_b), to as low as $<10^{\circ}\text{C}$, due to their high rates of heat loss (Lasiewski and Lasiewski, 1967; McKechnie and Lovegrove, 2002), however torpor also occurs in larger bird species such as passerines. T_b drops in the few passerines in which torpor does occur, but rarely to below 30°C (Prinzinger *et al.* 1991; Downs and Brown 2002; McKechnie and Lovegrove 2002).

Torpor can allow for substantial energy conservation; for example the metabolic rate of a 5g hummingbird when torpid at 6°C is only 20% that of a non-torpid bird resting at the

same temperature (Wolf and Hainsworth, 1972). The small body mass of the bird also allows for rapid heating and arousal at the end of a period of torpor (Heinrich and Bartholomew, 1971).

Flower Shape and Size

Bird-pollinated flowers have a variety of characteristics that suit them for pollination by birds. Bird-pollinated flowers are often large, or comprise a large inflorescence (Stiles, 1981). They possess a relatively hard flower wall, with stiff or united filaments as well as a protected ovary to offer protection from the hard, damaging bills of birds (Faegri and Van Der Pijl, 1979; Stiles, 1981). There is a positive correlation between high volumes of nectar, such as those found in hummingbird flowers, and the weight of flowers (Opler, 1981) and this increased weight is thought to come largely from harder, thicker protective tissues in the perianth, as well as the longer corolla tubes that are often found in bird-pollinated flowers.

Bird-visited flowers fall into two general categories: tubular and brush. Tubular flowers tend to have a long corolla tube down which the bird must stick its bill in order to feed. There is much variation in corolla length and shape amongst the bird-pollinated flowers and this has long been thought to correlate with the bill shape of bird visitors (e.g. Darwin, 1876; Snow and Snow, 1980, Stein, 1992, Temeles *et al.*, 2000, Temeles *et al.*, 2009). There are a few exceptions to this rule however, with hummingbirds being observed to visit flowers with corollas much longer or shorter than their bills (Feinsinger, 1976; Arizmendi and Ornelas, 1990; Cotton 1998). The hummingbird *Amazilia rutila*, classed as a medium to long-billed species, has been shown to visit a wide variety of flowers regardless of corolla tube length (Arizmendi and Ornelas, 1990). Longer bills give birds access to a wider range of flowers, with shorter-billed birds being excluded from flowers with long corolla tubes (Bleiweiss, 1999; Temeles and Kress, 2003). In laboratory and field experiments birds with longer bills feed more quickly from long artificial flowers than birds with shorter bills do; however birds with shorter bills do not feed more quickly from short artificial flowers than birds with longer bills

(Hainsworth, 1973, Hainsworth and Wolf, 1976; Montgomerie, 1984; Temeles and Roberts, 1993). The handling times of female *Selasphorus rufus* hummingbirds, known to have a bill around 10.5% longer than those of males of the species, while visiting flowers with long corollas, were shorter than those of the males visiting the same flowers; however no significant difference was observed between handling times at flowers with shorter corollas (Temeles and Roberts 1993).

Brush inflorescences are often regarded as generalists, possessing open flowers with nectar exposed to the environment and accessible to a variety of different pollinator species (Nicolson, 2002). In a typical brush inflorescence, there are many small, but distinctly herkogamous (with a spatial separation of the anthers and stigma), flowers, often with a much reduced perianth, and the stamens and stigmas exposed over a pollination surface (Webb and Llyod, 1986). Brush flowers house their nectar in cups or short tubes, out of which a brush of small stamens extends. Visitors feeding on the nectar are dusted with pollen and come into contact with the stigmas in a rather haphazard fashion to pollinate the flower. These (relatively large) brush blossoms visited by birds are thought to be more adapted toward 'large pollinators' rather than specifically bird pollinators (Stiles, 1981). The brush-blossom species *Eriotheca pentaphylla*, *Lafoensia glyptocarpa* and *Marcgravia polyantha* are pollinated by bats (Sazima *et al.*, 1999), while *Astrocaryum* sp., *Bactris* sp., *Crysophila* sp. and *Hydriastele* sp. and many others are pollinated by Coleoptera (Bernhardt, 2000).

Flowers pollinated by birds tend to not possess a lip or margin, as birds are generally too heavy to land on a flower. It is also assumed that birds will display a higher intelligence in comparison to insect visitors in finding an entrance to a flower (Faegri and Van der Pijl, 1979), though the shape of the corolla opening may also help to direct the birds to nectar (Smith *et al.* 1996).

Flower Colour

Bird-pollinated flowers often tend to be red in colour (Grant and Grant, 1968; Raven, 1972; Faegri and Van der Pijl 1979; Proctor *et al.*, 1996). Birds do not visit only red flowers, and are observed feeding on flowers of a variety of colours. Colour preference may be a learned predictor of reward, rather than an exclusive attraction agent. This means that birds associate the colour red with high nectar rewards and are therefore attracted to the colour in choice tests, however can be taught a preference for other colours if nectar rewards are altered (e.g. Goldsmith and Goldsmith, 1979; Waser *et al.*, 1996; Meléndez-Ackerman *et al.*, 1997; Altshuler, 2003). This supports the theory that bird-pollinated plants are not red in colour in order to attract birds, but rather to exclude other plant visitors.

The subject of red colour vision in insects is much debated. It was originally thought that insects could not see the colour red, or that most were unable to see colours past yellow or orange on the colour spectrum (e.g. Von Frisch, 1914, 1967; Bradshaw *et al.*, 1995; Proctor *et al.*, 1996), and therefore such flowers would not be as visible or attractive to insect visitors. Many studies however have found that some insects are capable of seeing the colour red (e.g. Chittka and Waser, 1997; Schaefer and Ruxton, 2008) and it is now more generally accepted that the colour red is visible to bees and other insects, although it may be less attractive to them than other colours, which they are more highly sensitive to (Bandai *et al.*, 1992; Peitsch *et al.*, 1992; Briscoe, 2000; Briscoe and Chittka, 2001).

Tests of hummingbird vision have shown, however, that hummingbirds do favour colours in the red to green spectrum as these subtend a higher chromatic contrast to background colours and allow flowers to be discriminated more effectively (Herrera *et al.*, 2008), however a lack of red colouring will not deter them from feeding on flowers with sufficient nectar concentration, sugar content and volume (e.g. Waser *et al.*, 1996; Melendez-Ackerman *et al.*, 1997; Altschuler, 2003).

Thus, in practice, most bird-pollinated flowers are red (Rodríguez-Gironés and Santamaría, 2004), and within a genus, species visited by hummingbirds are more likely to be red (Thomson *et al.*, 2000). Typical bird pollinated species such as *Ipomopsis aggregata*, *Justicia rizzinii*, *Silene laciniata*, *Ribes speciosum*, *Fouquieria splendens*, *Zauschneria latifolia*, *Zauschneria californica*, *Galvezia speciosa*, *Castilleja breweri*, *Penstemon bridgesii*, *P. centranthifolius*, *P. labrosus*, *Pedicularis densiflora*, *Monardella macrantha*, *Lobelia splendens*, *Brodiaea idamaia*, *Mimulus cardinalis*, *Aquilegia Formosa*, and many others, all possess red flowers, or some variation of red such as pink or orange (Grant, 1966; Chittka *et al.*, 1994; Chittka and Waser, 1997), although they may not be visited solely by hummingbirds (Mayfield *et al.* 2001). While red flower colour is often a good indicator of bird pollination, there are some species which are pollinated by birds yet do not produce completely red flowers, such as the bird of paradise flower *strelitzia reginae*, which produces a striking orange and blue display (Cronk and Ojeda, 2008).

Typical hummingbird-pollinated flowers also tend to be lacking in nectar guides, coloured stripes or spots as often found on insect pollinated flowers that point pollinators towards the nectar store. This is partially due to the lack of a lower margin or “landing platform” as mentioned above.

Flower Scent

Bird-pollinated flowers tend to produce little or no scent (Faegri and van der Pijl, 1979; Feinsinger, 1990; Vogel, 1990), or at least none that can be detected by human olfactory senses, though this does not necessarily mean the flower is completely lacking in scent (Ohloff, 1994). It is often assumed that birds, and in particular hummingbirds, have a poor sense of smell (Bang and Cobb, 1968; Knudsen *et al.*, 2004), but studies on the subject are conflicting. Early feeder experiments with hummingbirds showed no attractive, repellent, or learning-related roles for odour (Béne, 1945; Stong, 1960). However later studies have found that hummingbirds can discriminate between some scents (e.g. Loalé and Papi, 2003).

It is possible therefore, that the lack of scent in hummingbird-pollinated flowers has a similar explanation to the use of the colour red, in that it serves more to make flowers inconspicuous to insect pollinators, most of which rely on both visual and olfactory clues in foraging (Dobson, 1994; Wright and Schiestl, 2009).

Nectar Volume and Composition

Nectar is the only floral reward offered to birds. Ornithophilous birds have several adaptations to their tongues to facilitate nectar feeding. The tip of the tongue is grooved or fringed and is often able to roll into a tube to allow for feeding by capillary action, and the tongue is extensible beyond the tip of the bill (Stiles, 1981). The capillary action causes nectar to flow into the lateral grooves of the tongue, where it is then transported into the bill by the retraction of the tongue (Hainsworth, 1973). To achieve the highest energy intake rate when feeding using this method, low nectar concentrations are optimal (Roberts, 1995).

Bird-pollinated flowers tend to have higher volumes of nectar to meet the higher energy demands of birds, between 10 and 25 μ l (Castellanos *et al.*, 2002, Johnson and Nicolson, 2008) with slightly to markedly lower concentrations than those of insect-pollinated flowers, and considerably higher sugar production overall, in comparison to insect-pollinated flowers (Baker, 1975; Heinrich, 1975; Stiles, 1975, 1978; Opler, 1981; Cruden and Miller-Ward, 1981, Nicolson, 2002; Johnson and Nicolson, 2008). Nectar concentration is remarkably uniform in bird-pollinated flowers, ranging from 15-34%, though more commonly falling between 20 and 26% (Baker, 1975; Pyke and Waser, 1981; Stiles and Freeman, 1993; Proctor *et al.*, 1996).

Birds are endotherms, but they are small, leading to higher energy requirements than similar sized ectotherms. Hummingbirds in particular are the smallest endothermic vertebrates and have very high mass-specific metabolic rates, close to 215 cal g⁻¹hr⁻¹ regardless of body size (Pearson, 1950; Lasiewski, 1963; Calder, 1984; Bartholomew and

Lighton, 1986; Suarez, 1992). Both lab and field studies have shown that energetic criteria are the most important determinants of flower choice in nectarivorous birds (Hainsworth & Wolf, 1976; Stiles, 1976). The three dominant sugars in nectar (sucrose, glucose and fructose) are energetically equivalent, but sucrose is normally more prevalent in hummingbird pollinated flowers (Stiles, 1976; Baker and Baker, 1983). Old world flowers pollinated by passerine birds tend to be lower in sucrose as passerines are thought to have difficulty processing sucrose (del Rio, 1992; Baker & Baker, 1990), however there are exceptions to this rule. While preference for hexose over sucrose has been shown in hummingbirds, flowerpiercers, sunbirds, honeyeaters and lorikeets when they are offered a dilute diet, if a more concentrated diet is offered all show a preference for sucrose (Stiles, 1976; del Rio 1990; del Rio *et al.* 1992; Schondube and del Rio, 2003; Fleming *et al.*, 2004; 2008). The preference for hexose on dilute diets is thought to be related to the digestive capabilities of different taxa (del Rio, 1990; Fleming *et al.*, 2008).

Studies have shown that hummingbirds prefer nectar from feeders with a concentration of between 30 and 40% nectar (Roberts, 1996), and when flower and nectar handling costs are considered, the optimal nectar concentration for hummingbirds is over 40% (Kingsolver and Daniel, 1983). Why then do birds visit flowers of such low concentrations in the wild? This may be another case of floral characteristics whose purpose is to deter other visitors rather than to attract birds. Hummingbirds have extremely efficient kidney tubules that can excrete excess water rapidly, therefore they can handle highly diluted nectar better than insect pollinators (McWhorter and del Rio 1999). Nectar with sugar concentration below 18% is not beneficial to honeybees because of the high energetic cost of evaporating water in order to produce honey (Percival, 1965), and Bertsch, (1984) proposed that feeding a male bumblebee on nectar with concentration as low as 25% would be impossible because the water-load ingested to meet the daily energy requirements of 110mg of sugar would be too high. The optimal concentration for maximum net energy uptake in *Bombus* is between 50 and 65% (Harder, 1986), and for orchid bees between 30

and 40% (Borrell, 2007). Several species of Meliponinae and European *A. mellifera* have a maximum caloric uptake at a concentration of between 45% and 60% with lower uptake rates below these levels (Roubik and Buchmann, 1984).

Nectar concentration preferences are linked to nectar viscosity. The more concentrated a nectar solution, the more viscous and difficult it becomes to rise up or be sucked up a tube, as in the feeding systems of birds (Harder, 1986; Willmer, 2011) and although energy content increases linearly with concentration, viscosity increases exponentially. Therefore ingestion rates for hummingbirds should decline as nectar concentration increases and the optimum intake rate should occur at an intermediate sugar concentration (Kingsolver and Daniel, 1983; Nicolson and Thornburg, 2007).

Nectar production in bird-pollinated plants usually begins very early in the morning, so that plenty of nectar is available from dawn. Highest nectar production is in the morning, and production tails off around midday. This coincides with foraging patterns of bird flower visitors (Stiles, 1975; Bednekoff and Houston, 1994). In addition, many species of hummingbird-visited flowers are able to replenish the nectar of their flowers after they have been emptied by a flower visitor (Cruden *et al.*, 1983; Gill, 1988; Pyke, 1991; Galetto, *et al.*, 1994; Torres and Galetto, 1998; Navarro, 1999; Castellanos *et al.*, 2002).

Nectar also contains amino acids, lipids and polysaccharides (Baker and Baker, 1975) which have been suggested to be less nutritionally important to birds, who can find these substances in fruit or insects, unlike insect pollinators. Hummingbird flowers have been shown to have low concentrations of amino acids (Grant & Grant, 1968; Baker & Baker, 1975; 1990), and it was shown that low concentrations are not detected by hummingbirds, but high concentrations have a repellent effect, suggesting that hummingbirds do not usually use information on amino acid content when choosing flowers, however at high concentrations the “bad taste” will drive them away (Hainsworth and Wolf, 1976). Several studies have contradicted these findings however. Damage to flowers by either flower visitors

or nectar collection techniques such as microcapillaries may cause amino acids to leak from the plant tissue to the nectar (Willmer, 1980), and displacement of pollen into nectar may also cause faulty readings of amino acid concentration in nectar (Gottsberger *et al.*, 1990). In addition, due to the low viscosity of low concentration nectars, a larger diffuse “spot” is created on filter papers used for calorimetric amino-acid testing, even if constant volumes are collected and applied, therefore the more dilute nectars of bird flowers will tend to produce a lower concentration of amino acids (Willmer, 2011). There is little evidence to show that amino acids are important to, or detected by, many flower visitors such as tropical stingless bees (Gardener *et al.*, 2003) and sunbirds (Lesigneur *et al.*, 2007; Nicolson, 2007). Recent studies using more reliable HPLC techniques found no relationship between amino acid levels and life form or flowering season, and taxon showed very little effect (Petanidou *et al.*, 2006).

Placement of Reproductive Structures

The placement of anthers and stigmas on a bird-pollinated flower are ideally suited for depositing pollen onto the bird, and from there to the stigma of flowers. Long, protruding stamens dust the heads, backs and beaks of birds with pollen. When a bird next visits a flower, it brushes pollen onto the stigma, also long and protruding (Cronk and Ojeda, 2008). Timing of dehiscence follows nectar production, and therefore foraging patterns of birds, in that it occurs mainly in the early morning (van der Pijl, 1961; Proctor and Yeo, 1973; Stiles, 1981; Castellanos *et al.*, 2006). The filaments of stamens are generally tough to withstand the rough handling of birds, and the ovary is protected and usually inferior for the same reason. There is a large spatial separation between the nectar of bird-pollinated flowers and the anthers, when dehiscing, or the stigmas, when receptive (Faegri and van der Pijl, 1979).

Testing Pollinator Syndromes

In order to test the validity of determining the most effective pollinators of a flower species using floral traits corresponding to pollination syndromes, two species from Costa Rica with apparent hummingbird-pollination traits were investigated. The first, *Malvaviscus arboreus*, is well studied and has been shown to be pollinated most effectively by the cinnamon hummingbird, *Amazilia rutila*. This species was chosen as its most effective pollinator has already been subject to analysis (Webb and Bawa, 1983; Webb, 1984), therefore it makes for an effective test of the method of measuring pollinator effectiveness using single visit pollen deposition on stigmas, as defined in Chapter 2 (Assessing Single Visit Pollen Deposition).

The second species, *Helicteres guazumifolia*, is rarely mentioned in the literature, and no study of its pollinators has yet been carried out. It shares many traits with *Malvaviscus arboreus* and is again a plant indicative of hummingbird-pollination, according to the traits shown in Table 1. Should single-visit pollen deposition on stigmas be shown to be an effective means of determining pollinator effectiveness for the first species, I can test the flower visitors of *Helicteres guazumifolia* and attempt to identify its most effective pollinator species, and see whether this correlates with the predicted visitor based upon floral traits.

Materials and Methods

Study Site

The populations of hummingbird-pollinated plants chosen for this study were located in Parque Nacional Santa Rosa, Guanacaste Province (10° 50' N, 85° 40' W), in the North-West region of Costa Rica. The park covers about 495 square kilometres and contains savannah, deciduous forest, marshland and mangrove swamp habitats. The populations of both plants were both located in the dry, disturbed, deciduous forest area of the park.

Malvaviscus arboreus

Malvaviscus arboreus is a shrub from the Malvaceae family. Distribution is from northern South America to southern North America. In Costa Rica, it is found to flower throughout the dry season, from December to April.

Structure

Flowers are bright red in colour, with a corolla tube approximately 27mm long and approximately 15mm wide at the base, and a protruding style approximately 58mm long. The style comprises 10 branches, each terminating in a hairy stigma, and also bears between 20 and 30 anthers situated just below the stigmas. Large quantities of pollen grains are contained in small pod-like anther structures held on the style approximately 3mm from the stigmas (see Fig. 1). The ovary is positioned below the base of the corolla tube. Nectar is secreted from the base of the petals, pooling in the bottom of the corolla tube, between the staminal column and the petals (Webb, 1984).



Fig. 1: The bee *Trigona fulviventris* stealing nectar by piercing the base of the corolla of *Malvaviscus arboreus*. Stigma and anthers shown.

The placement of nectar, pollen and stigma in this flower allows for a hummingbird to feed on the nectar of the flower, coming into contact with the pollen, which is then transferred via the head of the bird to the next flower visited.

Nectar

Sugar concentration of nectar (w/w) was measured using a hand-held Bellingham and Stanley field sugar refractometer with a range of 0-50%. Nectar volume and concentration readings were taken every hour using 1µl glass capillary tubes to fully drain the nectar present in the corolla. These readings were taken throughout the day over three days to determine the nature of nectar replenishment and to confirm the observations of previous

studies (Webb, 1984) that nectar is replenished throughout the morning, with production tailing off around noon.

Timing

Flowers are viable for a single day, as stigmas and anthers turned black and withered on the second day, although the flower corolla remains until the end of the second day. This is thought to increase the attractiveness of a plant by adding to the overall floral display (Webb, 1984). Dehiscence begins before dawn, and the majority of anthers have dehisced by the time the sun has risen. Most of the pollen removal and dispersal occurs in the first 2-4 hours after dawn (Webb and Bawa, 1983). No temporal separation of sexual phases occurs in this species as both dehiscing anthers and apparently receptive (visually glossy) stigmas were observed on individual flowers at the same time, as confirmed by Webb (1984) by testing for receptive stigmas with peroxidase (as per Faegri and Van Der Pijl, 1979).

Previous studies have found this species to be self-compatible (Webb and Bawa, 1983; Webb, 1984). To test this, stigmas were brushed with pollen from the same flower and covered with netting to prevent further visitations. The flowers were then observed over several days and any incidence of seed set was recorded.

Helicteres guazumifolia

Helicteres guazumifolia is a shrub from the family Sterculiaceae. Its range covers South Mexico to Central America (Cristóbal, 2001). Flowering is typically from March to late June.

Structure

The bright red, erect, flowers are found in pairs, and possess a tubular corolla approximately 50mm long and approximately 15mm wide with a basal nectary. Each

possesses a protruding style approximately 70mm in length, terminating in a stigma consisting of two sticky frond-like structures (see Fig. 2). Also attached to this style, below the stigmas, are 8 club-shaped anthers containing large quantities of pollen.

As in *M. arboreus*, the placement of nectar, pollen and stigmas in this species is typical for a hummingbird flower, allowing for a hummingbird to feed on the nectar of the flower while pollen is brushed on to the top of the head by the anthers. This is then transferred via the head of the bird to the next flower visited.

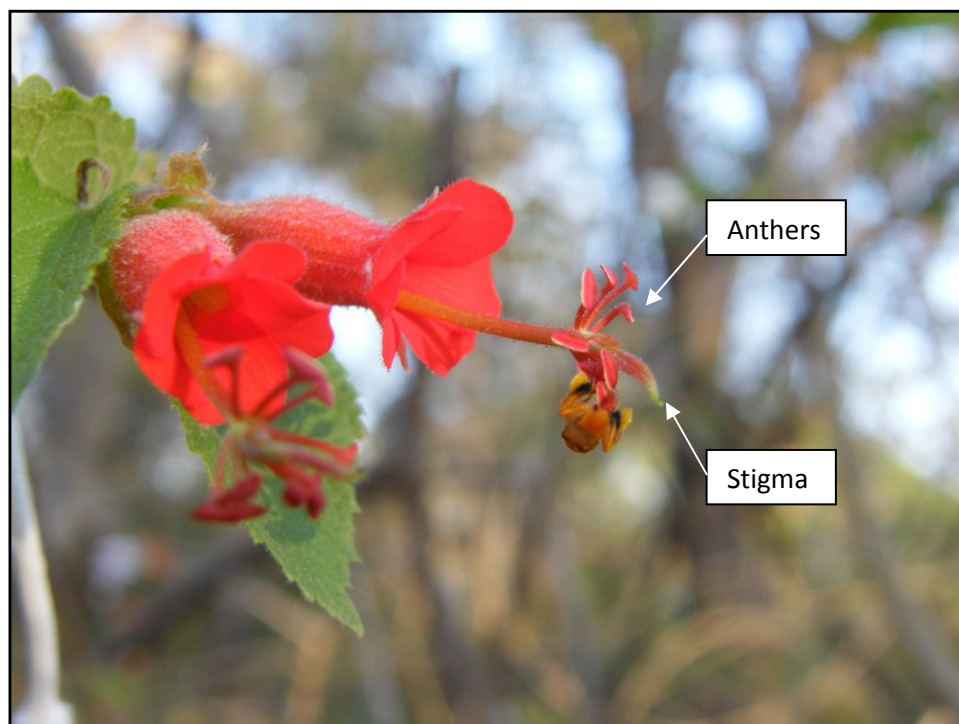


Fig 2: *Tetragonisca angustula* stealing pollen from flowers of *Helicteres guazumifolia* without coming into contact with the stigma. Stigma and anthers indicated.

Nectar

Nectar concentration was again measured using hand-held Bellingham and Stanley field sugar refractometers with a range of 0-50%. Nectar volume was measured at half hour intervals throughout the day over three days to determine the nature of production and replenishment and to confirm observations by Goldberg (2009) that nectar production occurred all day, from 06:00 to 18:00.

Timing

Dehiscence begins just before dawn and then pollen is available for the 2 or 3 days that the flowers are open. No temporal separation of sexual phases occurs in this species as both dehiscing anthers and receptive (visually glossy and sticky to the touch) stigmas were observed on flowers at the same time. Self compatibility of this species was tested as above for *M. arboreus*.

Sampling Periods

Field research occurred between January and April of 2009. During the dry season, daylight hours were between 06:00 and 18:00. To adequately sample throughout the day, daylight hours were split into 4 time periods: 06:00-09:00, 09:00-12:00, 12:00-15:00 and 15:00-18:00. In total 100 single-visit observations were made over several days. These were split into 25 observations from each of the different time periods. Each sampling session was between 1 and 3 hours long depending on the frequency of visitations and how long it took all opened flowers to be visited.

Results

Malvaviscus arboreus

Temperature and Humidity

Temperature and humidity readings were taken every 30 minutes during observation periods (see Fig. 3 and Fig. 4). For both species, mean temperature rose steadily from dawn until about midday, when it declined once more. Mean humidity started high and declined until about mid afternoon, when it began to rise again. Humidity readings for the study of *Malvaviscus arboreus* were lower than typical readings for the area, which tend to be taken

from denser areas of the forest, as this species favours more exposed, disturbed areas of the forest. Temperature and humidity readings for *Helicteres guazumifolia* were slightly higher and more variable at this time in comparison to readings taken during the study of *M. arboreus*. This was possibly due to the fact that during the study period of *H. guazumifolia* (two weeks later than the study of *M. arboreus*), the weather in Santa Rosa National Park was abnormally variable with several storms and unusual weather for the season. *Helicteres guazumifolia* also grows in disturbed, open areas of the forest which are more exposed to the elements, accounting for the variation in temperature and humidity in comparison to expected results based on typical measurements for the forest at that time of year.

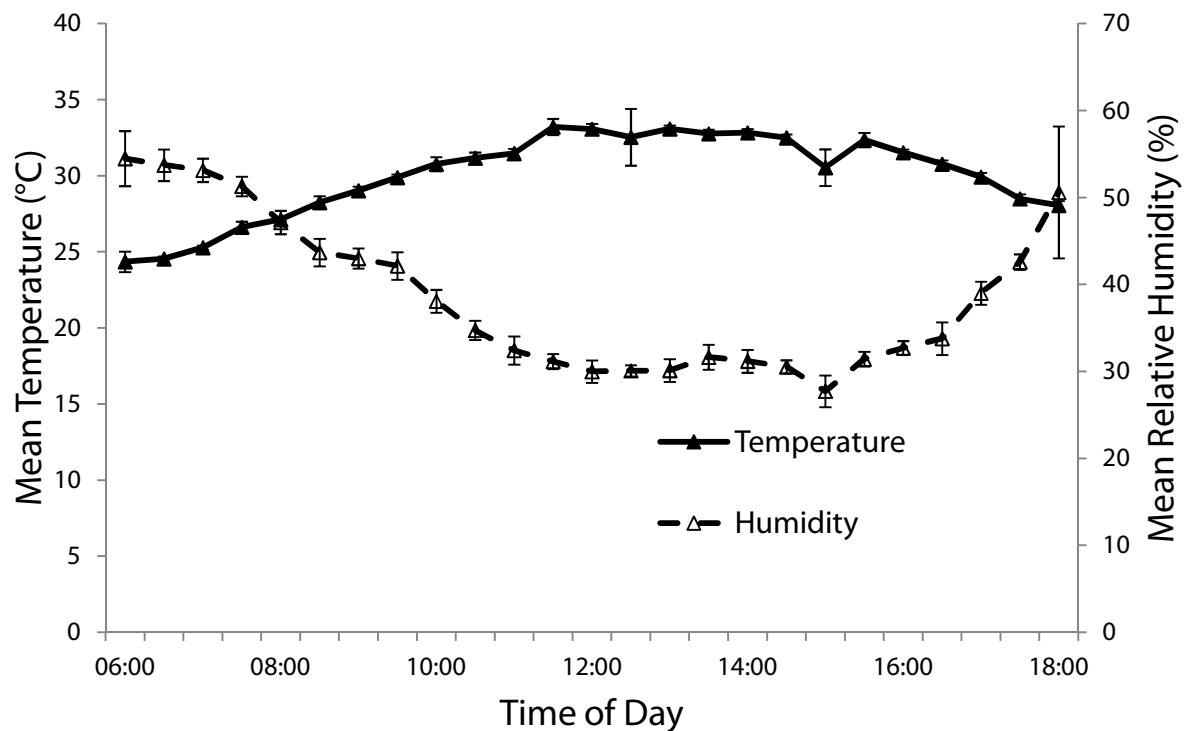


Fig. 3: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Malvaviscus arboreus*. Standard deviations are shown (N=4).

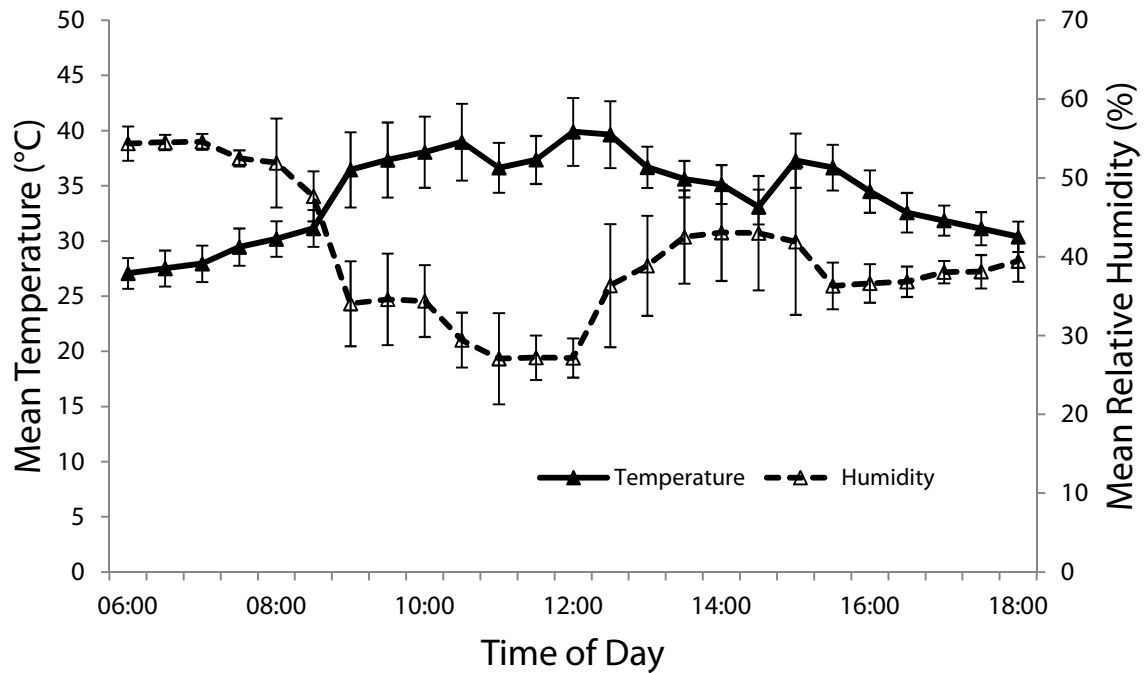


Fig. 4: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Helicteres guazumifolia*. Standard deviations are shown (N=4).

Nectar Concentration and Volume

The volume and concentration of nectar available in flowers of *Malvaviscus arboreus* and *Helicteres guazumifolia* was monitored every hour over 3 days (see Fig. 5 and Fig. 6). Nectar volume of *Malvaviscus arboreus* flowers is highest at dawn, with a mean of 7.5µl per flower. It drops quickly as the flowers are visited and emptied throughout the early morning. Volume is replenished slightly throughout the morning as flowers which were emptied were later found to have produced more nectar; however production stops almost completely after midday. Concentration varies slightly throughout the day, and is highest earlier in the morning, with an average of 26%.

Unlike *M. arboreus*, nectar production by *H. guazumifolia* continued throughout the day. Production began at dawn, with volume increasing throughout the day until late afternoon and production terminating at 18:00. Concentration was highest at dawn and showed a general decrease over the rest of the day, with a mean range of between about 15

and 30%. Nectar of *H. guazumifolia* therefore has high volume and low concentration, fitting the characteristics of typical bird-pollinated flowers (see table 1).

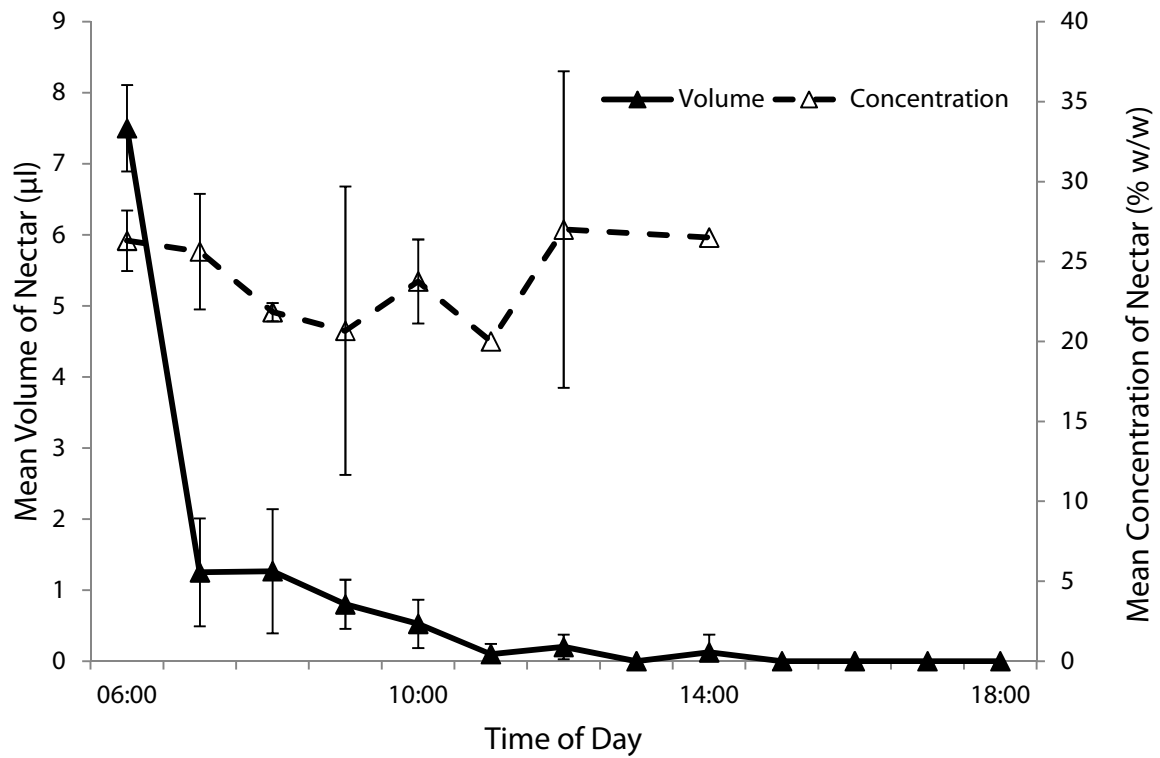


Fig. 5: Mean nectar volume (µl) and concentration (%w/w) of flowers of *Malvaviscus arboreus*. Standard deviations shown (N = 3).

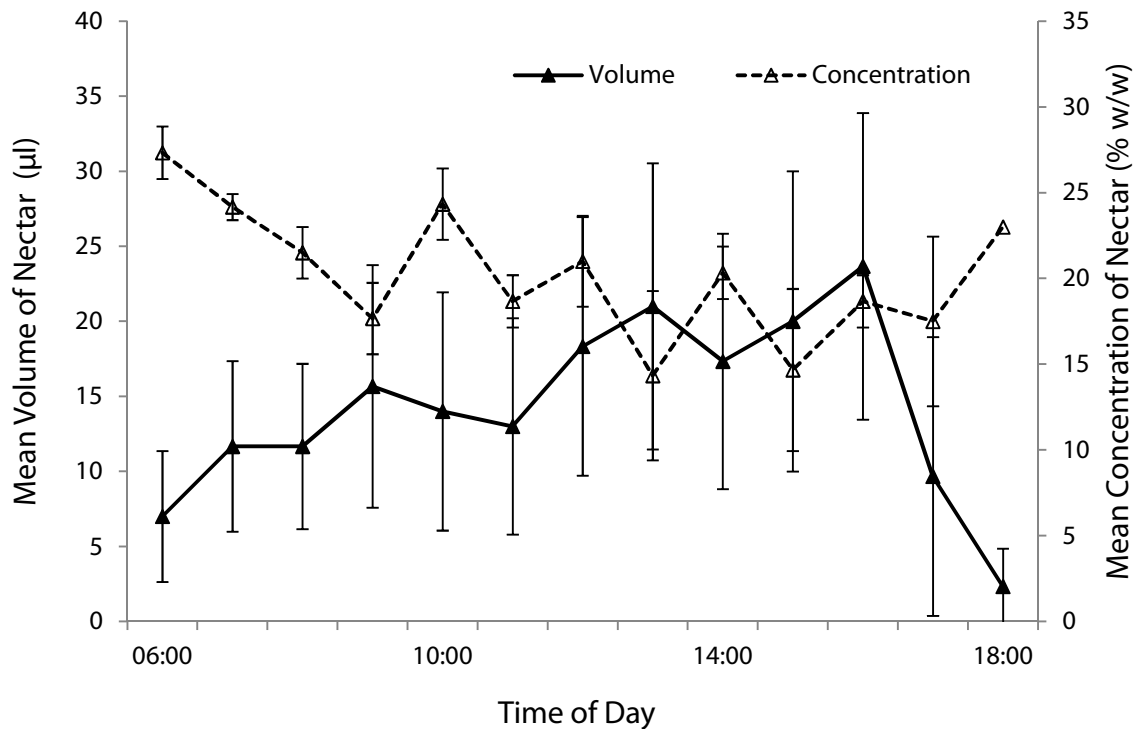


Fig. 6: Mean nectar volume (µl) and concentration (%w/w) of flowers of *Helicteres guazumifolia*. Standard deviations shown (N=3).

Self-Compatibility

After being dusted with self-pollen, the flowers of both species were observed over several days after which the ovaries were dissected and found to have set seed. This confirms that the flowers of *M. arboreus* are self-compatible, as shown in previous studies (Webb, 1983), as are those of *H. guazumifolia*.

Partitioning of Visitors over Time

Visitors to target flowers of *Malvaviscus arboreus* and *Helicteres guazumifolia* over the day were recorded throughout the study period. Visitors were first treated as functional groups (Fig. 7 and Fig. 9), and then by individual species (Fig. 8 and Fig. 10). Visits recorded do not provide a complete representation of the visitor assemblage of the plant species throughout the day, as only visits to target, previously bagged flowers were recorded; however some patterns of visitation can be seen in the limited data available.

Malvaviscus arboreus

Visitors to *Malvaviscus arboreus* were first treated as functional groups; in this case birds, bees, butterflies and ants (see Fig. 7), although only one species of bird (*Amazilia rutila*) and one species of ant (*Camponotus novograndensis*) were recorded, and two species of butterfly (*Eurema दौरा* and *Pieris अगरिथे*).

Visits noted were not an accurate representation of total visitation rates, as only single visits to previously bagged flowers were recorded. Some patterns regarding the earliest observations of different species, as well as times when certain species were absent, can be inferred from the results obtained, however more accurate conclusions would require a more intensive visitation study.

Bees were the earliest visitors to flowers, beginning their foraging at dawn, declining at midday as temperatures were at their highest and returning in the afternoon. Birds followed from about 07:30, again ceasing foraging at the hottest time of the day and returning in the afternoon. Butterflies began foraging around 09:00 and were not observed through the middle of the day and the afternoon. Ants were only found on flowers in the afternoons.

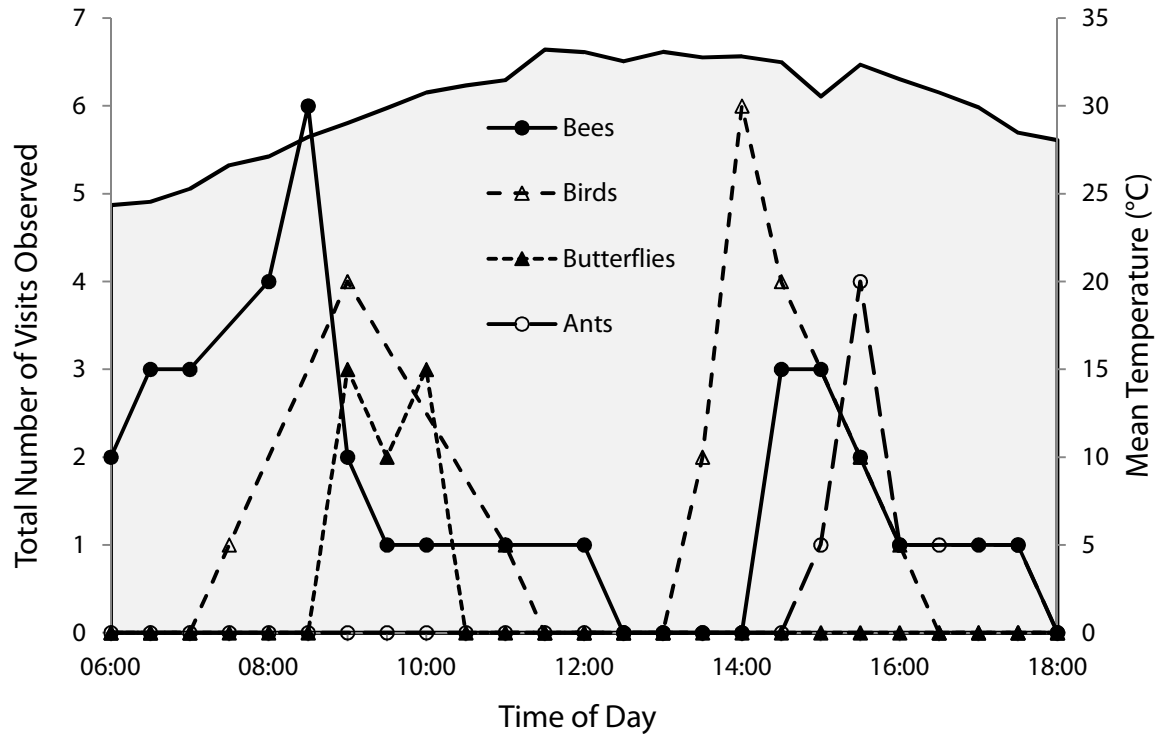


Fig. 7: Partitioning of flower visitor groups over time to flowers of *Malvaviscus arboreus*. Mean daily temperature (°C) is shown as an area plot.

Three different bee species visited the flowers (*Trigona fulviventr**is*, *Tetragonisca angustula* and *Agapostemon* sp.) and the partitioning over time of visits by each individual bee species was also investigated (see Fig. 8). The larger *Trigona fulviventr**is* and *Agapostemon* sp. began foraging at dawn while the smaller *Tetragonisca angustula* did not begin until about 07:30m. *T. fulviventr**is* and *Agapostemon* sp. stopped foraging around 10:30 as temperatures began to climb, returning around 14:00 as temperatures began to drop. *T. angustula* was able to continue foraging until midday, also returning in the afternoon.

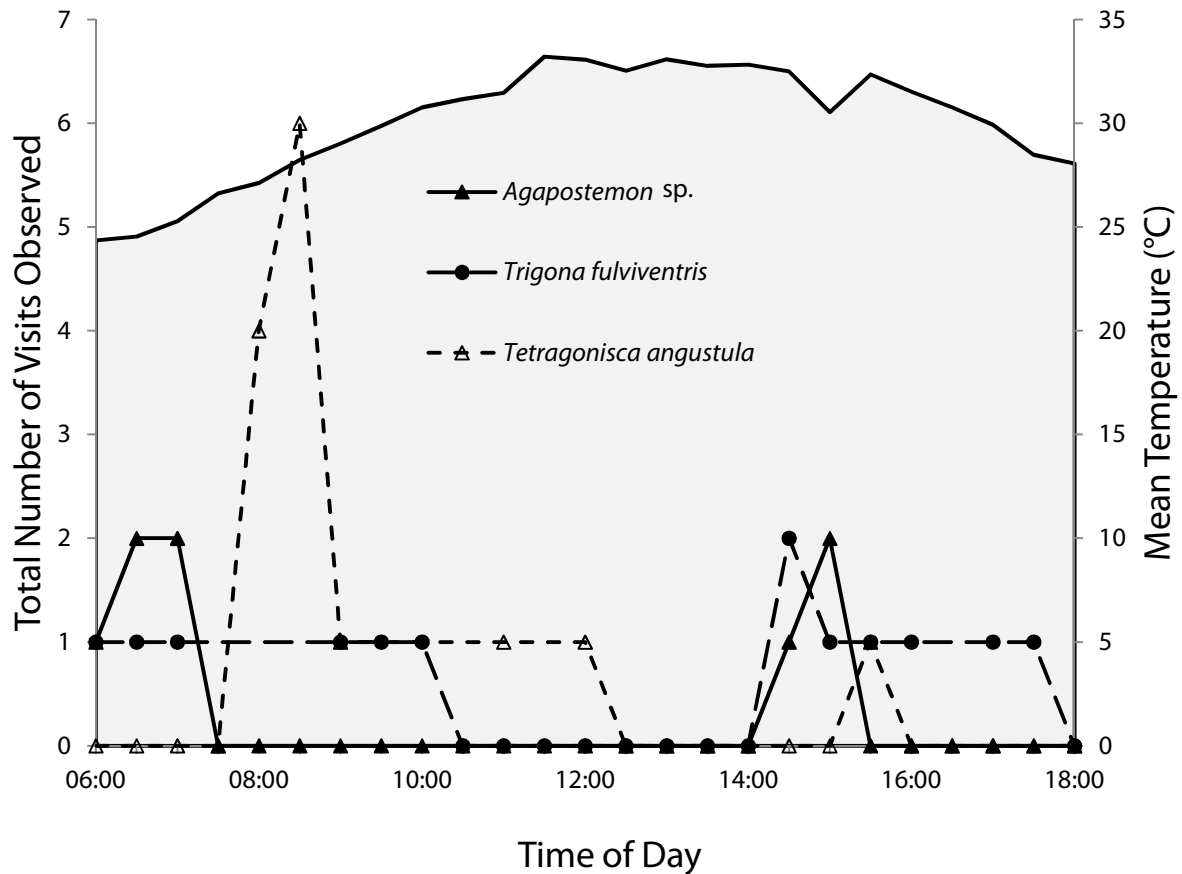


Fig. 8: Partitioning of individual bee species visiting flowers of *Malvaviscus arboreus* over the day. Mean daily temperature (°C) is shown as an area plot.

Helicteres guazumifolia

Visitors to *H. guazumifolia* flowers were at first analysed by the functional groups birds, bees and wasps (see Fig. 9) although only one species of hummingbird, *Phaethornis guy*, and one species of vespid wasp, were observed. The earliest flower visitors were the bees, which began foraging at 06:00, followed by the birds at 07:00. Only one visit by a wasp was recorded at 11:30. Birds were only recorded at flowers early in the morning, up to 10:30am.

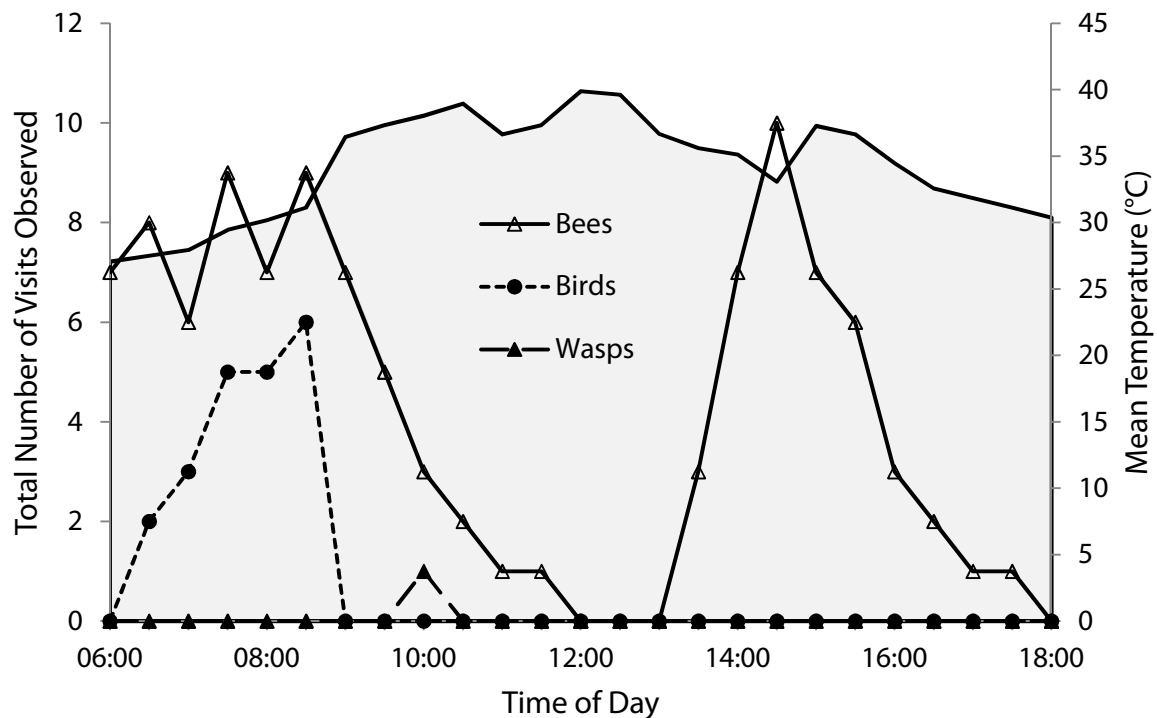


Fig. 9: Partitioning of flower visitor groups of *Helicteres guazumifolia* over time. Mean daily temperature (°C) is shown as an area plot.

As before, a more intensive visitation survey is required to make accurate conclusions as to the patterns of visitation, however first observations and times when certain species were absent can be noted. Bees visited throughout the day although there was a drop in visitation around noon when temperatures were at their highest. Three species of bee (*Trigona fulviventris*, *Tetragonisca angustula*, and *Agapostemon* sp.) visited *H. guazumifolia* (see Fig. 10). The earliest visitor was the medium sized *Trigona fulviventris* which began foraging at dawn and continued throughout the day, stopping only between 11:00 and 13:00. This was followed by the much smaller *Tetragonisca angustula* at 08:00 which foraged throughout the morning, continuing a little later until 12:00. *Agapostemon* sp. began foraging at 09:00 and continued until 12:00.

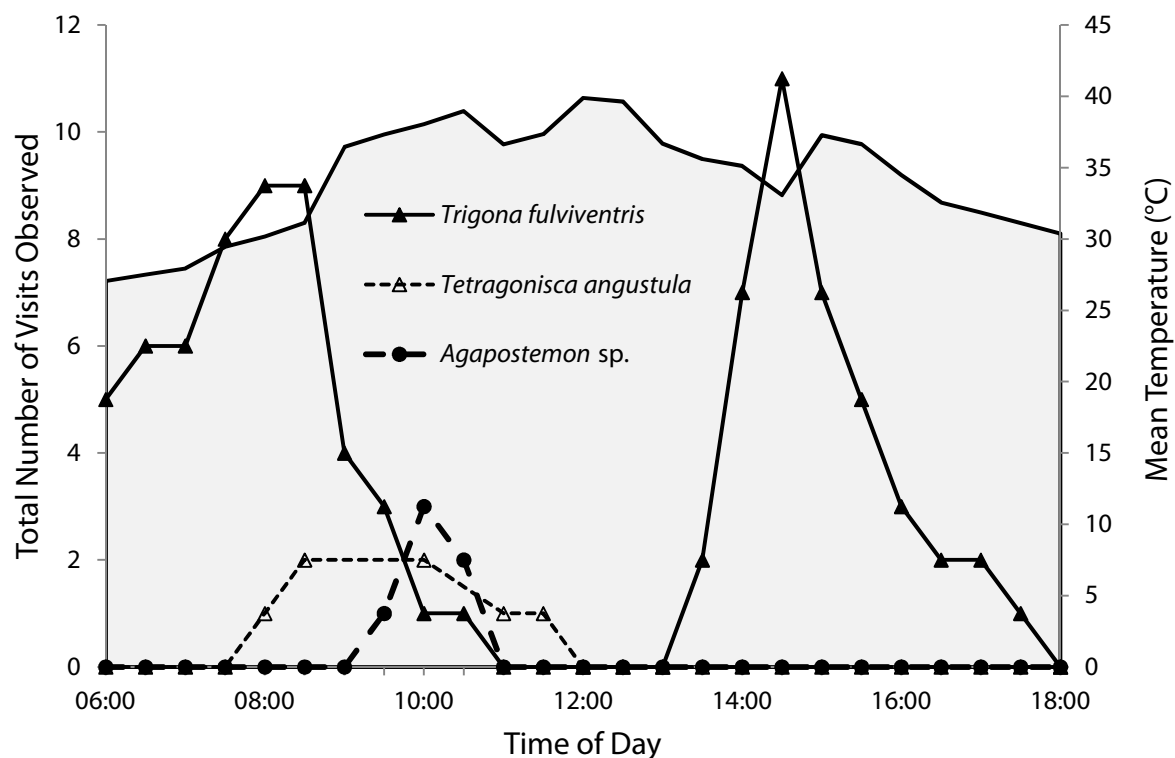


Fig. 10: Partitioning of individual bee species visiting flowers of *Helicteres guazumifolia* over the day. Temperature (°C) is shown as an area plot.

Pollen Deposition by Visitors

Malvaviscus arboreus

Group	MPS	Sig. of MPS (in comparison to MPS of Controls)
Bird	104.4	$P < 0.001$
Bee	29.0	$P = 0.003$
Butterfly	5.8	$P = 0.236$
Ant	11.1	$P = 0.351$

Table 2: Statistical analysis of visitor groups to *Malvaviscus arboreus*. Statistical analysis was performed by a Mann-Whitney U test with Bonferroni correction. In this case, $\alpha = 0.0125$.

The mean single visit stigmatic pollen deposition (MPS) by each visitor group (birds, bees, butterflies and ants) was calculated (see Fig. 11 and Table 2) and the difference in deposition between visitor groups was statistically significant. Both the hummingbird and bee groups deposited significantly more pollen grains on stigmas than present on control stigmas and were identified as pollinators. Butterflies and ants did not deposit a significant amount of pollen in comparison to the control stigmas.

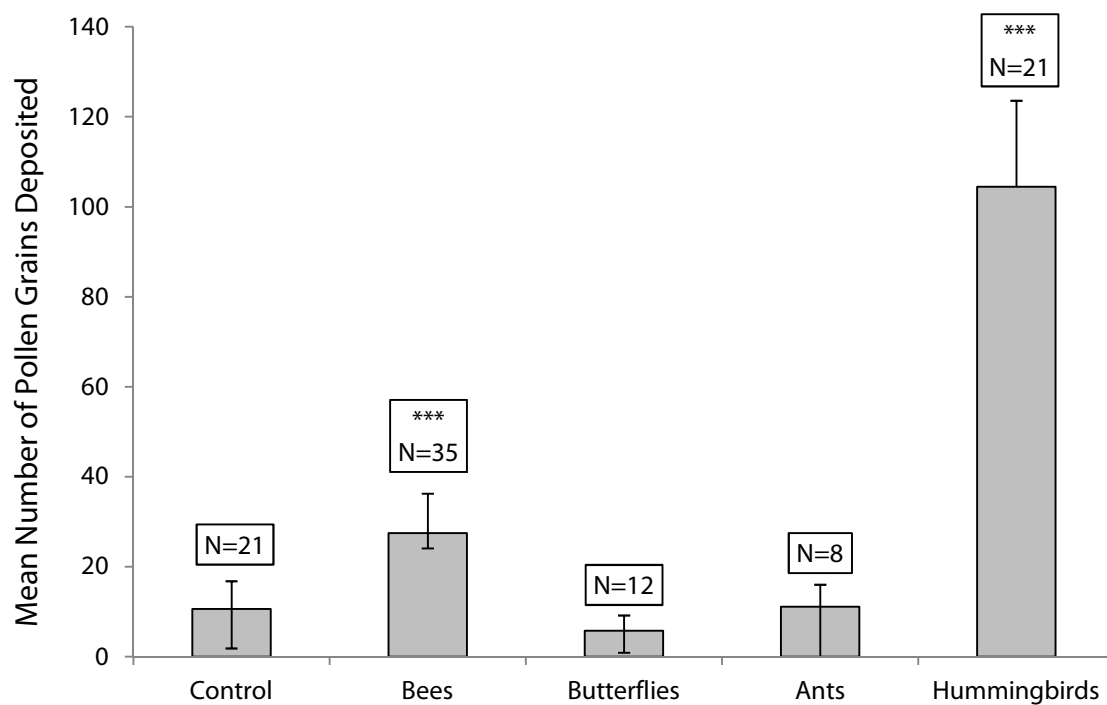


Fig. 11: Mean pollen deposition by visitor groups to *Malvaviscus arboreus*. N values and SD shown. Significance refers to the difference in pollen deposition between the groups and the unvisited control flowers. The difference in deposition between visitor groups was statistically significant (One-Way ANOVA: $P > 0.001$, $F = 43.639$, $df = 3$).

Group	Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
Bird	<i>Amazilia rutila</i>	104.4	P < 0.001
Bee	<i>Trigona fulviventris</i>	21.9	P = 0.095
Bee	<i>Tetragonisca angustula</i>	21.9	P = 0.040
Bee	<i>Agapostemon</i> sp.	53.1	P = 0.001
Butterfly	<i>Phoebis agarithe</i>	0.5	NA
Butterfly	<i>Eurema daira</i>	8.4	P = 0.090
Ant	<i>Camponotus novograndensis</i>	11.1	P = 0.351

Table 3: Statistical analysis of visitor species to *Malvaviscus arboreus*. Statistical analysis was performed by a Mann-Whitney U test with Bonferroni correction. In this case, $\alpha = 0.008$

Pollen deposition was then analysed by individual species to look for variation within groups of visitors (see Fig. 12 and Table 3). The butterfly *Phoebis agarithe* was excluded from statistical analyses as there were insufficient visits recorded. Variation between species was statistically significant. The hummingbird *Amazilia rutila* and the bee *Agapostemon* sp. deposited significantly more pollen on stigmas than controls. *Trigona fulviventris*, *Tetragonisca angustula*, *Eurema daira*, and *Camponotus novograndensis* did not deposit significant amounts of pollen.

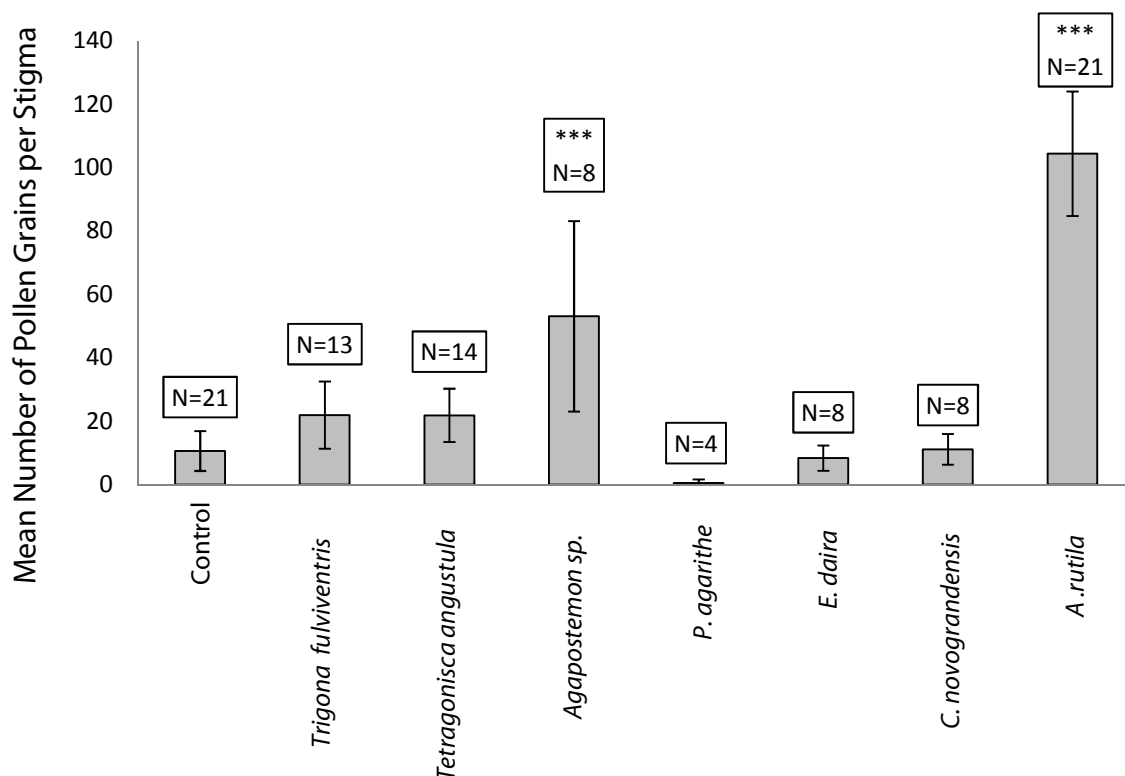


Fig. 12: Mean pollen deposition by visitor species to *Malvaviscus arboreus*. N values and SD shown. Significance refers to the difference in pollen deposition between the species and the unvisited control flowers. Variation between species was statistically significant (One-Way ANOVA: $P < 0.001$, $F = 23.256$, $df = 5$).

Helicteres guazumifolia

Group	MPS	Sig. of MPS (in comparison to MPS of Controls)
Bird	1517.1	$P < 0.001$
Bee	441.8	$P < 0.001$
Wasp	15	NA

Table 4: Statistical analysis of visitor groups to *Helicteres guazumifolia*. Statistical analysis was performed by a LSD Post Hoc Test.

The mean pollen deposition by each visitor group (birds, bees and wasps) was calculated (see Fig. 13). As there were insufficient data available for the vespid wasp, this visitor was omitted from statistical analyses. The difference in deposition between the bird and bee groups was statistically significant. Both the hummingbird and bee groups deposited significantly more pollen grains on stigmas than was found on control stigmas. The bird group deposited significantly more pollen on stigmas than did the bee group (LSD Post Hoc Tests: $P = < 0.001$).

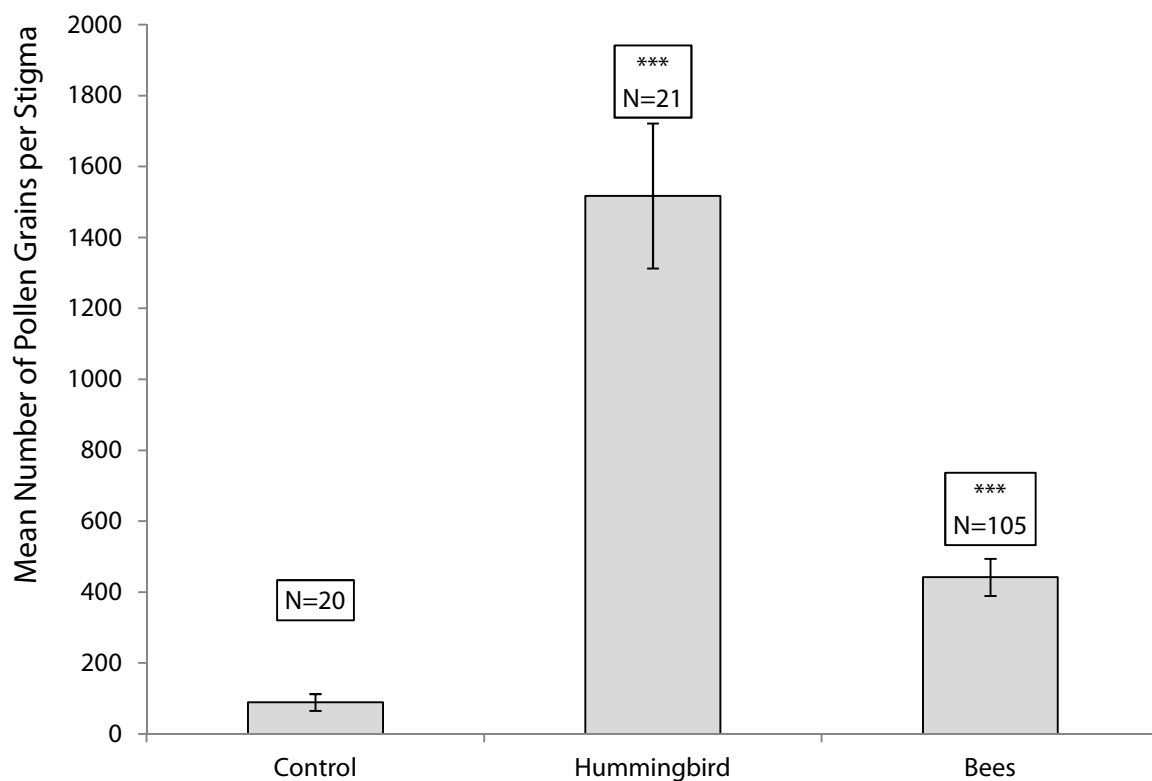


Fig. 13: Mean pollen deposition by visitor groups to *Helicteres guazumifolia*. N values and SD shown. Significance refers to the difference in pollen deposition between the groups and the unvisited control flowers. The difference in deposition between the bird and bee groups was statistically significant (One-Way ANOVA: $P > 0.001$, $F = 107.656$, $df = 1$).

Group	Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
Bird	<i>Phaethornis guy</i>	1517.1	$P < 0.001$
Bee	<i>Agapostemon</i> sp.	400.0	$P < 0.001$
Bee	<i>Trigona fulviventris</i>	443.4	$P < 0.001$
Bee	<i>Tetragonisca angustula</i>	162.9	$P = 0.060$

Table 5: Statistical analysis of visitor species to *Helicteres guazumifolia*. Statistical analysis was performed by a LSD Post Hoc Test.

Pollen deposition was then analysed for individual species to look for variation within functional groups (see Fig. 14 and Table 5). Variation between species was statistically significant. The hummingbird *Phaethornis guy*, and the bees *Agapostemon* sp. and *Trigona fulviventris*, deposited significantly more pollen on stigmas than was found on control groups; however, *P. guy* deposited significantly more pollen than both *Agapostemon* sp. (LSD Post Hoc Tests: $P = <0.001$) and *T. fulviventris* (LSD Post Hoc Tests: $P = <0.001$). *Tetragonisca angustula* did not deposit significant amounts of pollen.

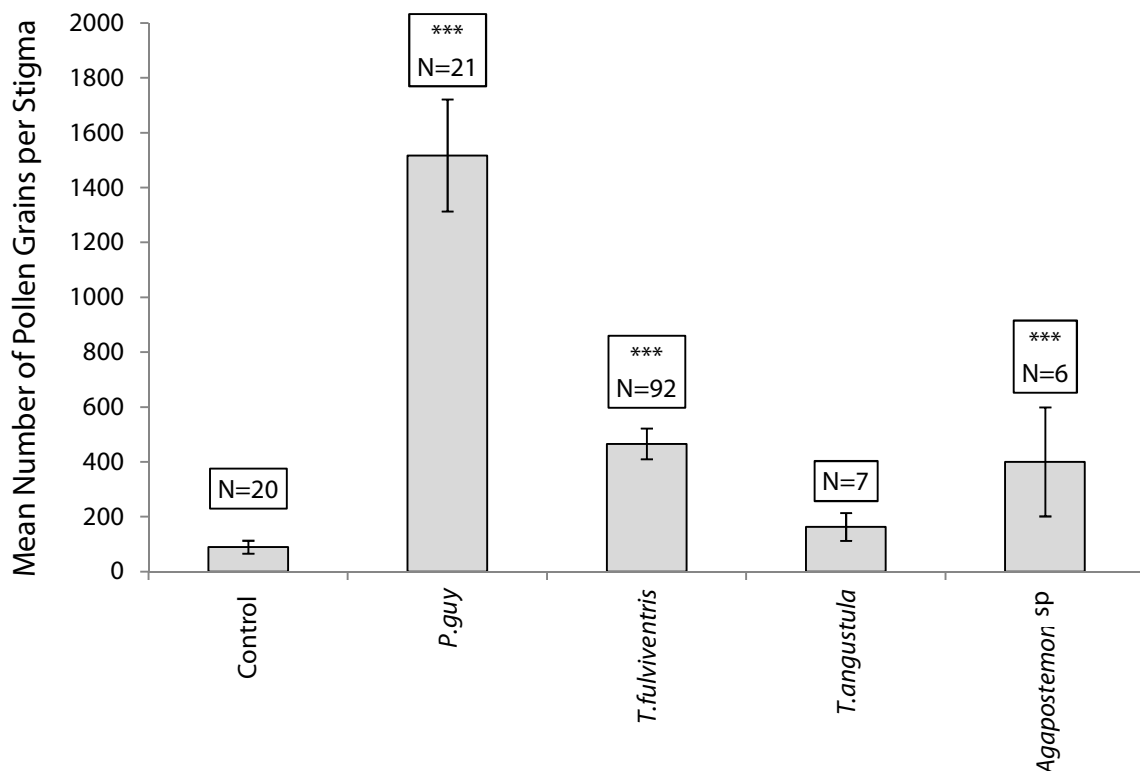


Fig. 14: Mean pollen deposition by visitor species to *Helicteres guazumifolia*. N values and SD shown. Significance refers to the difference in pollen deposition between the species and the unvisited control flowers. Variation between species was statistically significant (One-Way ANOVA: $P < 0.001$, $F = 43.062$, $df = 3$).

Discussion

Floral traits

Malvaviscus arboreus

Several features of *Malvaviscus arboreus* point towards it being a bird-pollinated flower. Its bright red colour, absence of strong scent, long corolla tube and protruding style are typical of the syndrome (see Table 1). The sugar concentration of nectar during peak production hours was between 20 and 35% (see Fig. 4), as found in previous studies (Webb and Bawa, 1983) and within the typical guidelines for a bird-pollinated flower (e.g. Wolff, 2006; Johnson and Nicolson, 2008). Nectar production continued throughout the morning, tailing off at noon as was suggested in previous studies of *M. arboreus* (Webb and Bawa, 1983) and other bird-pollinated flower species (e.g. Cruden et al., 1983, Castellanos et al., 2002; Wilson et al., 2006).

Helicteres guazumifolia

Helicteres guazumifolia has several traits indicative of hummingbird pollination. It has bright red, erect flowers with little obvious scent, a long corolla tube, approximately 50mm long, a basal nectary and a protruding style. Nectar production was measured and on average flowers contained 15-20µl of nectar, with production beginning at 06:00 and continuing throughout the day, stopping in the evening. Mean concentration of nectar was again around 19% (see Fig. 5). These results are similar to those found in a previous study of the species (Goldberg, 2009) and in line with typical records for hummingbird-pollinated flowers (Cruden et al., 1983, Castellanos et al., 2002, Wilson et al., 2006; Wolff, 2006; Johnson and Nicolson, 2008).

Visitor Assemblage

Malvaviscus arboreus

Webb (1984) used observations of flowering *M. arboreus* plants to determine the most frequent visitors and the likely pollinators of the species. The cinnamon hummingbird, *Amazilia rutila*, was the most frequent visitor, along with another unidentified species, thought to be the fork-tailed emerald hummingbird (*Chlorostibon canivetti*). Butterflies, in particular *Eurema daira* and species of *Phoebis* (both Pieridae), stole nectar using a method termed “base working”, where no hole is made in the flower, but the opening for pollinators is also not used to remove nectar, rather the visitor feeds through the gaps between petals in polypetalous flowers (Inouye, 1980a). The butterflies took nectar from between the petals of the flowers using their tongues but without coming into contact with the anthers or stigmas, and, when caught and examined, carried no grains of pollen. Ants, small bees and flies also crawled into the corolla tube to steal nectar, and trigonid bees stole pollen. Whole flowers were also eaten by iguanas and squirrels, possibly for their nectar content. Flowers were eaten by young white faced capuchin monkeys, again probably for the sweet nectar inside the corolla (Valerie Schoof, personal correspondence).

Past studies of the species (Feinsinger, 1978; Webb and Bawa, 1983; Webb, 1984; del Coro Arizmendi, 2001) have stated that hummingbirds are the most effective pollinators of *M. arboreus*, being the only visitors to come into contact with the stigmas and anthers of the flowers. Other visitors have been dismissed as “incorrectly” visiting the flower, without coming into contact with the anthers or stigmas (Feinsinger, 1978; Webb, 1984).

My observations of visitors to *M. arboreus* reflect those of Webb. The cinnamon-hummingbird, *Amazilia rutila*, was a moderately frequent visitor (N = 21 over 48 hours of observations of 101 flowers, see Figures 6 and 11) to *M. arboreus* and also appeared to be highly territorial at patches of flowers, often observing from nearby branches and chasing off

other hummingbirds and even butterflies that attempted to feed on the flowers. The butterfly visitors observed were identified as *Eurema daira* and *Phoebis agarithe*, and again I observed them to feed upon the nectar of the flowers through the base of the petals without coming into contact with the anthers or stigma (Fig. 15). A variety of bee species (Meliponinae, Halictinae, Xylocopinae) stole pollen from the protruding anthers, or stole nectar by perforating the base of the corolla tube (see Fig. 1), or (in the case of the smaller species) by crawling inside the corolla tube. None of the nectar-stealing bees were observed to make contact with the stigmas or anthers when visiting the flowers, and the pollen-eating bees made only incidental contact with either or both during the pollen-collecting visit. These other “flower visitors” were almost equal in their frequency, and much less frequent than the hummingbird visitors (Fig. 6).

No visitors other than the hummingbird *A. rutila* fed upon the nectar in the “correct” manner, via the corolla tube, while also making contact with both the stigmas and the anthers (Fig. 16).



Fig. 15



Fig. 16

Fig. 15: The butterfly *Phoebis agarithe* “base working” flowers of *Malvaviscus arboreus* in order to steal nectar.

Fig. 16: The hummingbird *Amazilia rutila* feeding upon the nectar of *Malvaviscus arboreus* in the “correct” manner, coming into contact with both stigma and anthers.

Helicteres guazumifolia

Despite its apparent hummingbird pollination syndrome, *Helicteres guazumifolia* is also visited by a range of different visitor species. The most frequent visitor was the stingless bee, *Trigona fulviventr**is*, which fed on the pollen of flowers, as well as occasionally piercing the corolla tube to steal nectar. Large numbers of *T. fulviventr**is* swarmed around bushes of *H. guazumifolia* from dawn and remained there throughout the day.

The Meliponinae bee *Tetragonisca angustula* also fed upon pollen (see Fig. 2), as well as crawling inside the corolla of the flowers to steal nectar, but without coming into contact with the stigmas or anthers. *Agapostemon* sp. and a species of vespid wasp were primary robbers, biting holes in the corolla in order to steal nectar.

The only visitor that visited the flowers “correctly”, feeding on nectar by inserting its long tongue into the corolla tube and coming into contact with both the anthers and the stigmas with the top of its head, was the green hermit hummingbird, *Phaethornis guy*. Unlike *A. rutila*, *P. guy* was not observed to take part in any territorial behaviour, and was not spotted perching in nearby trees. Due to the patchy distribution of *H. guazumifolia* it is likely that *P. guy* foraged in either a directional or random traplining manner, rather than guarding a rich patch of resources as in the case of *A. rutila* and *M. arboreus* (Baum and Grant, 2001).

Partitioning of Visitors across Time

Malvaviscus arboreus

When temperatures were low, bees were the most frequent visitor group to *M. arboreus*. Body size is known to have an effect on the ability of bees to function in extreme thermal conditions (Willmer and Unwin 1981; Stone and Willmer, 1989; Pereboom and Biesmeijer, 2003). In general, smaller insects can heat up and cool down more rapidly but do not attain excessively high body temperatures, and larger insects gain and lose heat more

slowly but can attain higher temperature excesses than smaller insects (Digby 1955; Willmer and Unwin 1981; Heinrich 1993; Bishop and Armbruster 1999). The foraging and flight of tropical and subtropical bees can therefore be constrained by high ambient temperatures and heat production (Chappell 1984; Armbruster and McCormick 1990) and overheating is a big risk. Smaller bees, for instance *Trigona* and *Tetragonisca* species, are able to fly in full sunlight when larger bees cannot due to the likelihood of overheating (Willmer and Corbet 1981). The bee species observed foraging on *M. arboreus* ranged in size from the very small *Tetragonisca angustula*, known to begin foraging at 08:00 (Biesmeijer 1997) to medium/large (*Ceratina* sp., Halictidae), which were able to start foraging earlier in the cool temperatures of the morning due to their ability to retain ambient body temperature from nesting overnight (Biesmeijer 1997, Pereboom and Biesmeijer, 2003). Visits from bees, especially the larger species, dropped between 12:00 and 15:00 when temperatures were at their highest and overheating would be an obvious risk (Fig. 3).

Hummingbirds are very small endotherms with high mass-specific metabolic rates as described above and their small size makes them vulnerable to thermal and energetic stresses, often becoming almost fully torpid overnight, only becoming active after dawn (Wolf and Hainsworth, 1983; McNab, 1988). Many species of hummingbird are known to have their peak activity between 08:00 and 12:00 (Smith-Ramirez, 1993; López-Calleja *et al.*, 1997) and this appears to be the case for *A. rutilia*. Due to its sensitivity to low ambient temperatures, the peak activity of this hummingbird occurs during warmer ambient temperatures (09:00-15:00) and they begin foraging later in the day than the bee species.

Butterfly foraging activity peaked between 09:00 and 12:00 and dropped through the afternoon. The flight activity of butterflies is also constrained by temperature and their foraging is limited to higher ambient temperatures, when they can bask in sunlight to gain heat (Heinrich 1986; Dennis 1993; Watt 2003).

Visitation by the ant species, *Camponotus novograndensis*, was infrequent until later in the afternoon. In this case it is unlikely that temperature is the restraining factor on visitation, as the activity of diurnal ant species in the tropics is known to increase in the hottest hours of the day, with reduction in periods of high humidity and during rains (Del-Claro and Oliveira 1999; Oliveira et al. 1999; Cogni and Freitas 2002; Yamamoto and Del-Claro, 2008). It is more likely that the loss of the majority of the pollen from the anthers by midday (Webb and Bawa, 1983) is the reason for the increase in ant activity. The pollen of *Malvaviscus arboreus* is repellent to *Camponotus novograndensis* (Ballantyne and Willmer, 2011), as is the case for a substantial number of temperate and tropical flower pollens (Junker and Blüthgen, 2007; 2010; Willmer et al, 2009), so ants are deterred from flowers while pollen is present, foraging only once it has been removed.

Helicteres guazumifolia

Despite its apparent hummingbird syndrome, and in contrast to *M. arboreus*, the most frequent visitors to *H. guazumifolia* were bees, in particular the stingless bee, *Trigona fulviventris*. This bee was active throughout the day, likely due to its small size and ability to quickly gain and lose heat in the hot temperatures of the tropics (Digby 1955; Willmer and Corbet 1981; Willmer and Unwin 1981; Heinrich 1993, Bishop and Armbruster, 1999) as explained in detail above.

Helicteres guazumifolia was studied later in the dry season than *Malvaviscus arboreus*. Temperatures at this time, in particular between 06:00 and 09:00, were higher than those for the *M. arboreus* study period, and humidity was slightly lower (see Fig. 4). This may explain why the abundance of hummingbirds was higher during the early morning than with *M. arboreus*. In addition, *Amazilia rutila* is smaller in wing length and weight than *Phaethornis guy* (Snow and Snow, 1972; Montgomerie et al., 1984), therefore *P. guy* is better able to generate and retain heat, and can be active earlier in the day than *A. rutila* as it has a much smaller surface area to body mass ratio over which heat can dissipate (Pearson, 1950;

Lasiewski, 1963; Calder, 1984; Bartholomew and Lighton, 1986; Atkinson, 1994; Blanckenhorn, 2000).

Pollinator Effectiveness

Malvaviscus arboreus

For my purposes, pollinators can be classified as visitors that deposit statistically significantly more conspecific pollen on stigmas than is found on unvisited control flowers. The most effective pollinator is then classified as the pollinator that deposits the most conspecific pollen on stigmas per visit. Due to the limitations of the experiment, no distinction could be made between self and non-self pollen, however the behaviour of the visitor species was used to infer the likelihood of deposited pollen being from the same flower.

Visitors were first analysed by functional groups: hummingbirds, bees, butterflies and ants. The hummingbirds and bees were the only groups that deposited a significant amount of pollen in comparison to unvisited control flowers, meaning these are the only groups classified as pollinators. The ants and butterflies are more accurately termed nectar robbers, as they fed upon the nectar of flowers but did not participate in significant pollen transfer. The hummingbird, *Amazilia rutila*, deposited significantly more pollen on stigmas than did the bees, and was the most frequent visitor species observed to visit flowers during the study period. It can therefore be classified as the most effective pollinator.

The bee group consisted of several different species of varying size, shape and foraging behaviour. To investigate the efficiency of individual bee species, pollinator effectiveness was analysed by species rather than functional group. Not all species of bee deposited a significant amount of pollen, and therefore not all visiting bee species could be classified as pollinators. Only *Agapostemon* sp. was a significant pollinator within the bee group.

Past studies of pollinator effectiveness have mainly analysed visitors according to functional groups rather than individual species (for example Galen and Stanton, 1989; Capellari *et al.*, 2009; Brown and McNeill, 2009; Marten-Rodriguez *et al.*, 2009), or have only investigated the pollination effectiveness of one or of a very few selected species (for example Motten *et al.*, 1981; Morandin *et al.*, 2001; Goodell and Thompson, 2007). By grouping visitors, some species may be inaccurately identified as pollinators, and others may not be identified because some species in their group are very poor pollinators. Wilson and Thomson (1991) showed that the pollen deposition by *Apis mellifera*, *Dialictus rohweri*, *Bombus impatiens* and *Bombus vagans* on *Impatiens capensis* was highly variable, when other studies may have lumped these species together as “Bees” with equal pollinator effectiveness. It is important, therefore, to allow for variation in pollen deposition between visitor species as a result of differing body size, foraging activity and other factors by analysing pollinator effectiveness of individual species rather than functional groups. In this manner, none of the variation in the results is lost and the determination of effective pollinators is more accurate.

Helicteres guazumifolia

There is little information available on *Helicteres guazumifolia*. The only previous study covers the nectar production of the species, and states that the most likely pollinators are hummingbirds (Goldberg, 2009), although this is only inferred from floral traits and nectar content and is not tested experimentally.

As with *M. arboreus*, the pollen deposition on stigmas by visitors to *Helicteres guazumifolia* was analysed by the functional groups, birds and bees (see Fig. 13). An effective pollinator was defined as before, and visitors that did not deposit a statistically significant amount of pollen were classified as nectar robbers. When the data were analysed according to these functional groups, both bees and birds deposited a statistically significant

amount of pollen above control levels. Therefore both the bee and bird groups could be described as being pollinators of *Helicteres guazumifolia*.

There was, however, significant variation in the body size, shape and foraging behaviour of the three bee species present and therefore it was likely that there was variation in their pollen deposition. When the data were analysed according to species rather than functional group, only *Trigona fulviventris* deposited significant pollen on stigmas, and it can therefore be classified as a pollinator (see Fig. 14). *Agapostemon* sp. and *Tetragonisca angustula* did not deposit significant amounts of pollen and must therefore be classed as nectar robbers.

Quality of Pollen Deposition by Pollinators

Malvaviscus arboreus

Each of the 20 to 30 anthers found on a flower of *M. arboreus* contains an average of 70 pollen grains (Webb, 1984). Therefore, each flower contains between 1400 and 2100 pollen grains. Figures 7 and 8 show that a hummingbird deposits on average 104.4 pollen grains per single visit, therefore each hummingbird deposited an average of between 7.5 and 5.0% of the total available pollen from one flower on to another flower that it visited. By contrast, *Agapostemon* sp. deposited an average of between 4.0 and 2.5%, and *Tetragonisca angustula* an average of between 1.5 and 1.0%, of the total available pollen per flower on each flower visited.

Agapostemon sp. and *Tetragonisca angustula* were mainly observed feeding upon the pollen of *M. arboreus* flowers, rarely crawling into the corolla tube to feed upon nectar. It is likely that the majority of pollen deposited by these species is self-pollen from the same flower transferred incidentally while feeding on pollen. However, since *M. arboreus* is self-compatible, this is still likely to result in seed set, although the quality of resulting seed and

flowers is likely to be lower than that from outcrossed pollen (Schemske and Pautler 1984; Bookman 1984; Vander Kloet and Tosh 1984; Waser and Price 1983; Price and Waser 1979).

The majority of pollen removal and dispersal from *Malvaviscus arboreus* occurs within the first 2-4 hours after sunrise (Webb and Bawa, 1983). Hummingbirds can disperse pollen of *M. arboreus* over great distances; the largest distance recorded by Webb and Bawa (1983) was 225.5m, with an average of 37.0m for small or medium plants and 38.2m for large plants. If the pollen dispersal behaviour of *Amazilia rutila* in the forests of Santa Rosa can be assumed to be similar to that of the species in Hacienda la Pacifica as in Webb and Bawa's study then it is likely that a large quantity of pollen deposited by *A. rutila* is outcrossed and will lead to higher quality seed set than that deposited by *Agapostemon* sp. and *Tetragonisca angustula*. The territorial behaviour of *A. rutila* may also serve to increase out-breeding, as while the resident hummingbird may only be depositing self-pollen and increasing geitonogamy, any intruders to the territory will only be able to visit one or a few flowers before being chased away by the resident, and may therefore bring in more outcrossed pollen (Grant and Grant, 1968; Feinsinger, 1990), as has also been found in cases of territorial bee species (Frankie *et al.*, 1976; Ghazoul, 2005b).

Helicteres guazumifolia

Despite its lower frequency of visits, the hummingbird *P. guy* deposited a significantly higher MPS than did the bee *Trigona fulviventr*is. Due to the pollen feeding and swarming behaviour of *T. fulviventr*is it is very likely that the majority of pollen deposited on stigmas is self-pollen from the same flower, although, despite the lower quality of self-pollen in comparison to outcrossed pollen as described above, this will still result in seed set due to the self-compatibility of the species. In contrast, *Phaethornis* is a typical far travelling trap-lining hummingbird species which visits many plants sequentially for short visits, flying from plant to plant, often over some distance (Janzen, 1971; Linhart *et al.*, 1987) and therefore it

is likely that much of the pollen deposited by this species is outcrossed pollen from other populations of *H. guazumifolia* (Snow and Snow, 1972; Stiles, 1975, 1981). Despite its low frequency of visitation in terms of number of visits to flowers observed during the study period in comparison to *T. fulviventris*, *P. guy* deposits statistically more, and higher quality, pollen on flowers. Typically, only one visit by a hummingbird to each flower was observed during the study period, although flowers were often visited by bees after hummingbird visits.

Conclusion

Both plant species studied showed typical traits of the hummingbird pollination syndrome, as described by Faegri and Van der Pijl (1979) and Proctor et al. (1996), but both were visited by a variety of other species not predicted by the syndrome. Using the deposition of pollen grains on stigmas as a measure of pollinator effectiveness, I have shown that the cinnamon hummingbird, *Amazilia rutila*, is the most effective pollinator species of *Malvaviscus arboreus*, and the green hermit hummingbird, *Phaethornis guy*, is the most effective pollinator species of *Helicteres guazumifolia*. This is in line with the predictions of the “pollination syndrome” approach for each species, and confirms the findings of previous studies (Feinsinger, 1978; Webb and Bawa, 1983; Webb, 1984; del Coro Arizmendi, 2001; Goldberg, 2009).

As described in chapter 1, Ollerton *et al.* (2009) tested the validity of pollination syndromes and whether they could predict the most frequent pollinators (though in this case the term visitors is more accurate) of flowers. Visitors were considered to be pollinators only after 5 or more legitimate visits, without nectar or pollen robbery, to different individuals of the given plant species, and after evidence of contact between the visitor and both the male and female reproductive organs of the flowers had been obtained. Pollinators were then grouped into functional groups of similar species such as birds, bees or butterflies. The study found that in few cases could the major pollinator of a plant species be predicted using pollination

syndromes, and in particular on 14.9% of the major pollinators of bird-pollinated syndrome flowers could be accurately predicted.

In this study, taking a more inclusive view of the Syndrome approach, the floral traits of both species tested do fit with those of a hummingbird pollination syndrome, which successfully predicts the most effective pollinator of each species. *M. arboreus* had very distinctive hummingbird pollination traits, and although visited infrequently by other species, the most frequent visitor, in terms of number of flower visits observed during the study period, was the hummingbird *A. rutila*, which was also the most effective pollinator in terms of single visit pollen deposition on stigmas. In the case of *H. guazumifolia*, the hermit hummingbird *P. guy* was again the most effective pollinator in terms of pollen deposition on stigmas, but it was not the most frequent visitor.

P. guy is one of the lek-mating species of hermit hummingbirds (Trochilidae and Phaethornithinae), known to be specialised traplining hummingbirds that visit isolated and undefended flowers, such as the isolated populations of *Helicteres guazumifolia*, containing large amounts of nectar (Feinsinger and Colwell, 1978; Stiles and Wolf, 1979; Snow and Snow, 1980; Gill, 1988), an alternative feeding strategy to the territorial behaviour of other species such as *A. rutila* (Stiles, 1975; Feinsinger and Colwell, 1978). Rather than chasing invaders, which incurs measurable costs (Paton and Carpenter, 1984), traplining hummingbirds invest their time and energy into repeated, sometimes unproductive, returns to flowers (Gill, 1988). There is evidence that many flowers adapted for hermit hummingbird pollination go unvisited, and populations of hermit hummingbirds may be sparse in relation to the number of flowers (Feinsinger, 1990). Flowers of *H. guazumifolia* are visited far more frequently by *T. fulviventris* than *P. guy*, so if we were to use Ollerton's (2009) definitions of an effective pollinator, then this small bee would be classed as an effective pollinator of *H. guazumifolia*, and the hummingbird would be dismissed. In terms of pollen deposition on stigmas however, the hummingbird is clearly the more effective of the two.

I have further shown that using visitation frequency to determine pollinators of a flower species is an ineffective procedure, and may not always indicate the most effective pollinator species. Observational values as a means of determining pollinators, such as the judging of “correct” pollination by observing visits and noting whether the visitor came into contact with anthers and stigmas in the intended manner (Feinsinger, 1978; Webb and Bawa, 1983; Webb, 1984; Ollerton, 2009), are also not necessarily an effective test for pollinator effectiveness. By using stigmatic pollen deposition as the primary measure, other variables such as loss of pollen through grooming, eating, deposition on other flower species or objects, etc. (Inouye *et al*, 1994) are eliminated and only the pollen available for fertilisation is counted. This shows how much pollen each species of visitor contributes to potential ovule fertilisation and together with information on pollen losses and vector movement is an effective means of determining pollinator effectiveness.

I have shown that when visitor species are grouped into functional groups for analyses, much of the variation within and between groups is lost and inaccurate conclusions about the effectiveness of certain pollinators could be drawn. In both plants studied here, lumping bees together as a functional group resulted in the whole group being termed pollinators, when in reality only one or two bee species were participating in pollination, and the rest were merely nectar or pollen robbers. The differing sizes and shapes of these bees may have an effect on pollen deposition, but it is likely that in this case, the foraging behaviour of the species has the greatest effect. Those that were found to deposit significant amounts of pollen were also those most often observed feeding upon the pollen of flowers, rather than (illegitimately) on the nectar.

Despite the most effective pollinators being determined as the two hummingbird species, *Amazilia rutila* and *Phaethornis guy*, the bee species *Trigona fulviventris* and *Agapostemon* sp. were also found to deposit significant amounts of pollen on stigmas, although due to the foraging behaviour of these species, this was most likely self-pollen. Both *M. arboreus* and *H. guazumifolia* were self-compatible; therefore, although the self-pollen

deposited by the bee species is likely to be of lower reproductive quality than the outcross pollen from the hummingbirds which serves to increase gene flow, it may still result in some seed set. Often species depositing only self-pollen incidentally during pollen feeding would be better termed as pollen thieves, but in the case of *Helicteres guazumifolia* and *Malvaviscus arboreus* they are perhaps better termed as “fall-back” pollinators for when pollination by hummingbirds is rare.

Both *M. arboreus* and *H. guazumifolia* have a patchy distribution, preferring disturbed habitats (Webb and Bawa, 1982; Cristóbal, 2001). While a scattered distribution such as this can promote outcrossing by forcing flower visitors to travel further to collect sufficient nectar, it can also result in poor pollination if floral constancy becomes low when other suitable plant species are present. In their extensive study of the pollen flow of *M. arboreus*, Webb and Bawa (1983) determined that only about 3% of the pollen of individual flowers reached the stigmas of the same or other flowers, and that 70-90% of pollen remained unaccounted for. Pollen can be lost from a pollination system during transport by a pollen vector in a number of ways:

Passively:

- Falling from a vector's body if adhered loosely
- Through the action of wind and rain

Actively:

- Eating pollen directly from the anthers of the flowers
- Packing pollen into a pollen-carrying structure such as a bee's corbiculum
- Discarding undesirable pollen (e.g. pollen unfit for consumption) from the body

(Inouye *et al.*, 1994).

It may be, therefore, that the nectar and pollen thievery of these flower visitors is tolerated because they ensure that, should pollination by the more effective hummingbirds

not occur, the species will still be able to reproduce due to the small amounts of self pollen being moved around incidentally by the bees during their foraging.

These two plant species cannot be considered generalists by any of the definitions of this described in Chapter 1, as they show clear adaptation towards specialised pollination by hummingbirds. They do, however, show flexibility in this pollination relationship which can tolerate pollinator extinctions or scarcity in the disturbed, patchy and unstable habitat in which they grow. Perhaps this chapter begins to show that it is not “generalisation” that is more frequent in plant-pollinator relationships, but this flexibility in syndromes and the existence of “fall-back” pollinators to ensure the continuation of specialised plant-pollinator interactions if pollinator extinctions or scarcities should occur.

Chapter 4: Testing Pollination Syndromes

The General Hoverfly-Pollination Syndrome

Introduction

Flies, of the order Diptera, are an incredibly diverse taxon of insects. They are characterised by a single pair of wings, rather than the two pairs of wings of the Hymenoptera and almost all other orders. The rear wings of the Diptera are instead modified into a pair of organs called halteres, used in balance and flight control. Because of this modified pair of wings, flies are extremely agile flyers, often able to hover, as well as to take off and land in any direction.

Within the order Diptera, the family Syrphidae, also known as the hoverflies, flower flies or syrphid flies, comprises about 6,000 species in 200 genera, and they are frequent flower visitors. The visiting of flowers involves the collecting of nectar and/or pollen, given that the hoverflies are among the relatively few Diptera which are able to digest pollen (Gilbert, 1981). Flower visiting may also serve a function in mate finding, either as simply a likely place for males to find females, or as a place to perform distinctive courtship behaviours (Stubbs and Falk, 2002).

Mouthparts of Diptera

Various types of mouthparts are found amongst the hoverflies, some adapted to take advantage of deep flowers, while others are only suited to those flowers with more exposed nectaries, and others still are adapted to feeding on the honeydew secretions of aphids rather than on flowers (Stubbs and Falk, 2002; Rotheray and Gilbert, 1999). The adult females of many holometabolous insects such as syrphids require protein to maximise reproductive success (Schneider, 1958). Nectar from flowers is a rich source of

carbohydrate (Percival, 1961) but provides at best only trace amounts of protein (Baker and Baker, 1973), whereas pollen contains substantial amounts of carbohydrate, protein and lipids (Stanley and Linskens, 1974; Roulston and Cane, 2000; Roulston and Buchman, 2000; Roulston *et al.*, 2000). Some fly species, in particular the syrphids, have therefore taken to feeding on flowers for pollen as well as nectar (Gilbert and Jervis, 1998; Willmer, 2011).

The mouthparts of Diptera as described by McAlpine (1981) form a tubular sucking organ, termed the proboscis, consisting of two main parts (see Fig.1). The rostrum is the basal part of the proboscis. In many taxa, for example the Infraorder Muscomorpha, the proboscis is extended mainly by the fulcrum shifting into the proboscis, forming the rostrum. The haustellum comprises two paired elements and three unpaired elements. The paired elements are the mandibles, usually absent except in the females of blood-feeding dipterans, and the maxillae, often consisting only of blade-like laciniae, bearing palps. The function of the laciniae has long been a puzzle (Schiemenz, 1957); however they have sometimes been described as implements for pushing aside floral structures or for forcing pollen into the labral food canal from the side (Menzbier, 1880; Gilbert and Jervis, 1998).

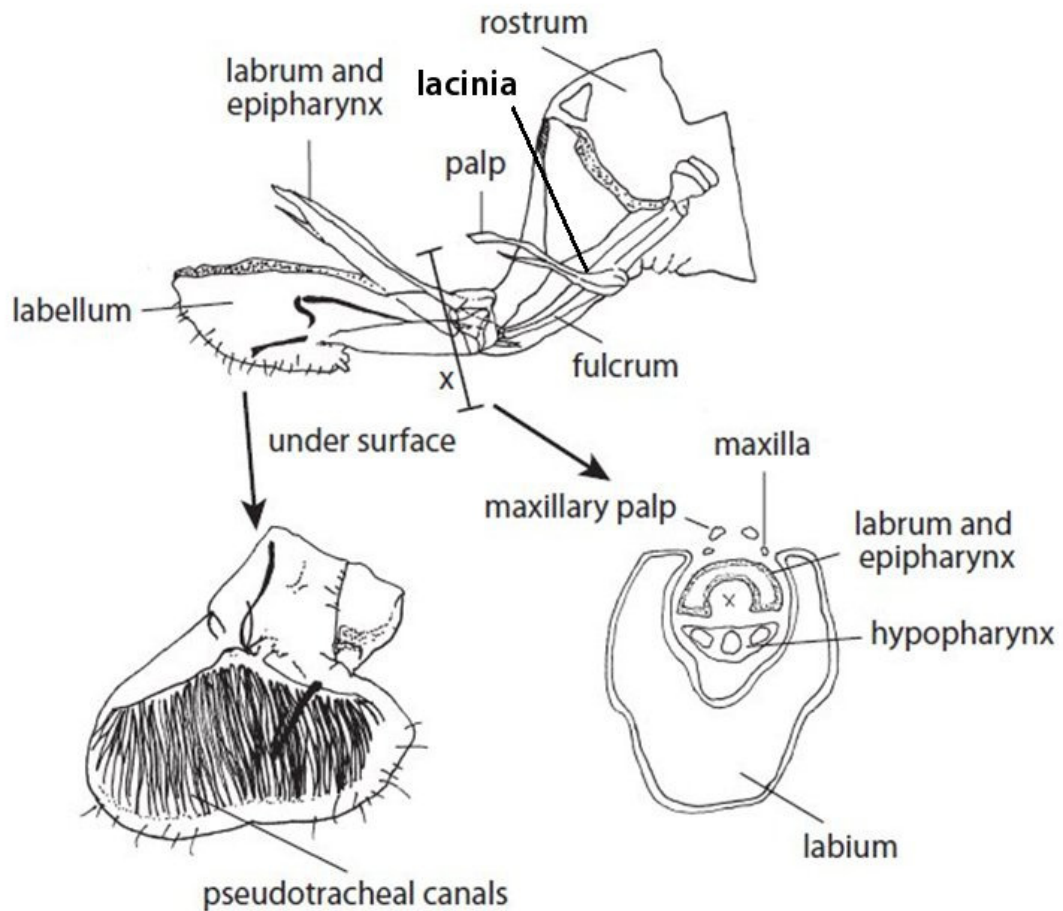


Fig. 1: The basic fly proboscis, with views of the labellar surface and pseudotracheae, and a transverse section showing the food channel at X. Dark areas are underlying sclerites. The paired mandibles of blood-feeding females are not shown. Willmer, 2011 (Largely modified from Gilbert and Jervis 1998.)

The unpaired elements are the labrum, which generally forms the dorsal and lateral sides of the food canal, often bearing tooth-like projections or brushes at the tip; the hypopharynx, containing the salivary duct opening at its tip, and forming the ventral part of the food canal; and the labium, the largest of the mouthparts, forming the ventral wall of the proboscis. The labium is usually formed like a gutter in which the other mouthparts lie.

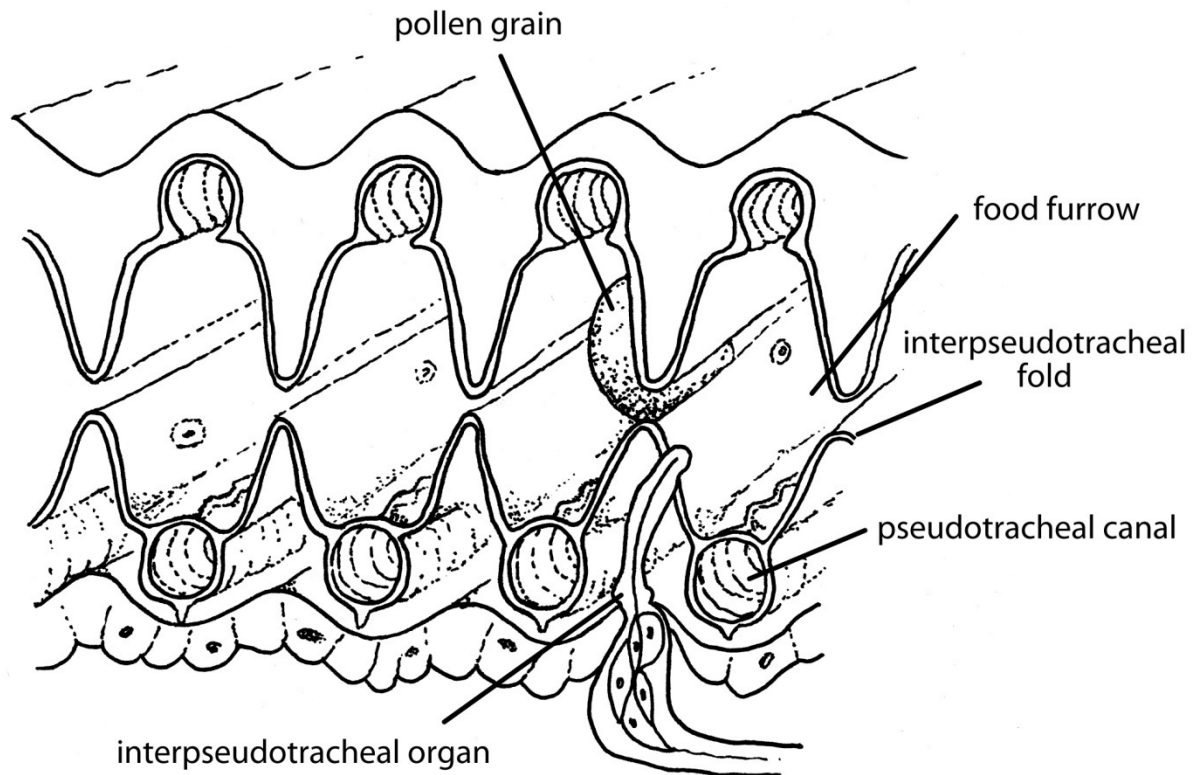


Fig 2: Fine structure of the inner labellar surface, indicating how pollen is collected in the food furrows (redrawn from Gilbert and Jervis, 1998).

The mouthparts of flies are fundamentally specialised for fluid-feeding, and in its most basic mode the proboscis is used to either mop up and/or suck up liquids (Vijaysegaran *et al.*, 1997), either as liquid food such as nectar or honeydew, or as solid material that is suspended or dissolved in salivary secretions (Gilbert and Jervis, 1998); therefore feeding on nectar does not require considerable specialised morphology or physiology (Gilbert and Jervis, 1998; Lundgren, 2009). The mouthparts of the Syrphidae were studied in great detail by Schuhmacher and Hoffman (1982) who showed that the main feature for pollen feeding was the inter-pseudotracheal folds maintained by haemolymph pressure (Gilbert and Jervis, 1998; see Fig.2). These folds create channels overlying the pseudotracheal canals in which fluids such as nectar, or pollen suspended in saliva, can be transported into the opening of the labral food canal. Once the sugar source is identified by the fly, the proboscis is extended until the labellum comes into contact with the fluid. The folds of the labella then separate so they can lie flush with the nectar droplet

and cibarial pumping motions then suck the fluid into the mouth. The labella do not always have to touch the fluid, as it can also be wicked up along creases in the corolla via capillary functions of the hydrophilic labella (Gilbert, 1981).

Different species of syrphid adults are known to specialise on specific flower types (Colley and Luna, 2000). As described above, pollen and nectar are the two main flower rewards gained from this feeding strategy (Gilbert, 1985; Lundgren, 2009). Ovarian development in certain female hoverflies is dependent on them consuming pollen soon after emerging from the puparium, as only pollen has the full range of nutrients required by females for egg production (e.g. Schneider, 1948; Stürken, 1964; Haslett, 1989b; van Rijn *et al.*, 2006). The diet of syrphids has been linked to their size, with larger species feeding more frequently on nectar and smaller species feeding more frequently on pollen (Gilbert, 1985). Longer winged species feed more frequently on pollen than nectar. Pollenivorous species which do not land on flowers require more time airborne in order to collect the pollen grains from anthers, therefore smaller body size in relation to wing span reduces energy demands and facilitates the hovering flight necessary for this task (Lundgren, 2009).

Visitation records of hoverflies on a vast array of plant species suggest a lack of constancy, though several important factors such as variation in the timing and location of flowering in different plant species, and the range of plants encountered by hoverflies at a given time or in a particular habitat, may mean that hoverflies show more constancy than was previously thought. Individual hoverflies or species may develop constancy in relation to the frequency with which they encounter flowers, or they may have innate preferences (Rotheray and Gilbert, 1999). High levels of floral constancy have been shown in at least some species of syrphids, for example *Episyrphus balteatus* and *Syrphus ribesii* (Goulson and Wright, 1998), though the subject is poorly researched in comparison to floral constancy studies of bees. Syrphids also move much more regularly and systematically through flower patches than other Diptera, and are well known as efficient and important

pollen vectors in temperate zones (Willmer, 2011). Weather conditions are known to affect hoverfly abundance. The optimal conditions for hoverfly visitation are typified by calm, humid conditions where the sky is bright yet overcast, or where there is a mixture of cloud and sunny intervals. If air temperature is low, hoverfly abundance will decrease as the sun disappears, however in warm conditions the flies will usually remain on flowers (Gilbert, 1985; Stubbs and Falk, 2002). Temperature and humidity are often only weakly correlated with hoverfly abundance however, and one of the main factors influencing their activity patterns is the abundance of flowers and flowering plant species (Sajjad *et al.*, 2010). Syrphid density is also higher in flower patches within greenhouses (Pineda and Marcos-García, 2008), and in sown flower strips rather than grass and crop land (Haenke *et al.*, 2009), therefore the appropriate management of field margins and crops play an important role in hoverfly abundance and biodiversity, allowing for more effective pollination as well as increased aphid management by aphidophagous hoverflies.

Syrphids as Pollinators

The importance of flower-visiting flies is poorly studied in comparison to other visitors such as bees (Ssymank *et al.*, 2008), but their benefits to ecosystems are twofold. Their larvae are very often important natural enemies of herbivorous arthropods, and their adults are important pollinators of many different plant species (Tooker *et al.*, 2006; Ghahari *et al.*, 2008). Syrphids are effective or frequent pollinators of many different plant species. Plantains, once thought to be exclusively wind-pollinated, have been shown to be visited extensively in the UK by *Melanostoma* sp. and *Platycheirus* sp. between the hours of 5am and 7am (Stelleman and Meeuse, 1976; Stelleman, 1978; 1981; 1984), and even produce stickier pollen, which is more easily adhered to hoverflies, when grown in sheltered areas where wind-pollination is unlikely, and hoverfly pollination is more frequent (Stelleman, 1984). Syrphids deposit high numbers of cross-pollen grains on the stigmas of wild Brassicaceae (Kobayashi *et al.*, 2010). The hoverfly *Eristalis tenax* was a principal

pollinator of the high-mountain perennial plant *Rhodiola dumulosa*, along with the bumblebee *Bombus pyrosoma*, contributing to higher outcrossing rates (Zhu and Lou, 2010). Using an index of pollen deposition potential, a combination of pollen load and population size, large tachinids, calliphorids and syrphid flies were the most important pollinators of *Eucalyptus regnans* (Griffin *et al.*, 2009). In a study not just of one species but of a whole alpine community, while Hymenoptera contributed the most in terms of numbers of visits recorded (43.3%), Diptera contributed 37% of visits, most of which were by syrphids, with flower abundance and length of flowering period thought to be the greatest influence on visitation (Makrodimos *et al.*, 2008).

While it is most often bees that are considered the key commercially relevant pollinators, hoverflies have also been shown to be important pollinators of crops. The extremely common British hoverfly *Episyrphus balteatus*, for example, is an effective pollinator of the crop oil-seed rape, *Brassica napus*, significantly increasing both the seed set and yield of the crop (Jauker and Wolters, 2008). Syrphid flies in northern Michigan are known pollinators of both crop and wild populations of the radish *Raphanus raphanistrum* (Lee and Snow, 1998). Syrphids are also pollinators of many crop species in Pakistan (Sajjad and Saeed, 2010), for example *Mangifera indica* (mango), *Citrus medica* (citron), *Grewia asiatic* (phalsa or falsa), *Raphanus sativus* (radish), *Momordica charantia* (bitter melon or bitter gourd), *Helianthus annuus* (sunflower), *Allium cepa* (allium) and *Coriandrum sativum* (Coriander).

Syrphids can also be caught out by deceptive flowers; populations of the orchid *Govenia utriculata* at Serro do Japi in South Eastern Brazil are visited and pollinated solely by two species of hoverfly in the genus *Salpingogaster* which are attracted to brownish yellow to orange spots on the lip apex and column base of the flower which mimic pollen clusters (Pansarin, 2008).

Floral Traits of Fly-Pollination Syndrome

The general fly-pollination syndrome, or myophily, was described by Faegri and van der Pijl (1979) and Willmer (2011) and its traits are summarised below (see Table 1).

Flower Shape	Radially symmetrical, simple, little or no depth effect, flat or bowl shaped
Colour	Generally light, but dull, white or cream, or sometimes greenish-yellow
Nectar Guide	Sometimes present
Odour	Imperceptible, or mild, sweet or musty, but not usually distasteful
Nectar	Exposed or easily obtainable
Sexual Organs	Well exposed
Arrangement	Often clustered in inflorescences
Timing	Opening in daytime, often producing nectar throughout the middle of the day

Table 1: Summary of the characteristics of fly-pollinated flowers, as described by Faegri and Van Der Pijl (1979) and Willmer (2011).

The above traits described a general fly-pollinated syndrome; however, in the same way that the mellitophilous syndrome can be further subdivided, the myophilous syndrome can also be divided further, and this chapter deals with the subdivision of hoverfly-pollination. The majority of the general myophilous traits mentioned above can be applied to this syndrome, with some additional distinctions described below.

The Hoverfly Pollination Syndrome

Flower Structure and Orientation

Hoverflies show preferences for flowers with certain traits (reviewed in Kevan and Baker, 1983; Proctor *et al.*, 1996; see Table 2), though flowers falling within the hoverfly-pollinated syndrome are often referred to as belonging to the fly-pollination syndrome or generalist-flower syndrome (e.g. Steinbach and Gottsberger, 1994; Lázaro *et al.*, 2008). Kugler (1938) investigated two flowers, *Veronica chamaedrys* and *Circaea luteliana*, which showed traits defined by earlier classic authors (Kirchner, Knuth and Müller) as being indicative of hoverfly-pollination, finding that these flowers were also visited by small bees and other dipterans; however the effectiveness of these other visitors in comparison to the syrphid visitors was not measured or defined.

Horizontal and upward facing flowers are more preferable to hoverflies over downward facing flowers. Flies have a more limited range of head movements than do beetles, wasps and bees, and often lack the ability to extend their heads and mouthparts very far forwards, therefore horizontal or upward pointing flowers may be more easily manipulated, and also allow for basking in the sun (Rotheray and Gilbert, 1999). Upward facing flowers however, may receive more illegitimate landings and a higher probability that the hoverflies would fail to touch the stigmas and anthers, at least in zygomorphic flowers (Ushimaru *et al.*, 2009). Hoverfly flowers are typically flat or bowl shaped, and hoverflies show a preference for actinomorphic flowers (Sajjad and Saeed, 2010). Hoverflies are frequently found on flowers such as *Lobularia maritima*, also known as Sweet Alyssum, and Buckwheat, also known as *Fagopyrum esculentum* (Lovei *et al.*, 1998; Stephens *et al.*, 1998; Hogg *et al.*, 2011), which have short corolla tubes (Vattala *et al.*, 2006), increasing nectar availability. As in other species however, tongue-length of hoverflies is often correlated with the corolla length of flowers visited (Gilbert, 1981), and flowers with long corollas can be manipulated by long-tongued syrphids (see Chapter 7).

Typically, the stamens of hoverfly-pollinated flowers are weak at the base and located above the stigma. When an insect clings to the anther or anthers to feed, the stamen droops and the underside of the insect's body comes into contact with the stigma. The anthers also touch the underside of the insect's body, so that repeated visits to different flowers of this species are likely to result in cross-pollination (Proctor *et al.*, 1996).

Flower Colour

Hoverflies possess relatively sophisticated colour vision. Preference for yellow is often shown (e.g. Kevan and Baker, 1983; Lunau, 1988; Rotheray and Gilbert, 1999; Sutherland, 1999; Campbell *et al.*, 2010; Sajjad and Saeed, 2010), thought to aid hoverflies in finding pollen which is often yellow (Lunau and Wacht, 1994); however, *Rhingia* has shown a preference for blue and violet and *Volucella* for white (Haslett, 1989a; Rotheray and Gilbert, 1999), and other hoverfly species have also shown some preference for these colours (Sajjad and Saeed, 2010). While yellow is often the most preferred colour of generalist, short-tongued hoverflies, a preference for colours in the pink, mauve and blue spectrum is often shown in long-tongued hoverflies such as *Rhingia*, *Volucella* and *Eristalis* (Rotheray and Gilbert, 1999) and the more specialised flower-visiting bombyliid bee-flies (Johnson and Dafni, 1998) and the non-yellow flowers of many plant species, for example the pink flowers of *Cirsium arvense*, are frequently visited by hoverflies (Theis *et al.*, 2007). Hoverflies are not thought to be influenced in their flower choice by UV reflectance (Campbell *et al.*, 2010), however they respond to sophisticated colour changes in flowers which are often indicative of whether flowers have been previously visited, and therefore the level of reward available. For example, *Rhingia campestris*, *Platycheirus* sp. and *Melanostoma* sp. fed more frequently, and for longer, at pre-colour-change flowers of wood forget-me-not, *Myosotis sylvatica* (Nuttman and Willmer, 2008).

Flower Scent

Olfactory cues also play a role in hoverfly attraction, and Odour is likely to be involved in the detection of flowers by hoverflies (Molleman *et al.*, 1997), as floral scent is clearly attractive to hoverflies (Laubertie *et al.*, 2006). Catches of hoverflies in sticky yellow traps have been increased by adding volatile organic compounds (Zhu and Park, 2005), and such compounds can also elicit searching behaviour for oviposition sites for larva in some species (Harmel *et al.*, 2007; Almohamad *et al.*, 2009). This host-searching behaviour has been exploited by deceptively pollinated plants, for example the orchid *Epipactis veratrifolia*, which uses the aphid-mimicking volatile compounds α - and β -pinene, β -myrcene and β -phellandrene to attract female hoverflies, which in turn pollinate the orchid (Stökl *et al.*, 2011). The volatile compounds produced by flowers visited by hoverflies are similar to those of the sweet, fruity or typically floral odours of bee-flowers (Majetic *et al.*, 2009; Primante and Dötterl, 2010). Flies possess long-range chemosensors on the antennae that are receptive to floral odours, although until recently little was known about the importance of olfactory cues in comparison to visual cues when finding nectar or pollen (Majetic *et al.*, 2009), and even now our knowledge of the subject is far from extensive. Though the use of deceptive volatiles for attraction of predatory hoverflies, which then take part in pollination is well studied (see Almohamad *et al.*, 2009 for a review), there have been very few studies into the use of floral odours for pollen and nectar detection by hoverflies. Hoverfly visitors of *Hesperis matronalis* visited flowers with a higher emission rate more frequently, leading to higher fitness of the flowers (Majetic *et al.*, 2009). Primante and Dötterl (2010) described the first instance of a syrphid fly using the olfactory cues of the non-yellow flower *Cirsium arvense* rather than visual cues to find a host plant, where the hoverfly *Episyrphus balteatus* showed antennal response to the volatile compounds phenylacetaldehyde, methyl salicylate dimethyl salicylate and pyranoid linalool oxide emitted by flower heads. This response however, has not yet been shown for other plant species.

In addition, hoverflies possess taste receptors on the mouthparts and feet that are particularly attuned to detecting the presence of sucrose and/or glucose (Hood Henderson and Wellington, 1982; Lundgren, 2009; Willmer, 2011). The flower-visiting adults of the hoverfly *Eristalis tenax*, for example, possess labellar taste hairs that only detect the presence of sucrose solutions (Wacht *et al.*, 1996; 2000).

Summary of the Traits of the Hoverfly Pollination Syndrome

From the above, a series of traits associated with hoverfly pollination can be assembled as in Table 2.

Flower structure	Actinomorphic, often flat or bowl-shaped, though sometimes with long corollas
Flower orientation	Upward or horizontally facing
Colour	Usually yellow, though sometimes pink, purple or blue
Odour	Aphid-mimicking volatiles <i>or</i> compounds similar to the pleasant floral scents of bee-visited species
Reproductive structures	Stamens weak at base, positioned above stigma.

Table 2: Traits of typical hoverfly-pollinated flowers (Kevan and Baker, 1983; Proctor *et al.*, 1996; Willmer, 2011).

Testing Pollination Syndromes

To test the validity of determining the most effective pollinators of a plant species using floral traits corresponding to pollination syndromes, the plant species *Agrimonia eupatoria*, (also known as Yellow Agrimony), showing typical characteristics of hoverfly-pollination, such as yellow colouring and bowl shaped, horizontal flowers (see below) was investigated. This species has been shown to be visited by Diptera in earlier studies

(Memmot, 1999; Fründ *et al.*, 2010), but those studies do not identify visitors to groupings smaller than the order, making no mention of the species of dipterans involved or whether syrphids in particular are involved; and the pollination effectiveness of these visitors has not yet been investigated.

Materials and Methods

Study Site

The population of *Agrimonia eupatoria* investigated was located at West Quarry Braes, a Scottish National Heritage Site in Fife (NO 597 088). The reserve was once a set of quarries which for many years were filled in as a refuse dump. Approximately ten years ago it was landscaped and planted with a mix of trees and now consists of a mixture of scrub and woodland habitats, with many species from the native British flora and fauna present.

Agrimonia eupatoria

Agrimonia eupatoria is a herbaceous perennial of the family Rosaceae, also known as Common Agrimony, or Yellow Agrimony. Distribution is throughout North America, Eurasia, Brazil, Argentina, South Africa and Europe (Kline and Sørensen, 2008). In Scotland, the species is not common and is centralised in its distribution. In Britain, flowering begins on average at the end of June, continuing until about August (Keble Martin, 1972), and first flowering date is related to temperature, occurring earlier when temperatures are increased (Fitter *et al.*, 1995).

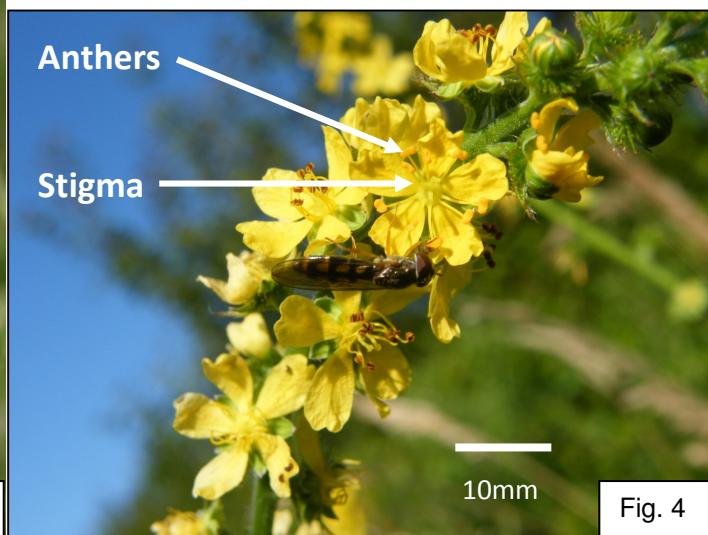
Structure

Plants of *Agrimonia eupatoria* grow to between 30 and 60cm high. They usually possess between one and several stems that are branched at the flowering portion. Inflorescences are typically racemes (unbranched and indeterminate), though sometimes compound (branched); and terminal (arising from the end of a stem), although sometimes also axillary (arising from the base of a leaf; see Fig. 3). Each axis possesses between 9 and 120 flowers.



Fig 3: Simple raceme inflorescence of *Agrimonia eupatoria*

Fig. 4: Syrphid fly feeding upon pollen of an inflorescence of *Agrimonia eupatoria*. Stigma and anthers indicated.



The flowers are usually 10mm or less in diameter with five petals, elliptical to obovate (ovate with a narrower end at the base) in shape, and yellow in colour. Flowers possess a receptacle containing either one or two separate ovaries, only one of which produces a ripened seed (Lindman, 1974; Kiviniemi, 2001). Flowers also possess between 5 and 15 stamens, and styles are exserted (Kline and Sørensen, 2008). The flowers are open and bowl-like in shape with clear access to both the stigmas and anthers (see Fig. 4).

Flowers give off a mildly fruity odour that could be described as spicy like apricots (personal observations).

Nectar

Information on the nectar production of the species is not readily found, though some studies have found no measurable quantities (Raine and Chittka, 2007a; 2007b). In my study, small amounts of liquid, assumed to be dew, though possibly nectar, were visible at the base of the corolla of flowers; however there were not substantial volumes available for nectar analysis and visitors were not observed to feed upon this fluid.

Timing

Flowers are generally viable for a single day, with anthers and stigmas withering after this point, although the corolla remains for one or two days after this, becoming orange in colour as the flower ages. Dehiscence begins at dawn and pollen is available throughout much of the day (personal observations). No temporal separation of sexual phases is apparent in this species, as dehiscing anthers and glossy, receptive stigmas are observed on individual flowers at the same time.

Sampling Periods

Field research occurred between July and August of 2009. Observations were taken throughout the morning and afternoon to gain an accurate representation of visitors throughout the day. In total 133 single-visit observations were made over approximately 12 days. Each sampling session was between 1 and 3 hours long depending on the frequency of visitations and how long it took all previously protected newly-opened flowers to be visited. Temperature and humidity readings were taken from a shaded area of the study site using a HM34 Vaisala Pocket Size Relative Humidity Meter every half hour during each sampling session.

Results

Temperature and Humidity

Mean temperatures over the course of the study were low throughout the morning, rising to their peak in the middle of the day; staying relatively constant throughout the afternoon and dropping in the evening (see Fig. 5). Mean humidity was high in the early morning, declining over the middle of the day and rising again in the afternoon. As observations were limited to days with relatively high temperatures, low cloud cover, wind and rainfall the measurements recorded will vary from the typical means for the region over the study months.

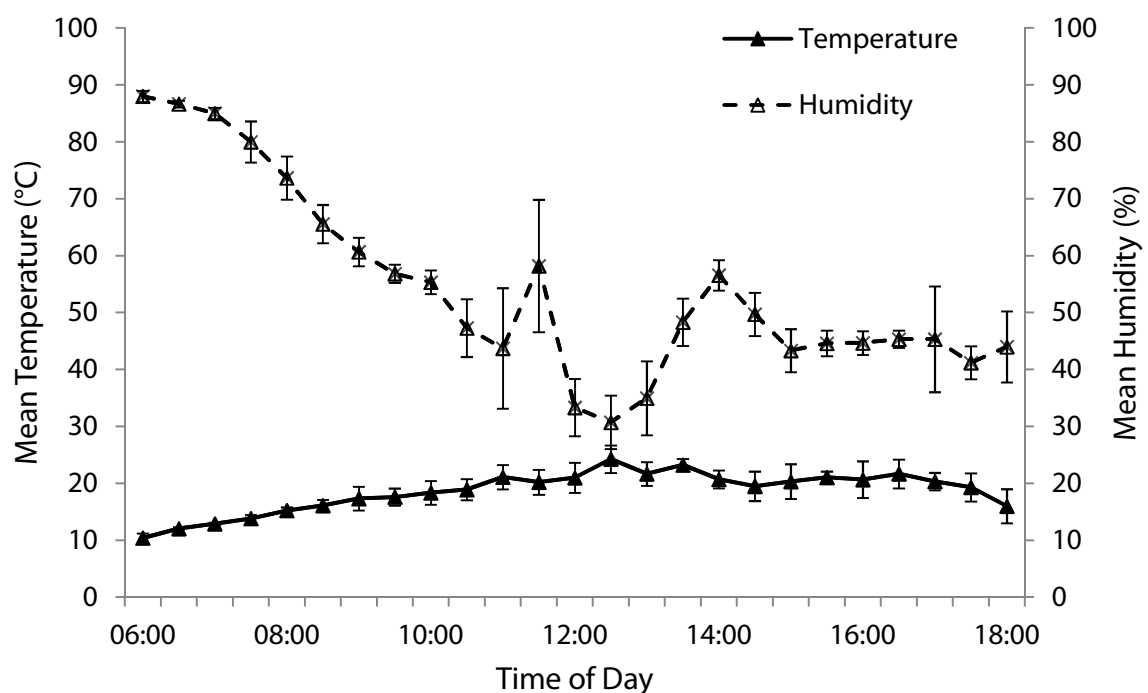


Fig. 5: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Agrimonia eupatoria*. Standard deviations are shown.

Self-Compatibility

Flowers were observed over several days after which the ovaries were dissected and found to have set seed. This confirms that the flowers of *Agrimonia eupatoria* are self-compatible, as shown in previous studies (Kline and Sørensen, 2008).

Partitioning of Visitors over Time

The partitioning of visitors to *Agrimonia eupatoria* over daily time in a separate visitation study, conducted over the course of a full day, is discussed in further detail in Chapter 8. However, with the exception of one unknown fly species, *Agrimonia eupatoria* was visited solely by hoverflies during the pollinator effectiveness study, and their partitioning over time is shown in Fig. 6. The earliest flower visitor was *Episyrphus balteatus* which was present on flowers from 06:30 and throughout the day, showing a slight dip in abundance over the hottest parts of the day. *Rhingia campestris* was the next species observed on flowers at 07:00, followed by the smaller species, *Leucozona laternaria*, *Meliscaeva auricollis*, *Platycheirus albimanus* and *Platycheirus scutatus*. *Parasyrphus punctulatus* was observed rarely, and only at 11:30. The unknown fly species was also observed only at 11:30, while the unknown hoverfly species was observed between 11:00 and 12:00. The larger species, *Episyrphus balteatus* and *Rhingia campestris*, experienced either a drop in abundances or an absence altogether over the hottest periods of the day, though they persisted later into the afternoon as temperatures cooled, while the smaller species were often present on flowers throughout midday though they ended foraging earlier in the afternoon.

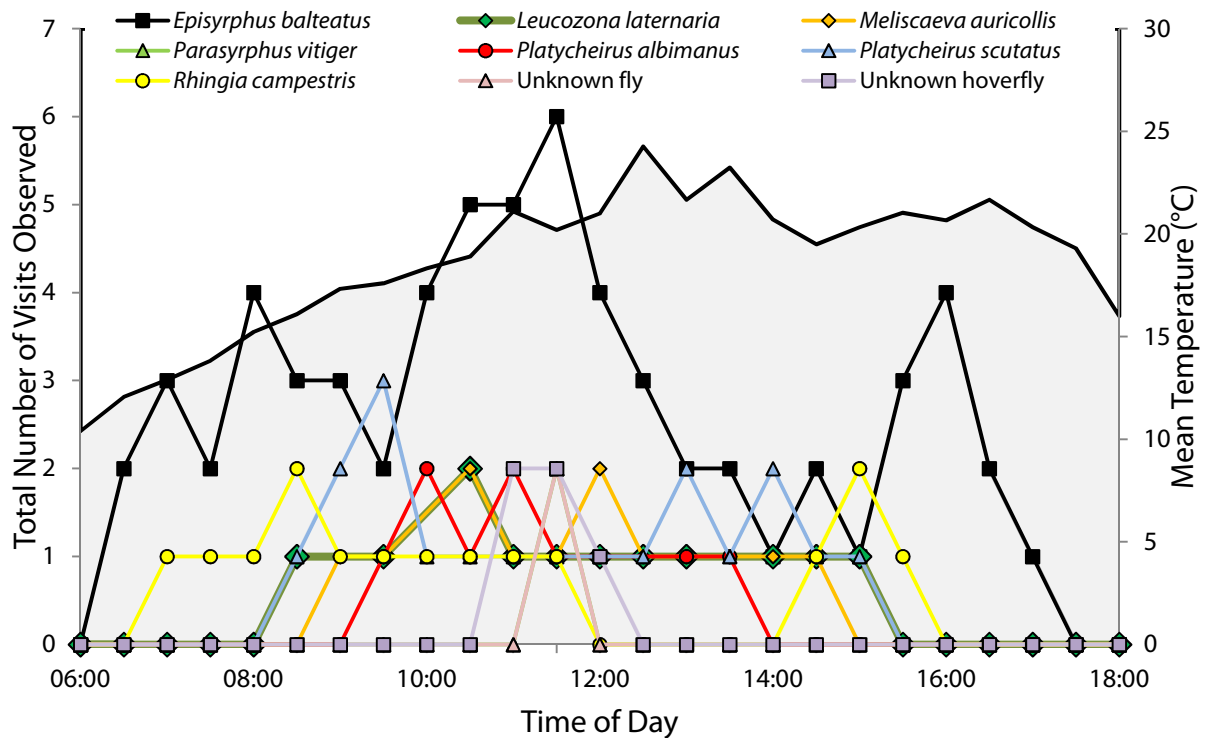


Fig. 6: Partitioning of visitor species to *Agrimonia eupatoria* over daily time. Mean temperature (°C) shown as an area plot.

Pollen Deposition by Visitors

Group	Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
Hoverfly	<i>Episyrphus balteatus</i>	27.6	P < 0.001
Hoverfly	<i>Leucozona laternaria</i>	43.5	P = 0.005
Hoverfly	<i>Platycheirus albimanus</i>	47.6	P = 0.005
Hoverfly	<i>Platycheirus scutatus</i>	52.8	P < 0.001
Hoverfly	<i>Rhingia campestris</i>	55.2	P = 0.016
Hoverfly	<i>Meliscaeva auricollis</i>	23.2	P = 0.032
Hoverfly	<i>Parasyrphus punctulatus</i>	57.5	NA
Hoverfly	Unknown hoverfly	10.8	NA
Other dipteran	Unknown fly	31.0	NA

Table 2: Statistical analysis of visitor species to *Agrimonia eupatoria*. Statistical analysis was performed by a Mann-Whitney U test with Bonferroni correction. In this case, $\alpha = 0.008$.

The mean pollen deposition by each visitor species was calculated (see Fig. 7 and table 2) and the difference in deposition between species was statistically significant.

There were insufficient observations for *Parasyrphus punctulatus*, the unknown fly species

and the unknown hoverfly species to carry out further analysis. Almost all visitor species deposited significantly more pollen grains on stigmas than was found on control stigmas, with the exception of *Rhingia campestris* and *Meliscaeva auricollis*. Of the species which deposited a significant amount of pollen, the most effective pollinator was *Platycheirus scutatus*.

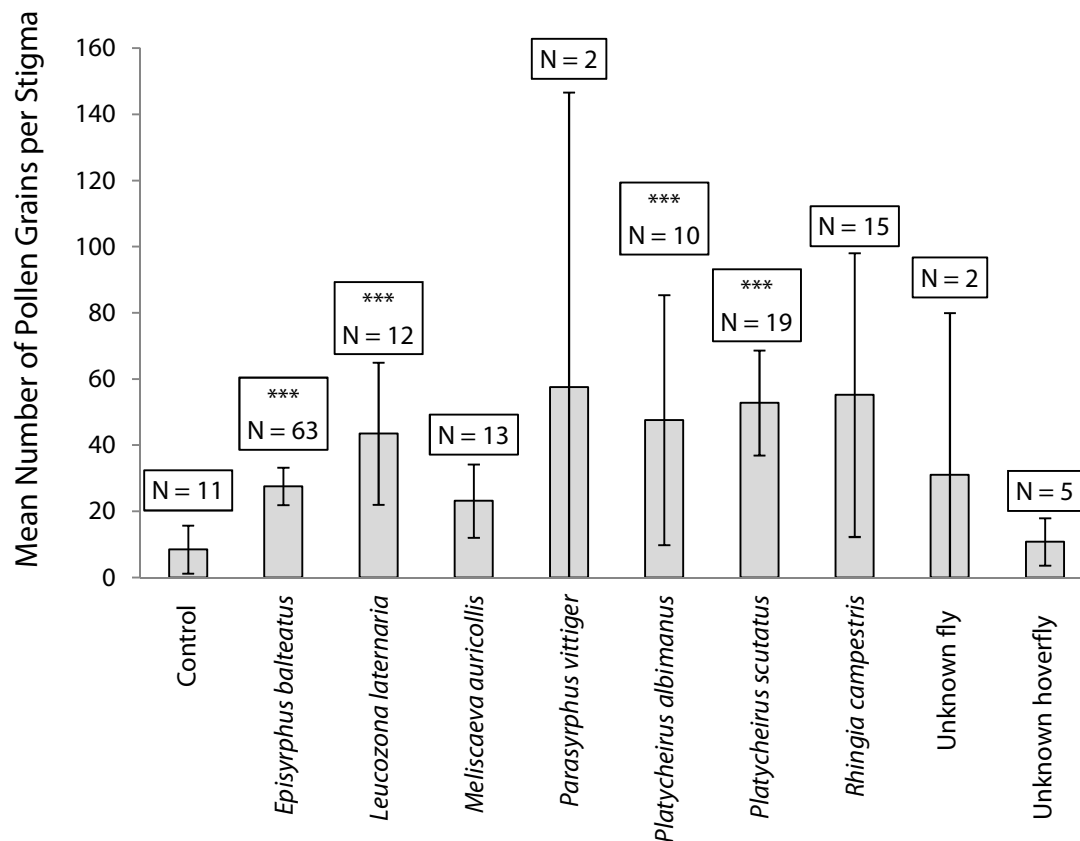


Fig. 7: Mean pollen deposition by visitor groups to *Agrimonia eupatoria*. N values and SD shown. Significance refers to the significant of number of pollen grains deposited in comparison to control flowers. Variation between visitors was significant (One-Way ANOVA: $P = 0.046$, $F = 2.336$, $df = 5$).

Discussion

Floral traits

Several features of *Agrimonia eupatoria* point towards it being a hoverfly-pollinated flower. Its yellow flower colour, “fruity” odour, inflorescence arrangement and floral structure are typical of the syndrome (see table 2). Flowering times and dehiscence followed patterns of dipteran activity as dehiscence began early in the morning, with pollen being available throughout most of the day (discussed further in Chapter 8). In addition, a lack of obvious nectar production indicates pollination by a pollen-eating species, and the lack of observation of species other than hoverflies, and a few rare sightings of other dipterans, further suggests that this species is pollinated effectively by hoverflies (though there must be some caution here given the paucity of all bees during the study period, as referred to in the Introduction).

Visitor Assemblage

Previous studies have shown dipterans to be the sole visitors of *Agrimonia eupatoria* (Memmot, 1999; Fründ *et al.*, 2010). My observations of *A. eupatoria* during the course of the pollinator effectiveness study confirm that it is almost solely visited by hoverflies, with the occasional visit from other flies (an unknown fly making up 1.41% of the visits).

Group	Species	N	Mean Size (mm)
Hoverfly	<i>Episyrphus balteatus</i>	63	WL 6.00-10.25 ¹
Hoverfly	<i>Platycheirus scutatus</i>	19	WL 5.5-7.5 ¹
Hoverfly	<i>Rhingia campestris</i>	15	WL 6-9.5 ¹
Hoverfly	<i>Meliscaeva auricollis</i>	13	WL 6-9.5 ¹
Hoverfly	<i>Leucozona laternaria</i>	12	WL 7-10 ¹
Hoverfly	<i>Platycheirus albimanus</i>	10	WL 5-8 ¹
Hoverfly	<i>Parasyrphus punctulatus</i>	2	WL 5.5-7.75 ¹
Hoverfly	Unknown hoverfly	5	NA
Dipteran	Unknown fly	2	NA

Table 3: Sizes of visitor assemblage of *Agrimonia eupatoria*. WL refers to wing length. ¹ Stubbs and Falk, 2002

By far the most frequent visitor was *Episyrphus balteatus* (see table 3). This is a common, medium-sized species across Britain and Ireland, and the most frequently recorded in Britain into which it migrates annually (Rotheray and Gilbert, 1999). This was followed by *Platycheirus scutatus*, a smaller species, and *Rhingia campestris*, a long-tongued, mid-sized hoverfly species. The next most frequent visitors were the two mid-sized species, *Meliscaeva auricollis* and *Leucozona laternaria*; and the smaller species *Platycheirus albimanus*. *Parasyrphus punctulatus*, a small species, the unknown fly and the unknown hoverfly were too rare in their visitations to be included in further analysis. The visitor assemblage of this species is discussed in more detail in Chapter 9.

The hoverfly species visiting flowers were observed to feed on the pollen of the flowers only. This suggests that nectar is not available as a flower reward from this species, or is not used as such by hoverfly visitors to the species in Northern Britain. Given the size of both visitor and flowers however, it is also possible that any nectar feeding was subtle and not easily observed. As the hoverflies fed upon the flowers, their bodies came into contact with both the male and female sexual organs and therefore they could be said to be pollinating the flowers in the “correct” manner, at least from an observational standpoint.

Partitioning of Visitors over Time

The partitioning of visitor species over time appears to be related to body size. The earliest flower visitors were the largest hoverflies, in particular *E. balteatus* and *R. campestris*, and the latest flower visitor to arrive on the flowers was the smallest species, *M. auricollis* (see Fig. 6 and Table 3). The larger species were able to visit later into the afternoon as temperatures cooled, in particular *E. balteatus* and *R. campestris*, though they did experience a drop in visitation over the hottest period of the day while the smaller species were better able to withstand the highest temperatures.

Pollinator Effectiveness

For my purposes, pollinators are classified as visitors that deposit statistically significantly more conspecific pollen on stigmas than is found on unvisited control flowers. The most effective pollinator is then classified as the pollinator that deposits the most conspecific pollen on stigmas per visit. Due to the limitations of the experiment, no distinction could be made between self and non-self pollen, however the behaviour of the visitor species was used to infer the likelihood of deposited pollen being from the same flower.

Almost all of the visitor species, with the exception of *Rhingia campestris*, *Meliscaeva auricollis* and those for which insufficient observations were available for further analysis, were found to deposit a statistically significant amount of pollen on the stigmas of *Agrimonia eupatoria* flowers, therefore almost all visitors could be classified as pollinators (see Fig. 7). The most effective visitor, in terms of amount of pollen deposited, was *Rhingia campestris*, a long-tongued syrphid, though there was much variation in the amount of pollen deposited by this species and this pollen deposition could not be analysed statistically, therefore it was not possible to confirm the status of *R. campestris* as a pollinator. Species in the genus *Rhingia* are known to be selective pollen feeders with a significant avoidance of certain pollens (Haslett, 1989a). *R. campestris* is unusually long-tongued (up to 11mm; Ssymank, 1991) and

shows some preference for blue or violet flowers and long corolla tubes (Speight, 1978; Gilbert, 1981); however, it is also known to frequent shallow flowers from other colour groups (Haslett, 1989a), as in this case, though it is able to feed from flowers without effectively pollinating them, possibly because its long tongue allows it to reach the pollen of flowers without necessarily coming into contact with the stigma. This may explain the high level of variation in pollen deposition for this species, as in some visits it will crawl over flowers and make contact with the reproductive organs, thereby depositing pollen; and in others it will rob pollen from the anthers without making substantial contact with the stigma, thereby depositing very little pollen. Of the pollinating species, *Platycheirus scutatus* was the most effective pollinator in terms of single-visit pollen-deposition.



Fig. 8



Fig. 9

Fig. 8: Small, short-tongued *Platycheirus* sp. feeding on the pollen from flowers of *Agrimonia eupatoria*.

Fig. 9: The larger, long-tongued *Rhingia campestris* feeding on pollen from the flowers of *Agrimonia*

The other visitor species were highly variable in their size and form (see Fig. 8 and Fig. 9; and table 3), but almost all were found to deposit sufficient pollen on stigmas to be classified as pollinators (with the exception of *Meliscaeva auricollis*). There was significant variation between pollen depositions of the different species.

Conclusion

Agrimonia eupatoria possesses the typical floral traits of a hoverfly-pollinated syndrome, and the observations of flower visitors confirm this. The traits of *Agrimonia eupatoria* could be considered indicative of a “generalist-fly” pollinator syndrome; however, while there were a few rare observations of other families of fly observed to visit this flower, the main flower visitors are from the Syrphidae family, therefore this plant species may be considered more specialised than originally thought.

There is significant variation in the quality of these different pollinator species. Many pollination studies focus on parameters such as visitor frequency and the observation of “correct” pollination as determinants of the most effective pollinator species of a plant. A more detailed investigation of visitor frequency of *Agrimonia* is discussed in further detail elsewhere (Chapter 9). All visitor species observed during the pollinator effectiveness study were observed to pollinate flowers in the “correct” manner, and all were identified as effective pollinators in terms of pollen deposition on stigmas in a single visit. If we are to use the amount of pollen as a determinant of pollinator quality however, it is clear that not all pollinators are equally effective. Without analysing in detail the pollen deposition of individual species, the variation in pollinator quality cannot be determined, and the most effective pollinator may be misidentified, or a plant that is in fact rather specialised in terms of pollinators may be misidentified as a generalist. As not all pollinators are equal in their pollen deposition, some species may be more beneficial to a plant than others. This could prove extremely important in conservation efforts of rare and threatened species. This importance cannot be determined by observational means alone; therefore analysis of pollen deposition by individual species is an extremely important method of studying pollinator effectiveness.

This study offers another confirmation of the predictive powers of pollinator syndromes as described by Faegri and van der Pijl (1979). In this case, a plant showing a clear hoverfly-pollination syndrome was effectively pollinated exclusively by insects of the family Syrphidae.

Chapter 5: Testing Pollination Syndromes

The Bee-Pollination Syndrome

Introduction

Pollination by Bees

Pollination by bees, or mellitophily, is perhaps the best known, and most researched, of the pollination syndromes. Bees make ideal pollinators as they are completely reliant on flowers for nectar and pollen, the sole sources of nutrition for both adults and larva (Roubik, 1989; Westrich, 1989; Dobson and Peng, 1997). Bees forage not only for their own nutritional requirements, but also for those of their offspring, in the case of solitary species, or the offspring of the queen in social species (Haydak, 1970). As much as 95.5% of pollen produced by flowers of *Campanula rapunculus*, for example, was removed by bees, and only 3.7% contributed to pollination (Schlindwein *et al.*, 2005). In another study, 85% of 41 bee species required the whole pollen content of more than 30 flowers to rear a single larva, and some bee species even used the pollen of 1000 plant species to rear a single larva (Müller *et al.*, 2006). Due to their reliance on flowers for nutrition, the number of floral visits by bees is much greater than for other taxa. The foraging distances of bees strongly influences the sexual reproduction of most flowering plant species and can determine the genetic structure of plant populations (Campbell, 1985; Waser *et al.*, 1996). Foraging distance increases non-linearly with body size in bees (van Nieuwstadt and Iraheta, 1996; Gathmann and Tscharnthke, 2002; Westphal *et al.*, 2006; Greenleaf *et al.*, 2007) and the distances covered can be substantial; for example bumblebees do not necessarily forage close to their nests (Osborne *et al.*, 1999) and have been observed flying 20km from the nearest land over an 80km stretch of water (Mikkola, 1984), and mean honeybee foraging ranges are from 1-13km (Von Frisch, 1967; Visscher and Seeley, 1982; Schneider, 1989; Schneider and McNally, 1993; Schneider and Hall, 1997;

Beekman and Ratnieks, 2000) therefore possible pollen dispersal distances for bee-pollinated species are high (Willmer, 2011).

In addition to this, bees are commonly able to produce and control extra internal metabolic heat, also known as endothermy, allowing them to warm their bodies enough to perform flight in little or no sunlight. This means bees can commonly be active around dawn and dusk (or even in to the night, for example the nocturnal carpenter bee *Xylocopa tranquebarica* (Somanathan *et al.*, 2008)); and similarly bees are not restricted in their foraging by weather in the same way as most other flower visitors (Stone & Willmer, 1989; Goulson, 2003; Willmer, 2011).

There are perhaps 20,000 to 40,000 bee species worldwide (Parker *et al.*, 1987; Arbuckle, *et al.*, 2001), with a high level of variation in both morphological and behavioural features such as size, tongue length, endothermic abilities, social structures and flower visiting patterns and behaviours (Willmer and Stone, 2004). This means that there is also much variation in the characteristics of flowers visited by bees, and the bee syndrome could readily be split into several sub-categories (Willmer 2011). This chapter investigates a general bee-pollinated flower syndrome, which could also be described as a large-bodied bee-pollinated syndrome. Many of the general mellitophilous traits defined by Faegri and van der Pijl (1979; reviewed in Willmer, 2011) apply to this syndrome, with some modifications. These traits are summarised further below.

Mouthparts of Bees

Variation in bee tongue length is independent of body size, therefore small bees may have long tongues and vice versa. Bee tongues have relatively uniform construction (see Fig 1), with variation in length coming from differing proportions of each section (Michener, 2000; Krenn, *et al.*, 2005). The basic feeding mechanism of most bees is a combination of licking and sucking functions of the labiomaxillary complex (Krenn *et al.*, 2005). The labiomaxillary complex rests within the head cavity until a turning of the cardines, which articulate within

the head, causes the main body to protract, where it is suspended between the extended and more or less stationary maxillae. The ligula (glossa and paraglossa) produces a licking motion by repeated extension and contraction. When the labium is fully protracted, the ligula extends beyond the apical ends of the galeae where its exposed and hairy surface comes into contact with the liquid food material. When the ligula is retracted, the liquid that has adhered to it is drawn into the food canal, where it is unloaded and transported further by capillarity, by labial movements, and also by suction force from the muscular cibarial or pharyngeal pumps.

Modification of the labiomaxillary complex towards nectar feeding occurs primarily through a lengthening of the main axis, with the labrum or head capsule sometimes contributing to this elongation. From a morphological and functional point of view, nectaring proboscides can be classified as short, medium or long.

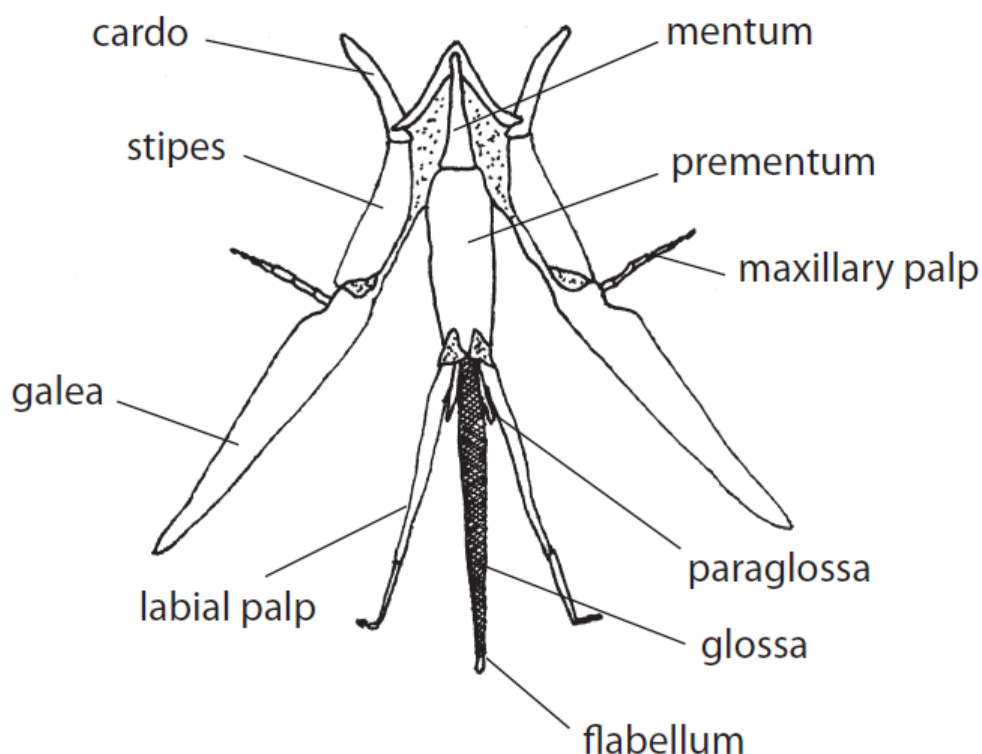


Fig. 1: Tongue structural components in bees, spread apart. Willmer, 2011 (Modified from Michener 2000.)

A short proboscis is defined as being slightly or moderately elongated in comparison to unspecialised hymenopteran mouthparts, with the glossa generally equal in length to, or shorter than, the prementum. The feeding strategy of species with short proboscides is primarily by lapping and/or sucking. The Andrenidae, Colletidae, Mellitidae and Halictidae are classified as “Short-Tongued Bees”. Characteristics of long-tongued bees are explored further in Chapter 7.

Bee Flower Syndrome

Flowers pollinated by bees often share several generic characteristics (see Table 1). Aspects of flower shape, colour, size, scent, reproductive structure placement and nectar volume and concentration can all serve to attract bees to flowers, and effect efficient pollination (Faegri and van der Pijl, 1979; Willmer, 2011).

Flower Structure	Medium to long corolla, often pendant, usually zygomorphic (i.e. bilaterally symmetrical rather than radial)
Landing Platform	Often with a complex texture or ridging so that a bee may hang on easily
Arrangement	Often in spike inflorescences
Timing	Flowers usually open in early morning and offer main rewards before midday, though some may be rewarding in the evening
Colour	Blue, pink, purple or white, sometimes yellow
Nectar Guides	Often present on petals
Odour	Usually sweet, typically “floral”
Nectar	Medium concentration nectar, typically 30-60%, in medium volumes, often located quite deeply in the base of the flower
Pollen	Between 15 and 60µm, easily picked up by the feathery bodies of bees

Table 1: Summary of the characteristics of Bee-pollinated flowers according to Faegri and van der Pijl (1979) and Willmer (2011).

Floral Shape and Size

Corollas of bee-flowers are often medium to long in length, though the corollas of flowers in the large-bodied bee syndrome are more often bowl-or disc shaped or bell shaped with wide corollas, as the visitors are expected to climb inside or onto the flowers in order to feed, rather than inserting their proboscis to reach nectar. Floral structure is usually zygomorphic, often bilaterally symmetrical rather than radially symmetrical. Flowers are mechanically strong and often also possess a ridged or textured landing platform for bees to hang from when visiting flowers. Bee flowers are regularly arranged in inflorescences, typically spikes which the bees can climb, visiting each flower in turn (Faegri and van der Pijl, 1979; Willmer, 2011) and often from the bottom up, reducing geitonogamy and increasing pollen export per flower in species with sexual segregation (e.g. Harder *et al.*, 2000; Routley and Husband, 2003).

Floral Colour

Bees are known to use their sophisticated colour vision to detect flowers at a distance (e.g. Lunau and Maier, 1995; Chittka and Raine, 2006), and, in particular, those colours which form a strong contrast with the background, whether that is the ground or other foliage (Lunau *et al.*, 1996; Spaethe *et al.*, 2001). Many species of bee-visited flowers also display colour patterns on their corollas which can attract and guide pollinators (Manning, 1956; Free, 1970; Dafni and Giurfa, 1999; Lunau, 2000; 2006; 2009). These patterns consist of a large-scale coloured corolla for long-distance attraction, and a contrasting, sometimes diminutive floral guide, often spots or stripes, indicating the site of access to the floral reward (e.g. Waser and Price, 1985; Hempel de Ibarra and Vorobyev, 2009; Lunau *et al.*, 2009).

Floral Odour

Bees also use olfactory cues to distinguish between different plant species (e.g. Dobson *et al.*, 1999; Dobson and Bergström, 2000; Howell and Alarcón, 2007). Floral odours can trigger landing behaviour in bees at close range (Lunau, 1992), as well as playing an important role in long-range orientation to flowers (e.g. Kunze and Gumbert, 2001; Dötterl and Schöffler, 2007).

Nectar Volume and Composition

Bee-visited flowers often produce a medium volume of nectar, between about 0.2-5µl in temperate flowers and up to 50µl in tropical flowers (Perret *et al.*, 2001), with a medium concentration, which can range from about 25-60% (Pyke and Waser, 1981; Harder, 1986; Baker and Baker, 1990; Perret *et al.*, 2001). However factors such as tongue length will affect the concentration of nectar that can be imbibed and a longer proboscis requires a lower nectar concentration for optimum nectar uptake (e.g. Borell, 2007); thus short-tongued bees may prefer 40-60% concentrations, while longer-tongued bees take nectars at 25-50% (Willmer, 2011).

Nectar often accumulates at the base of the flower, and its accessibility varies with corolla length and width. Flowers usually open in the early morning and produce rewards until around midday, which coincides with the peak activity times of bees, although some floral species may offer substantial rewards in the evenings.

Placement of Reproductive Structures

The sexual organs of general bee-flowers are often orientated in such a way that only a relatively large-bodied visitor will come into contact with them. The stamens of bee flowers are typically few in number (Faegri and van der Pijl, 1979; Willmer, 2011), but the ovaries often contain many ovules. Pollen grains are small, typically between 15 and 60µl, which

allows for them to easily adhere to the usually hairy bodies of bees (Faegri and van der Pijl, 1979; Willmer, 2011).

Testing the Large-Bodied Bee-Flower Syndrome

To test the syndrome proposed above, two British wildflower species showing traits indicative of pollination by large-bodied bees were investigated. *Geranium pratense* and *Digitalis purpurea* are well-studied species said to be pollinated by various species of bee (*Geranium pratense*: Brian, 1957; Berg, 1960; Kozuharova, 2002; Chapman *et al.*, 2003; *Digitalis purpurea*: Berg, 1960; Best and Bierzychudek, 1982; Grindeland *et al.*, 2005), however no study as yet into the effectiveness of different bees, or other visitors, at pollinating either of these species has been carried out.

Materials and Methods

Study Sites

The population of *Geranium pratense* was studied at West Quarry Braes between June and September of 2008 and 2009, a Scottish National Heritage Site in Fife (NO 597 088) with a diverse range of native British flora and fauna present, consisting primarily of scrub and woodland habitats. *Digitalis purpurea* populations were studied in a meadow habitat on the banks of Loch Tay in Perth and Kinross, Scotland (NN 669 358) in June of 2010.

Geranium pratense

Geranium pratense, of the Geraniaceae, also known as meadow cranesbill, is found in meadows and along roadsides throughout England and the South and East of Scotland. It is found more rarely in Ireland, Wales and North and West Scotland. Flowering occurs between June and September (Walters, 2002).

Structure

Individuals have long flower stalks, up to 1m high, possessing large, violet-blue flowers up to 4cm across with rounded petals (personal measurements). Corollas also possess paler nectar guides in the form of narrow stripes leading towards the centre where nectar is secreted at the base of the style (see Fig. 2). The flower structure is dish-shaped rather than tubular, with radial symmetry and lateral orientation, where flowers are held away from the stem (Berg, 1960). Flowers possess between 7 and 10 anthers approximately 18mm in length, and a protruding stigma approximately 23mm in length.

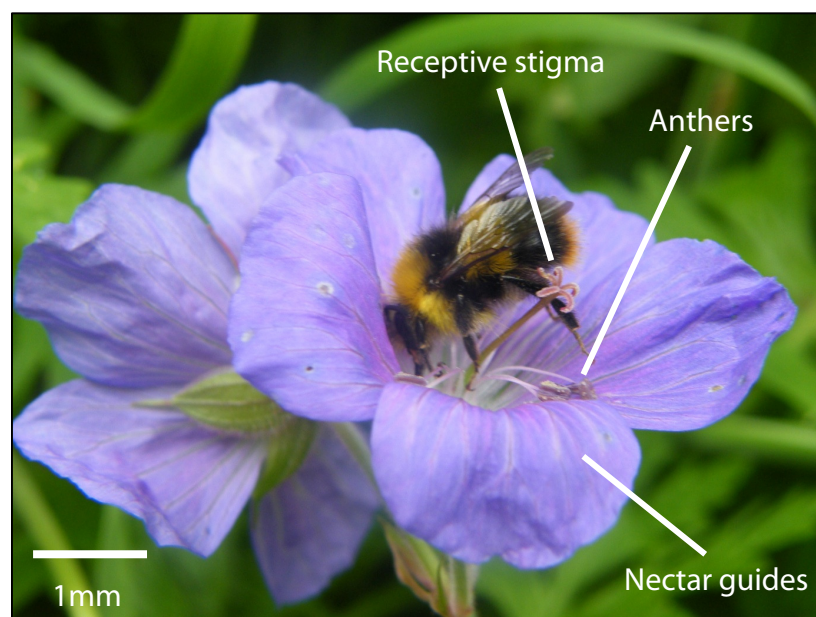


Fig. 2: *Bombus pratorum* feeding on flowers of *Geranium pratense*. Stigma, anthers and nectar guides indicated.

Nectar

No studies on the nectar reward of *Geranium pratense* are available, though personal observations showed that nectar was secreted from the base of the style. However this nectar was difficult to collect and evaporated quickly due to the open structure of the

flowers and the exposed conditions of the field site, making further analysis of its composition impossible. Data gathered by P. G. Willmer (pers. comm.) indicate a mean concentration of 38% and a mean volume of 0.13ul in the morning hours in late June in a Fife garden, rising to over 55% with many empty flowers in the afternoon.

Timing

Again, no studies are available on the timing of dehiscence and stigma receptivity of *Geranium pratense*, however from personal observations it could be seen that anther dehiscence began shortly after flowers became fully opened and continued for between 1 and 2 days. Stigmas remained closed until the second day of flower opening. At this time the tip of the stigma splits into 5 fronds with a hairy and sticky surface, which splay and curl downwards as the stigma becomes receptive.

Digitalis purpurea

Digitalis purpurea, of the Scrophulariaceae, also known as foxglove, is a biennial, native to Europe and commonly found along shaded roadsides and other disturbed habitats. Flowering is from June to September in the UK (Walters, 2002).

Structure

Flowers of *Digitalis purpurea* are large, bell shaped, and usually either purple or white in colour, often with spotted nectar guides on the lower lip (see Fig. 3). A basal rosette of leaves produces a vertical spike inflorescence up to 1m tall (Best and Bierzychudek, 1982). Stigmas and anthers are obscured, located inside the corolla tube, lying along the upper surface. The stigmas extend past the anthers, and are separated from them by approximately 10mm. This effectively prevents self-fertilisation, although flowers are self-compatible (Stead and Moore, 1979; Best and Bierzychudek, 1982).

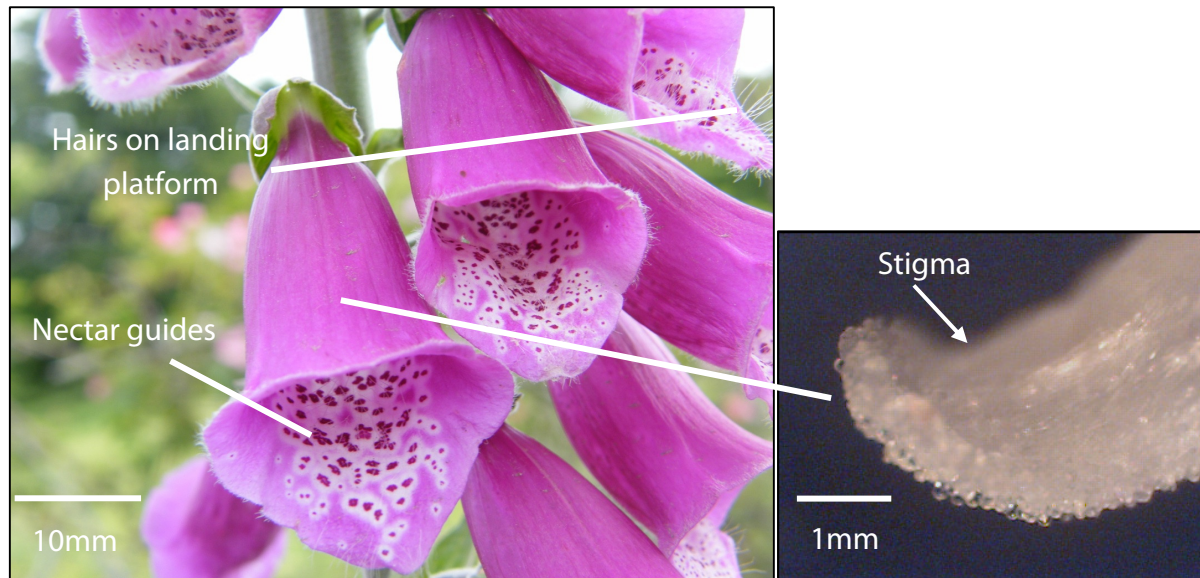


Fig. 3: Flowers of *Digitalis purpurea* and receptive stigma of (x10 magnification, Watson Barnet Microsystem 70 Compound Light Microscope). Nectar guides and hairs on landing platform indicated.

Nectar

The floral nectary of *Digitalis purpurea* is located at the base of the ovary. Nectar secretion begins shortly after flower opening, and continues for the duration of anthesis (Percival and Morgan, 1965; Stead and Moore, 1977; Gaffal *et al.*, 1998). Flowers can produce up to 10µl of nectar (Percival and Morgan, 1965; Assmann, 1968; Gaffal *et al.*, 1998), with concentration ranging from 16-27% (Percival, 1961; Gaffal *et al.*, 1998). *Digitalis* nectar is primarily sucrose, but also contains some fructose (Percival, 1961). The calorific value of nectar contained in flowers is independent of inflorescence size, however lower, older flowers contain a higher calorific reward than higher, younger flowers (Percival and Morgan, 1965; Best and Bierzychudek, 1982). By secreting nectar in such a way, the plant ensures that visitors feed from female flowers first, depositing the pollen they have picked up from previous flower spikes visited, before visiting younger, male flowers higher up the inflorescence and collecting pollen to carry to later visited flowers (Best and Bierzychudek, 1982).

Timing

Individual flowers of *Digitalis purpurea* remain open for approximately 10 days. Each day of the flowering of the spike inflorescence, the lowermost flower withers and drops off, while a new bud at the top of the spike unfolds (Best and Bierzychudek, 1982). Flowers are protandrous (Percival and Morgan, 1965); the anthers mature first and only after dehiscence does the stigma appear to become glossy and sticky, indications of receptivity (see Fig. 3). Flowers therefore pass through male and female phases with little overlap. At any one time, an inflorescence will then consist of, from the top, a cluster of closed buds, several newly-opened buds, male flowers, female flowers, and maturing seed capsules, with a mean of 10 flowers open per inflorescence (Best and Bierzychudek, 1982; and personal observation).

Sampling Period

Field research for *Geranium pratense* occurred between July and August of 2009 and 2010. Temperature and humidity readings were taken from a shaded area of the study site using a HM34 Vaisala Pocket Size Relative Humidity Meter every half hour during each sampling session. *Digitalis purpurea* was studied over 4 days in June 2010. Temperature readings only were collected continuously over a period of 4 days using two Tinytag TGP-4017 data loggers, which were placed in undergrowth beside the study site. For both species, each sampling session was between 1 and 3 hours long depending on the frequency of visitations and how long it took all formerly protected newly opened flowers to be visited.

Results

Temperature and Humidity

Mean temperature (°C) and relative humidity (%) readings for *Geranium pratense* are shown in Fig. 5. Mean temperature was lowest in the morning and evening, peaking between 13:00 and 14:30. Relative humidity was variable throughout the day, though in general highest in the morning and evening, with lowest readings over midday.

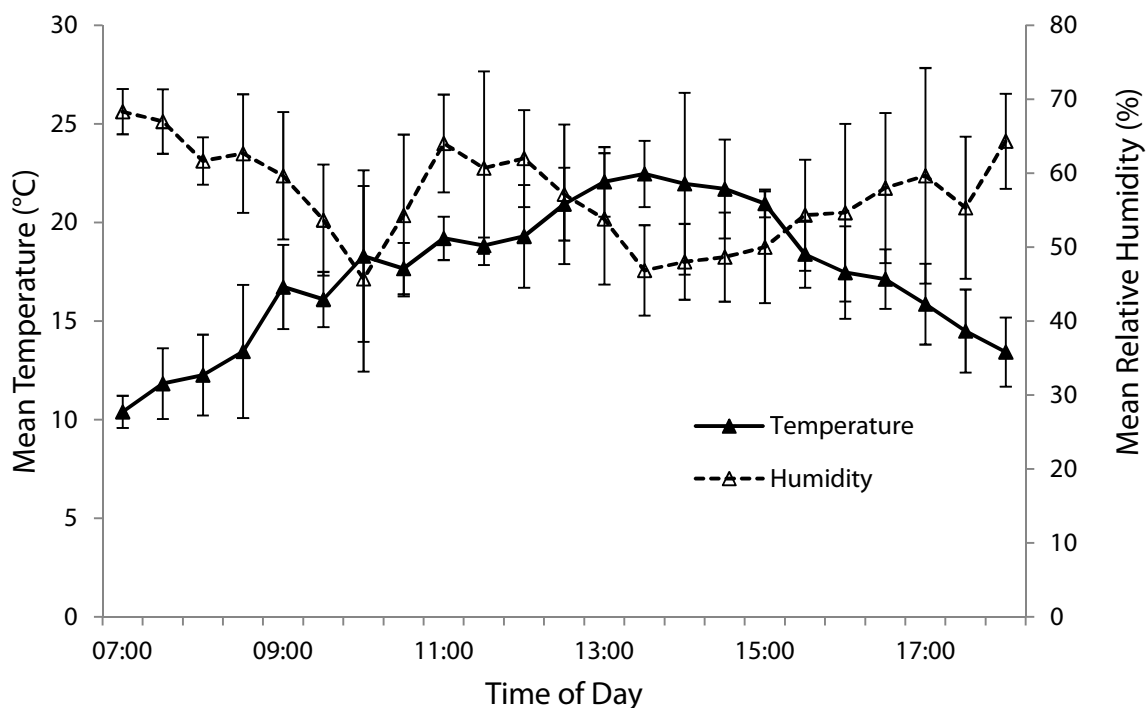


Fig. 5: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Geranium pratense*. Standard deviations are shown.

Mean temperature readings for *Digitalis purpurea* are shown in Fig. 6. In general, temperatures were lowest early in the morning, peaking around midday and falling in the evening. Temperatures for this study period were less variable than those of *Geranium pratense*, possibly due to the shorter study period, as well as the sheltered study site at Loch Tay, which was located in a valley and surrounded by trees on all sides. In addition, weather conditions, in particular evening temperatures, during this week of study were surprisingly

favourable in comparison to general weather conditions over the summer of 2010. Variable heavier cloud cover may have contributed to the declining temperatures before 09:30 and between 14:30 and 15:30.

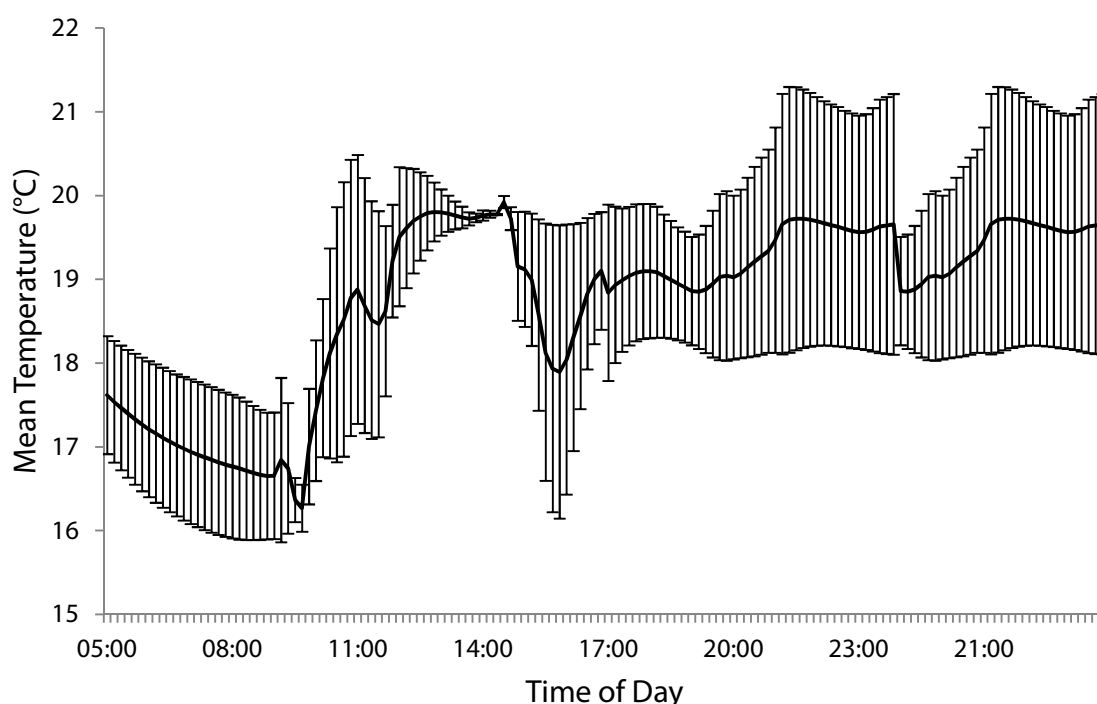


Fig. 6: Mean daily temperature (°C) readings during the study period of *Digitalis purpurea*. Standard deviations are shown.

Partitioning of Visitors over Time

Visitors to target flowers of both *Geranium pratense* and *Digitalis purpurea* throughout the day were recorded across the study period. As the study periods of *D. purpurea* were only between 10:30 and 14:30 however, it is not possible to show visitation across the full day. Visitors to *G. pratense* were first allocated to functional groups (Fig. 7) and then analysed by individual species (Fig. 8). As *D. purpurea* was solely visited by bumblebees and no other groups were observed visits were analysed by individual species only (Fig. 9). Visits recorded do not provide a complete representation of the visitor assemblage of the plant species throughout the day, as only visits to target, previously bagged flowers were recorded; however some patterns of visitation can be seen from the data available.

Geranium pratense

Bumblebees were the earliest recorded visitors to *Geranium pratense*, first observed at 07:00 (Fig. 7). Bumblebees were present throughout the day, except between 14:00 and 15:00, when mean temperature was at its highest. Dipterans arrived at flowers from 08:00 and were present throughout the day, although numbers declined from 11:00 onwards.

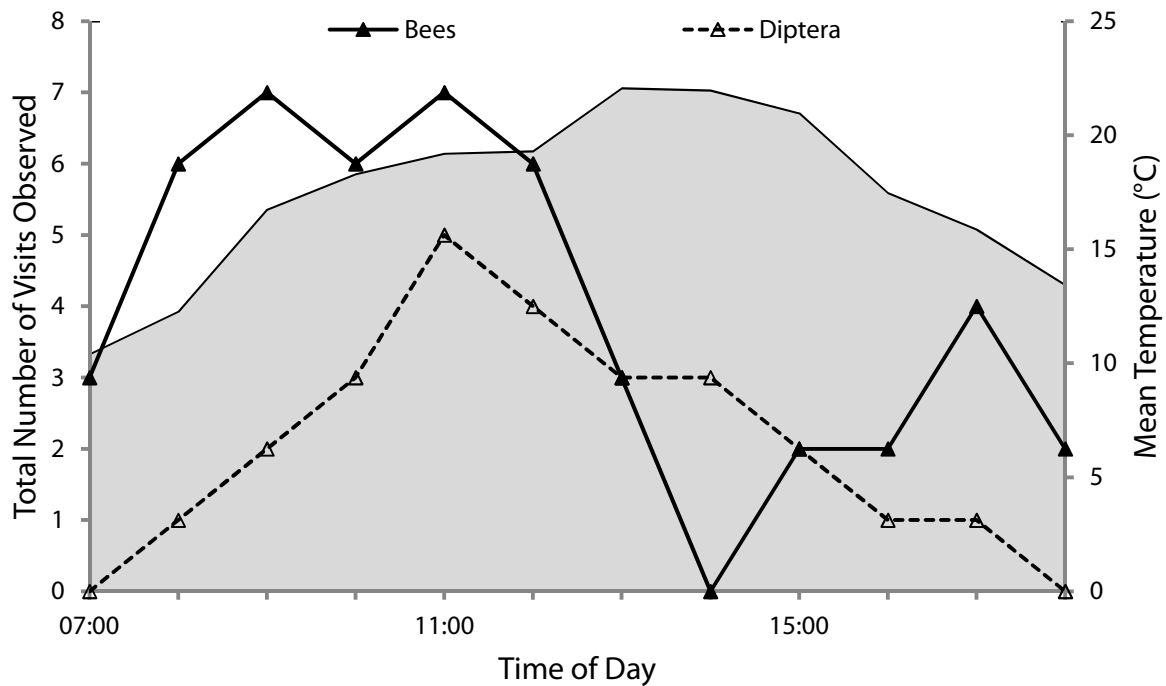


Fig. 7: Partitioning of visitor groups to *Geranium pratense* over daily time. Mean temperature (°C) shown as an area plot.

When analysed by individual species (Fig. 8), the earliest flower visitor was *Bombus pratorum* at 07:00. This was followed by the hoverfly *Rhingia campestris* at 08:00. *Bombus lapidarius* arrived at flowers from 09:00 but was only recorded again at 10:00. *Melanostoma mellinum* and the unknown fly species arrived at flowers from 10:00, though this was the only time these species were observed. *Episyrphus balteatus* was observed once at 11:00, as was *Meliscaeva auricollis* at 14:00, and *Platycheirus occultus* was observed twice at 13:00. *Bombus pratorum* and *Rhingia campestris* were the only species to visit frequently, and throughout the day. *R. campestris* arrived later in the morning and ended foraging earlier in the evening, though it was present consistently throughout the day, while *B. pratorum* arrived earlier in the

morning and foraged later into the evening, and was not present on flowers between 14:00 and 15:00.

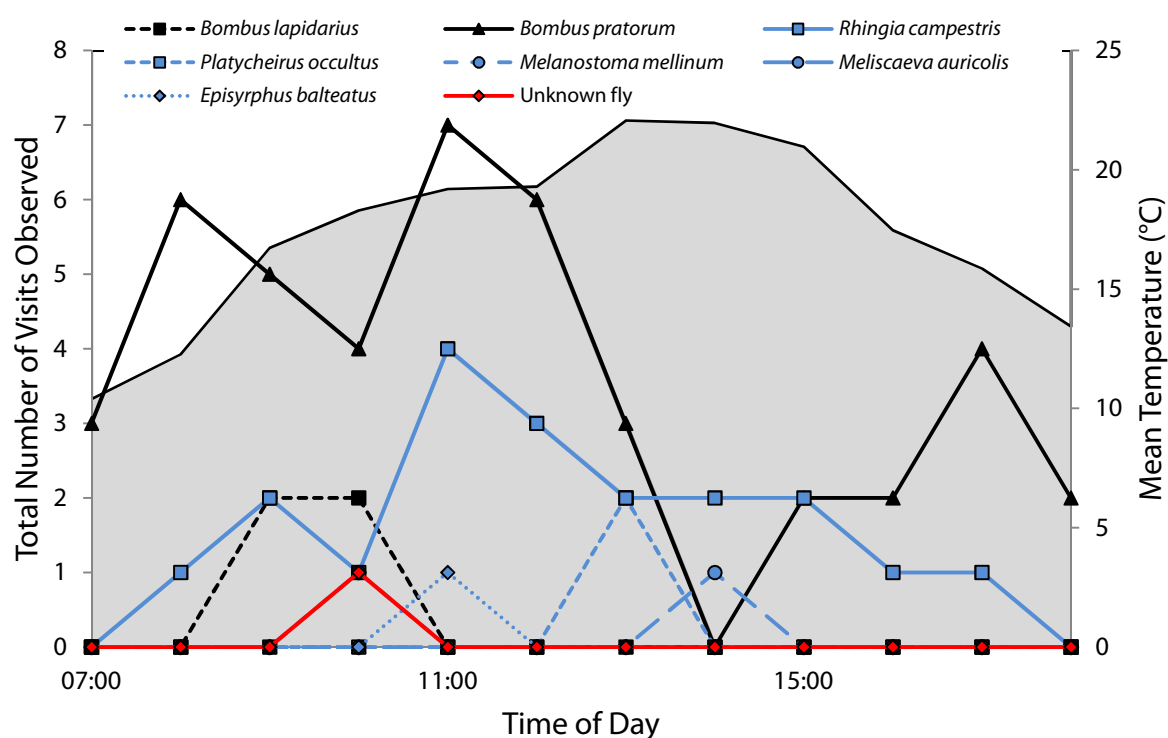


Fig. 8: Partitioning of visitor species to *Geranium pratense* over daily time. Mean temperature (°C) shown as an area plot. Bees indicated in black, hoverflies in blue and other dipterans in red.

Digitalis purpurea

Recordings of visitors to *Digitalis purpurea* began at 10:30, and the only visitor recorded at this time was *Bombus muscorum* (Fig. 9). *Bombus hortorum* was observed on flowers from 11:00, and *Bombus terrestris* was only recorded at 11:30. *B. muscorum* and *B. hortorum* both showed a decline in visits at 12:30, and again at 14:00, when the highest mean temperatures were recorded.

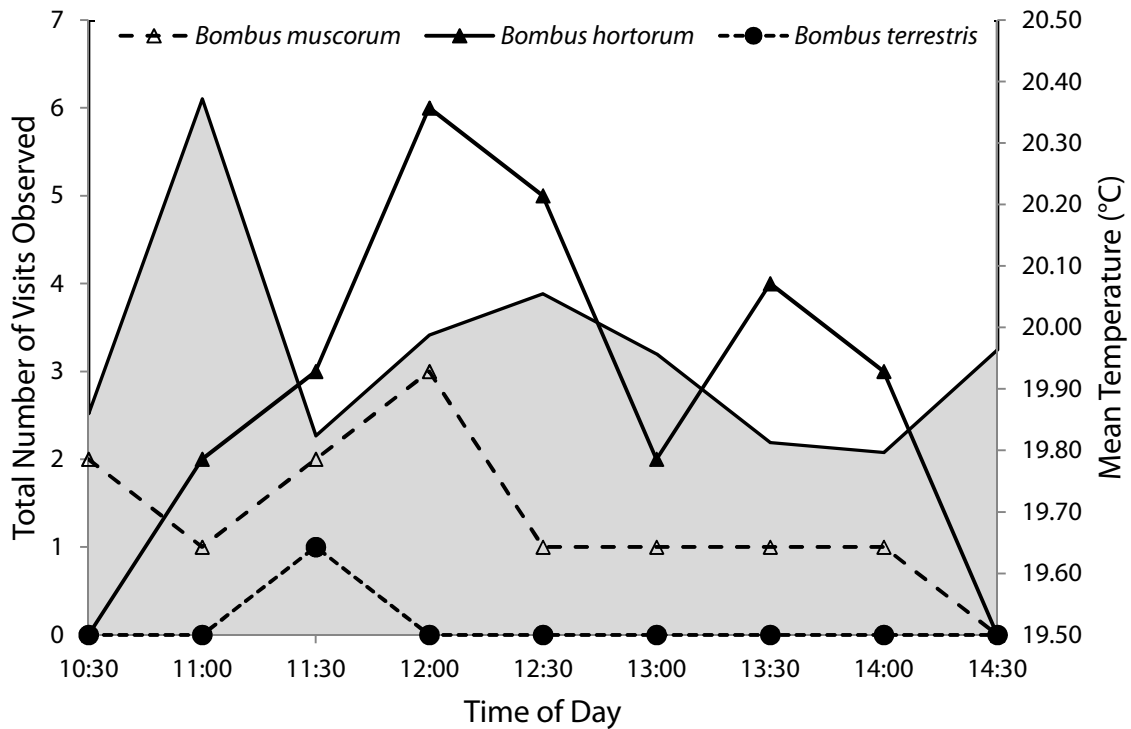


Fig. 9: Partitioning of visitor species to *Digitalis purpurea* over daily time. Mean temperature (°C) shown as an area plot.

Pollen Deposition by Visitors

The mean number of pollen grains per stigma (MPS) of *Geranium pratense* was first calculated by visitor functional group (Fig. 10) and then by individual species (Fig. 11). As *Digitalis purpurea* was only visited by bumblebees, the pollen deposition of visitors to this species was only analysed by individual species (Fig. 12). As in other chapters, a pollinator was defined as a species that deposited a statistically significantly greater amount of pollen on stigmas in comparison to the unvisited control stigmas.

Geranium pratense

Group	MPS	Sig. of MPS (in comparison to MPS of Controls)
Bees	33.9	$P = 0.572$
Dipterans	19.8	$P = 0.863$

Table 2: Statistical analysis of visitor groups to *Geranium pratense*. Statistical analysis was performed by with Post Hoc LSD tests.

The bee group had a higher MPS on *Geranium pratense* than the dipteran group (see Table 2) but the difference was not significant (see Fig. 10). Neither species deposited a significant MPS in comparison to controls therefore neither were classed as pollinators.

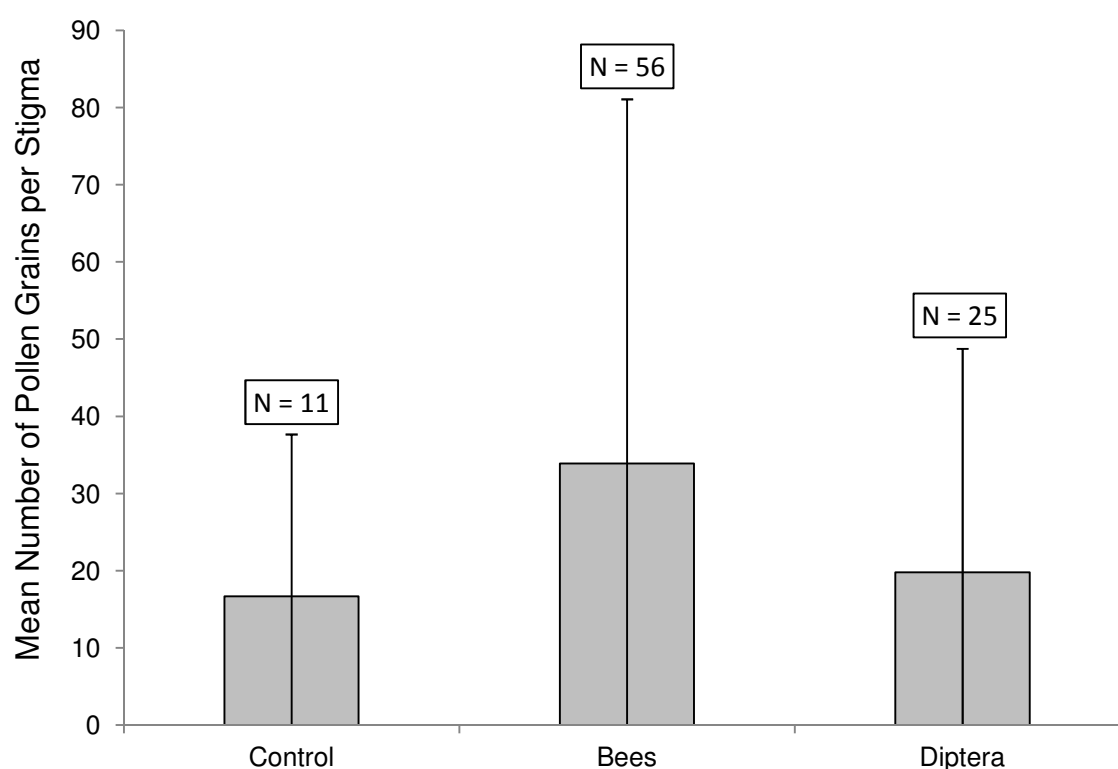


Fig. 10: Mean pollen deposition by visitor groups to *Geranium pratense*. N values and SD shown. Significance refers to the significant of number of pollen grains deposited in comparison to control flowers. Variation in deposition between the Bee and Dipteran groups was significant (LSD Post Hoc Tests $P = 0.305$).

Group	Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
Hoverfly	<i>Rhingia campestris</i>	19.0	P = 0.958
Hoverfly	<i>Platycheirus occultus</i>	56.0	NA
Hoverfly	<i>Melanostoma mellinum</i>	6.0	NA
Hoverfly	<i>Meliscaeva auricollis</i>	15.0	NA
Hoverfly	<i>Episyrphus balteatus</i>	0.0	NA
Other Dipterans	Unknown muscid	0.0	NA
Bee	<i>Bombus pratorum</i>	31.2	P = 0.389
Bee	<i>Bombus lapidarius</i>	1.0	P = NA

Table 3: Statistical analysis of visitor species to *Geranium pratense* Statistical analysis was performed by LSD Pot Hoc Tests.

When analysed by species, *Platycheirus occultus* had the highest MPS (see Fig. 11 and Table 3), however, the low number of observed visits by *Platycheirus occultus*, *Melanostoma mellinum*, *Meliscaeva auricollis*, *Episyrphus balteatus* and the unknown fly species meant that these species were excluded from further analysis. Of the species analysed, *Bombus pratorum* had the highest MPS, followed by *Rhingia campestris* and *Bombus lapidarius*. Variation in MPS between species was not significant. None of the species analysed deposited a significant number of pollen grains in comparison to control flowers, therefore no pollinator species were identified according to the criteria used in this thesis.

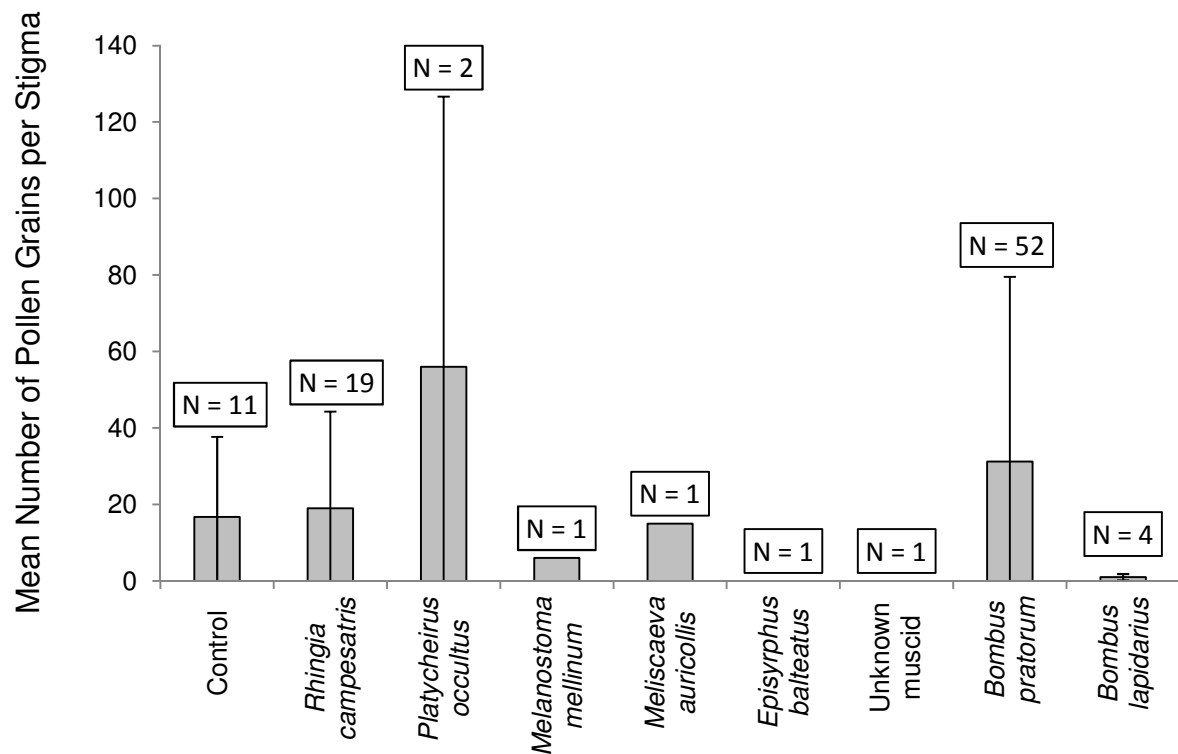


Fig. 11: Mean pollen deposition by visitor species to *Geranium pratense*. N values and SD shown. Significance refers to the significantly greater number of pollen grains deposited in comparison to control flowers. Variation in MPS between species was not significant (LSD Post Hoc Tests: $P = 0.438$).

Digitalis purpurea

Group	Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
Bee	<i>Bombus hortorum</i>	73.2	$P = 0.002$
Bee	<i>Bombus muscorum</i>	31.0	$P = 0.082$
Bee	<i>Bombus terrestris</i>	9.0	NA

Table 4: Statistical analysis of visitor species to *Geranium pratense*. Statistical analysis was performed by a LSD Post Hoc Test.

Bombus hortorum had the highest MPS of visitors to *Digitalis purpurea*, followed by *Bombus muscorum* and *Bombus terrestris* (see Fig. 12 and Table 4). *Bombus terrestris* was excluded from further analysis as only one visit was observed. Variation between species was significant (One-Way ANOVA: $F = 5.503$; $df = 2$; $P = 0.007$). Of the species analysed, *Bombus*

hortorum deposited a significantly greater MPS in comparison to controls while *Bombus muscorum* did not, therefore *Bombus hortorum* was the only pollinator identified.

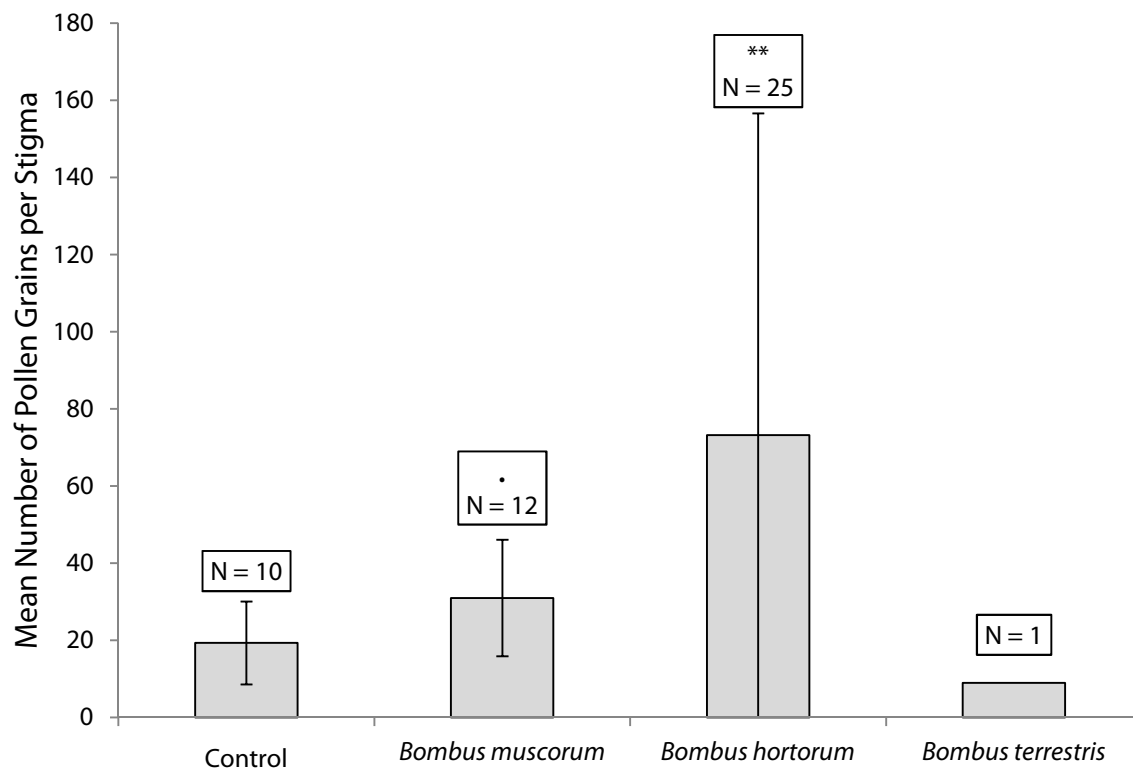


Fig. 12: Mean pollen deposition by visitor species to *Digitalis purpurea*. N values and SD shown. Significance refers to the significant of number of pollen grains deposited in comparison to control flowers. Variation between species was significant (One-Way ANOVA: $F = 5.503$; $df = 2$; $P = 0.007$).

Discussion

Floral Traits

Both *Geranium pratense* and *Digitalis purpurea* possess traits indicative of a general Bee-Pollinated Flower, in particular one pollinated by rather large-bodied visitors. The flowers were coloured appropriately for the syndrome, and possessed nectar guides, which are not normally associated with other species of visitor. Timing of nectar production and dehiscence is also correlated with the activity patterns of bees, as described above. *G.*

pratense possessed fairly open dish-shaped flowers, while *D. purpurea* had enclosed bell-shaped flowers, however the width of the corolla of these flowers allowed for fairly easy access to visitors and did not require adaptations such as long tongues to reach the nectar. The placement of the reproductive structures of these flowers meant that small-bodied visitors might enter the corollas but would not come into contact with the anthers or stigmas while feeding, however larger-bodied visitors such as bumblebees would be able to effectively pollinate flowers.

Visitor Assemblage

Geranium pratense

Group	Species	N	Mean Size (mm)
Bee	<i>Bombus pratorum</i>	52	TW 4.21 ¹
Bee	<i>Bombus lapidarius</i>	4	TW 4.46 ¹
Hoverfly	<i>Rhingia campestris</i>	19	WL 6-9.5 ²
Hoverfly	<i>Platycheirus occultus</i>	2	WL 5.5-6.5 ²
Hoverfly	<i>Melanostoma mellinum</i>	1	WL 5.0 ²
Hoverfly	<i>Meliscaeva auricollis</i>	1	WL 6-9.5 ²
Hoverfly	<i>Episyrphus balteatus</i>	1	WL 6-10.75 ²
Dipteran	Unknown fly	1	NA

Table 5: Visitor assemblage of *Geranium pratense*. WL refers to wing length. TW refers to thorax width.

¹ Peat *et al.*, 2005; ² Stubbs and Falk, 2002.

Geranium pratense was visited by several different insect species, including bumblebees, hoverflies and other dipterans. The most frequent visitor to the study flowers was *Bombus pratorum*, a relatively small bumblebee species, followed by *Rhingia campestris*, a fairly large hoverfly species, and the bumblebee *Bombus lapidarius*, a mid-sized species (see Table 5). Other visitors were much less frequent (*Platycheirus occultus*, *Melanostoma mellinum*, *Meliscaeva auricollis*, *Episyrphus balteatus* and Unknown fly).

Digitalis purpurea

Group	Species	N	Mean Size (mm)
Bee	<i>Bombus hortorum</i>	25	TW 4.74 ¹
Bee	<i>Bombus muscorum</i>	12	TW 4.94 ¹
Hoverfly	<i>Bombus terrestris</i>	1	TW 4.88 ¹

Table 6: Visitor assemblage of *Digitalis purpurea*. TW refers to thorax width. ¹ Peat *et al.*, 2005

Visits to *Digitalis purpurea* were solely by bumblebees (see Table 6). The most frequent bumblebee visitor was *Bombus hortorum*, a fairly large species, followed by *Bombus muscorum*, another fairly large species (cf Table 5). *Bombus terrestris*, also a large bumblebee, was rare and visited only once during the course of the study. *B. terrestris* and *B. muscorum* are typically classed as short-tongued bees (see Chapter 7), while *B. hortorum* is a typically long-tongued bumblebee species.

Partitioning of Visitors over Time

Geranium pratense

When analysed by groups (see Fig. 7), bees visited flowers of *Geranium pratense* earlier than dipterans. While many insects, and in particular the smaller species, are ectothermic, larger flying insects such as bumblebees can generate substantial amounts of metabolic heat which allows them to maintain stable body temperatures above that of ambient temperatures. The upward and downward strokes of the wings are controlled by two sets of muscles which contract alternately in flight, but can contract at the same time during warm up, generating heat but no movement (Heinrich, 1979; Goulson, 2003) and giving the effect of ‘shivering’. They also exhibit substrate cycling in these muscles (Newsholme *et al.*, 1972; Goulson, 2003), and both effects together can raise the temperature of their flight muscles to over 30 °C. In bumblebees, because of this they are able to forage earlier in the day than other insects, which are unable to warm up adequately for flight during the cooler temperatures of early morning (Goulson, 2003).

Bumblebees did experience a drop in visitation over midday, when temperatures were at their highest, while hoverflies did not show any obvious decline. Again this can be explained by size, as the larger size and smaller surface area to volume ratio of bees in comparison to most dipterans makes them less able to withstand the high temperatures of the day by dissipating heat and therefore makes them more susceptible to overheating, while in general dipterans are better able to withstand the higher temperatures of the midday hours (Willmer, 1983).

When we analyse visitor partitioning by species (see Fig. 8) we see that the first bee to visit, *Bombus pratorum*, is in fact the smaller of the two bee species (see Table 5), which is not as we would expect. This may be attributable to the low visitation frequency of the larger species *Bombus lapidarius*, as it may have been active much earlier in the morning, but not present on *Geranium pratense* flowers (either any of these flowers, or specifically the observed ones). *Rhingia campestris*, one of the largest hoverflies to visit, was also the earliest of the dipteran visitors, and, while it did show a slight decline in visitation over the hotter parts of the day, it was not completely absent. The low frequency of visitations for the other species of dipteran visitors makes it impossible to draw any conclusions about their partitioning of visitation across the day.

Digitalis purpurea

Bombus muscorum was the largest (see Table 6) and earliest of the bee visitor species to *Digitalis purpurea* (see Fig. 9), which is as we would expect. This species experienced a marked drop in visitation after 12:00, when mean temperatures were at their highest, while the smaller bumblebee species, *Bombus hortorum*, also experienced a drop, but to a lesser extent. Again, this is as we would expect given that smaller visitors are better able to withstand high temperatures and avoid overheating than larger visitors (Willmer, 1983). As *Bombus terrestris* made only one visit we cannot determine anything about its daily partitioning with the available data.

Pollinator Effectiveness

Geranium pratense

According to previous studies of *Geranium* species with similar traits, the main pollinators are bumblebees and occasionally honeybees (Berg, 1960; Brian, 1957; Chapman *et al.*, 2003; Kozuharova, 2002). These studies have made such determinations from visitation to flowers only however, and have made no attempt to ascertain whether effective pollination by these species is occurring. An effective pollinator of *Geranium pratense* would be expected to be fairly large in size so that it would contact both the anthers and stigma of flowers visited; something smaller species would be unlikely to do.

In addition to bumblebees, some hoverflies and one unknown fly species were also observed to visit *Geranium pratense*. When analysed by the functional groups (bees and dipterans), the visitors within the bee group were defined as effective pollinators, while the dipterans were not (Fig. 10 and Table 2).

Pollinator effectiveness was next analysed by individual visitor species, and none of the visitors were identified as pollinators according to the definition of such in this thesis (Fig. 11 and Table 3). It was clear, however, that within the dipteran group there was much variation in MPS. Categorising the visitors by functional group meant that the variation within groups was lost, and species which were not effective pollinators, could be classified as such, and vice versa.

The results of the pollinator effectiveness were inconclusive when it came to identifying the most effective pollinator of *Geranium pratense*. Bee populations during the time of the study were relatively low in comparison to normal levels; therefore it is likely that, given a more intensive study of *Geranium pratense*, we would find that more bumblebee species are active visitors, though they may not necessarily be equal in their effectiveness.

Digitalis purpurea

Previous studies of *Digitalis purpurea* have indicated the principle pollinators to be bumblebees (Brian, 1957; Best and Bierzychudek, 1982; Grindeland *et al.*, 2005). As before, these studies did not take any measure of pollinator effectiveness and relied solely on visitation to flowers as a determinant of important pollinators. Given the placement of the reproductive structures of *Digitalis purpurea*, an effective pollinator would have to be relatively large to consistently contact both the stigma and anthers and pollinate the flower.

Bumblebees were the only species observed to visit *Digitalis purpurea* during the course of the study. *Bombus hortorum* deposited the highest MPS, and was also the only species identified as a pollinator (Fig. 12 and Table 4). There was significant variation between MPS of the different bumblebee species. Due to the shape of the flowers, it was not possible to observe pollinator behaviour while inside the flowers. Thus the variation in MPS could be due to differing actions of the visitors, as there is little variation in size between the bee species that could otherwise account for such variation (see Table 6). The order in which flowers of *Digitalis purpurea* are visited will affect the amount of pollen, and in particular the amount of outcross pollen, deposited by a visitor (Grindeland *et al.*, 2005), so it is possible that *B. hortorum* has a high level of floral constancy and is visiting inflorescences in the “correct” order, increasing its pollinator effectiveness, while other species are visiting more sporadically, travelling in the “wrong” direction on a spike and therefore visiting in a less effective manner for pollination.

Conclusion

Both species studied showed traits indicative of a general (or large-bodied) Bee-Pollinated Flower Syndrome as described by Faegri and van der Pijl (1979) and reviewed by Willmer (2011). The main characteristic distinguishing this syndrome from others within the mellitophilous syndrome is the placement of reproductive structures in such a manner that only larger-bodied species may come into contact with both the stigma and anthers, thereby effectively pollinating the flower. Both flowers tested here were visited by bumblebees, and in the case of *Digitalis purpurea* exclusively so. Using pollen deposition of stigmas as a measure of pollinator effectiveness, the bumblebee species *Bombus hortorum* was identified as the most effective pollinator species of *Digitalis purpurea*, though the most effective pollinator of *Geranium pratense* was not found.

Given the limitations of the studies, and the low local populations of bees around the time of the investigation, it is possible that further study of the species would identify additional bee species as effective pollinators in addition to those identified above. What is clear from the study, however, is that assumptions cannot be made as to the effectiveness of a species using visitation frequency or other means without conducting some measure of effectiveness. In addition, lumping visitors into functional groups may result in labelling non-pollinators as pollinators, and will mask the variation between species. Not all bee species, regardless of similarities in size and shape, are equally effective pollinators, as factors such as behaviour will also have an effect on the pollination effectiveness of a given species. Particularly in species such as *Digitalis purpurea*, where the order in which flowers of an inflorescence affects the quantity and quality of pollen deposited, the behaviour of a visitor will have an important influence on its effectiveness at pollinating the flower.

While this study may not definitively identify the effective pollinators and appropriate syndrome of *Geranium pratense* and *Digitalis purpurea*, it again demonstrates that the classical definitions and partitioning of pollinator syndromes are in need of updating. In

particular, the mellitophilous syndrome possesses many subdivisions which could benefit from more recognition. The following chapter (Chapter 6) describes a further offshoot of the mellitophilous syndrome, and Chapter 7 includes consideration of a long-tongued bee syndrome. However these three are by no means the only 'sub-syndromes' associated with bee pollination, and many more types (see Willmer 2011) can be identified and further defined.

Chapter 6: Testing Pollination Syndromes

The Oil-Flower Pollination Syndrome

Introduction

Floral rewards are any substance or component offered by a flower or inflorescence that are used by animals and encourage repeat visits, increasing the likelihood of effective pollination. Usually this reward is offered in the form of nectar or pollen, but some flowers offer different substances as encouragement to pollinators.

Flowers can offer non-nutritive or nutritive rewards to pollinators. Non-nutritive rewards can be incidental by-products of floral structure such as floral trichomes (used in nest construction), sleeping places, heat sources or mating sites. Rewards can also be intentionally secreted for animal visitors, such as odours used as sexual attractants; or resins, waxes or chemical mixtures used in nest construction.

Rewards can be provided for larval stages, in the form of brood sites, or for adults, in the form of fatty oils (lipids), stigmatic secretions, food tissues (food scales, food bodies, sweet tissues and pseudopollen), nonfertile “food” pollen (Simpson and Neff, 1981) and nectar, often the primary offering of a flower. Nectar, a high sugar concentration solution derived from the phloem of a plant (De La Barrera and Nobel, 2004), is usually secreted through nectaries, specialised superficial glands found in a few species of ferns, gymnosperms and many species of angiosperms (Koptur *et al.*, 1982; Pacini *et al.*, 2003; De La Barrera and Nobel, 2004).

This chapter focuses primarily on nutritive rewards, in particular lipids as a food source for animal visitors.

Oils as a Floral Reward

The secretion of oils as a reward for animal visitors was first recognised by Vogel (1969), and since then has been intensively studied (Vogel, 1974, 1981, 1986, 1990; Simpson *et al.* 1977; 1979; Seigler *et al.*, 1978; Neff and Simpson, 1981; Simpson *et al.*, 1990; Dumri *et al.*, 2008; Renner and Schaefer, 2010). There is a distinction recognised between essential oils used as odour or sexual attractants and non-volatile oils, for which the term “floral oils” is reserved.

The secretion of floral oils occurs in at least 80 genera over several angiosperm families, comprising about 1% of flowering plants (Buchmann 1987; Steiner & Whitehead 1991). Although oil is known to be produced in these families, it is not necessarily the primary reward offered. Flowers of the Memecylaceae and Gesneriaceae families are cited as producing floral oil (Buchman, 1987), however pollen is thought to be the main pollinator reward in these species (Steiner, 1985; Renner, 1989). Examples of oil-producing flowers are common in the Neotropics and South Africa, most commonly found in moist forest and savannah habitats, but also in Holarctic and Paleotropical regions as well as in Australia (Steiner and Whitehead, 2002). In the Iridaceae and Scrophulariaceae, these oils are released from trichome elaiophores, glandular trichomes that secrete lipids; however, in the Malpighiaceae, Orchidaceae, and Krameriaceae the oil is formed in epithelial elaiophores, areas of glandular tissues with lipid secreting epidermal cells (Vogel, 1974; Neff and Simpson, 1981; Simpson and Neff, 1981; Buchmann, 1987; Vinson *et al.*, 1997).

There are similarities across families in the major chemical components of floral oil, which include monoglycerides, diglycerides and triglycerides with long chain (C₁₆-C₂₀) saturated or unsaturated fatty acids (Vogel, 1974; 1986; 1990; Cane *et al.*, 1983; Seigler *et al.*, 1978; Vinson *et al.*, 1997, Reis *et al.*, 2000, 2003, 2006).

Collection and use of Floral Oils by Animal Visitors

In almost all cases the oil is collected by female solitary bees and incorporated with pollen into nest cell provisions for their larvae. It is thought that the primary function of oil is as an energy-rich supplement to nectar and pollen (Vogel, 1974; Vinson *et al.*, 1997), although some bees also incorporate it into the nest cell lining (Cane *et al.*, 1983).

The majority of oil-collecting bees are from four tribes of the Apidae; Centridini, Tetrapedini, Ctenoplectrini and Tapinotaspini (Vogel, 1974; 1990; Neff and Simpson, 1981; Cocucci *et al.*, 2000), but oil collection is also well-developed in the Melittidae (Michener, 1981; Vogel, 1986; Steiner and Whitehead, 1988; 1990; 1991a; 1996; Whitehead and Steiner, 2001) and is known in at least two species of Colletidae (Houston *et al.*, 1993).

Some species of oil-collecting bees have special body adaptations for the purpose. Elaborate setal combs and pads found on the forelegs and midlegs of certain members of several genera of New World anthophorid bees have been shown to serve the purpose of collecting floral oils (Vogel, 1971; 1974; Simpson *et al.*, 1977, Simpson and Neff, 1981).

The Oil-Flower Pollination Syndrome

Oil flowers can be considered an offshoot of the “Bee Pollination Syndrome”, sharing several features with “bee flowers” offering nectar or pollen as a reward. As bees are an extremely diverse pollinator group, with variation in size, shape, feeding behaviour, tongue length and other factors relation to pollination, it is hard to define bee pollination, or melittophily, as a single syndrome. Thus it is helpful to split the category, and one sub-category would be the oil-gathering bee.

The “Pollination Syndrome” of a group of 15 oil-producing orchids with shared characteristics indicative of a shared pollinator was described by Pauw (2006). He theorised that the presence of yellow-green coloration, oil secretion, pungent scent, shallow flowers,

and a September peak in flowering in all species indicated that they were specialised and visited by a particular shared pollinator, which was shown to be the oil-collecting bee *Rediviva peringueyi* of the Melittidae. The same relationship with oil-collecting bees has been found in several other oil flower species (e.g. Vinson *et al.*, 1997; Steiner and Whitehead, 2002; Bezerra *et al.*, 2009; Nattero *et al.*, 2010; Steiner, 2010). Traits such as corolla colour, shape, scent and the presence of oil as a reward serve to attract oil-feeding bees to the flowers, and traits such as corolla length, placement of reproductive structures and location of reward serve to ensure that the insect is in the correct orientation to facilitate effective pollination.

Traits of the Oil-Flower Syndrome

There are many similarities between the traits of oil-flowers. The Malpighiaceae are a mostly tropical flowering plant species showing much diversity in their habit, fruit, pollen and chromosome number. Despite this variation, the flowers of Malpighiaceae are remarkably similar in structure. The calyx comprises five sepals, four or all five of which usually possess fatty oil producing elaiophores, with the exception of most species of *Galphimia* and all species of *Coleostachys*, *Echinopterys*, *Lasiocarpus*, *Ptilochaeta* and *Thryallis* in which these structures are reduced or absent. The five free petals are clawed, and often reflex between the sepals allowing access to the elaiophores for insects that land in the centre of the flower. Floral colour is usually carrot-yellow, lemon-yellow, white or pink, with blue petals being found only in a few species of *Mascagnia*. Colour changes from yellow, white or pink to deep red sometimes occur with age, thought to be a means of diverting pollinators away from old flowers. Flowers often have a “flag” petal whose purpose is to provide a structure for pollinators to grip on to. There is no disc present, nor any of the structures normally associated with producing sugary nectar in flowers. The androecium comprises 10 free stamens, the anthers often of different shapes and sizes within the same flower. The gynoecium comprises three superior uniovulate carpals, which may be free to connate in the ovary and free to connate in the style. The stigmas vary from minute to fairly large and from

terminal to internal, with the styles bearing apical-dorsal extensions or appendages. The flower as a whole shows bilateral symmetry, running from front to back (Anderson, 1979). Pollination of these species is known to be by Hymenopterans of a few families, mainly Centridini and Exomalopsini, with several other species, such as Trigonid bees, known to collect pollen from flowers (Vogel, 1974; Machado, 2004).

Colpias mollis is a perennial lithophyte that produces oil as a floral reward. It has white to yellow tubular (approximately 12-15mm long) flowers bearing two narrow pouches lined with glandular oil-secreting trichomes. Stamens are didynamous and lie against the upper inside of the corolla tube. The staminoide is short, inconspicuous and usually present at the base of the stamens. The style lies along the upper corolla surface between the two sets of stamens, curving down near the tip and causing the stigma to emerge from between the anthers. Flowers have a pleasant, spicy fragrance. The Mellitidae bee *Rediva albifasciata* collects oil from flowers using specialised hairs on its forelegs and midlegs, rubbing them against the glands (Steiner and Whitehead, 2002).

Relationships between oil flowers and their bees are often very specialised. The genus *Diascia* comprises about 70 genera, 20 of which are found in the eastern parts of Southern Africa. Most species are characterised by twin floral sacs (Steiner and Whitehead, 1988), with trichome eliaophores located within the tips of paired spurs. The females of *Rediva* bees are the only visitors able to exploit this oil as a result of specially adapted elongated forelegs. The bees are able to transfer the oil to their hind legs and carry it to the nest, where it is used as larval food and presumably also for construction and lining of nest cells. Leg length of *Rediva* bees has been correlated to spur length of *Diascia* (Steiner and Whitehead, 1990, 1991). Corolla size of *Colpias mollis* was correlated with the size of the *Rediva albifasciata* species that visited it, excluding larger pollinators (Steiner and Whitehead, 2002).

Attraction of pollinators at a distance is often by floral odour, and the detection of oil on flowers is a trigger for oil-collecting behaviours. *Lysimachia punctata*, an oil-producing plant species found in Europe, was found to attract oil-collecting bees primarily through the scent compounds emitted by the flowers and vegetative parts. Oil-collecting behaviour was triggered by the detection of oil by chemoreceptors on the legs (Dötterl and Schöffler, 2007).

These studies, and that of Pauw (2006) show that oil-secreting flowers share a variety of characteristics indicative of their syndrome, such as flower colour, shape, reward production and odour. However, possession of these traits should not lead one to assume that pollination is by an oil-collecting bee species without further testing the pollination effectiveness of flower visitors.

Testing the Oil-Flower Pollination Syndrome

According to the syndrome, a flower producing oil as a reward should be pollinated most effectively by a specialised oil-collecting bee species. In order to test this prediction, a flower species with oil-flower traits, *Byrsonima crassifolia*, was chosen for investigation. Oil-collecting *Centris* bees are known to collect the oil of this flower (Frankie *et al.*, 1988; Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Sazima and Sazima, 1989; Sigrist and Sazima, 2004; Rego *et al.*, 2006), and it has been shown that oils from this flower make up the main nest provision, along with pollen, of several species of *Centris* in the dry forests of Costa Rica (Vinson *et al.*, 1997). The effectiveness of these bees and any other visitors at pollinating the flowers of *Byrsonima crassifolia* however has not yet been shown and is investigated further in this chapter.

Materials and Methods

Study Site

The oil-flower trees chosen for this study were located in Parque Nacional Santa Rosa, Guanacaste Province, in the North-West region of Costa Rica (10° 53' 1" N, 85° 46' 30" W). The park covers about 495 square kilometres and contains savannah, deciduous forest, marshland and mangrove swamp habitats. The population of *Byrsonima crassifolia* studied was both located in the dry, disturbed, deciduous forest area of the park.

Study Species

Byrsonima crassifolia, of the family Malpighiaceae, is a Neotropical tree found widely distributed across regions of Central and South America as well as Trinidad, Barbados, Curaçao, St. Martin, Dominica, Guadeloupe, Puerto Rico, Haiti, the Dominican Republic and throughout Cuba and the Isle of Pines (USDA, ARS, National Genetic Resources Program). Flowering is typically between July and October, depending on the climate where the plant is situated (Frankie *et al.*, 1974; Neto *et al.*, 1994).

Structure and Rewards

The flowers of *Byrsonima crassifolia* are bright yellow in colour when freshly opened, becoming more orange as they age. They are presented in vertical racemes. The flower shape is relatively simple and open, and oil is secreted from eliaophores just under the epidermis of the calyx. Each flower possesses five claw-shaped petals, typically arranged so that four of these petals curve down over the calyx, while one petal, the “flag” petal, is thrust away from the calyx (Anderson, 1979; Buchmann, 1987; Vinson *et al.*, 1997). The flower structure of *Byrsonima crassifolia* is shown in Fig. 1.

Each flower possesses three stigmas, approximately 10mm in length, and 10 stamens, clustered in the centre of the flower (Anderson, 1979). On the end of each stamen are the anthers, which produce large quantities of pollen grains when ripe (see Fig. 1).

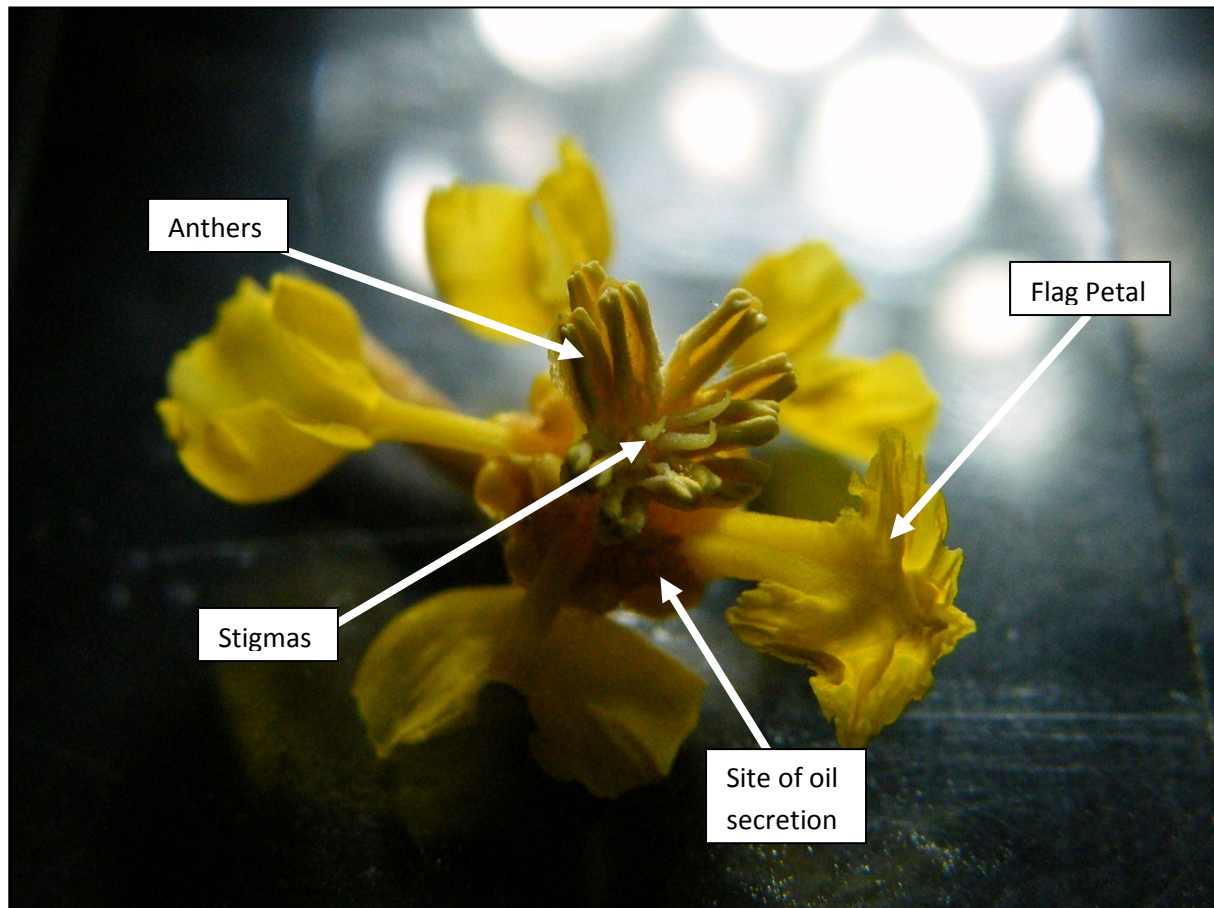


Fig. 1: Flower of *Byrsonima crassifolia*. Anthers, stigmas, flag petal and site of oil secretion indicated.

The placement of oil, anthers and stigma on this flower allows for oil-collecting bees to grasp the flag petal and collect oil from the flowers using their hind legs while transferring pollen to stigmas via the underside of their bodies (Vinson *et al*, 1997).

Oil is produced from elaiophores beneath the epithelium of the calyx. The amount of oil produced by individual flowers of *Byrsonima crassifolia* averages 1.66 μ l per day and consists primarily of mono-glycerides (70%) and di-glycerides (15-20%) with less than 10% tri-glycerides and 5% or less that could be attributed to free fatty acids. No evidence of nectaries or nectar production has been found in this species (Vinson *et al*, 1997).

Timing

Dehiscence begins just before dawn and continues for the 2 or 3 days that the flowers are open. No separation of sexual phases occurs in this species as both dehiscing anthers and glossy, receptive stigmas were observed on flowers at the same time.

To confirm the self-compatibility of this species (Bawa, 1974; Anderson, 1979; Sigris and Sazima, 2004), stigmas were dusted with pollen from the same flower and covered with netting to prevent further visitations. The flowers were observed and any incidence of seed set was recorded.

Sampling Periods

Field research occurred between April and May of 2009. During the dry season, daylight hours were between 06:00 and 18:00. To adequately sample throughout the day, daylight hours were split into 4 time periods; 06:00-09:00, 09:00-12:00, 12:00-15:00 and 15:00-18:00 and sampling was split between these time periods. In total 102 single-visit observations were made over several days. Each sampling session was between 1 and 3 hours long depending on the frequency of visitations and how long it took all opened flowers to be visited. Temperature and humidity readings were taken from a shaded area of the study site using a HM34 Vaisala Pocket Size Relative Humidity Meter every half hour during each sampling session.

Results

Temperature and Humidity

Measurements of temperature (°C) and relative humidity (%) were taken every 30 minutes during each observation period of *Byrsonima crassifolia* (see Fig. 2). Temperatures were low in the morning, reaching their peak between 12:00 and 1:30 and falling again over the course of the afternoon. Humidity was highest in the morning, dropping to its lowest around midday and then rising again over the afternoon. The *Byrsonima crassifolia* individuals studied were located in very exposed, often elevated, parts of the park; therefore temperature and humidity readings varied from those typically found in more sheltered parts of the forest.

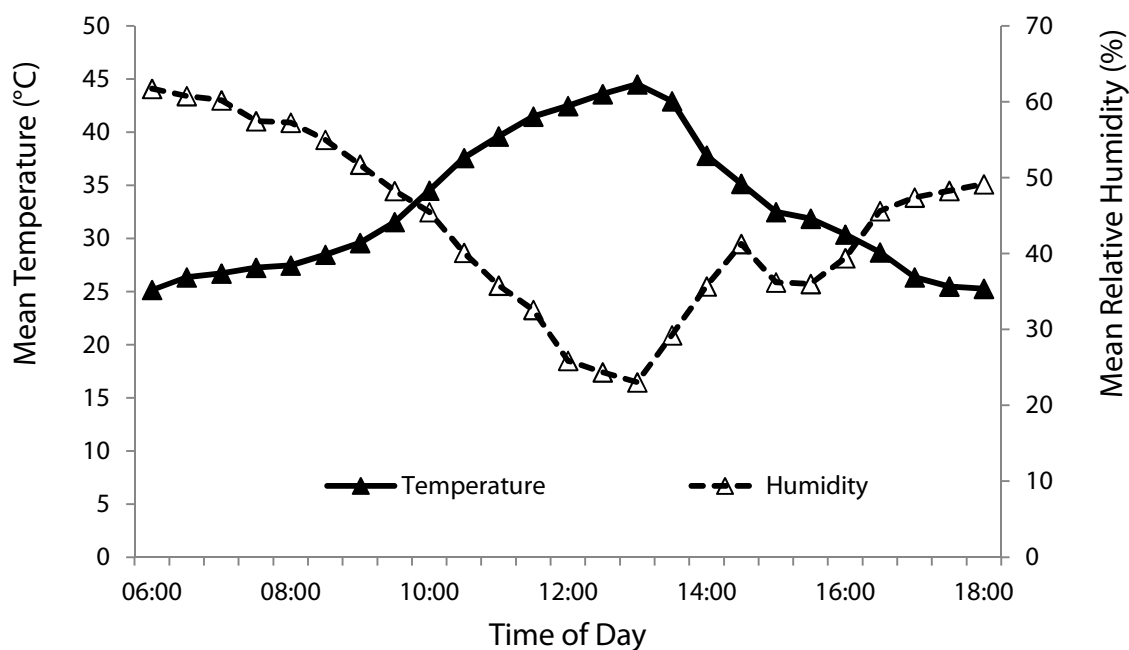


Fig. 2: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Byrsonima crassifolia*. Standard deviations are shown.

Self-Compatibility

Flowers that were dusted with self-pollen were observed over several days and were observed to have set seed. This confirms that the flowers of *B. crassifolia* are self-compatible, as shown previously (Bawa, 1974; Anderson, 1979; Sigrist and Sazima, 2004).

Partitioning of Visitors over Time

Visitors to *Byrsonima crassifolia* were recorded throughout the day (see Fig. 3). Four different species of bees were observed visiting flowers of *Byrsonima crassifolia*, and all visitors were identified to species where possible. The number of visits to flowers by each was recorded for every half hour time period. *Tetragonisca angustula* was the earliest flower visitor. It was observed rarely but was present throughout the morning and afternoon, with the exception of midday when temperatures were at their highest, and was only observed to be feeding upon the pollen of flowers, rather than the oil produced at the base of the sepals. *Trigona fulviventris* was the next earliest flower visitor, and was also observed feeding on pollen of flowers only throughout the day, in much greater numbers, with the exception of midday. The oil-collecting bee species *Centris nitida*, larger in size than *Trigona fulviventris*, was observed collecting oil only from flowers between 07:30 and 08:30 but was not observed during the rest of the study period. *Exomalopsis* sp., larger in size than *Trigona fulviventris*, though slightly smaller than *Centris nitida*, was also observed collecting oil only from flowers between 14:00 and 14:30 and *Tetragonisca angustula*, the smallest of all observed species, was observed feeding on pollen between 14:00 and 16:30.

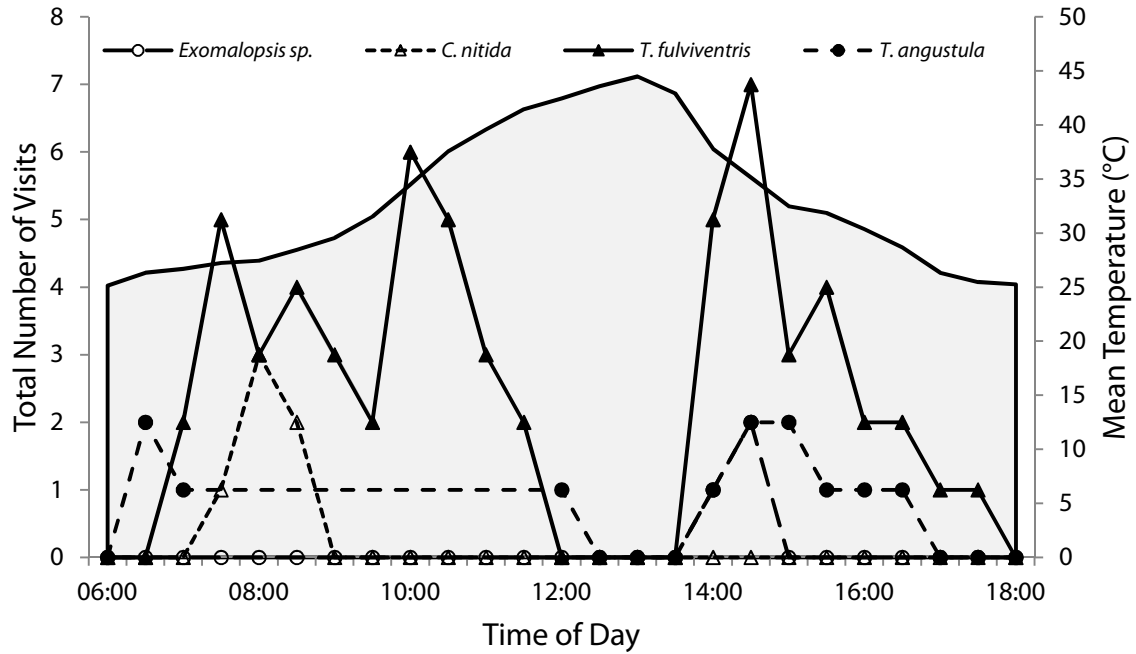


Fig. 3: Partitioning of visitor groups to *Byrsonima crassifolia* over daily time. Mean temperature (°C) shown as an area plot.

Pollen Deposition by Visitors

Group	Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
Bee	<i>Exomalopsis</i> sp.	1686.7	P < 0.001
Bee	<i>Centris nitida</i>	381.7	P < 0.001
Bee	<i>Trigona fulviventris</i>	254.5	P < 0.001
Bee	<i>Tetragonisca angustula</i>	238.8	P < 0.001

Table 1: Statistical analysis of visitor species to *Byrsonima crassifolia*. Statistical analysis was performed by a LSD Post Hoc Test.

The MPS of each visitor species was calculated (see Fig. 4 and Table 1) and the difference in deposition between visitor species was statistically significant. All four species deposited a significant MPS in comparison to controls and can therefore be classified as pollinators of *Byrsonima crassifolia*. The oil-collecting *Exomalopsis* sp. deposited significantly more pollen than all three other visitor species (LSD Post-Hoc testing: P < 0.001

for each). The second highest single-visit deposition was by *Centris nitida*, followed by *Trigona fulviventris*, and *Tetragonisca angustula* depositing the smallest MPS. The remaining visitor species did not differ significantly from each other in pollen deposition (LSD Post Hoc testing: *C. nitida* vs. *T. fulviventris*: $P = 0.184$; *C. nitida* vs. *T. angustula*: $P = 0.335$; *T. fulviventris* vs. *T. angustula*: $P = 0.781$).

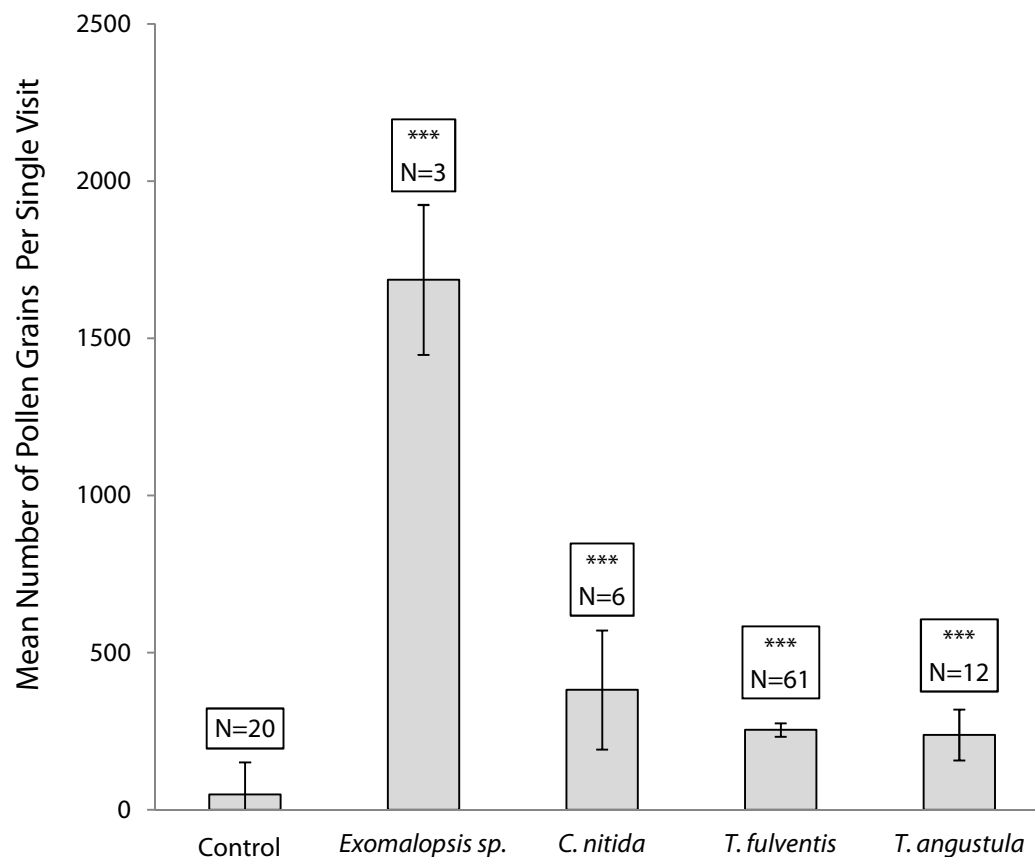


Fig. 4: Mean pollen deposition by visitor species to *Byrsonima crassifolia*. N values and SD shown. Significance refers to the significant of number of pollen grains deposited in comparison to control flowers. Variation in MPS between visitor species was statistically significant (One-Way ANOVA: $P < 0.001$, $F = 9.845$, $df = 3$).

Discussion

Floral traits

Flowers of *Byrsonima crassifolia* possess several traits indicative of an oil-flower pollination syndrome. Clearly the main feature of this syndrome is the production of oil as a reward to flower visitors; however several other floral traits serve to attract bee visitors and facilitate the effective pollination of the species. Yellow colouring of flowers has been shown to be attractive to bees (Menzel, 1985; Jones and Reithel, 2001), although it is often associated with attracting a more generalised insect visitor assemblage (Lunau and Wacht, 1994). *Byrsonima crassifolia* also possesses the strong “floral” odour; usually produced by terpenoids and less frequently by aliphatic compounds such as hydrocarbons, esters, ketones and alcohols (Willmer, 2011); typically associated with bee flowers (Dobson, 1987; 2006). The chemical composition of the odour of *Byrsonima crassifolia* flowers has not yet been analysed, however the aroma of its fruits is composed of ethyl butanoate (fruity, sweet), ethyl hexanoate (fruity), 1-octen-3-ol (mushroom like), butyric acid (rancid, cheese), hexanoic acid (pungent, cheese) and phenylethyl alcohol (floral) (Rezende and Fraga, 2003).

The shape of *B. crassifolia* flowers allows for effective pollination by oil-collecting bees (see Fig. 1). The bees grip the “flag petal” with their forelegs while collecting oil from pores at the base of the corolla using their hind legs. This positions them in a way that effectively removes pollen from anthers and transfers it to the stigmas of subsequently visited flowers via the underside of the body.

Visitor Assemblage

Previous studies have indicated that *Byrsonima crassifolia* is primarily pollinated by specialist oil-collecting *Centris* bees (Vogel, 1990; Vinson *et al.*, 1997). *Centris nitida* was

observed rarely, but when sighted it was observed collecting oil from the flowers in the “correct” manner described above. *Exomalopsis* bee species are also known to collect oil from certain flowers and have specialised hairs on the scope of their hind tibia to facilitate this (Thorp, 1979). *Exomalopsis* sp. was rarely active on *Byrsonima* flowers, but was did visit these flowers in the “correct” manner. Flowers of *Byrsonima crassifolia* were also visited by *Tetragonisca angustula*, and the most frequent visitor was the generalist bee *Trigona fulviventris*. Both species were observed to feed upon pollen only, rarely positioning themselves in the “correct” manner.

It should be noted that the visitor assemblage and frequency of visitors is not an absolute representation of flower visitors to *Byrsonima crassifolia* due to the limitations of the study. Only buds on the lower branches of *Byrsonima crassifolia* could be easily reached for the purposes of bagging and observation, therefore the denser inflorescences of higher branches were not studied. The relative frequencies of visitors may not be accurate representations of this pollination system and this study is perhaps better considered as a comparison of pollinator effectiveness rather than an absolute record of visitors to *Byrsonima crassifolia*.

Partitioning of Visitors across Time

Variation in the activity peaks of different bee visitors (See Fig. 3) to *Byrsonima crassifolia* are probably related to temperature restraints, as described in Chapter 2. *Tetragonisca angustula* individuals are very small indeed, much smaller than the other visitor species and would be able to tolerate the higher temperatures of midday. Conversely however, they cool down rapidly and end foraging earlier in the evening than the larger species. *Trigona fulviventris* individuals are next in size, and stop foraging earlier in the morning as temperatures rise above a tolerable level. *Centris nitida* and *Exomalopsis* sp. are larger still in size and less tolerant of high temperatures, as reflected by their activity peaks.

Pollinator Effectiveness

For the purposes of this study, pollinators were classified as those visitors which deposited significantly more pollen on stigmas per visit in comparison to the unvisited control flowers. The most effective flower visitor is therefore classified as the pollinator that deposited the greatest average amount of pollen on stigmas per visit.

Visitors were grouped according to species, although in this case it may be beneficial to sort the visitors according to their flower-feeding habits. *Centris nitida* and *Exomalopsis* sp. are specialist oil-collecting bees of the family Anthophoridae, with structural modifications to suit the purpose of effective oil-collection. Female *Centris* bees possess greatly modified, blade-like setae on their fore and mid tarsi which function to rupture lipid-filled elaiophores, especially in the Malpighiaceae, as well as a distinctive oil-harvesting comb formed of a single row of giant, flattened and apically curved, overlapping setae, along with two to five giant spatulate setae, on the ventral surfaces of the anterior and middle basitarsi in opposition to the primary anterior comb (Vogel, 1974; Neff and Simpson, 1981; Buchmann, 1987). Bees of the tribe Exomalopsini also possess modified setal combs for this purpose (Vogel, 1974; Buchmann, 1987).

Both *Tetragonisca angustula* and *Trigona fulviventris* were observed feeding on or collecting pollen of flowers but never on the oil, and neither species possesses any adaptations towards oil-collecting (Neff and Simpson, 1981).

Despite their varying feeding habits, all four visitor species deposited a significant amount of pollen on flowers per visit (see Fig. 5) and can therefore all be classified as pollinators of *Byrsonima crassifolia*. This pollen deposition was not equal between species, and showed significant variation, therefore species could not be classed as equal in their pollen deposition and some, in particular *Exomalopsis* sp. and *Centris nitida*, were more effective pollinators than others.

Quality of Pollen Deposition by Pollinators

It is likely that the pollen transferred by *T. fulviventris* and *T. angustula* is incidental pollen transfer from the same flower knocked from anthers during foraging due to the method of flower feeding. The bees were only observed on the anthers of the flowers, and did not collect pollen from the anthers with the underside of their bodies as the oil-collecting species did, rather pollen was brushed into the corbiculae of the legs, thereby becoming unavailable for transfer to stigmas (Inouye *et al.*, 1994). The stigmas of the flowers rarely came into contact with the pollen-feeding bees, and little pollen was available on the bodies of these bees, therefore it is likely that pollen found on stigmas after visits was self pollen that had fallen from the anthers during the collection process. The flowers of *Byrsonima crassifolia* are however self-compatible, therefore it is likely that this pollen transfer will still result in seed set, though it is likely the offspring of self-fertilised flowers will be of low genetic quality in comparison to those of out-crossed flowers (Price and Waser, 1979; Waser and Price, 1983; Bookman, 1984; Schemske and Pautler, 1984; Vander Kloet and Tosh, 1984; Griffen and Eckert, 2003; Herlihy and Eckert, 2004; Aizen and Harder, 2007).

Centris nitida and *Exomalopsis* sp. were observed to visit rarely, and were only seen to collect oil from one or a few flowers per visit, in contrast to the more extensive foraging behaviour of *T. angustula* and *T. fulviventris*. Foraging flights of marked *Centris* bees on *Krameria lanceolata* have been shown to include hundreds of flowers, including plants several hundreds of metres apart (Neff and Simpson, 1981). Although the foraging flights of *Centris nitida* feeding on *Byrsonima crassifolia* has not yet been studied, if the behaviour of this species can be extrapolated to the other flower species it utilises, the outcrossing potential of *Centris nitida* could be high indeed. Observations of *Centris nitida* and *Exomalopsis* sp. to only feed on a few flowers per tree would suggest that they are collecting oil from other individuals of the species, rather than foraging extensively on one individual, and therefore creating a high potential for outcrossing.

Conclusion

The floral traits of *Byrsonima crassifolia* indicate an oil-flower pollination syndrome. Following the predictions of this syndrome, we would expect the most effective pollinator species of *Byrsonima crassifolia* to be a specialised oil-collecting bee. In reality, two specialised oil-collecting bee species and two generalist bee species were observed visiting flowers. Both oil-collecting bee species were observed to visit flowers in the “correct” manner, collecting oil with their hind legs and transferring pollen from anther to stigma via the underside of the body, whereas the generalist bee species were observed to thief pollen from the anthers of dehiscent flowers, without interacting with the flowers in a manner that would facilitate efficient pollination. Inouye (1980a) classed a pollen thief as a visitor that collects pollen in a manner that precludes the possibility of pollination, but does not damage floral tissues, as did the bee species collecting pollen from *Byrsonima crassifolia*.

Several features of the *Byrsonima crassifolia* oil-collecting bee system indicate it to be a specialised pollination system, despite the presence of other, more generalised, flower visitors. The specialised oil-collecting body adaptations of the bees species, such as the extremely specialised setae of *Centris* bees adapted specifically to the lipid filled pores of plants of the family Malpighiaceae, indicate a close relationship between flower and pollinator. We would therefore expect a higher pollen deposition, and therefore pollinator effectiveness, from the oil-collecting species in comparison to the more generalised pollen-feeding species.

While all flower visitors were effective pollinators of *Byrsonima crassifolia*, it was also determined that their effectiveness varied between species, and therefore not all flower visitors should be assumed to be equally effective pollinators of a species. Observations of the feeding behaviour of visitors suggested that, while some (*Exomalopsis* sp. and *Centris nitida*) were visiting the flowers in the “correct” manner and facilitating pollination in the intended manner, other species (*Trigona fulviventris* and *Tetragonisca angustula*) were more

likely to be transferring self-pollen from the same flower. The self-compatibility of *Byrsonima crassifolia*, however, would suggest that this pollen deposition would still result in seed set, albeit of lower quality than outcrossed offspring (Price and Waser 1979; Waser and Price 1983; Bookman 1984; Schemske and Pautler 1984; Vander Kloet and Tosh 1984; Griffen and Eckert, 2003; Herlihy and Eckert, 2004; Aizen and Harder, 2007). While the “intended” pollinator species is clearly a specialised oil-collecting bee, it is possible that these pollen thieves are tolerated, and perhaps even encouraged, by *Byrsonima crassifolia* to facilitate pollination in times where visits from the intended pollinator are rare. They do not indicate that *Byrsonima crassifolia* is part of a “generalised” pollination syndrome, despite its pollination by generalist visitor species. A more precise description may be that *Byrsonima crassifolia* has a specialised pollination syndrome with “back-up” pollinators to ensure the continuation of reproduction of the species in the face of preferred pollinator scarcity or extinction, or adverse conditions.

Previous studies (Herrera, 1996; Waser *et al.*, 1996; Hingston and McQuillan, 2000; Consiglio and Bourne, 2001; Zhang *et al.*, 2005; Valdivia and Niemeyer, 2006; Ollerton and Watts, 2007; Ollerton *et al.*, 2009) have questioned the validity and predictive powers of pollination syndromes. Ollerton *et al.* (2009) suggested that for approximately 2/3 of all flower species the correct pollinator could not be predicted using pollinator syndromes. If we consider the “correct” pollinator to be the most effective pollinator however, we find that in this case, the predictions of the pollinator syndrome match the indicated pollinator species.

In the past, studies of pollinator syndromes (Schemske and Horvitz, 1984; Galen and Newport, 1987; Ollerton *et al.*, 2009) have used visitor frequency as a measure of pollinator effectiveness. It is difficult to draw conclusions on the validity of this method due to the limitations of this study as mentioned before. Were we to extrapolate from the results obtained however, the most frequent visitor to *Byrsonima crassifolia* was one of the least effective pollinators, and the most effective in terms of pollen deposition was one of the rarer flower visitors. Other studies (Feinsinger, 1978; Webb and Bawa, 1983; Webb, 1984;

Ollerton, 2009, and see Table 1 of Chapter 1) have used visual observations to identify whether the pollinator was visiting the flower in the “correct” manner, and, while this is a more effective means than visitor frequency, it is difficult to quantify and not completely accurate. While visual observations of “correct” pollination are more accurate than other measures of pollinator effectiveness, they fail to account for the variation in pollen deposition between species, even those which are identified as “good pollinators”. In the case of *Byrsonima crassifolia*, two species were observed to visit flowers “correctly”, and while both were effective pollinators, they varied significantly in the average amount of pollen deposited per visit, and could therefore be said to vary in pollination effectiveness. Despite both being effective and “correct” pollinators, they were not completely equal in this regard and one species was significantly more effective at depositing pollen than the other.

I therefore suggest that, as in other cases described in this thesis, the most effective means of evaluating pollinator effectiveness, and thus determining the “correct” pollinator of a flower species, is the measurement of stigmatic pollen deposition in a single visit.

Chapter 7: Testing Pollination Syndromes

The Long-Tongued Insect-Pollination Syndrome

Introduction

Some flowers possess traits indicative of pollination by visitors with long proboscides. In particular, a long corolla tube with concealed or semi-concealed nectar is typical of the long-tongued insect pollination syndrome. Darwin (1862) suggested that the evolution of deep flowers represented an “evolutionary arms race” between plants and pollinators; with corollas lengthening as a response to pollinator tongues increasing in length, whether through a general increase in size, or because longer tongues led to greater nectar-collecting efficiency. As this occurred, those plants with shorter corollas could be disadvantaged, as pollen transfer, effected by physical contact between the anthers or stigma of the plant, could be reduced when the insect tongue was long relative to flower depth. Insects have been shown to insert their tongues deeper into flowers than is necessary for nectar feeding, and experimental shortening of corolla tubes resulted in a decrease in both male and female components of fitness for the plant (Nilsson, 1988). Selection was proposed to favour longer corolla tubes or spurs when they cause the pollinator to insert its entire proboscis into the flower, and thus pick up and deposit pollen firmly via its face (Nilsson, 1978; 1988).

Reciprocal coevolution between spur length and pollinator proboscis length could result in the evolution of extreme traits, for example the very long orchid spurs and proboscides of some moths in Madagascar (Darwin, 1862; Nilsson *et al.*, 1985; 1987; see also Steiner and Whitehead, 1990; 1991). Divergence in floral spur length has resulted from selection exerted through pollinator proboscis length, leading to variation in spur length when plant species are pollinated in different habitats by pollinator assemblages with varying

proboscis length (Johnson and Steiner, 1997).

Among many plant genera, there are evident shifts in pollinator syndromes. Whittall and Hodges (2007) showed that in the genus *Aquilegia*, the ancestral pollination syndrome is bee-pollination, however hummingbird and hawkmoth-pollinated examples are also found to have derived from this syndrome. To explain these shifts, they proposed that, in some part of its range, an *Aquilegia* taxon with ancestral bee-pollination and a relatively short nectar spur, began to receive visits from hummingbirds rather than bees. As hummingbirds possess longer tongues than bees, the body of the hummingbird would not make adequate contact with the reproductive structures of the flower when feeding, resulting in inefficient pollination. A flower with longer spurs, therefore, would be at a selective advantage, forcing the hummingbird to probe deeper and make contact with the stigmas and anthers, increasing the plant's reproductive fitness. Over time, spur length would increase to match the length of the hummingbird's tongue. The same process would occur in a shift from hummingbird-pollination to pollination by longer-tongued hawkmoths.

This theory accounts for the observed uni-directional pollination syndrome shifts and lengthening spurs observed in some genera, however it requires a rather implausible ecological scenario (Thomson and Wilson, 2008). Crucially, a short-spurred, bee-pollinated *Aquilegia* population must be maintained over many generations in the absence of an effective pollinator, otherwise bees would remain the more effective pollinators in the presence of hummingbirds, and no selection for longer spurs would occur. An alternative hypothesis, first proposed by Darwin (1862), and refined by Ennos, (2007), avoids these difficulties and remains compatible with the data. He argued that when plants possessing spurs interacted with specialist pollinators, co-evolution should lead to a lengthening of both plant spurs and pollinator tongues. Those plants which possess a slightly longer spur would gain a reproductive advantage because pollinators probing deeper into flowers would lead to increased contact with the reproductive structures, increasing pollination efficiency (Nilsson, 1988; Alexandersson and Johnson, 2002). In turn, selection would act to increase the

tongue length of the pollinator as this would allow for more effective emptying of lengthening nectar spurs. Lengthening of both nectar spurs and pollinator tongues would continue until a limit to further increase, either in spur length, or more plausibly tongue length, is reached. Therefore, if the habitat of a population of bee-adapted *Aquilegia* was invaded by a hummingbird with a similar tongue length of the bee, an ecologically plausible situation, hummingbird pollination may be as effective, or more so, than bee pollination (Castellanos *et al.*, 2003). Co-evolution between the hummingbird and *Aquilegia* could then occur in the presence of the bee. The necessary conditions for the “co-evolutionary” hypothesis therefore are more ecologically realistic than those required for the “pollinator-shift” hypothesis.

Long tongues for nectar feeding can be found in several different flower visitor groups: hummingbirds, for example the sword-billed hummingbird (*Ensifera ensifera*), which possesses a 10cm bill (Snow and Snow, 1980); flies, for example the mega-nosed fly (*Moegistorynchus longirostris*) with a 5.7cm long proboscis (Johnson and Steiner, 1997); moths, such as the giant hawkmoth (*Xanthopan morgani praedicta*); bats, such as the nectar bat *Anoura fistulata*, with a 8.5cm long tongue (Muchhala and Thomson, 2009); and also some butterflies, for example *Eurybia lycisca*, with a tongue length of up to 45.6mm (Bauder *et al.*, 2010) and bees such as the orchid bee *Euglossa samperi*, with a tongue length of approximately 16.84mm (Ramírez, 2006). Butterfly and moth pollination is not considered here, however hummingbird pollination was described in Chapter 3 and long-tongued bee and long-tongued hoverfly pollination is described further below.

Long-tongued Bees

Variation in bee tongue length is independent of body size, therefore small bees may have long tongues and vice versa (e.g. Harder, 1983). Bee tongues have relatively uniform construction, described in Chapter 5.

A short proboscis is defined as being slightly or moderately elongated in comparison to unspecialised hymenopteran mouthparts, with the glossa generally equal in length or

shorter than the prementum. Examples of short-tongued bees include most of the Andrenidae, Colletidae, Mellitidae and Halictidae.

The development of a medium to long proboscis is achieved by innovation and variation in the design of the food tube, methods of extension and retraction, storage positions and feeding movements. The lapping/sucking feeding mechanism in this case is often replaced by a purely suctorial feeding mode. A medium proboscis is generally defined as having a glossa longer than the prementum, and a long proboscis is defined as one which, when extended, is longer than the head. The majority of bees in the Megachilidae and Apidae are termed “long-tongued” bees and most possess a food tube consisting of elongated galeae and labial palps which align together to form a temporary canal that completely ensheathes the linear and hairy glossa (Krenn *et al.*, 2005).

Tongue Length and Flower Handling

There is much evidence of a correlation between bee tongue length and corolla length of flowers visited (Heinrich, 1976a,b; 2004; Inouye, 1978; 1980b; Pyke 1982; Graham & Jones 1996; Arbulo *et al.*, 2010). Proboscis length determines the depth at which a bee can reach nectar within a flower, as well as the flower handling time, and therefore the number of flowers that can be visited in a given unit of time (Holm 1966, Inouye 1980b, Harder 1983, 1985, Graham and Jones 1996). While it is often the case that long-tongued bees visit flowers with long corollas and short-tongued bees visit flowers with short corollas (Holm 1966, Heinrich 1976a,b, 1979, Inouye 1978, 1980b, Harder 1985, Graham & Jones 1996), the relationship between tongue length and flower choice is complex. Short-tongued bees are also able to take nectar from flowers with long corollas at certain times of day, when the flowers are full. Long-tongued bees may be expected to make use of a wider range of flowers, not being excluded from flowers with short corollas in the same way short-tongued bees are excluded from long corollas; however it is often the case that long tongues become unwieldy on flowers with short corollas, and the nectar of such flowers is often too

concentrated to be sucked up a long tubular tongue (Willmer, 2011). Tongue length therefore shows an interaction with time spent per flower. Medium-tongued species spend longer on a long corolla flower than do long-tongued species; and short-tongued species work faster than all other species on short corolla flowers (Heinrich, 1976; Inouye, 1980b; Harder, 1985; Graham and Jones, 1996; Arbulo *et al.*, 2011). In addition to time constraints, long corolla flowers often have much larger nectar rewards making visits to them more profitable for those species whose tongues allow them to reach the rewards than visits to a shallower flower which may have a lower handling time (Pleasants, 1983; Willmer, 2011).

Long-Tongued Hoverflies

While most syrphid species are relatively uniform morphologically, there is a 400-fold range in body weight, from 0.5mg to more than 200mg (Rojo *et al.*, 2003). Species can also vary in their mobility, ranging from rather ineffective flyers that rarely move far from their larval habitats, sometimes less than 2m (Schönrogge *et al.*, 2006), to highly effective flyers which can cover distances of more than 2km a day (Schneider, 1958; Gatter and Schmid, 1990).

There is also much variation in the mouthparts of syrphids, some of which are adapted towards feeding on flowers with long corolla tubes. Nectar from flowers provides a rich source of carbohydrate but pollen contains substantial amounts of carbohydrate, protein and lipids for syrphids which feed upon flowers (Gilbert and Jervis, 1998; Willmer, 2011). The mouthparts of syrphids were described in further detail in Chapter 4.

Tongue Length and Flower Handling

The syrphid database, “Syrph the Net” (Speight, 2003) provides information on the biological traits of European hoverflies, allowing for the classification of hoverflies into functional groups with feeding styles encompassing saprophagy, phytophagy and zoophagy (Schweiger *et al.*, 2007). However, among the phytophagous functional group further

distinctions can be made as to the mode of flower feeding. Hoverfly adults can display a wide range of lifestyles and adaptations (Gilbert 1990; 1993; Gilbert *et al.*, 1994) and different species of syrphid adults are known to specialise on specific flower types (Colley and Luna, 2000). Tongue length in hoverflies is correlated with corolla length of flowers visited (Gilbert, 1981). *Rhingia* species, which are long-tongued syrphids, are known to specialise on flowers with long corolla tubes, and blue or purple flowers (Haslett, 1989a). While often thought of as a “nectar-specialist”, it has been shown that *Rhingia campestris* also makes considerable use of flower pollen (Haslett, 1989a) and the species was observed to feed on the pollen of *Agrimonia eupatoria* in the previous chapter.

The diet of syrphids, and therefore their feeding strategy, has also been linked to their size, with larger species feeding more frequently on nectar and smaller species feeding more frequently on pollen (Gilbert, 1985). Species with a larger wing length feed more frequently on pollen than nectar (Lundgren, 2009).

Given such variation among the syrphids, it is clear that, in addition to the general hoverfly-pollination syndrome mentioned previously (see Chapter 4), there is a possibility for more specialised hoverfly-pollinated syndromes to be distinguished. One such distinction is the long-tongued hoverfly syndrome investigated here.

Long-Tongued Insect-Flower Syndrome

We would expect that flowers with long corolla tubes are visited primarily, if not exclusively, by visitors with sufficiently long tongues to allow access to the concealed nectar rewards. A lengthening of the corolla tube of a flower will restrict access to nectar for shorter-tongued insects and only species with sufficiently long tongues will be able to feed from such flowers. Flower species of composites such as *Senecio jacobea* (corolla length 2.75mm), *Aster cf. novae-angliae* (corolla length 3.0mm) and *Centaurea nigra* (corolla length 4.0mm) for example, have relatively long corolla tubes, and are visited more frequently by long-tongued hoverflies such as *Eristalis tenax* (5.06mm) than by shorter-tongued species

(Gilbert, 1980). Flowers visited by long-tongued insects would likely have the characteristics of generic bee, hoverfly or butterfly flowers as described earlier (see Chapters 4 and 5) but with an elongated, and possible narrowed, corolla tube to exclude shorter tongued visitors. If such flowers are visited by other, shorter-tongued species, we would expect that the Pollinator Effectiveness of these species would be lower than that of longer tongued species.

Testing the Long-Tongued Insect-Flower Syndrome

To test these predictions, four British wildflower species showing traits indicative of pollination by long-tongued insects were investigated. *Cirsium arvense*, *Knautia arvensis* and *Trifolium pratense* are well-studied species known to be pollinated by various species of bee (Shuel, 1951; Bond and Fyfe, 1968; Coomba *et al.*, 1999; Theiss, 2006; Tiley, 2010), or long-tongued butterflies or moths (Clausen *et al.*, 2001; Plepys *et al.*, 2002). *Centaurea nigra* also shows traits indicative of pollination by long-tongued insects, and previous studies (Gilbert, 1980) have indicated the species to be visited more frequently by long-tongued hoverfly species over shorter-tongued species, as well as being visited by butterflies (Corbet, 2000) and bees (e.g. Lack, 1976). However no study into the effectiveness of different insect species, with different tongue lengths, at pollinating these flower species has as yet been carried out.

Materials and Methods

Study Sites

The populations of *Cirsium arvense*, *Knautia arvensis* and *Centaurea nigra* investigated were located at West Quarry Braes, a Scottish National Heritage Site in Fife (NO 597 088) consisting mainly of scrub and woodland habitats, with a diverse range of native British flora and fauna present. The population of *Trifolium pratense* studied was located in a meadow habitat on the banks of Loch Tay in Perth and Kinross, Scotland (NN 669 358).

Study Species

Cirsium arvense

Also known as Creeping Thistle, Canada Thistle and Field Thistle, *Cirsium arvensis* is a member of the Asteraceae. It is native to Europe, Western Asia and North Africa, is an invasive species in the Eastern United States, and was probably introduced into Canada in the early 17th century (Moore, 1975; Theiss, 2006; Tiley, 2010). Flowering is normally between July and September (Nuzzo, 1997; Tiley, 2010).

Structure

The species is an erect perennial between 0.3 and 2.0m high at reproductive age. The dioecious flower heads hold disk florets that are pink-purple in colour. Staminate flowers are oblong to spherical in shape with projecting corollas. Pistillate flowers are ovoid or flask-shaped, with shorter corollas and protruding stigmas (see Fig. 1). Corollas of florets are approximately 13-18mm in length and 0.36 – 0.57mm in breadth at their narrowest. Flowers give off a strong, honey-like or vanilla odour (Tiley, 2010) composed mainly of (\pm)-Linalool and *cis*-linalool oxide (Plepys *et al.*, 2002) which attracts pollinators (Theiss, 2006). The stamens of male flowers each produce 500-800 pollen grains (Tiley, 2010), typically about 43 μ m in diameter (Hanley *et al.*, 2008). In male flowers, the style possesses a sterile appendage at its tip and its base, which form a tube through which the stamen protrudes at dehiscence, as described below (Tiley, 2010).

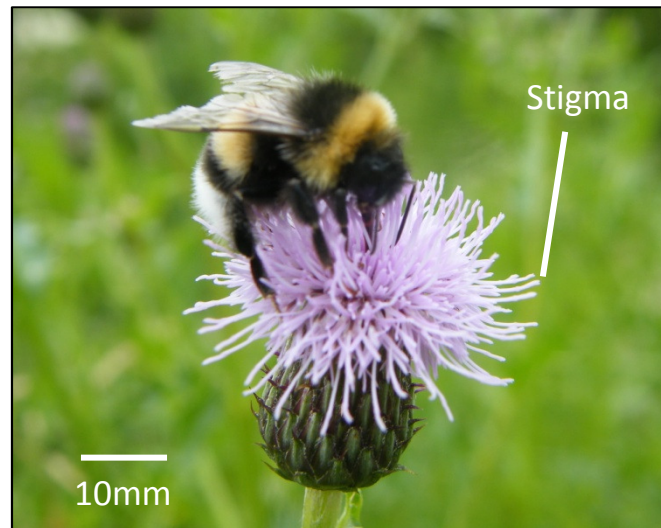


Fig. 1: *Bombus terrestris* feeding on a female capitulum of *Cirsium arvense*. Protruding stigmas indicated.

Nectar

Nectar rises up the corolla tube to the base of the throat, making it easily accessible to a wide range of insects with either short or long proboscides (Müller, 1883; Knuth, 1908; Tiley, 2010). Information on the concentration and volume of typical nectar rewards from *Cirsium arvense* is not available, not least because the tiny volumes of nectar contained within composite flowers are notoriously difficult to sample and measure accurately (Willmer, 2011).

Timing

Flower buds open in cymose order from the top of the plant to progressively lower axillary branch capitula. All the flowers on a capitulum may open in a single day, especially in warm weather. In male flowers, an abrupt, forceful lengthening of the style, along with a contraction of the stamen filaments, causes a sweeping of pollen from the anthers by a collar of pointed, unicellular hairs at the base of the stamen thus producing a secondary pollen presentation system (Tiley, 2010).

After emergence of the style in female flowers, the branches of the stigma widen to expose their dorsal surfaces, clothed with unicellular papillae on which pollen grains adhere

(Tiley, 2010). For fertilisation and production of viable seeds, both pistillate (female) and staminate (male) flowers must be growing in close proximity to one another, and stigma receptivity and pollen presentation must coincide. Isolated clones are unable to reproduce (Kay, 1985). Where pollen deposition is low, the period of stigma receptivity is increased to enhance the chances of effective pollination (Lalonde and Roitberg, 1994).

Knautia arvensis

Knautia arvensis, or Field Scabious, is a member of the Dipsacaceae. It is found across Europe and adjacent areas of Africa and Asia (Hultén and Fries, 1986). Flowering in the UK occurs between July and September (Lack, 1982a, b, c, d; Walters, 2002).

Structure

Knautia arvensis is a perennial, gynodioecious species that can grow to approximately 1m tall, possessing approximately 55-100 flowers arranged in a dense capitulum. Stems are simple or branched, and hold one or several capitula (Vange, 2002). Capitula are approximately 30-40mm across (Walters, 2002) and flowers are protandrous and self-compatible (Vange, 2002). Corolla tubes are long, approximately 6mm (Comba *et al.*, 1999), and narrow, approximately 1.5mm wide; they are pale lilac to light blue in colour, and with a protruding filament possessing four anthers (see Fig. 2). The anthers produce approximately 250 pollen grains each and each hermaphroditic flower possesses one ovule (Larsson, 2005). Flowers exude a strong, floral odour dominated by benzenoid compounds, monoterpenes and irregular terpenes (Andersson, 2003).

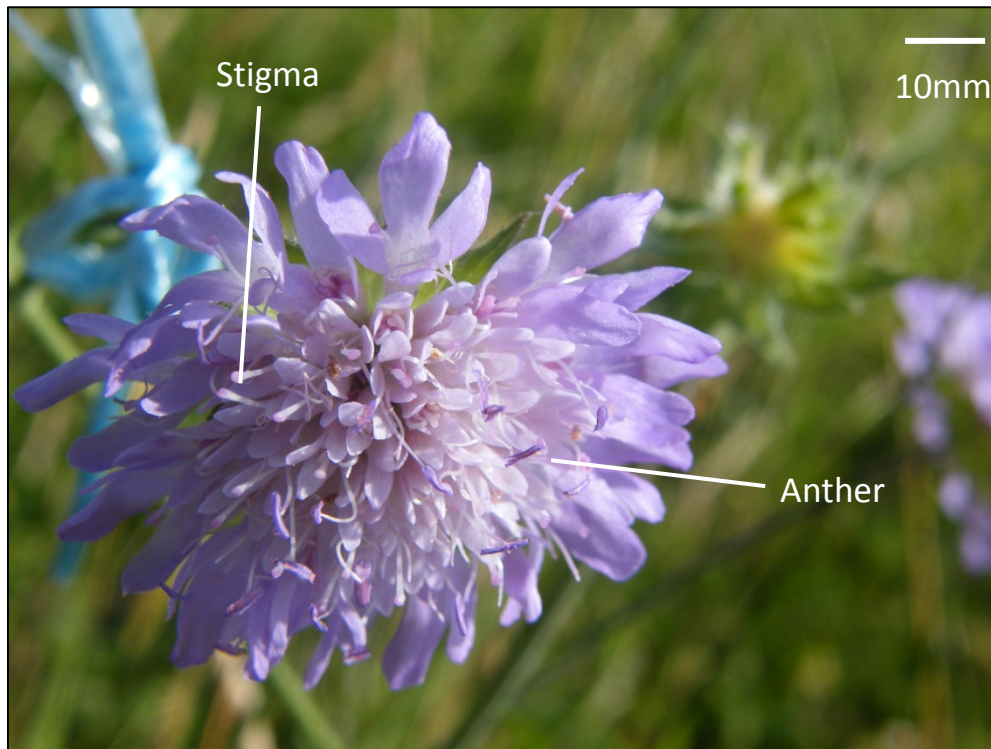


Fig. 2: Capitula of *Knautia arvensis*. Stigmas and anthers indicated.

Nectar

Nectar collects at the base of the long corolla and can be reached by visitors with long tongues. Female flowers produce more nectar when their stigmas are presented than hermaphroditic flowers (Larsson, 2005). The nectar of *Knautia arvensis* is sucrose dominant (Percival, 1961). In a field study of between 30 and 60 flowers over a 3 hour period with visitors excluded, flowers of *Knautia arvensis* have been recorded as producing approximately 130-150µg of sugar per 24 hours (Raine and Chittka, 2007a). For a variety of reasons however (summarised in Willmer, 2011), nectar readings are not necessarily reliable between studies, especially when taken over a short period of time, at different times of day and in a different habitat, therefore nectar volumes and concentrations for my site may vary from those quoted above.

Timing

Within a hermaphroditic inflorescence, filaments gradually present the anthers approximately 4-5mm above or outside the corolla. This gradual pollen presentation lasts for

between 1 and 4 days depending on the frequency of flower visitations (Cresswell, 1999; Larsson, 2005). The filaments are attached to the anthers by a thin joint on the dorsal side, allowing the anthers to perform a “seesaw mechanism”, stamping pollen onto visitors (Larsson, 2005). When all or most of the pollen is gone, the stigma-presenting stage begins.

Trifolium pratense

Trifolium pratense, or Red Clover, is a legume of the family Fabaceae. The species is native to central Europe, the Mediterranean region, Balkans, Asia Minor, Iran, India, Himalayas, Russia from Arctic south to east Siberia, Caucasus, and the Far East. It spread to England ca 1650 and was carried to America by British colonists (Taylor and Smith, 1981). Flowering in the UK is from May to September (Walters, 2004).

Structure

Trifolium pratense is a perennial species growing between 1 and 5cm tall. Florets are campanulate, with a lip composed of keel petals joined together, pink to purple in colour and arranged in a corymb inflorescence. Corolla tube length is between 10 and 15mm, although there is much variation between individuals and even within an inflorescence (Bond and Fyfe, 1968). *Trifolium pratense* is self-incompatible (Heslop-Harrison and Heslop-Harrison, 1982). The stigma and style form a crozier-shaped projection approximately 10mm long, with the stigmatic head curved upwards in the flower (Heslop-Harrison and Heslop-Harrison, 1982; see Fig. 3). The androecium consists of two whorls of stamens, one antesealous and one antepetalous, each with approximately 5 anthers (Retallack and Willison, 1990). Pollen grains are approximately 45µm in diameter (Hanley *et al.*, 2008). Flowers of *Trifolium pratense* give off a honey-like odour composed mainly of acetophenone, methyl cinnamate and 1-phenyl-ethanol (Buttery *et al.*, 1984).

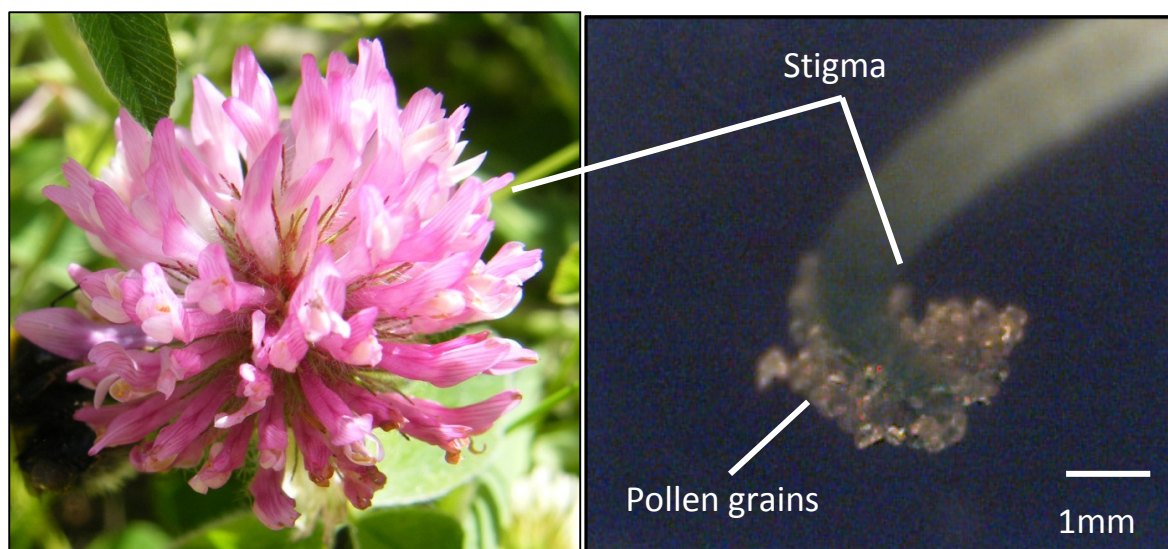


Fig. 3: Capitula of *Trifolium pratense* and close up of the crozier shaped stigma and style with pollen grains adhered (x10 magnification, Watson Barnet Microsystem 70 Compound Light Microscope). Position of stigma and style within floret indicated.

Nectar

Nectar of *Trifolium pratense* is contained at the base of the 5 to 70 narrow tubular florets of the inflorescence (Marden, 1984), and only extends for 1-2 mm up the tube (McGregor, 1976). Studies on the nectar volume and concentration of this species mainly involve artificial manipulation of nectar secretion using factors such as nutrient availability and environmental conditions such as temperature (e.g. Schuel, 1951; Bukhareva, 1960), however these studies probably do not give an accurate indication of nectar concentration and volume in natural conditions. There is almost certainly only a fraction of a μl per floret (pers obs).

Timing

Receptive stigmas and dehiscing anthers of *Trifolium pratense* are present in florets at the same time. The self-incompatibility of the species prevents selfing of flowers occurring (Heslop-Harrison and Heslop-Harrison, 1982). When a pollinator lands on the keel petals, they spring apart distally allowing the pollinator to contact the stigma and transfer pollen from

a previously visited flower. The pollinator is then dusted with pollen, and, once it vacates the flower, the keel petals rejoin and the “spring-loaded” (‘tripping’) mechanism resets, ready for another visitor (Clark and Malte, 1913; Retallack and Willison, 1990).

Centaurea nigra

Centaurea nigra is self-incompatible, vegetative reproduction is slow and localised, and plants are not thought to live for more than 5 years, and therefore the species is almost completely reliant on insect-pollination for its reproduction (Marsden-Jones and Turrill, 1954; Lack, 1976). Flowering in this species is from June to September in the UK, though when it is competing with *Centaurea scabiosa* flowering onset can be delayed until as late as mid-July (Lack, 1982b). The species is widespread in its distribution across Central and Western Europe, as far North as Scandinavia but essentially absent in the Mediterranean (James and Hammond, 2002).

Structure

Centaurea nigra is an upright perennial, roughly hairy with grooved stems that branch near the top (James and Hammond, 2002). Individuals of *Centaurea nigra* can grow to between 30 and 100cm in height and are often found clumped together in groups (Brodie, 1996). Florets are arranged in capitula approximately 1.5-2cm across (Lack, 1982d) and have a mean corolla depth of 4.79mm (Corbet, 2000), usually less than 1mm wide (Brodie, 1996). The florets are purple in colour, arising from a ball of brown bracts (James and Hammond, 2002), and with a faintly sweet odour. Capitula possessed between 15 and 30 florets per capitula (personal observation).

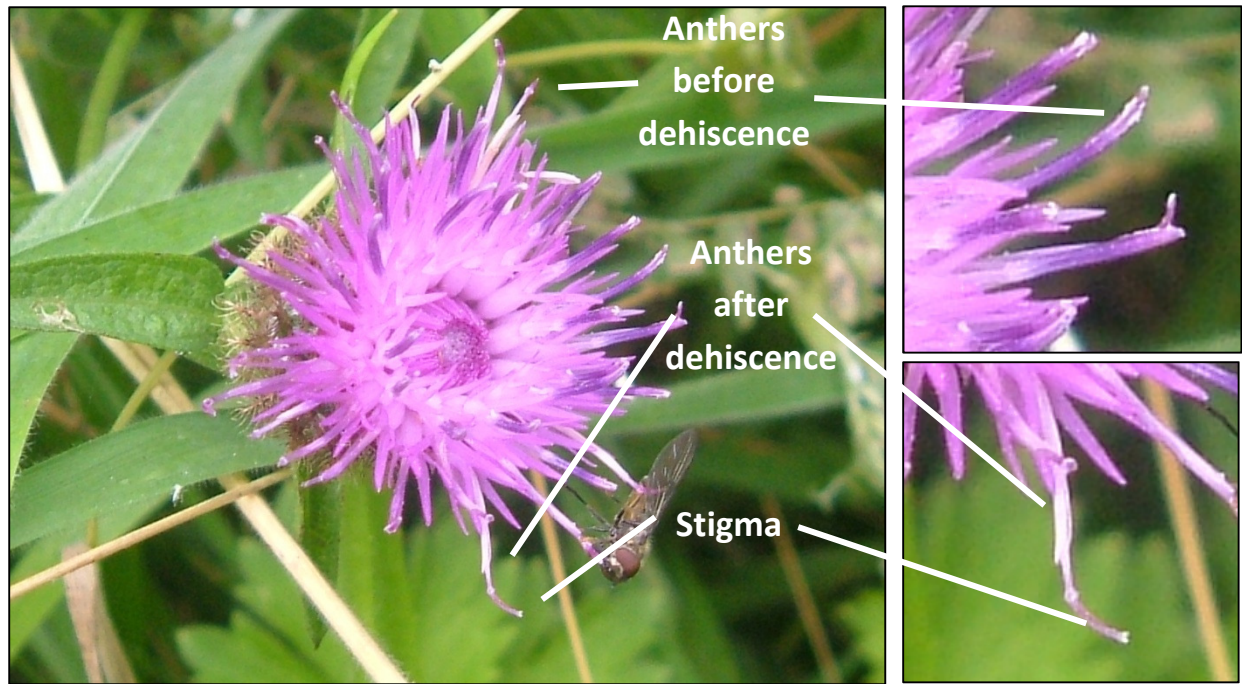


Fig. 4: Hoverfly feeding on capitula of *Centaurea nigra*. Protruding stigmas and location of anthers before dehiscence (when stigma is obscured) and after dehiscence (when stigma has pushed through) indicated.

Nectar

Nectar secretion begins soon after florets open, and continues at a fairly constant rate until between 15:00 and 16:00, when secretion slows. Some plants, however, may show a slight peak in production around 13:00 and there is some variation in secretion both between individuals and florets (Lack, 1982c). Nectar secretion rarely continues past the first day, and when it does it is much reduced. Capitula can produce 84 to 134 μ g of sucrose in a 24hour period, with concentrations ranging from below 30% to above 70%, varying with time of day and temperature (Lack, 1982c).

Timing

Florets open between 08:00 and 10:00, beginning with those on the outside of the capitulum and progressing towards the centre over 2 to 4 days. Flowers are protandrous and stimulation of the filaments triggers the ejection of pollen grains (approximately 33 μ m in

diameter (Hanley *et al.*, 2008)), from the 5 stamens soon after the opening of the florets. The stigma is usually partially hidden by the anthers and is not receptive until pollen is depleted, sometimes around 16:00 on the first day, or as late as the second day of flowering. The stigma then swells and elongates and pushes through the anthers (Lack, 1982c). The last florets to open on a capitulum undergo anthesis in the afternoon, rather than the morning, and near the end of the flowering period some capitula produce flowers which do not contain all the sexual parts, or remain unopened (Lack, 1982c).

Sampling Period

Field research occurred between the spring and summer months of 2009 and 2010. *Cirsium arvense* was studied from July to August in 2009 and 2010. *Knautia arvensis* and *Centaurea nigra* were observed from June to August in 2009 and 2010. Each sampling session was between 1 and 3 hours long depending on the frequency of visitations and how long it took all previously protected newly-opened flowers to be visited. Temperature and humidity readings were taken from a shaded area of the study site using a HM34 Vaisala Pocket Size Relative Humidity Meter every half hour during each sampling session, with the exception for *Trifolium pratense* (studied for 4 days in June 2010), for which temperature readings only were collected continuously using two Tinytag TGP-4017 data loggers, placed in undergrowth beside the study site.

Results

Temperature and Humidity

Mean temperature (°C) and relative humidity (%) for the study periods of three of the species were calculated and plotted (Fig. 5-Fig. 7). For *Trifolium pratense* continuous temperature readings were plotted (Fig. 8). In general, mean temperatures were low in the early mornings, climbing steadily to their highest levels around noon. Afternoon temperatures were much more variable than those of the morning. Mean relative humidity was variable across the day, though in general the lowest levels were around noon. Mean temperatures were slightly higher for the study period of *Knautia arvensis* and *Centaurea nigra*, and lowest for the study period of *Trifolium pratense*. Temperatures recorded during this period were less variable, particularly during the evening and night, than those of both *Knautia arvensis* and *Cirsium arvense*, possibly due to the more sheltered location of the study site at Loch Tay, in addition to the shorter length of study.

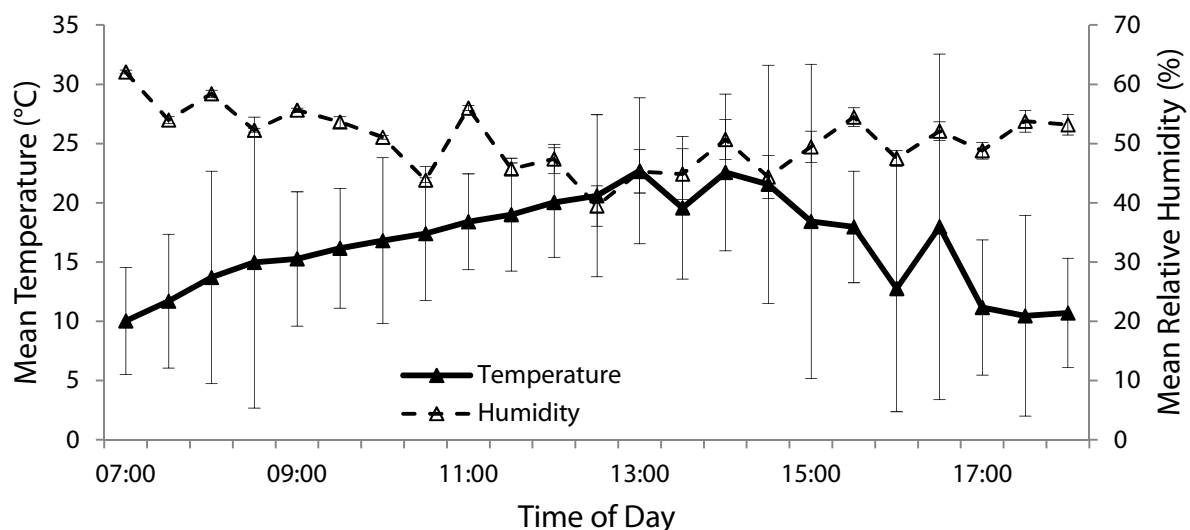


Fig. 5: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Cirsium arvense*. Standard deviations are shown.

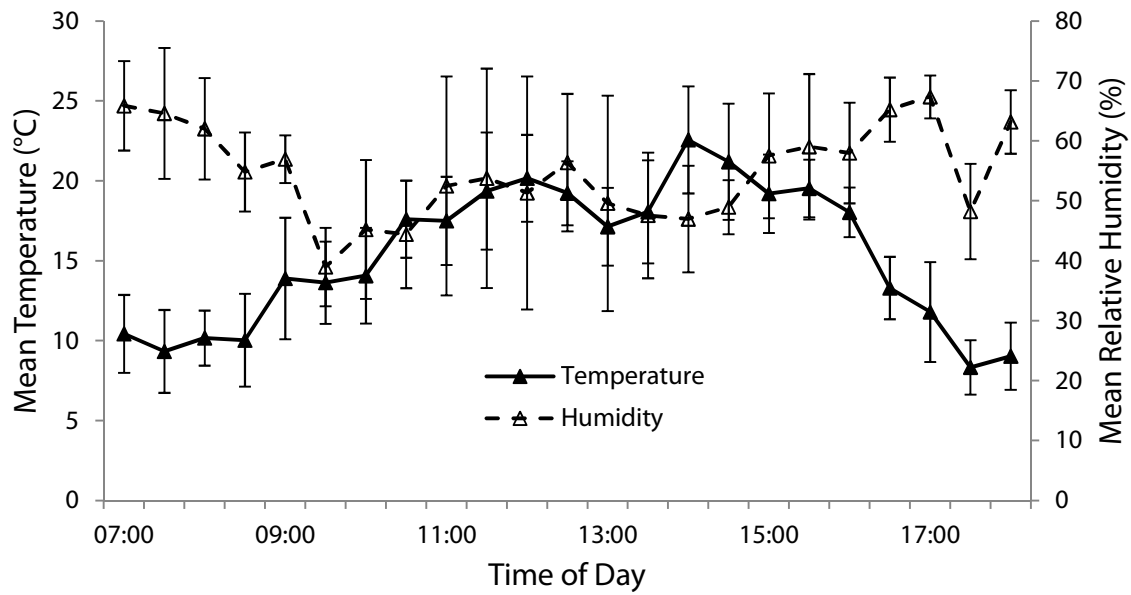


Fig. 6: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Knautia arvensis*. Standard deviations are shown.

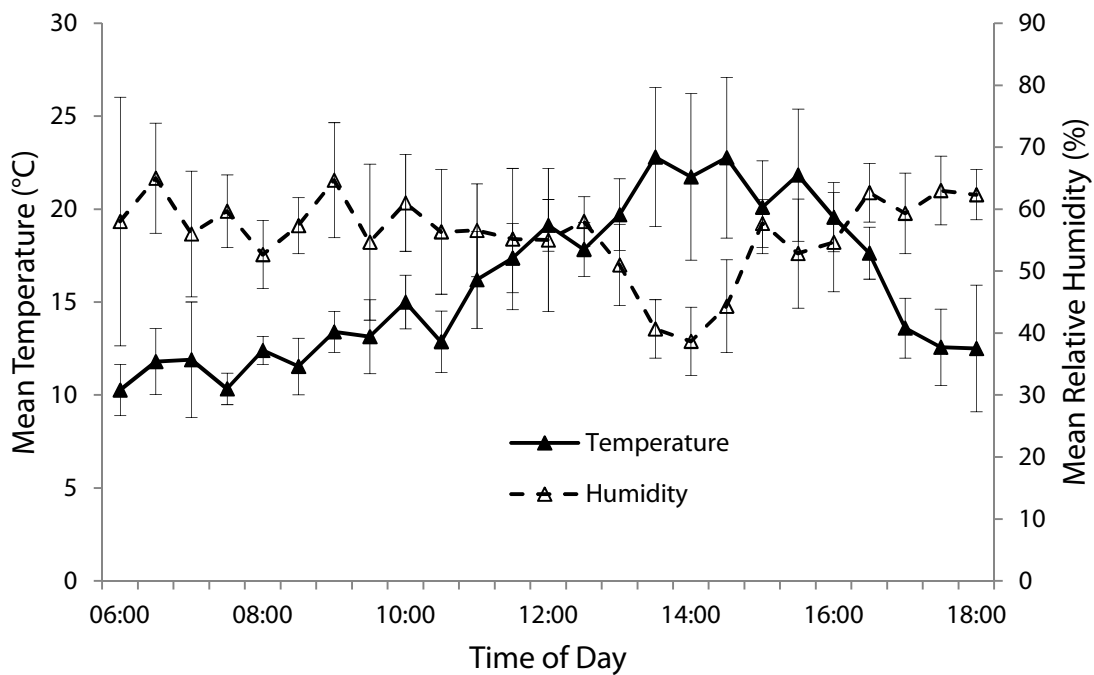


Fig. 7: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Centaurea nigra*. Standard deviations are shown.

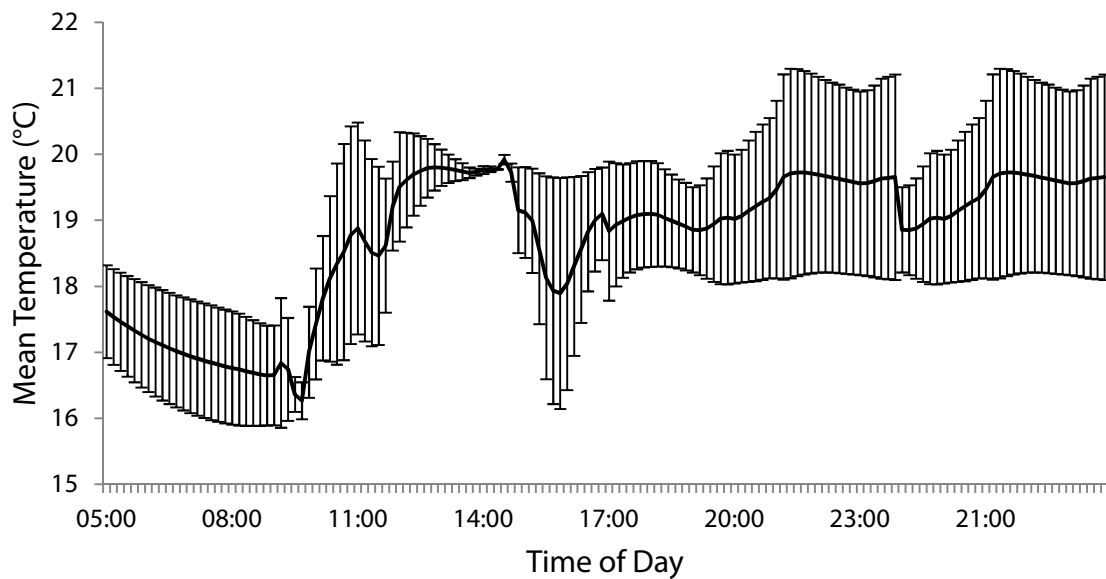


Fig. 8: Mean daily temperature (°C) readings during the study period of *Trifolium pratense*. Standard deviations are shown.

Partitioning of Visitors over Time

Visitors to target flowers of *Cirsium arvense*, *Knautia arvensis* and *Centaurea nigra* over the day were recorded throughout the study period. The study periods of *Trifolium pratense* were between 10:30 and 14:30, not substantial enough to show visitation across the whole day. Visitors were first treated as functional groups (Fig. 9, Fig. 11, Fig. 14), except for *Centaurea nigra*, which was visited by only one functional group; and then by individual species (Fig. 10, Fig. 12, Fig. 13 and Fig. 15). Visits recorded do not provide a complete representation of the visitor assemblage of the plant species throughout the day, as only visits to target, previously bagged flowers were recorded; however some patterns of visitation can be seen in the limited data available.

Cirsium arvense

Bumblebees were the earliest flower visitors to *Cirsium arvense*, followed by hoverflies and other dipterans (Fig. 9). The peak activity levels of the flower visitors coincide with the higher mean temperatures of the day, however visitation was lower around noon during the highest mean temperatures, in particular for the bumblebees.

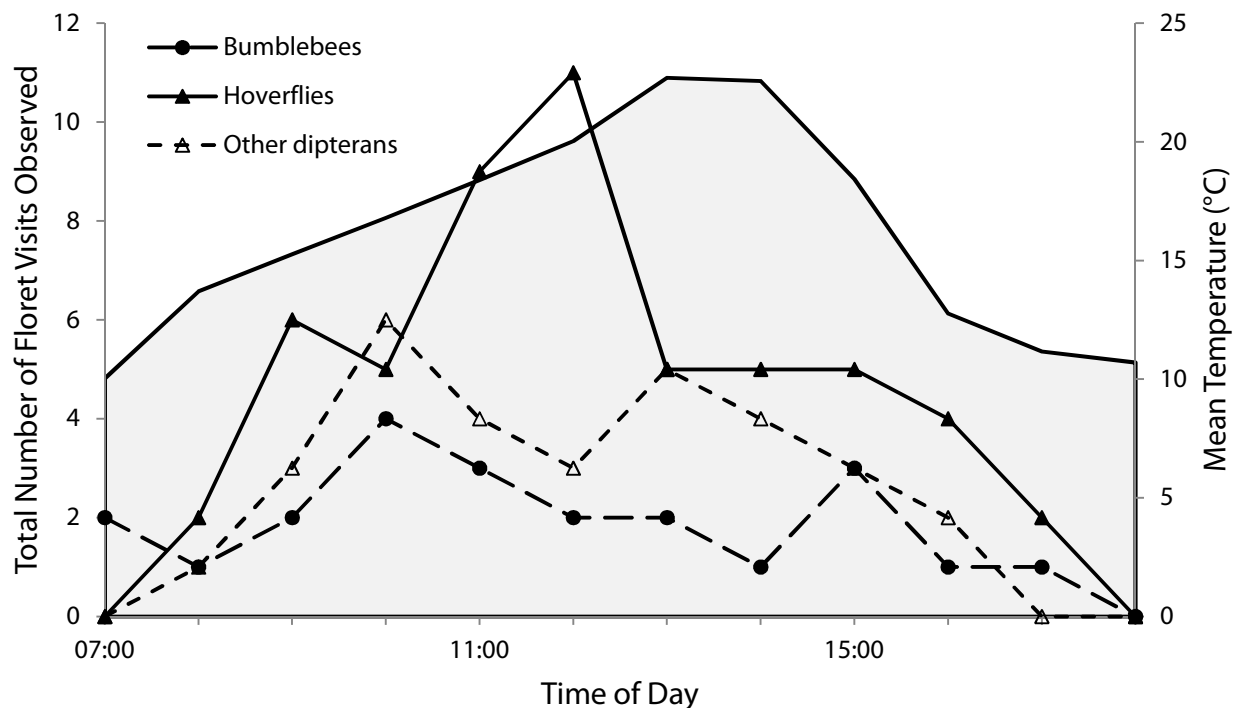


Fig. 9: Partitioning of visitor groups to *Cirsium arvense* over daily time. Mean temperature (°C) shown as an area plot.

When analysed by species (Fig. 10), the earliest flower visitor was *Bombus terrestris*, followed by the hoverfly *Episyrphus balteatus* and the unknown muscid species. Other, smaller hoverfly species and the bluebottle *Calliphora vomitoria* arrived at flowers from around 09:00. Again, peak visitation levels of the visitors coincided with higher temperatures; however the highest temperatures of the day, around noon, coincided with a drop in visitation, in particular for the bumblebee *Bombus terrestris* and the larger dipterans.

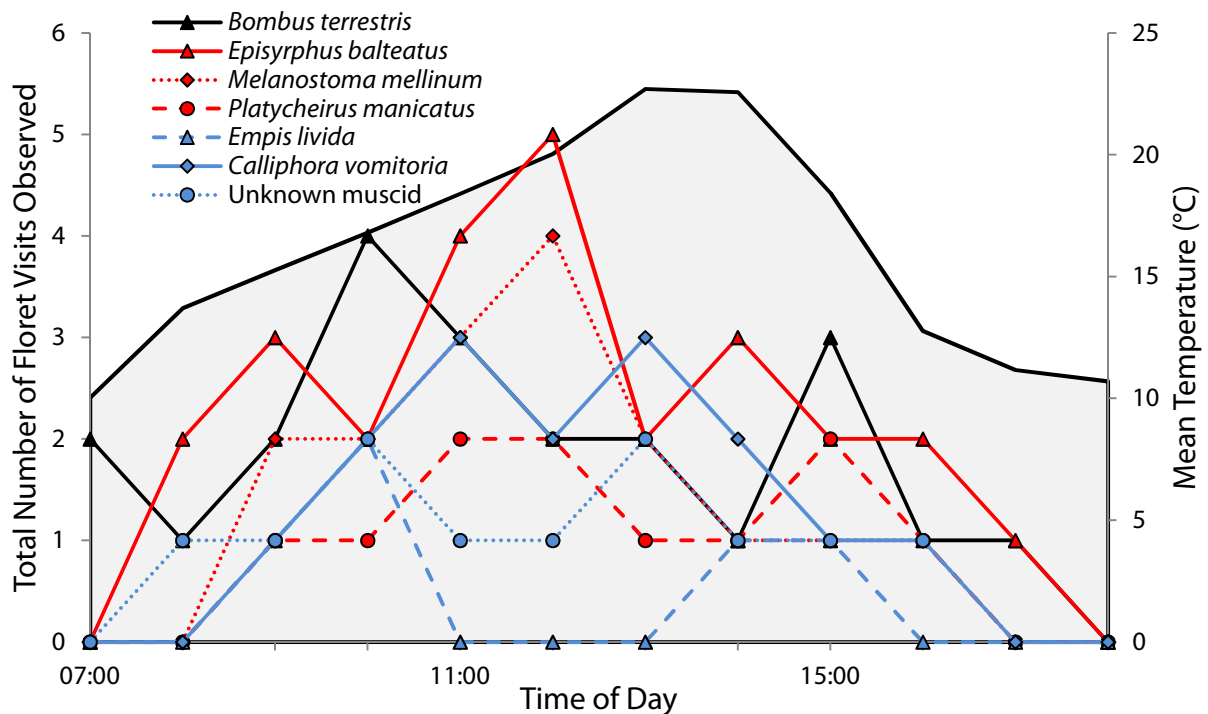


Fig. 10: Partitioning of visitor species to *Cirsium arvense* over daily time. Mean temperature (°C) shown as an area plot. Bees are shown in black, hoverflies in red and other dipterans in blue.

Knautia arvensis

Both hoverflies and bumblebees were observed earliest in the morning on flowers of *Knautia arvensis* (Fig. 11). Other dipterans followed later in the morning. Cuckoo bumblebees, once considered members of a separate genus *Psithyrus*, but now defined as a subgenus of *Bombus* (Williams, 1998), look very much like true bumblebees; however there is no worker caste and all individuals develop into reproductive males or females. Each of the six British species of cuckoo bumblebee is an inquiline of one or a few species of other bumblebees. The offspring of cuckoo bumblebees are reared by bumblebee workers. In this case, *Bombus* (*Psithyrus*) *bohemicus* takes over the nests of *Bombus lucorum* and possibly also *Bombus magnus* (Prŷs-Jones and Corbet, 1991). Cuckoo bumblebees were only observed on flowers between 09:00 and 13:00. Visitation rates were positively correlated with mean temperature; though rates dropped significantly for all groups (exception hoverflies) over noon when mean temperatures were at their highest.

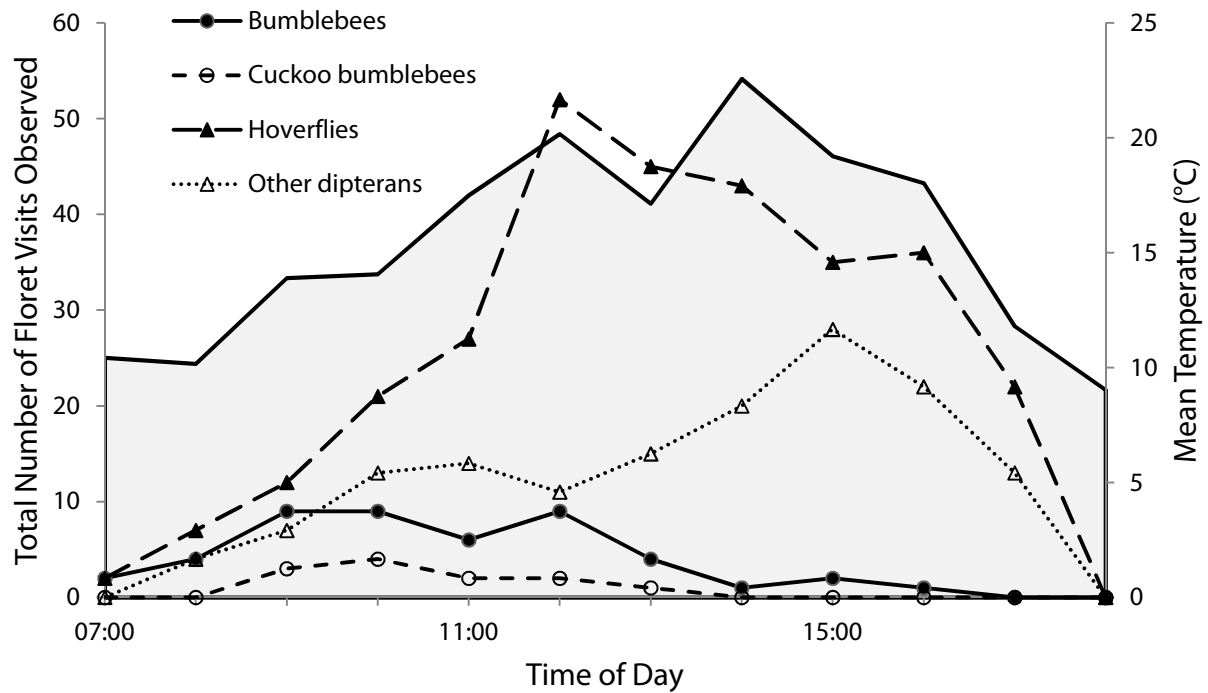


Fig. 11: Partitioning of visitor groups to *Knautia arvensis* over daily time. Mean temperature (°C) shown as an area plot.

When split into individual species (Fig. 12), we see that the earliest flower visitors are the bumblebee *Bombus terrestris* and the hoverfly *Episyrphus balteatus*. The other bumblebee species *Bombus lucorum* and *Bombus pratorum* arrived at flowers from 08:00 as did the hoverflies *Rhingia campestris* and the dipteran *Empis livida*, with the larger hoverfly *Syrphus ribesii* following at 09:00. The small hoverfly *Eupeodes corollae* was only recorded on flowers between 13:00 and 15:00. Activity levels were again consistent with mean temperature, however all species experienced a drop in visitation over the highest mean temperatures of the day at noon, with the exception of *Episyrphus balteatus*, which was present on flowers throughout the day.

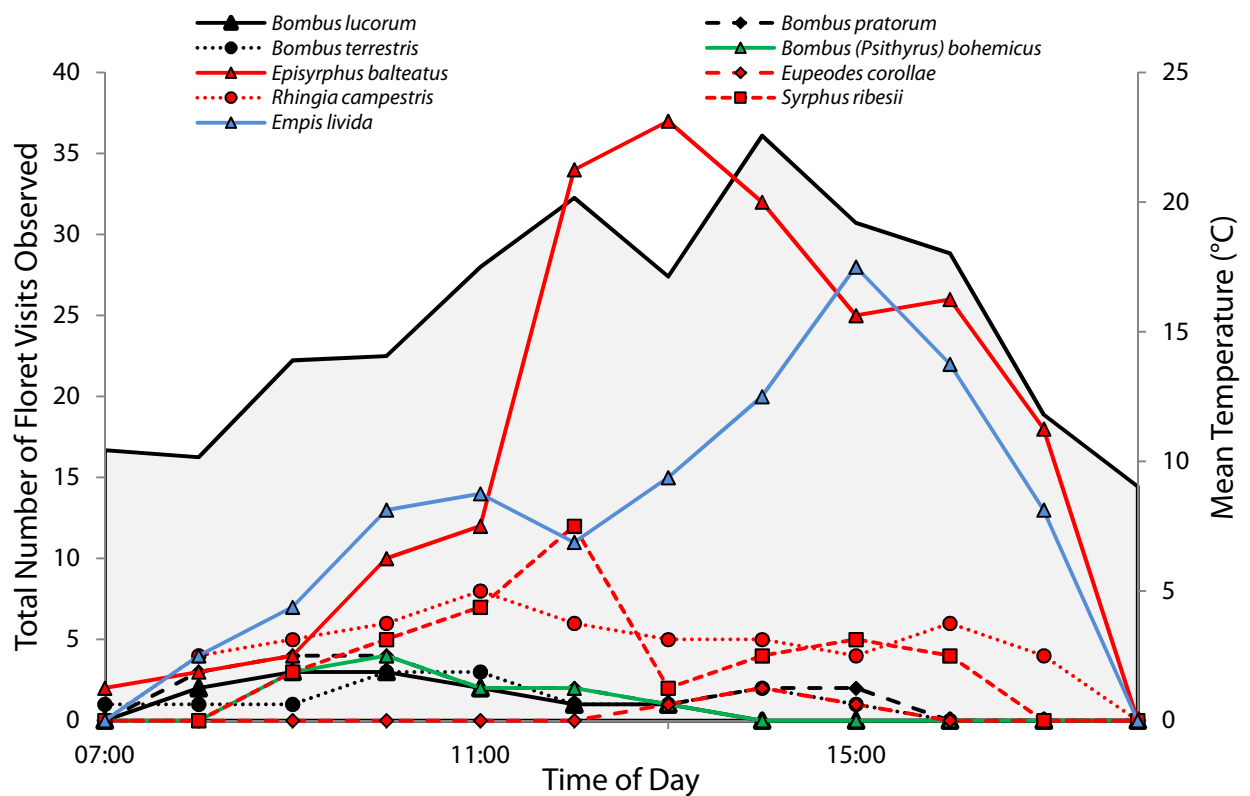


Fig. 12: Partitioning of visitor species to *Knautia arvensis* over daily time. Mean temperature (°C) shown as an area plot. True bumblebees are indicated in black, cuckoo bumblebees in green, hoverflies in red and other dipterans in blue.

Centaurea nigra

During the course of this study, *Centaurea nigra* was visited solely by hoverflies. The partitioning of the visits of these species over time, alongside the mean temperature for each time period, is shown in Fig. 13. The earliest flower visitor was *Episyrrhus balteatus*, which was present on flowers from 06:30 throughout the day. The second earliest visitor was *Rhingia campestris* at 07:00, which was also present throughout most of the day. *Eupeodes corollae* was the next flower visitor, observed on flowers from 07:30, and ended foraging earlier in the evening than the other species. *Platycheirus manicatus* was observed rarely from 08:00 until around 15:00. All four showed a visible drop in visitation between 12:00 and 14:00, the hottest part of the day.

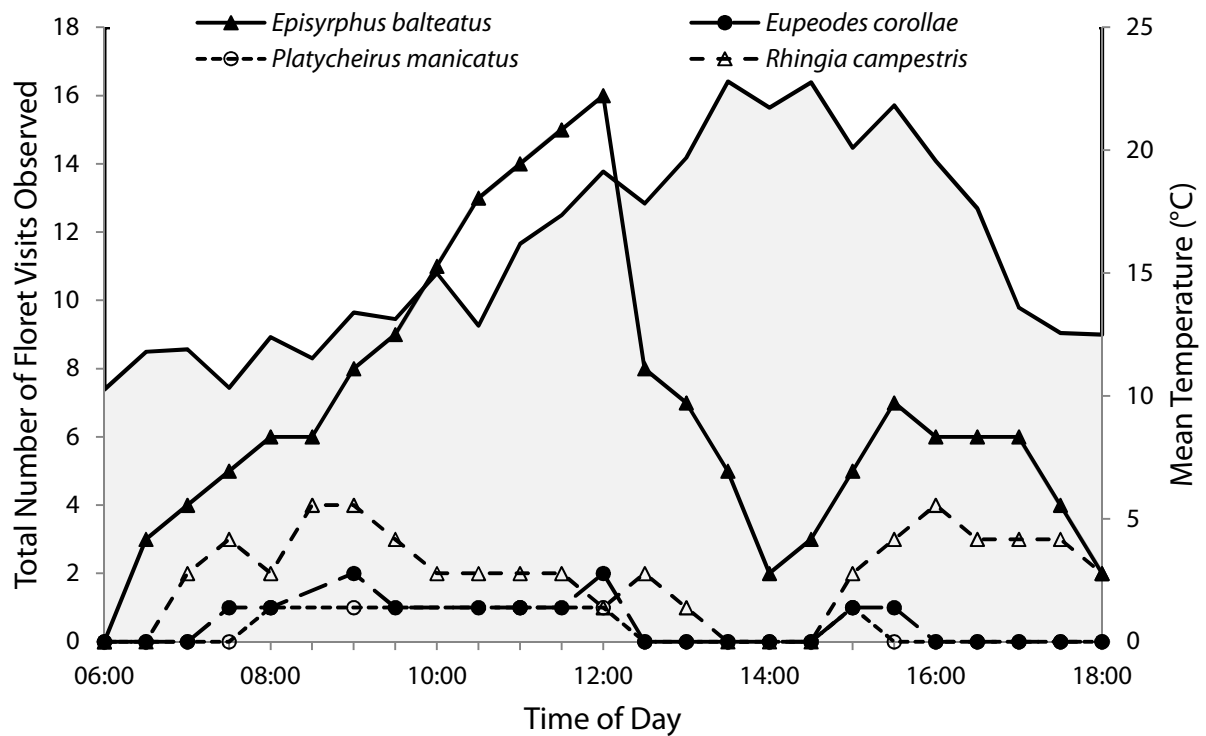


Fig. 13: Partitioning of visitor species to *Centaurea nigra* over daily time. Mean temperature (°C) shown as an area plot.

Trifolium pratense

Both bumblebees and hoverflies were observed on flowers of *Trifolium pratense* from 10:30 (Fig. 14). Due to the limitations of the study period of *Trifolium pratense* it was not possible to determine the earliest flower visitor. Highest visitation rates coincided with the mid-ranges of mean temperature, however, as before, the high temperatures of midday coincided with reduced visitation rates for both hoverflies and bumblebees.

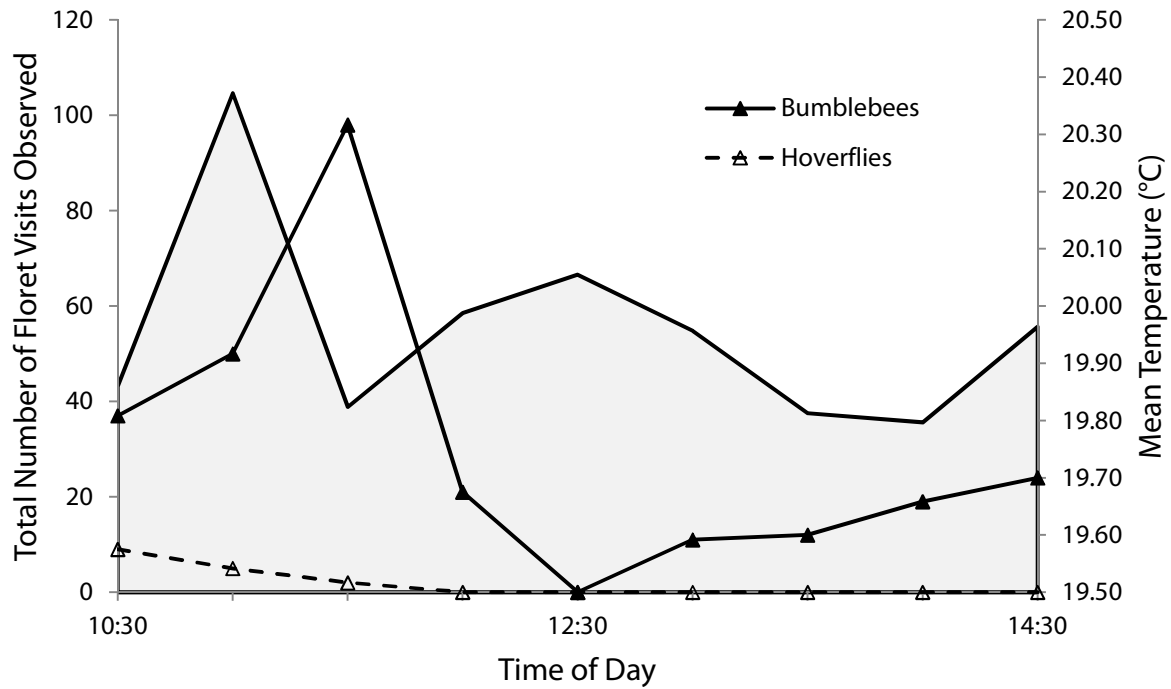


Fig. 14: Partitioning of visitor groups to *Trifolium pratense* over daily time. Mean temperature (°C) shown as an area plot.

When visitors were analysed by species (Fig. 15) the bumblebee *Bombus hortorum* was the only species to be observed in both the morning and afternoon portions of the study period. The other bumblebee species, *Bombus terrestris*, *Bombus muscorum* and *Bombus lucorum*, and the hoverfly species *Criorhina* sp., were observed on flowers throughout the morning only. No visitors were observed on target flowers between 12:00 and 12:30, a time with the one of highest mean temperatures of the day. Another peak in temperature occurred at 11:00, though only the hoverfly *Criorhina* sp. showed a drop in visitation at this time.

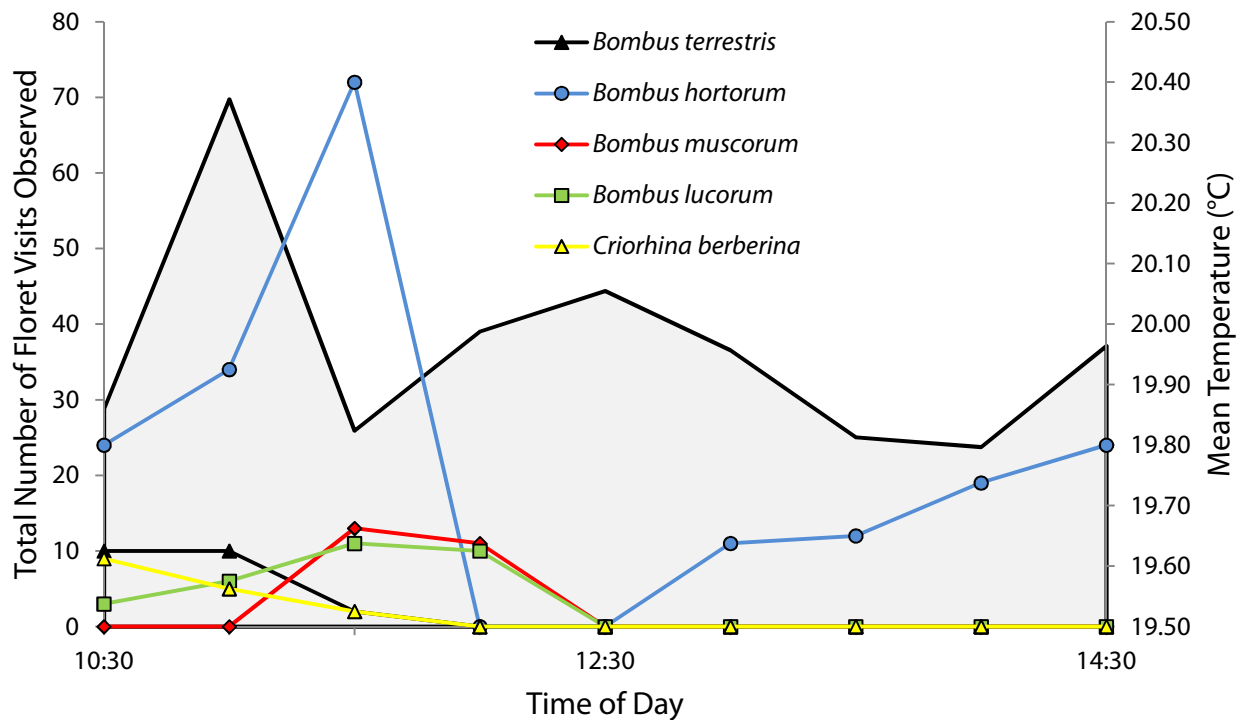


Fig. 15: Partitioning of visitor species to *Trifolium pratense* over daily time. Mean temperature (°C) shown as an area plot.

Pollen Deposition by Visitors

The mean number of pollen grains per stigma (MPS) for each flower species was first calculated by visitor functional group (Fig. 16, Fig. 18 and Fig. 21), except for *Centaurea nigra* as above, and then by visitor species (Fig. 17, Fig. 19, Fig. 20 and Fig. 22). As before, a pollinator was defined as a species that deposited a statistically significantly greater amount of pollen on stigmas in comparison to the unvisited control stigmas.

Cirsium arvense

Group	MPS	Sig. of MPS (in comparison to MPS of Controls)
Hoverfly	2.9	$P < 0.001$
Bee	1.8	$P < 0.001$
Other dipterans	1.2	$P < 0.001$

Table 1: Statistical analysis of visitor groups to *Cirsium arvense*. Statistical analysis was performed by a Mann-Whitney U Test with Bonferoni correction. In this case, $\alpha = 0.0167$.

The hoverfly group had the highest MPS for *Cirsium arvense* (see Fig. 16 and Table 1), followed by the bee group. Variation in MPS between visitor groups was statistically significant. All visitor groups deposited a significant MPS in comparison to controls; therefore all groups would be classified as pollinators.

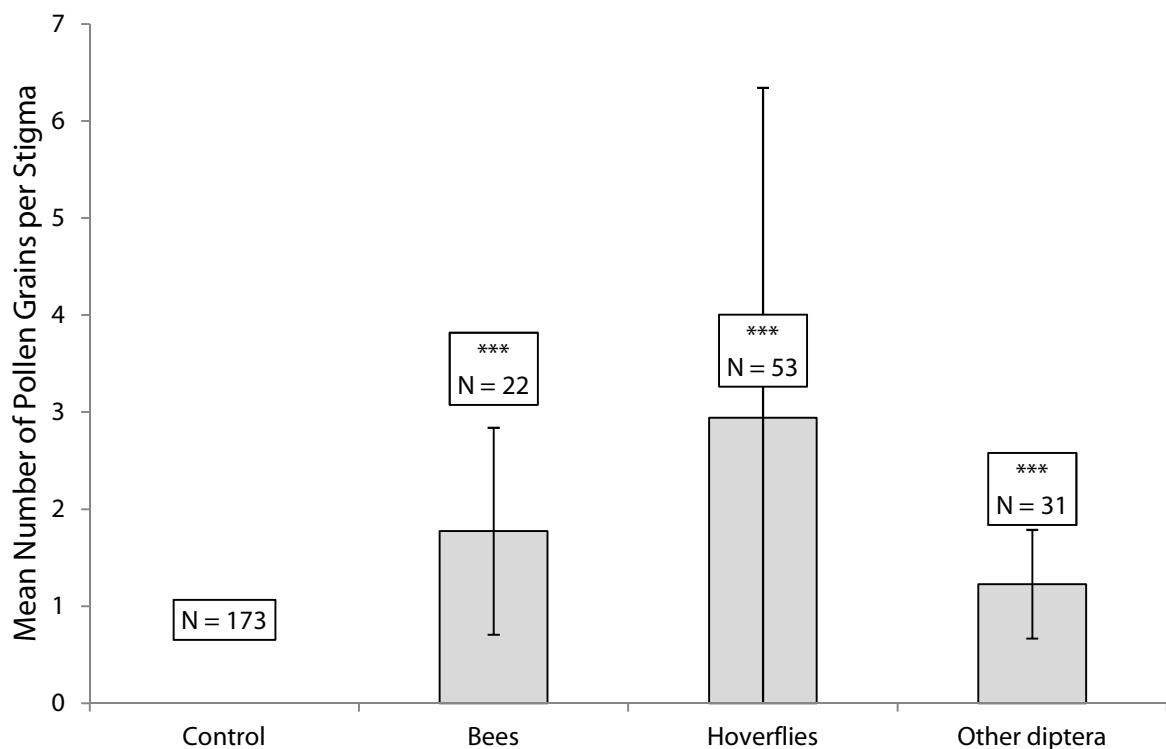


Fig. 16: Mean pollen deposition by visitor groups to *Cirsium arvense*. N values and SD shown. Significance refers to the significant of number of pollen grains deposited in comparison to control flowers. Variation in MPS between visitor groups was statistically significant (Kruskal-Wallis Non-Parametric Test: Chi-Square = 13.584; DF = 2, $P = 0.001$).

Group	Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
Hoverfly	<i>Episyrphus balteatus</i>	3.8	P < 0.001
Hoverfly	<i>Melanostoma mellinum</i>	2.1	P = 0.001
Hoverfly	<i>Platycheirus manicatus</i>	2.1	P < 0.001
Other dipterans	<i>Empis livida</i>	1.8	P < 0.001
Other dipterans	<i>Calliphora vomitoria</i>	1.2	P < 0.001
Other dipterans	Unknown muscid	1.0	NA
Bee	<i>Bombus terrestris</i>	1.8	P < 0.001

Table 2: Statistical analysis of visitor species to *Cirsium arvense*. Statistical analysis carried out by Mann-Whitney U Test with Bonferoni correction. In this case, $\alpha = 0.007$.

Analysis of MPS by individual species was then carried out (see table 2). The highest MPS on *Cirsium arvense* was deposited by the hoverfly *Episyrphus balteatus* (see Fig. 17 and table 2). Variation between species was statistically significant. All species deposited significantly more pollen grains than was found on control flowers, and were therefore classed as pollinators (see table 2). The Unknown Muscid was excluded from statistical analysis as all values of pollen deposition for this species were constant (one single grain per visit).

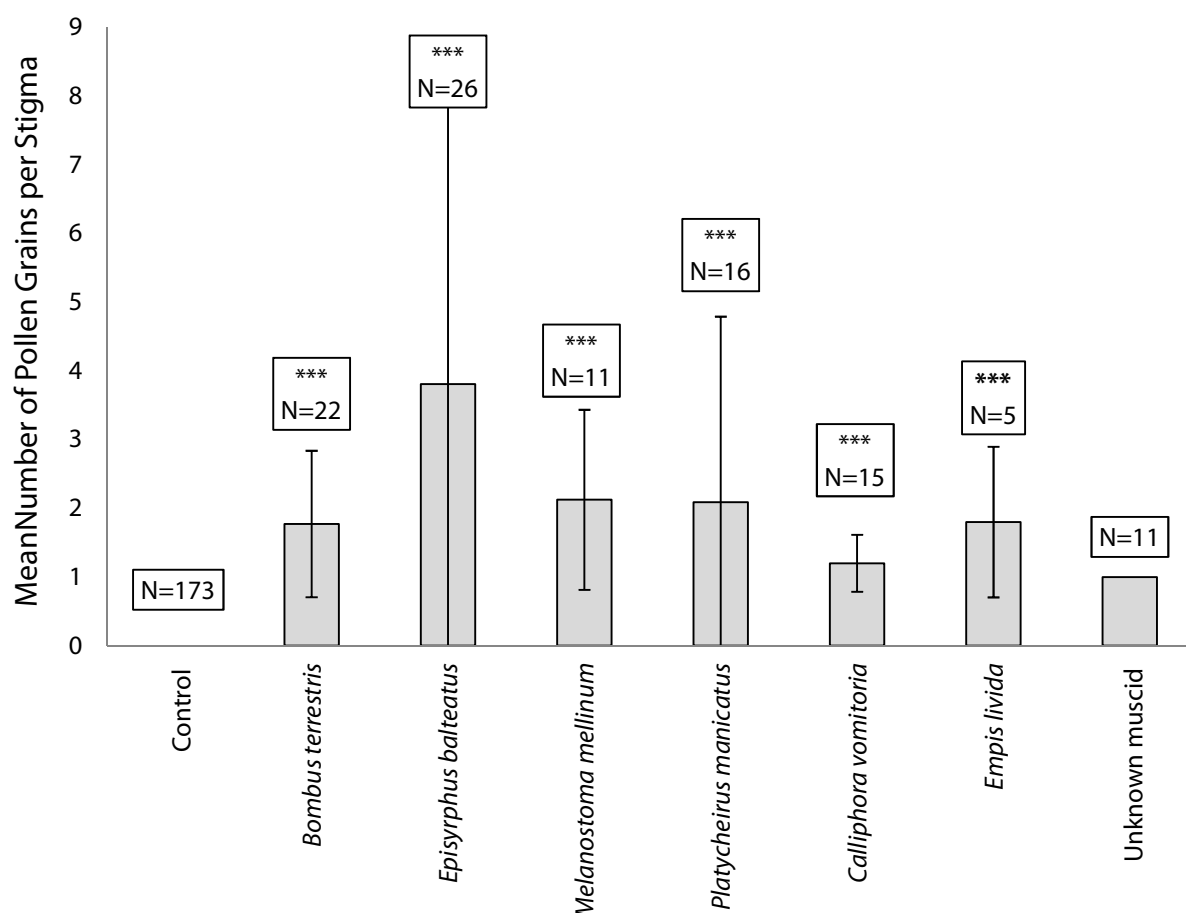


Fig. 17: Mean pollen deposition by visitor species to *Cirsium arvense*. N values and SD shown. Significance refers to the significant of number of pollen grains deposited in comparison to control flowers. Variation in MPS between visitor species was statistically significant (Kruskal-Wallis Non-Parametric Test: Chi-Square = 20.488, DF = 6, P = 0.002).

Knautia arvensis

Group	MPS	Sig. of MPS (in comparison to MPS of Controls)
Hoverfly	8.7	P < 0.001
Cuckoo bumblebees	8.0	P = 0.001
Other dipterans	7.4	P < 0.001
Bumblebees	5.6	P < 0.001

Table 3: Statistical analysis of visitor groups to *Knautia arvensis*. Statistical analysis was performed by a Mann-Whitney U Test with Bonferoni correction. In this case, $\alpha = 0.0125$.

The hoverfly group deposited the most pollen on stigmas of *Knautia arvensis* in comparison to the other groups, followed by the cuckoo bumblebees, the dipterans and the bumblebees (see Fig. 18 and table 3). All visitor groups deposited a significant amount of pollen in comparison to controls and were therefore classified as pollinators of *Knautia arvensis*. Variation in MPS between groups was not statistically significant.

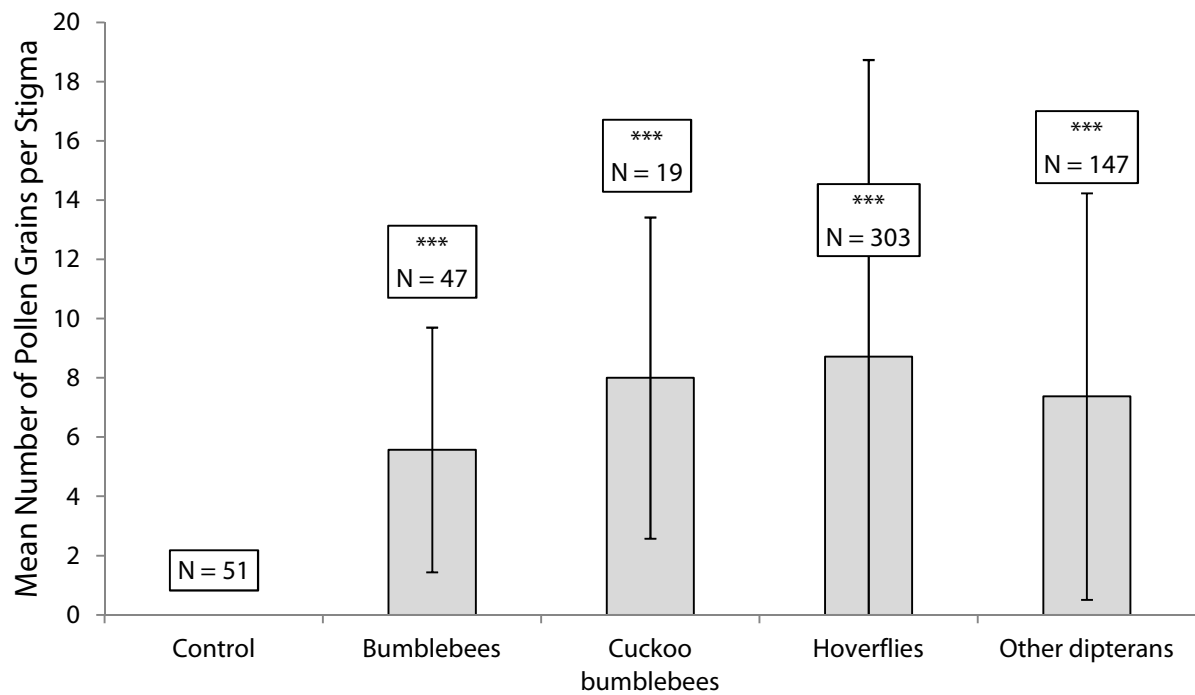


Fig. 18: Mean pollen deposition by visitor groups to *Knautia arvensis*. N values and SD shown. Significance refers to the significant of number of pollen grains deposited in comparison to control flowers. Variation in MPS between groups was not statistically significant (Kruskal-Wallis Non-Parametric Test: Chi-Square = 2.348, DF = 3, P = 0.503).

Group	Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
Hoverfly	<i>Rhingia campestris</i>	7.4	P < 0.001
Hoverfly	<i>Episyrphus balteatus</i>	6.4	P < 0.001
Hoverfly	<i>Syrphus ribesii</i>	1.0	P < 0.001
Hoverfly	<i>Eupeodes corollae</i>	2.3	NA
Other dipterans	<i>Empis livida</i>	6.1	P < 0.001
Cuckoo bumblebees	<i>Bombus (Psithyrus) bohemicus</i>	5.9	P < 0.001
Bumblebees	<i>Bombus terrestris</i>	2.1	P < 0.001
Bumblebees	<i>Bombus pratorum</i>	6.0	P < 0.001
Bumblebees	<i>Bombus lucorum</i>	4.8	P < 0.001

Table 4: Statistical analysis of visitor species to *Knautia arvensis*. Statistical analysis was performed by a Mann-Whitney U Test with Bonferoni correction. In this case, $\alpha = 0.00625$.

Visitors were then further analysed by species. The hoverfly *Rhingia campestris* deposited the highest MPS on *Knautia arvensis* (see Fig. 19 and table 4). Variation in MPS on *Knautia arvensis* between visitor species was statistically significant. All visitor species deposited significantly more pollen grains than was found on control flowers, and were therefore deemed pollinators. *Eupeodes corollae* was excluded from statistical analyses due to the low number of recorded visits for this species.

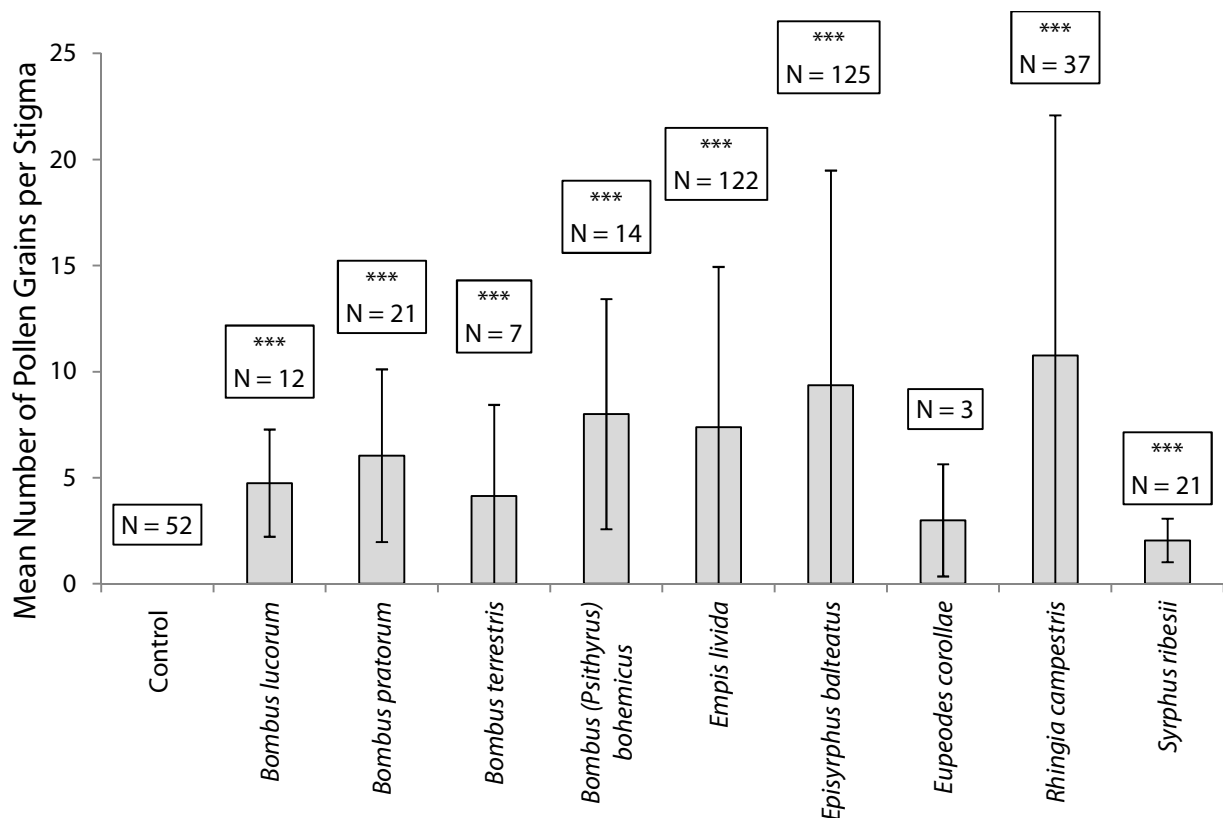


Fig. 19: Mean pollen deposition by visitor species to *Knautia arvensis*. N values and SD shown. Significance refers to the significant of number of pollen grains deposited in comparison to control flowers. Variation in MPS between visitor species was statistically significant (Kruskal-Wallis Non-Parametric Test: Chi-Square = 27.773, DF = 7, $P < 0.001$).

Centaurea nigra

Group	Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
Hoverfly	<i>Rhingia campestris</i>	58.9	$P < 0.001$
Hoverfly	<i>Episyrphus balteatus</i>	273.7	$P < 0.001$
Hoverfly	<i>Eupeodes corollae</i>	180.0	$P < 0.001$
Hoverfly	<i>Platycheirus manicatus</i>	50.4	$P = 0.161$

Table 5: Statistical analysis of visitor species to *Centaurea nigra*. Statistical analysis was performed by a Mann-Whitney U Test with Bonferoni correction. In this case, $\alpha = 0.0125$.

Visitation to *Centaurea nigra* was analysed by individual species only, as all visitors were from the hoverfly functional group. *Episyrphus balteatus*, *Rhingia campestris*, and *Eupeodes corollae* all deposited a significantly greater amount of pollen in comparison to control stigmas and were identified as pollinators (see Fig. 20 and table 5). *Platycheirus manicatus* did not deposit a significant amount of pollen and was therefore not classified as a pollinator. Variation between species was significant. *Episyrphus balteatus* was the most effective pollinator in terms of MPS. *Rhingia campestris* was least effective of the pollinators, though still depositing a significant MPS in comparison to controls.

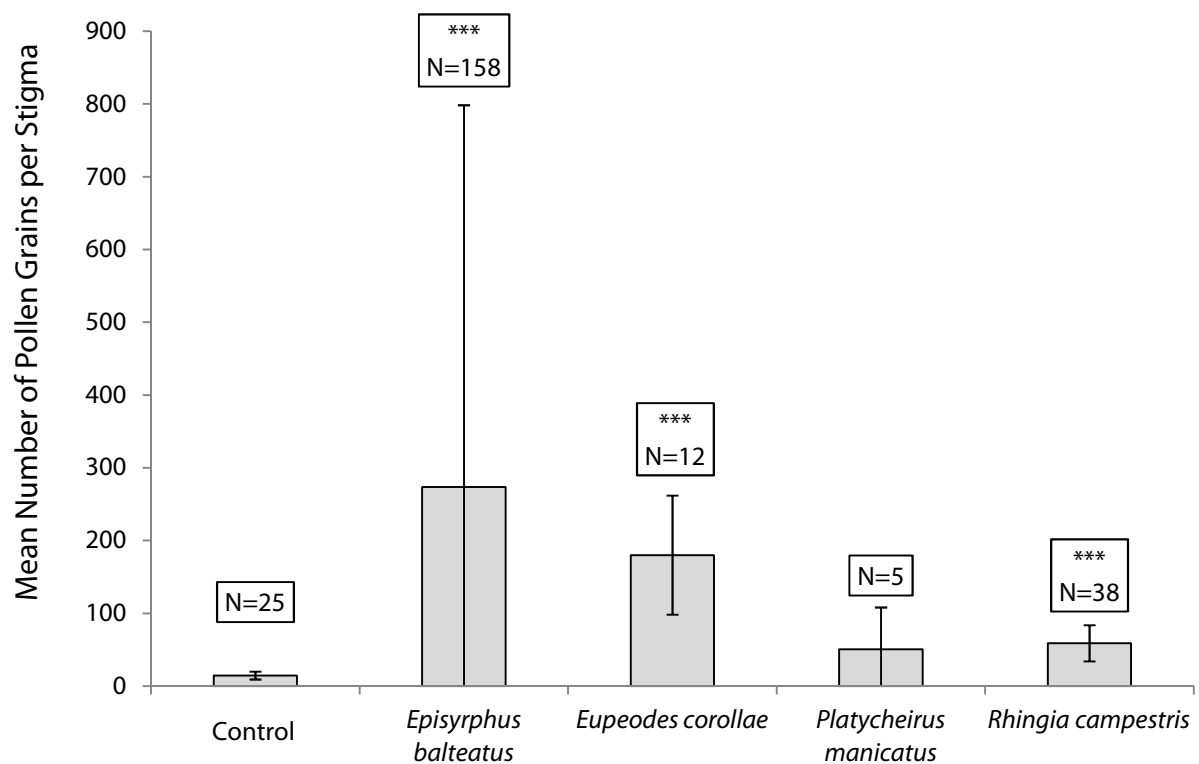


Fig. 20: Mean pollen deposition by visitor species to *Centaurea nigra*. N values and SD shown. Significance refers to the significant of number of pollen grains deposited in comparison to control flowers. Variation between visitors was significant (Kruskal Wallis Non-Parametric Test: Chi Square = 33.725, df = 3, $P < 0.001$).

Trifolium pratense

Group	MPS	Sig. of MPS (in comparison to MPS of Controls)
Hoverfly	28.75	P < 0.001
Bees	12.2	P < 0.001

Table 6: Statistical analysis of visitor groups to *Trifolium pratense*. Statistical analysis was performed by a Mann-Whitney U Test with Bonferoni correction. In this case, $\alpha = 0.025$.

When analysed by visitor groups, the highest pollen deposition on *Trifolium pratense* stigmas was by the hoverfly group (see Fig. 21 and table 6). Both the hoverfly and bee groups deposited a significant amount of pollen in comparison to control stigmas, and both groups were classified as pollinators. The variation in MPS between groups was not significant (One-Way ANOVA: $F = 3.693$, $DF = 1$, $P = 0.056$).

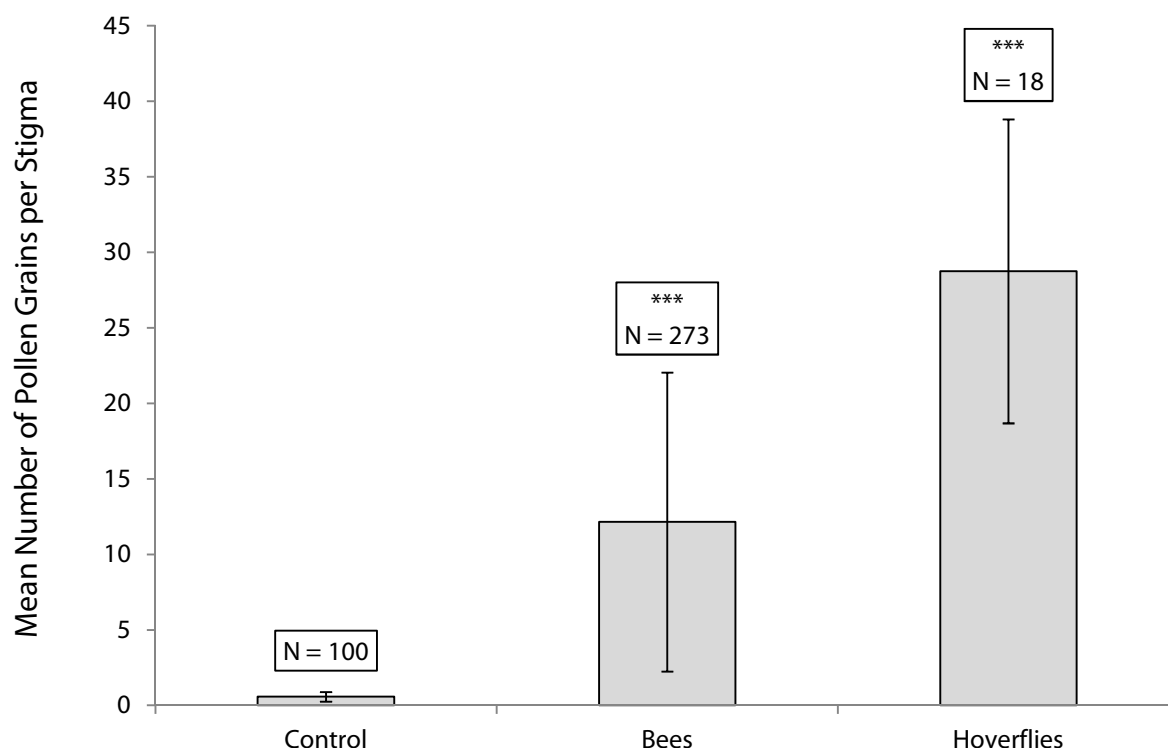


Fig. 21: Mean pollen deposition by visitor groups to *Trifolium pratense*. N values and SD shown. Significance refers to the significance of number of pollen grains deposited in comparison to control flowers. The variation in MPS between groups was not significant (One-Way ANOVA: $F = 3.693$, $DF = 1$, $P = 0.056$).

Group	Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
Hoverfly	<i>Criorhina</i> sp.	28.8	$P < 0.001$
Bee	<i>Bombus lucorum</i>	25.1	$P < 0.001$
Bee	<i>Bombus hortorum</i>	10.0	$P < 0.001$
Bee	<i>Bombus terrestris</i>	13.3	$P < 0.001$
Bee	<i>Bombus muscorum</i>	10.0	$P < 0.001$

Table 7: Statistical analysis of visitor species to *Trifolium pratense*. Statistical analysis was performed by a Mann-Whitney U Test with Bonferoni correction. In this case, $\alpha = 0.01$.

Visitors were then split into individual species for further analysis. The highest MPS for *Trifolium pratense* was deposited by the hoverfly *Criorhina* sp., followed by the bumblebee *Bombus lucorum* (see Fig. 22 and table 7). All visitor species deposited significantly more pollen on stigmas than was found on controls, therefore all species were classified as pollinators. The variation in stigmatic pollen deposition between visitor species was significant.

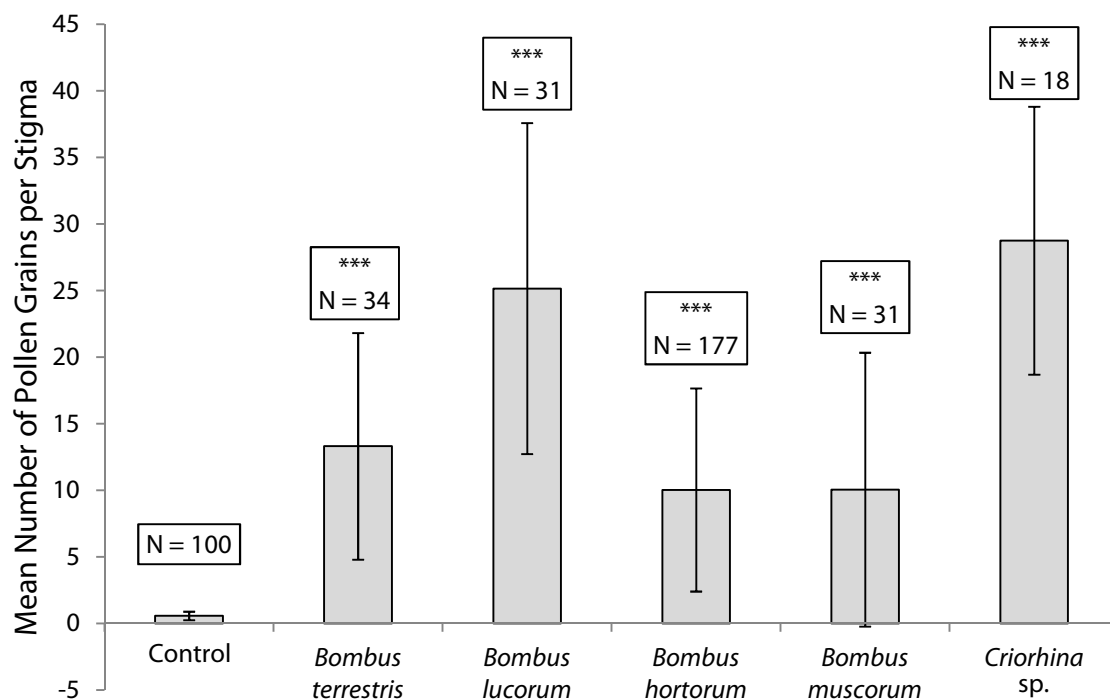


Fig. 22: Mean pollen deposition by visitor species to *Trifolium pratense*. N values and SD shown. Significance refers to the significant of number of pollen grains deposited in comparison to control flowers. The variation in stigmatic pollen deposition between visitor species was significant (One-Way ANOVA: $F = 27.089$, $DF = 4$, $P < 0.001$).

Discussion

Floral Traits

Each of the flower species investigated showed traits indicative of a Long-Tongued Insect-Flower syndrome. All flowers were tubular, and partially closed in shape, requiring some manipulation by the visitors to gain access to the nectar rewards at the base of the

corollas. In particular, the corollas of these species were relatively long in length. The flowers were arranged in inflorescences; *Cirsium arvense*, *Trifolium pratense* and *Centaurea nigra* in a simple cyme arrangement and *Knautia arvensis* in a capitulate inflorescence. All species were coloured appropriately for the syndrome; *Cirsium arvense* and *Knautia arvensis* possessed lilac to blue coloured corollas, and *Centaurea nigra* and *Trifolium pratense* had a darker, reddish purple colouring. Floral odours in each species were strong, sweet and honey or “floral” in description. While it was not possible to effectively measure the nectar volume and concentration of these species, the results of previous studies (Raine and Chittka, 2007a) are in line with those of typical bee and hoverfly flower nectar concentration and volume values.

Visitor Assemblages

Cirsium arvense

Group	Species	N	Mean Size (mm)	Mean Tongue Length (mm)
Hoverfly	<i>Episyrphus balteatus</i>	26	WL 6.00-10.25 ¹	2.9 ²
Hoverfly	<i>Melanostoma mellinum</i>	16	WL 6.75-8.00 ¹	1.7 ⁵
Hoverfly	<i>Platycheirus manicatus</i>	11	WL 7.25-8.25 ¹	2.4 ⁵
Other dipteran	<i>Calliphora vomitoria</i>	15	WL 10.6 ⁵	2.3 ⁵
Other dipteran	Unknown muscid	11	NA	NA
Other dipteran	<i>Empis livida</i>	5	WL 17.4 ⁵	15.3 ⁵
Bumblebee	<i>Bombus terrestris</i>	22	TW 4.99 ⁴	7.6 ³

Table 8: Visitor assemblage of *Cirsium arvense*. WL refers to wing length. TW refers to thorax width.

¹ Stubbs and Falk, 2002; ² Gilbert *et al.* 1985; ³ Goulson *et al.*, 2005; ⁴ Peat *et al.*, 2005; ⁵ Personal measurements.

Several different species of insects, including bumblebees, hoverflies and other dipterans, were observed to visit flowers of *Cirsium arvense* (see Table 8). The most frequent floret visitor was the hoverfly *Episyrphus balteatus*, a medium sized hoverfly, with a tongue length that is insufficient to allow effective feeding from, or at least to fully drain, the 13-18mm long *Cirsium arvense* corollas. Other, less frequent, hoverfly visitors included *Melanostoma mellinum*, a relatively small species and *Platycheirus manicatus*, a medium-sized species, both species also with tongues much shorter than the corolla length of *Cirsium arvense*. The hoverflies were seen to feed upon the nectar of flowers, but were perhaps favouring the study flowers because these flowers had been bagged before observations and were previously unvisited, therefore full of nectar allowing the hoverflies to feed without the necessity of a long tongue to reach the bottom of the corolla. When flowers visited by the smaller-tongued hoverfly species were collected for removal of the stigma, they still contained nectar at the base of the corolla and were never fully emptied.

The second most frequent floret visitor was the bumblebee *Bombus terrestris*, a widespread British bumblebee with a relatively short tongue length in comparison to other bumblebee species; however its tongue more than sufficient to reach at least some of the nectar from flowers of *C. arvense* (see Fig. 1). Again this species did not fully empty corollas of *Cirsium arvense*; however it did deplete nectar more than the shorter-tongued hoverflies. Other bumblebees were observed in the area, but their populations were low throughout the course of the investigation, likely due to environmental factors and the sub-optimum weather conditions experienced during the time of this study.



Fig. 23: *Empis livida* feeding upon florets of a *Cirsium arvense* inflorescence.

Other visitors to *C. arvense* included the bluebottle *Calliphora vomitoria*, a medium-sized, bristly surface-feeder of the family Calliphoridae with a relatively short tongue, and an unknown muscid species, both of which did not fully empty flowers visited. The dance fly *Empis livida* (see Fig. 23), of the Empididae, is a medium-sized fly with a long, horny proboscis which was able to probe deeper into flowers for nectar, however flowers were not always emptied fully.

Knautia arvensis

Group	Species	N	Mean Size (mm)	Mean Tongue Length (mm)
Hoverfly	<i>Episyrphus balteatus</i>	125	WL 6.00-10.25 ¹	2.89 ²
Hoverfly	<i>Eupeodes corollae</i>	3	WL 6.75-7.75 ¹	3.00 ²
Hoverfly	<i>Syrphus ribesii</i>	21	WL 7.25-10.25 ¹	2.99 ²
Hoverfly	<i>Rhingia campestris</i>	37	WL 6-9.5 ¹	11.00 ⁴
Other dipterans	<i>Empis livida</i>	122	WL 17.4 ⁵	15.3 ³
Bee	<i>Bombus pratorum</i>	21	TW 4.21 ⁶	7.30 ³
Bee	<i>Bombus lucorum</i>	12	TW 4.70 ⁶	7.50 ³
Bee	<i>Bombus terrestris</i>	7	TW 4.99 ⁶	7.60 ³
Cuckoo bumblebee	<i>Bombus (Psithyrus) bohemicus</i>	14	TW 4.79 ⁷	7.00 ⁵

Table 9: Visitor assemblage of *Knautia arvensis*. WL refers to wing length. TW refers to thorax width.

¹ Stubbs and Falk, 2002; ² Gilbert *et al.* 1985; ³ Goulson *et al.*, 2005; ⁴ Ssymank, 1991; ⁵ Goulson *et al.*, 2008; ⁶ Peat *et al.*, 2005; ⁷ Løken, 1984

The most frequent floret visitor to *Knautia arvensis* was, once again, the hoverfly *Episyrphus balteatus*, followed by the dance fly *Empis livida* (see Table 9). The long tongued hoverfly *Rhingia campestris* was also a frequent visitor to *K. arvensis*. This is a very common species throughout Britain and Northern Ireland, fairly large with an extremely long tongue which is more than adequate to reach the bottom of *K. arvensis* corollas. Other, less frequent, short-tongued hoverfly visitors included *Eupeodes corollae* (a medium-sized hoverfly with a relatively short tongue), and *Syrphus ribesii* (a relatively large hoverfly with a relatively short tongue length).



Fig. 24: *Bombus pratorum* feeding upon florets of an inflorescence of *Knautia arvensis*

Of the bee species that visited *K. arvensis*, *Bombus pratorum* was the most frequent (see Fig. 24). All the bumblebee flower visitors had relatively short tongues in relation to other bumblebees, however the tongue lengths of these species are again adequate to reach the bottom of corollas of *K. arvensis*. In addition to the bumblebee species, *K. arvensis* was also visited by the short-tongued cuckoo bumblebee, *Bombus (Psithyrus) bohemicus*.

Centaurea nigra

While other studies have shown this species to be pollinated by butterflies (Corbet, 2000) and bees (e.g. Lack, 1976; 1982d), it has also been shown that *Centaurea nigra* competes for pollinators with *Centaurea scabiosa*, and possibly other species (Lack, 1982a,b,c,d). The nectar reward of *C. nigra* is lower than that of *C. scabiosa* (Lack, 1982d), and its lack of attractive ray florets, except in some individuals where the ray florets of *C. scabiosa* are mimicked, may mean that *C. nigra* is less attractive to bee visitors. Bees visit *C. scabiosa* up to two or three times as often as *C. nigra* (Lack, 1982d), therefore it is possible that *C. nigra* is being outcompeted in this habitat. The site was populated by many apparently bee-pollinated species, including *C. scabiosa*, which may be more attractive to the small numbers of bees available in the area; therefore *C. nigra* is perhaps more actively pollinated by hoverflies in this particular habitat.

Group	Species	N	Mean Size (mm)	Mean Tongue Length (mm)
Hoverfly	<i>Episyrphus balteatus</i>	158	WL 6.00-10.25 ¹	2.89 ²
Hoverfly	<i>Rhingia campestris</i>	38	WL 6-9.5 ¹	11.00 ³
Hoverfly	<i>Eupeodes corollae</i>	12	WL 6.75-7.75 ¹	3.00 ⁴
Hoverfly	<i>Platycheirus manicatus</i>	5	WL 6-7.25 ¹	2.70 ⁴

Table 10: Visitor assemblage of *Centaurea nigra*. WL refers to wing length. ¹ Stubbs and Falk, 2002; ² Gilbert *et al.* 1985; ³ Ssymank, 1991; ⁴ Personal measurements

All species observed to feed upon the flowers of *Centaurea nigra* were feeding on the nectar (see Fig. 25) of flowers, inserting their tongues into the corolla tubes of the florets. Most of the flower visitors, with the exception of *Rhingia campestris*, were relatively short-tongued, as in previous sections above.



Fig. 25: *Episyrphus balteatus* feeding on the nectar of florets of *Centaurea nigra*. Protruding receptive stigma indicated.

Trifolium pratense

Group	Species	N	Mean Size (mm)	Mean Tongue Length (mm)
Hoverfly	<i>Criorhina</i> sp.	18	WL 8-12 ¹	6.1 ⁴
Bumblebee	<i>Bombus hortorum</i>	177	TW 4.74 ³	12.50 ²
Bumblebee	<i>Bombus terrestris</i>	34	TW 4.99 ³	7.60 ²
Bumblebee	<i>Bombus muscorum</i>	31	TW 4.94 ³	8.90 ²
Bumblebee	<i>Bombus lucorum</i>	31	TW 4.70 ³	7.50 ²

Table 11: Visitor assemblage of *Trifolium pratense*. WL refers to wing length. TW refers to thorax width. ¹ Stubbs and Falk, 2002; ² Goulson *et al.*, 2005; ³ Peat *et al.*, 2005; ⁴ Francis Gilbert, personal communication.

The most frequent visitor to *Trifolium pratense* was the bumblebee *Bombus hortorum* (see Fig. 9 and Table 11). This species is classed as a very-long-tongued bee species (Goulson *et al.*, 2008), allowing *B. hortorum* individuals to easily reach the nectar contained in the bottom of the long (between 10 and 15mm) corollas of *T. pratense* (see Fig. 26).



Fig. 26: *Bombus hortorum* feeding on florets of an inflorescence of *Trifolium pratense*.

As before, *Trifolium pratense* was visited by short-tongued species as well as long-tongued species. *Bombus muscorum* was the only other long-tongued bee to visit, while the shorter-tongued bee and hoverfly species failed to empty many of the florets visited.

Partitioning of Visitors over Time

In each of the four plant species studied, the timings of visitors largely followed the thermally-related and size-related patterns described and referenced in earlier chapters. In general, larger flower visitors were active earlier in the morning, but experienced declines over the hottest parts of the day, while smaller visitors were active later in the morning and were better able to cope with high temperatures.

While the visitation rates mentioned relate to a proportion of target flowers rather than

giving an absolute value, and are therefore not representative of the full visitor assemblage and activity patterns of visitor species, it is possible to make some determinations from the data available. In particular, a decline in a particular species does not necessarily mean a decline in the number of individuals active, and could perhaps mean that the species is being outcompeted by others at that particular time; or, conversely, an increase in visits may mean this species is able to outcompete other species at this particular time and gain access to more flowers.

Analysing visitor partitioning by functional groups (see Figs. 9, 11, 14) did not show an accurate depiction of the visitor activity patterns on flowers, as there was variation in body size and other factors within these groups which would influence the thermodynamics of visitors and therefore the timing of their activity. Analysing visitor partitioning by individual species gave a better picture of the patterns of visitor activity (see Figs. 10, 12, 13, 15)

Pollinator Effectiveness

Cirsium arvense

Whereas previous studies have shown the main pollinators of *Cirsium arvense* to be butterflies (Clausen *et al.*, 2001; Tiley, 2010), moths (Plepys *et al.*, 2002), honeybees (Theis, 2006) and other bees (Tiley, 2010), no butterflies or moths were observed to visit target flowers of *Cirsium arvense* during the study period, and indeed very few butterfly or moth visitors were seen at the study site throughout the course of my investigations. This is possibly due to sub-optimum weather conditions or some other factor affecting local populations of butterflies, and I cannot show that butterflies and moths are not pollinators of *Cirsium arvense*. Several butterfly species, in particular the Peacock butterfly *Inachis io*, did visit flowers of *Cirsium arvensis*, however no visits to previously covered target flowers were observed. A more intensive study of *Cirsium arvensis* could provide more information as to the pollinator effectiveness of butterfly and moth visitors to this species.

Visitors were first analysed according to functional group (Fig. 16), all of which deposited significant amounts of pollen (see Table 1). Hoverflies deposited the largest MPS of all groups and were therefore classified as the most effective pollinator group of *Cirsium arvense*. However there is much variation in characters such as body size, behaviour and, most importantly in this case, tongue length, amongst different visitor groups (see Table 8), therefore it is more accurate to analyse pollen deposition by individual species (Fig. 17). *Bombus terrestris* was the only bee species to visit *Cirsium arvense* and was an effective pollinator, though not the most effective (see Table 2). However, when hoverflies and other dipterans were analysed by species we see that not all are effective pollinators. The bluebottle *Calliphora vomitoria* was the only effective pollinator species in the 'other dipteran' group. Overall, the most effective pollinator species was *Episyrphus balteatus*, followed by *Melanostoma mellinum*, *Bombus terrestris* and *Calliphora vomitoria*.

The floral traits of *Cirsium arvense* would indicate that the most effective pollinator of this species would be nectar-collecting, as both male and female flowers produce nectar, but only males produce pollen, and a longer tongue length is required to manipulate male flowers than to manipulate female flowers. While pollen deposition by visitors was significantly more than for control flowers, the stamens of male flowers produce 500-800 pollen grains (Tiley, 2010), so the recorded pollen deposition in *C. arvense* was surprisingly low, with even the "Most Effective Pollinator" depositing a MPS of 3.8 grains. While the relatively short tongues of the visitors enabled them to feed upon the nectar of the shorter female corollas of *Cirsium arvense*, the low pollen deposition may be explained by the fact such visitors would be unable to effectively feed correctly from the longer corollas of male flowers and therefore would be unable to pick up large amounts of pollen (or may have picked up pollen in the wrong place for effective deposition if visiting incorrectly); or they may have been less attracted to the male flowers as a result of the lower nectar reward in relation to flower handling time.

In addition to this, the large number of florets per flower-head combined with the

often short durations of visits made it difficult to determine which florets had been visited and which had been “skipped over” by the visitor. An estimate was made, and pollen grains deposited on stigmas were counted, however many stigmas with no pollen present were found in florets which were suspected to have been visited. These “empty” stigmas were often discounted unless it was certain that they had been visited, therefore it is possible that the MPS for some visitors may have been overestimated. Conversely, some visited florets may have been missed and stigmatic pollen not counted, therefore some species may have been assigned underestimated MPS values.

The pollinators of *C. arvense* do not appear to fit with the syndrome that the floral traits suggest. However given the low local populations of butterflies, moths and bumblebees at the time of the study it is likely that the ‘intended’ pollinators of *C. arvense* were missed by this study. While the shorter-tongued visitors were able to pollinate female flowers, their ineffective manipulation of male flowers meant they were unable to deposit high numbers of pollen grains. When *C. arvense* is subject to low pollination levels, the receptivity period of stigmas can increase from 3 or more days to over 5 days to maximise chances of effective pollination (Lalonde and Roitberg, 1994). Studies of pollen limitation have usually involved manipulating the number of male flowers present, or the distance between male and female flowers (Lalonde and Roitberg, 1989; 1994). A local decline in pollinators which are able to effectively collect pollen from male flowers however, would also have a substantial effect on the reproduction of this species. The shorter-tongued visitors may allow for some pollination of the species, but a more intensive study of this species at a time and place where visitors are much more abundant may find the true Most Effective Pollinator(s) of *C. arvense*.

Knautia arvensis

Previous studies of *Knautia arvensis* have indicated that it is pollinated predominantly by bees and butterflies (Lack, 1982; Coomba *et al.*, 1999), although these conclusions were based upon visitations to flowers and effective pollination was not determined. Larsson

(2005) used pollen removal from anthers and deposition on stigmas as a measure of pollinator effectiveness comparing one individual solitary bee species, *Andrena hattorfiana*, to several functional groups of flower visitor; bumblebees, other bees, furry dipterans, non-furry dipterans, lepidopterans and beetles. The study found that female *A. hattorfiana* removed and deposited a higher amount of pollen than any of the other visitor groups.

As described above, there were few butterfly and moth visitors during my study period, and none were observed to visit *K. arvensis*, therefore it is not possible to determine the effectiveness of these species at pollinating the flowers.

In a similar manner to Larsson's study, the pollen deposition on stigmas of observed visitors was first analysed by functional groups; bumblebees, cuckoo bumblebees, hoverflies and other dipterans (see Fig. 18). All three groups deposited a significantly greater MPS in comparison to control stigmas, and were therefore classified as pollinators (see Table 3). The highest MPS was deposited by the hoverfly group, followed by the cuckoo bumblebees, the other dipterans and the bumblebees. This is at first sight surprising given the apparent long-tongued bee syndrome of the species, and previous studies indicating bees to be the predominant pollinators of *K. arvensis*.

As described above (see Table 9), there is great variation within the functional groups which visited *K. arvensis* in terms of tongue-length, body size, hairiness and behaviour. Given such variation within the functional groups, it is difficult to justify analysing pollinator effectiveness in this manner. As I have shown above, and in previous chapters, it is much more accurate to analyse pollinator effectiveness by species (see Fig. 19). Variation in MPS between visitor species is then statistically significant. *Bombus terrestris*, *Bombus lucorum*, *Bombus pratorum*, *Psithyrus bohemicus*, *Empis livida*, *Episyrphus balteatus*, *Rhingia campestris* and *Syrphus ribesii* all deposited a significant MPS and could be classified as pollinators (see Table 4), and most of these are relatively long-tongued. The Most Effective Pollinator species of *K. arvensis* was the hoverfly *Rhingia campestris*,

followed by *Empis livida*. Within the bumblebee group, *Bombus pratorum* had the highest MPS, followed by *Bombus lucorum*. This looks surprising given the relatively short tongue of *B. pratorum*; however the variation between the two bee species was not significant, and it should be remembered that all *Bombus* are reasonably 'long-tongued' relative to most other visitor groups. What is clear is that not all the species within a functional group can be classed as pollinators, and variation between identified pollinators of a group can often be significant. Analysing pollinator effectiveness according to functional groups, as in Larsson's study, can therefore be misleading and inaccurate.

In terms of the most effective pollinator species of *K. arvensis*, the results above may not be as expected. While bumblebees did deposit significantly more pollen than several other observed species, they were not the most significant in terms of pollen deposition. *Rhingia campestris* and *Empis livida* are extremely long-tongued flies in comparison to others within their functional groups however, and possess more than adequate tongues to manipulate the corollas of *Knautia arvensis*, therefore their relatively high pollen deposition is not surprising.

Again, it should be noted that local bumblebee populations around the time of my observations were low, (in line with the low butterfly and moth populations), and this study is not necessarily an accurate representation of the 'normal' visitor assemblage of *K. arvensis*.

Centaurea nigra

The most effective pollinator species of *Centaurea nigra* in terms of MPS was *Episyrphus balteatus*, (see Fig. 20 and Table 5); however the individual variation in MPS within this species was extremely high, and a major reason for the non-normal distribution of the data. *Eupeodes corollae* was the second most effective pollinator and *Rhingia campestris* was the least effective.

In comparison to other hoverflies, the species that visited *Centaurea nigra* had relatively long tongues (see Table 10); however, given the length of corolla of *Centaurea nigra*

(about 4.5-5 mm, Corbet, 2000; and between 4 and 5.5mm from personal observations) it is surprising that the most effective species had tongues much shorter than the length of the corollas. In the same way that shorter-tongued visitors were able to effectively pollinate the long-corolla flowers of the long-tongued bee-pollinated flowers of *Cirsium arvense* and *Knautia arvensis*, these shorter-tongued visitors may be able to feed on full corollas, obtaining some nectar without fully draining flowers, while still coming into contact with the reproductive structures; the receptive stigma protruding from the corolla tubes (see Fig. 3) and the anthers located just below the lip of the corolla tube. It would appear therefore that corolla tube lengthening does not necessarily affect the ability of visitors to reach reproductive structures in flowers such as these, though it may serve to deter visitors with extremely short tongues that would be unable to effectively feed on sufficient amounts of nectar.

Rhingia campestris had the longest tongue length of the pollinators; however it had the lowest pollen deposition. Long-tongued insects may be expected to make use of a wider range of flowers, not being excluded from flowers with short corollas in the same way that those with shorter tongues can be excluded from long corollas; but long tongues become unwieldy on flowers with relatively short corollas. Furthermore the nectar of such flowers is often too concentrated to be sucked up a long tubular tongue (Willmer, 2011). The excessive length of the proboscis of *Rhingia campestris* may therefore act as a hindrance in this case.

While no distinction could be made between self and non-self conspecific pollen, the self-incompatibility of this species means that self pollen will either not germinate on stigmas, or if it does, the pollen tube will be blocked by a callose plug (Heslop-Harrison, 1975; de Nettancourt, 1977). A further study of these species investigating which, if any, or the deposited pollen grains germinate or remain unblocked would allow for a more detailed analysis of the pollinator effectiveness of visitors to *Centaurea nigra*, distinguishing between visitors that are promoting outcrossing, and those which are merely moving around self pollen which will not contribute to fertilisation.

Trifolium pratense

Previous studies have indicated that flowers of *T. pratense* are pollinated by bees (Plowright and Hartling, 1981; Free, 1993; Coomba *et al.*, 1999). Most studies have based such conclusions on visitations, although Plowright and Hartling (1981) showed significant seed set in flowers of *T. pratense* visited by bumblebees.

The pollinator effectiveness of visitors to *Trifolium pratense* was first analysed by the two functional groups, bees and hoverflies (see Fig. 21), and both deposited a significant MPS in comparison to control stigmas, and were therefore classified as pollinators (see Table 6). Of the two, the hoverflies deposited a higher number of pollen grains than the bee group, but variation between the two groups was not significant.

When the functional groups were split and pollinator effectiveness was analysed by individual species (see Fig. 22 and Table 7), we see that the previous method obscures much of the variation in pollinator effectiveness. Only one hoverfly species, *Criorhina* sp., was observed to visit *T. pratense*. This is a bee-mimic hoverfly with a tongue length of approximately 6.1mm, relatively long in comparison to other hoverfly species, and this length appears to allow the species to effectively feed from flowers of *T. pratense*.

Within the bee group however, there were 4 different species; *Bombus terrestris*, *Bombus lucorum*, *Bombus hortorum* and *Bombus muscorum*. Variation in MPS between visitor species was significant, and all visitor species had a significant MPS in comparison to the control flowers and were classed as pollinators. *Bombus lucorum* had the highest MPS. Given the relatively short tongue length of *B. lucorum*, *B. terrestris* and *B. muscorum* in comparison to *B. hortorum* (see Table 11), and the long corolla length of *T. pratense* florets, the high pollinator effectiveness of these 3 species is surprising. They did not have adequately long tongues to effectively manipulate the florets of *T. pratense* yet they appear to be able to effectively pollinate the species. When we consider the placement of pollen and stigmas within the 10-15mm long corollas however, at the end of a style approximately

10mm long, it is clear that while a long tongue is required to reach all of the nectar contained within a floret, it is not necessary to reach the anthers and stigmas, therefore pollination can still occur even if a flower is not fully drained. Very short-tongued visitors were not recorded, though a number of medium-length to long-tongue length visitors were recorded, and were all classed as effective pollinators. It may be that in the case of *T. pratense* the length of corollas deters short-tongued species as the reward available to them (i.e. what can be reached from the top of full corollas) is not great enough to offset the energy expended in foraging from such flowers; whereas species with a medium to long tongue may not be able to completely empty flowers, but can gather enough nectar to make a foraging bout worthwhile. In this case, the height of the reproductive structure ensures that, should a visitor be enticed to a flower it will be able to effectively pollinate it; and if it does not completely empty said flower, further visitations by longer-tongued species are then possible, increasing the likelihood of outcrossing and successful pollination.

My study period of *T. pratense* was short though, and a longer, more intensive study may offer more information on the effectiveness of other visitor species. It is rather likely that the limitations of the study have “missed” other active flower visitors to *T. pratense*. Nevertheless this study does show that, even within a functional group in which all species are effective pollinators, variation in this effectiveness is present.

Conclusion

All four flowers species studied showed traits indicative of a Long-Tongued Insect-Pollinated syndrome as described by Faegri and Van der Pijl (1979) and others (e.g. Willmer, 2011), the main characteristic being a long corolla tube excluding shorter-tongued visitors from effectively extracting nectar. In all cases, however, the flowers were visited by species not necessarily predicted by this syndrome. The deposition of pollen on stigmas was once again used as a measure of effective pollination of each species, and showed that, in many cases, the most effective pollinator species was not as expected.

There are several possible explanations for these results. The first is an acknowledgement of the limitations of the study. Local populations of several key visitor species were low during the time of the study, possibly due to adverse weather conditions for all taxa, and compounded by the recent well-documented declines in bee numbers. In particular, butterfly, moth and bee populations were lower than would be expected. This may have led to an under-representation of certain species, and an over-representation of others which may have taken advantage of vacated niches. In addition to this, a more accurate representation of the visitor fauna of each species could be gained with a longer, more intensive study.

While unexpected, the results of the studies described here can be accounted for. Hoverflies were surprisingly effective pollinators of all species. Some specialised hoverflies possess relatively long tongues for flower feeding, and can manipulate flowers that would appear to be suited towards bees. Hoverflies are known to be important pollinators, with many overlaps between “bee-flowers” and “specialised hoverfly-flowers”. Hoverflies often become important pollinators on typical bee flowers at times when bees are scarce (Freitas and Sazima, 2003; Willmer, 2011). Hoverflies are able to manipulate complex zygomorphic corollas such as the ones possessed by *Cirsium arvense*, *Knautia arvensis*, *Centaurea nigra* and *Trifolium pratense* more effectively than most other insects, and often work systematically around the capitula of composite flowers (Gilbert, 1983). Given the low local populations of bee species around the time of the study, the effectiveness of hoverfly visitors is not in fact surprising.

Another factor is the placement of reproductive structures in the species studied. While the length of the corolla excludes the majority of visitors from being able to fully empty flowers of nectar, many others are still able to feed from full corollas at least partially. By excluding flower visitors using netting, the methods of this study ensured that target flowers were full of nectar when visited, and therefore could be more easily exploited by shorter-tongued visitors. As the stigmas and anthers are placed high in the corolla, even visitors that

are only partially able to remove nectar from corollas are able to effectively pollinate flowers. In addition, by leaving some nectar in the corolla the flower can be further visited by longer-tongued visitors, thereby increasing outcrossing potential and pollination success. While tongue length may be a factor in the decision of a visitor to feed from a flower, by increasing cost of foraging and reducing the quantity of reward gathered, a short tongue does not necessarily prevent effective pollination of a species.

This study has shown again that when visitors are analysed according to functional groups rather than by individual species much of the variation within groups is missed. Some species may be incorrectly identified as pollinators, while some may be missed if others within their functional group are poor pollinators. I have shown that variation in pollinator effectiveness between species and within functional groups is common and that collating species into functional groups is an ineffective means of study.

While this study does not completely refute or support the Long-Tongued Insect-Flower syndrome, it does perhaps suggest that our classification of such syndromes should be extended beyond the constraints of functional groups. Such flowers may often be classified as purely bee-pollinated (in particular long-tongued bees) based upon their floral traits, without considering the substantial overlap between specialised bee-flowers and specialised hoverfly-flowers. While the most effective pollinators of these flowers are not necessarily long-tongued bees, they are long-tongued insects with similar morphologies. This is why the syndrome is perhaps better considered a Long-Tongued Insect-Flower syndrome incorporating both long-tongued bees and long-tongued flies, rather than one which is exclusively bees; these visitor species often show much overlap in their morphologies and flower visiting behaviour and can therefore effectively share a pollination syndrome. Despite the number and variation in pollinator species, *Cirsium arvense*, *Knautia arvensis*, *Centaurea nigra* and *Trifolium pratense* are by no means “Generalised” flowers, and show adaptations that attract certain pollinator types, and exclude others. They are therefore more specialised than a visitation survey might suggest, further reinforcing the

concept that a measure of Pollinator Effectiveness is an important part of any pollination study.

Chapter 8: Testing Pollination Syndromes

The Generalist Pollination Syndrome

Introduction

It is often assumed that the existence of “generalist flowers” provides an argument against the concept of pollinator syndromes. It can also be argued, however that this floral type is a syndrome in itself. While these flowers have been described as catering for the “mass market” (Proctor *et al.*, 1996), their visitor assemblage is not quite as broadly generalist as one would assume, given that long-tongued visitors or larger vertebrates are excluded and visitation is most often by shorter-tongued or small insects. Again, as seen in other chapters, this shows the problem associated with applying pollinator syndromes to taxonomic groups rather than to functional groups. While the visitors to “generalist” flowers cross a number of different taxonomic groups, they could be considered part of the same functional group of small, short-tongued insects, in the same way that insects of different taxonomic groups could be considered long-tongued, or large-bodied (Corbet, 2006).

“Generalist” Flower Visitors

Certain species of visitor, while also possessing their own, more specialised “syndromes”, are considered “generalist” visitors and frequently visit flowers of the generalist flower syndrome as well as flowers from other syndromes. There are many insects that will make occasional visits to flowers to feed on nectar in addition to their core diet, and can therefore be occasional, opportunistic pollinators. Not all insects within the “generalist” functional group are occasional flower visitors however, and some are regular flower feeders covering a range of taxonomic groups such as beetles, wasps, and other more unusual taxa.

While they create highly variable visitation patterns, there are several shared features, both of the visitors and the flowers they frequent, that can allow us to further define the syndrome.

Coleoptera

The order Coleoptera is the largest insect order currently described, and about 30 families contain at least a few flower-visiting species. Beetles are estimated to be responsible for the pollination of 88% of all known angiosperms (Buchmann and Nabhan, 1996), and 184 angiosperm species are pollinated almost exclusively by beetles, while 98 are pollinated by a combination of beetles and other visitors (Bernhardt, 2000).

While beetles are important pollinators, they are often overlooked in the literature, due in part to the fact they are more obvious in warm Mediterranean habitats, or tropical and arid habitats of the Southern hemisphere, where pollination ecology lags behind the fieldwork of the temperate regions of the Northern Hemisphere (Momose *et al.*, 1998; Willmer, 2011).

The mouthparts of beetles are primarily for chewing, and they can therefore be quite destructive flower feeders, often consuming whole flowers, including petals and ovule tissues. Regardless of their destructiveness however, some beetle types are able to disperse moderate amounts of pollen to successive flowers, often several metres, or up to tens of metres away. Some of the more effective pollen-moving species possess relatively hairy bodies to aid with the adherence of pollen. Beetles do not always act in the same way on different flowers, and some may completely destroy certain flower species, while non-destructively pollinating others (Hawkeswood, 1989).

Some flower-visiting beetles possess adaptations towards pollen-collecting, such as the long, “pollen-brush” hairs of the maxillae in some chafer beetle and cerambycids, or the spoon-like bristles of *Malachius* used for scooping up pollen (Barth, 1985; Bernhardt, 1996). While these adaptations are primarily for the consumption of pollen, they may also play a

role in its transportation between flowers. Some flower-visiting species also show adaptations towards nectar feeding, such as the elongation of the rostrum in some *Lycus* species, or the elongated proboscis of *Nemognatha* (Hawkeswood, 2002; Hawkeswood and Turner, 2004; Krenn *et al.*, 2005; Nicolson, 2007).

As a consequence of these relatively specialised feeding habits, some beetles show high levels of floral constancy, for example the long-range pollen dispersal (sometimes up to 18m) and floral constancy of *Cetonia* beetles visiting *Viburnum opulus* (Englund, 1993). Floral constancy has also been shown for alleculid (de los Mozas Pascual and Domingo, 1991) and byturid beetles (Pellmyr, 1985; Willmer *et al.*, 1996) as well as for some beetle visitors of palm flowers (e.g. Eriksson, 1994; Listabarth, 1996).

Odour is thought to be the most important cue for beetles when searching for flowers, with colour playing a secondary role at close range, though perhaps being more important in some of the more specialised interactions between specific beetle species and particular flowers (Pellmyr and Patt, 1986; Eriksson, 1994; Weiss, 2001).

There are relatively few studies on the effectiveness of beetles as pollinators, though some have shown that beetle pollinators are capable of transporting pollen over fairly large distances (Englund, 1993), or carrying large amounts of pollen between flowers (Kwak and Bekker, 2006). In general however, beetles are not as highly mobile as bees or other flower visitors, and are more likely to sit passively on flowers for long periods of time due to the increased protection of their hardened elytra which allows them to remain unthreatened by disturbances which would disperse other insects (Willmer, 2011). Beetles are therefore thought to usually move relatively small amounts of pollen between only a few flowers; however their relatively high levels of floral constancy make it more likely that such pollen will be transported to an appropriate place.

Beetles therefore have their own pollinator syndrome, cantharophily, although many of the features attributed to cantharophily are not unique to this syndrome. Many species

visited by beetles are also visited by other animals of different taxa, and beetles are also known to visit flowers characteristic of the generalist syndrome, as described below.

Hymenoptera: Wasps

The term “wasps” is a broad one, which technically also involves bees and ants, given that they are derived from the ancestral wasp lineage; however these shall be considered separately as their interactions with flowers are considerably different. The remaining species within the wasp classification can be further divided into sawflies, of the sub-order Symphyta, defined as those without a wasp-waist, and the Apocrita, which possess a classic wasp-waist. The Apocrita can be further divided into the Parasitica, possessing an ovipositor used for laying eggs in hosts, and the Aculeata, where the ovipositor is modified into a sting.

Some sawflies consume nectar, and may also consume pollen and honeydew. In particular, the females are often regular flower visitors, and will eat not only the intended floral rewards, but also the petals and stamens (e.g. Willis and Burkill, 1895; 1903a, b; 1908; Jervis *et al.*, 1993; Jervis, 1998). Therefore, in the same way as beetles, the pollinator effectiveness of sawflies may be undermined by the damage they do to floral tissues. Some species, however, have modified mouthparts allowing for less damaging flower feeding, such as the pollen-feeding mouthparts of Xyelidae, or the long proboscides of some other families which allow for the extraction of semi-concealed nectar (Jervis and Vilhelmsen, 2000). Most species of sawflies possess relatively short, unspecialised mouthparts however, and are most commonly seen on open flowers with exposed rewards.

Of the Parasitica, some ichneumon and braconid wasps are common flower visitors, feeding on both floral and extrafloral nectar, sap or honeydew (e.g. Noordijk *et al.*, 2009). The majority have extremely short mouthparts, often less than 1mm, therefore are most commonly found on open flowers with exposed rewards, similar to those frequented by sawflies, though they may be more restricted in their flower visits to only a few species

(Tooker and Hanks, 2000). There are a few plant species identified as being pollinated by ichneumons, such as the twayblade orchids of the genus *Listeria* (Sprengel, 1793; Müller, 1878; Brys *et al.*, 2008), though these orchids are also attractive to other insects (Nilsson; 1981), or the pseudocopulatory deceit pollination of the orchid species *Cryptostylis* by an ichneumon wasp (Roberts, 2003).

Gall wasps (Cynipidae) are very tiny insects possessing extremely short mouthparts, and are therefore not common flower visitors. While some do use flowers as an occasional food source, their effectiveness as pollinators has not been shown. One exception however, are the fig wasps, of the related Agaonidae family, which have an extremely specialised relationship with the flowers of figs, as described in more detail in chapter 1.

Within the Aculeata, the “true wasps”, many species are known to be flower visitors. The chrysidids, “rubytail wasps”, and scolioids, “velvet ants”, are visitors of flowers; however their shiny surfaces and lack of wings respectively render them largely ineffective as pollinators (Willmer, 2011).

Members of the solitary wasp groups sphecids, pompilids, tiphids and eumenids are known to feed on nectar between prey-gathering foraging trips. They can be seen feeding from open bowl shaped flowers and generalists such as those in this chapter, as well as from some species with more concealed nectar. There are also some examples of more specialised relationships between flowers and solitary wasps, for example the pollination of orchid species by sphecid and pompilid wasps (Johnson, 2005), and some sphecids show remarkable floral constancy to asclepiads (Theiss *et al.*, 2007). The thynnine wasps of the Tiphidae are known for pseudocopulatory interactions with hammer orchids (Peakall, 1990), and the larvae of the sphecid *Krombeinictus* feed solely on nectar and pollen, paralleling the feeding habits of bees (Krombein and Norden, 1997).

The social wasps, vespids, are known to take some nectar from flowers for feeding their young. They have similar flower preferences to generalist flies, and can be effective

pollinators of species such as ivy (Ollerton *et al.*, 2007). The common yellow jacket wasps have longer tongues than most of the other wasps mentioned, and are able to frequent more specialised flowers. Some flower species show specialised relationships with vespids, such as the pollination of the deceptive orchid *Dendrobium* by the hornet species *Vespa bicolor* (Brodmann *et al.*, 2008).

Finally, the masarids are a group closely related to the vespids, and known for pollen-collecting. They can be abundant on flowers, collecting pollen on their hairy faces and transferring it to subsequently visited flowers (Cooper, 1952). Their flower preferences are similar to those of short-tongued bees, though there are examples of more specialised interactions such as the pollination of *Nigella arvensis* flowers by *Ceramius bureschi* (Mauss *et al.*, 2007).

Hymenoptera: Ants

Ants (Formicoidea), evolved from wasps, are closely related to bees and the wasps mentioned above. They are extremely abundant in almost all habitats, and could be important to flowers on the basis of sheer number alone. They do however possess several traits making them ineffective as pollinators. Their small size and lack of wings make them a poor physical fit for many flowers, and ill-suited to transporting pollen over great distances. Their shiny and hairless surfaces are not conducive to pollen adhesion, and the anti-bacterial and anti-fungal secretions of their metapleural glands are damaging to pollen longevity and fertility (Beattie *et al.*, 1984; Hull and Beattie, 1998; Galen and Butchart, 2003), while their elongated mandibles are ill-adapted towards pollen and nectar feeding.

Despite this, ants are highly attracted to sugary solutions such as honeydew and sometimes nectar, and can be common visitors of open, bowl-shaped flowers with exposed rewards, such as those within the generalist syndrome. Generally, ants are considered poor pollinators, and more often nectar thieves than effective pollinators. However some plant genera such as *Herniaria*, *Paronychia*, *Trinia glauca*, *Diamorpha smallii* and *Polygonum*

cadense, do show adaptations towards ant-pollination, including small, open flowers close to the stem with almost no stalk, prostrate and often being intertwined with other plants (Hickman, 1974; Wyatt, 1981; Peakall *et al.*, 1991; Proctor *et al.*, 1996; Carneiro *et al.*, 2008).

Examples of ant-pollination can also occur in plant species that do not appear to be specially adapted towards ants, for example the Mediterranean parasitic plant *Cytinus hypocistus*, pollinated by various species of ant (de Vega *et al.*, 2009), or in more montane habitats the pollination of *Paronychia pulvinata* (Puterbaugh, 1998).

Ants are thought to be “fall-back” pollinators in cases where other pollinators are rare, such as ant-induced self-pollination in *Blandfordia grandiflora* when bees or birds fail to be effective pollinators (Ramsey, 1995). In some cases, for example in the mass flowering species *Hormathophylla*, the sheer abundance of ants allows them to be effective pollinators so long as the pollen does not remain on their bodies for too long (Gomez and Zamora, 1992).

Hymenoptera: Bees

Generalist flowers may also be visited by small, short-tongued, relatively unspecialised bees. The majority of solitary bees in Europe and North America are short to medium-tongued: small halictid and andrenid bees are common in Palaearctic and Nearctic habitats, and the very short-tongued colletids occur in most communities and are dominant in Australasia. Small bees differ from large bees in their behaviour, flying slower and lower, and being more attracted to radial flowers with a dissected rim (Dafni *et al.*, 1997). Such bees are common on the more generalist flower types such as hawthorn, bramble and bindweed (reviewed in Willmer, 2011).

In warmer and more tropical climes, small solitary bees are common on small, often white, radially symmetrical flowers of trees, shrubs, climbers and herbs. The more social

species of stingless bees (Meliponinae) are often also important pollinators in tropical and sub-tropical ecosystems. They are usually small in size, and most have short to medium-length tongues, and require small amounts of low to medium-concentrated nectar, often from small or short-corolla flowers. They are frequent visitors to many flowers, often acting as nectar or pollen thieves on larger flowers (see Chapter 3); however they have been shown to be effective pollinators of at least 18 crop species (Slaa *et al.*, 2006).

In temperate climates, some of the shorter-tongued bumblebee species such as *Bombus lucorum* and *Bombus bifarius* may visit more generalist flower types than the longer-tongued species, being unable to forage effectively from those flowers typically considered as bumblebee-visited (see chapter 5) and exploiting flowers with short corollas or open, bowl-shaped flowers.

Honeybees, of the genus *Apis*, are fairly “average” bees, medium in size, tongue-length and endothermic abilities and they will visit almost any flower in the habitats they frequent, often being termed “super-generalists”. They will often select more open or radial flower designs, and frequently visit white, yellow and orange flowers that are not so frequently visited by other bees. Collectively, as a genus, honeybees are the most generalist and polylectic of all pollinator species, although individuals can be rather specialised and show strong floral constancy in a single foraging trip or across a whole day (e.g. Basualdo *et al.*, 2000; Montgomery, 2009; Fohouo *et al.*, 2010).

Many of the above bee types will visit the open, generalist flower forms described below, sometimes in addition to preferred, more specialised flower types. They are often a good physical fit to these flower forms, and their high floral constancy increases their pollinator efficiency.

Thysanoptera

Thrips, also known as thunder flies, are tiny insects approximately 1-2mm long. They possess piercing and sucking mouthparts and are often thought of as significant plant pests due to their virus-transmitting potential. They can be incredibly abundant within flowers and feeding on nectar, as well as sucking the liquid from pollen grains. For this reason they are normally considered pollen destroyers and accidental pollinators, transporting small amounts of pollen on their bristly bodies (Kirk, 1984; 1985; 1987).

In some cases, however, thrips can be important pollinators, for example of the diptereocarp trees (Appanah and Chan, 1981) and some of the Annonaceae (Momose *et al.*, 1998) of the South East Asian tropical rainforest, as well as some ant-plants (Moog *et al.*, 2002) and endemic moraceous plants of New Guinea (Zerega *et al.*, 2004). In lowland New Zealand forests (Norton, 1984) and in some cycads of Australia (Mound and Terry, 2001; Terry *et al.*, 2005) thrips can be reasonably effective pollinators. They are also thought to be effective pollinators in colder climes where bees and butterflies are rare (Hagerup, 1950; Baker and Cruden, 1991; Garcia-Fayos and Goldarazena, 2008) and may be important commercial pollinators on crops such as onion, bean, sugar beet, plum, cacao and certain chilli plants (Saxena *et al.*, 1996).

Diptera

Many flowers with a generalist form are visited by Diptera, both hoverflies, as described in chapters 6 and 7, and more “general” flies. The primitive flies of the suborder Nematocera, which include midges, mosquitoes, gnats and crane flies, are mostly small with short mouthparts, lacking the qualities of effective pollinators but nonetheless common flower visitors. They mainly take nectar, though some may also feed on pollen (Willis & Burkill, 1895-1908, UK flower visiting records; Vogel, 1978a,b; Mesler *et al.*, 1980; Sugawara, 1988; Olesen and Warncke, 1989; Kato *et al.*, 1990; Proctor *et al.*, 1996; Vogel

and Martens, 2000; Okuyama *et al.*, 2004), and they are particularly active on flowers around dusk.

The suborder Brachycera include several important flower-visiting families, for example the Stratiomyidae (soldier flies), Rhagionidae (snipe flies), as well as the acrocerids, species of which show strong floral constancy to geraniums and similar flowers (Borkent and Schilinger, 2008). and the families of the more advanced Muscomorpha and Heterodactyla Infra-orders (Nemestrinidae, Apioceridae, Bombyliidae, Asilidae, Threvidae) Some of the most important flower visitors come from the more advanced Muscomorpha and Infra-order, such as the Bombyliidae, or bee-flies, and the nemestrinids, both reasonably long-tongued and important pollinators of many long-corolla flowers, as well as the Apioceridae (flower-loving flies), the Asilidae (robber flies) and the Threvidae (stiletto flies).

Alongside the specialised flower-feeding Syrphidae (see Chapters 4 and 7), the infra-order Cyclorrhapha also includes the Phoridae (scuttle flies), which are important potential pollinators of flowers such as Araceae and some Aristolochiaceae (Rulik *et al.*, 2008). Finally, the infra-order Schizophora includes the “higher flies”, within which are a number of smaller families such as the Drosophilidae, (fruit flies), Muscidae (house flies), Fannidae (house flies), Anthomyiidae (lesser house flies), Calliphoridae (blowflies and bluebottles) and Scathophagidae (dungflies), many of which show common flower visiting behaviour.

The general structure of the mouthparts of flies, and the more specialised Syrphidae, is described in further detail in Chapter 4. In flies with a relatively short proboscis, exposed fluids can be exploited with a dabbing or lapping motion, which draws fluid into the pads of the labium (see Fig. 1 of Chapter 4), but they can also use quite solid materials by first suspending the particles in saliva regurgitated through the hypopharynx (Gilbert and Jervis, 1998; Krenn *et al.*, 2005; Willmer, 2011). In those families with more elongated proboscides, feeding from long, tubular corollas becomes possible, as well as from more generalist open flowers. In Bombyliid flies, for example, the ventral part of the proboscis base is lengthened,

and the suctorial mechanism is also more powerful, leading to a tongue which can penetrate and suck fluid from long corolla tubes. The labellar musculature is also altered in a way that allows these flies to feed from laterally opening flowers as well as those with frontal and dorsal openings (Szusich and Krenn, 2002). Where pollen is taken in (suspended in nectar, especially in the syrphids), mouthparts tend to be shorter with broader labella and more pseudotracheae, the width of the furrows possibly reflecting the size of the pollen grains (Gilbert and Jervis, 1998; see Fig. 2 of Chapter 4).

Flowers traditionally classified as myophilous, or fly-pollinated, share many traits with the generalist flower syndrome, and overlap with it considerably, as described below.

Other Insects

In addition to the above, there are some sparse records of pollination by cockroaches (Nagamitsu and Inouye, 1997), termites (Dixon *et al.*, 1990), grasshoppers (Philipp *et al.*, 2006), crickets (Micheneau *et al.*, 2010), lacewings (Altieri and Whitcomb, 1979; Al-Doghari and Cranshaw, 1999) and hemipteran bugs (Ishida *et al.*, 2009), which have all been shown to move considerable amounts of pollen between certain flowers.

The Generalist Flower Syndrome

Flowers of the generalist flower syndrome share a number of typical characteristics, described by Willmer (2011). These are summarised in the table below.

Flower Structure	Small flowers. Open, radial, bowl-shaped or flat
Arrangement	Often grouped into inflorescences
Flower colour	White, cream or yellow-green
Nectar	Exposed, high concentration and low volume
Pollen	Easily accessible
Odour	Mild; sweet or musty

Table 1: Summary of the typical traits of the generalist flower syndrome (described by Willmer, 2011)

Floral Shape and Size

The open, disc, flat or bowl shape of these flowers makes them easily accessible to a wide variety of flower visitors. Long tongues are often considered unwieldy and ineffective on short corolla tubes or open flowers (Plowright and Plowright, 1997; Peat, *et al.*, 2005; Willmer, 2011; see Chapter 7) and intense competition with short-tongued visitors will drive long-tongued visitors to flowers with longer corollas (Rodríguez-Gironés and Santamaría, 2004), therefore these flower forms are more often frequented by short-tongued visitors.

Floral Colour

Many different types of visitors are known to visit flowers with white flowers, in particular beetles, dipterans, butterflies, moths, bats and bees (reviewed by Willmer, 2011). Other flower colours such as red, yellow, blue or purple tend to attract specific pollinators based on their colour vision and innate preferences (see earlier chapters) while white colouring serves as more of a “catch all” attracting a variety of generalist species, while possibly excluding more selective and specialised visitor species. It should be noted however, that flower which seem white by human perceptions, are rarely observed as white by insect eyes. “Human-white” flowers are nearly always UV-absorbent and appear highly chromatic to insect eyes (Daumer, 1958; Kugler, 1963; Kevan, 1972; 1978; 1983; Menzel and Shmida, 1993; Chittka *et al.*, 1994; Kevan *et al.*, 1996), and therefore may be more attractive to insect visitors than they at first appear.

Odour

Flowers of a generalist form often have a mild sweet or musty odour (Willmer, 2011). As they attract visitors from a wide range of different taxonomic groups with different odour detecting capabilities and preferences, odour is perhaps not so strong an attractant as other traits.

Nectar Volume and Composition

Generalist flowers are often small, and therefore nectar rewards are not normally large in volume. Being open with exposed nectar subject to evaporation, the flowers tend to offer more concentrated rewards, which may be more difficult for long-tongued insects to consume through their tubular tongues (Willmer, 2011), as, while there is a positive correlation between bee tongue length and ingestion rate (Harder, 1983), fluid viscosity negatively affects ingestion rate at concentrations greater than 35-40% sucrose (Harder, 1986). Generalist flowers with concentrated rewards can therefore exclude long-tongued visitors while providing sufficient rewards for shorter-tongued, smaller species, especially short-tongued flies which are able to spit into the nectar (even when it is crystalline) and then take up the resulting somewhat diluted fluid.

Placement of Reproductive Structures

The reproductive structure of generalist flowers should be easily accessible and close to the flower reward, allowing for the visitors to contact both stigmas and anthers when they feed upon flowers, reducing the possibility of nectar robbery. The open form of the flower makes concealment of both nectar and reproductive structures difficult; therefore it is in the plant's interests to ensure that, when visits are made, effective pollination can occur relatively easily by a variety of variable visitor types, though in most cases small-bodied and short-tongued.

Variation in Traits and Visitors between Populations

The visitor assemblages encountered by generalist flowers can vary between populations, which can have a significant effect on reproductive output (Davila and Wardle, 2008). Some generalist species have diverged in the visitors they attract, without permanently specialising towards them (Dilley *et al.*, 2000). If, for example, the visitor assemblage of a plant species is diverse and similar in its pollinator effectiveness, but with

visitors exerting different selection pressures on the plant, the significant differences between different insect types in patterns of variation in visitation rates in response to floral design and display may act to diversify selection on floral traits, and thereby constrain specialisation of the plant to particular pollinators (Thompson, 2001).

The Geographic Mosaic Theory of Coevolution predicts the occurrence of mosaics of interaction-mediated local adaptations and maladaptations (Thompson, 2005), empirical support for which has come mostly from specialist interactions. It is now becoming clearer, however, that this theory also extends to generalist flowers (Gomez *et al.*, 2009). Corolla shape in *Erysimum mediohispanicum* is found to vary spatially depending on whether the visitor assemblage is dominated by bees, or bee flies (Gomez *et al.*, 2008), and flowers of *Paeonia broteroi* vary in characters such as the number of flowers per plant, petal size, number of stamens per flower and ovules per carpel between populations where visitors differed in size and assemblage (Sanchez-Lafuente, 2002), however, in this case it was also suggested the influence of pollinator selection was not the only factor contributing to differences in flower size and integration (Sanchez-Lafuente and Parra, 2009).

While the above traits (Table 1) are common among generalist flowers, it is often the case that “generalist” species show variation in characters such as flower size and nectar production in different locations, therefore it is important to note that the following study applies only to the given populations of plant species studied, and result may not be universally true for the species.

Testing the Generalist Flower Syndrome

To test the existence of a generalist flower syndrome, two British wildflower species and one tropical example showing traits indicative of the syndrome were investigated. *Heracleum sphondylium* is a well-studied British species known to be visited by members of the orders Ephemeroptera, Dermaptera, Hemiptera, Thysanoptera, Neuroptera, Mecoptera, Lepidoptera, Diptera, Hymenoptera and Coleoptera (e.g. Sheppard, 1991), and *Rubus*

fruticosus is another well-studied British species visited by bees and hoverflies (e.g. Yeboah Gyan and Woodell, 1987a). Flowers of the tropical *Ipomoea trifida*, related to the sweet potato, are well-documented to be self-incompatible (e.g. Kowyama *et al.*, 2000), and while studies of the pollinator assemblage of *I. trifida* in particular are absent, the pollinator assemblage of *Ipomoea* as a genus is known to include beetles, flies, bees, butterflies, moths, hummingbirds and bats (McDonald, 1991; Chemás-Jaramillo and Bullock, 2002; Galetto and Bernardello, 2004; Wolfe and Sowell, 2006). No studies into the effectiveness of the various visitors of the above species have taken place, and while I would expect that many of them are effective pollinators given the features of the generalist syndrome above, I would also expect that, given the vast differences in their form and behaviour, the visitors would also vary in their pollinator effectiveness. To test this theory, the effectiveness of the visitors to these species was calculated using single-visit stigmatic pollen deposition as a measure of effectiveness as in previous chapters (see Chapter 2 for protocols).

Materials and Methods

Study Sites

The population of *Ipomoea trifida* studied was located in the dry, disturbed, deciduous forest area of Parque Nacional Santa Rosa, Guanacaste Province (10° 50' N, 85° 40' W), in the North-West region of Costa Rica. The park covers about 495 square kilometres and contains savannah, deciduous forest, marshland and mangrove swamp habitats. The population of *Heracleum sphondylium* investigated was located at West Quarry Braes, a Scottish National Heritage Site in Fife (NO 597 088) consisting mainly of scrub and woodland habitats, with a diverse range of native British flora and fauna present. Two *Rubus fruticosus* populations, from West Quarry Braes and from a meadow habitat on the banks of Loch Tay in Perth and Kinross, Scotland (NN 669 358), were investigated.

Study Species

Ipomoea trifida

Ipomoea trifida is a herbaceous vine also known as Morning Glory (as are many species from this and related genera). It is a member of the Convolvulaceae family, and closely related to the sweet potato *Ipomoea batatas*. It is widespread in America and the West Indies. Little information is available specifically on *Ipomoea trifida*, hence measurements of its features and traits were collected during the study period.

Structure

Sepals are green, and approximately 9mm in length. Corollas are tubular, approximately 30mm long, 10mm wide at their narrowest portion, and 40mm wide at their broadest. They are pale pink to white in colour, sometimes with darker nectar guides inside the corolla tube (see Fig. 1). Flowers give off a light, sweet, typically floral odour. The stigmas are white in colour, and held on a style approximately 12-18mm long. The stamens of mature flowers are attached to the gamopetalous corolla and are approximately 8-10mm long. Flowers possess a two-chambered ovary, each chamber usually containing two ovules (Kenyan, 1928). Flowers are known to be self-incompatible (e.g. Kowyama *et al.*, 2000).

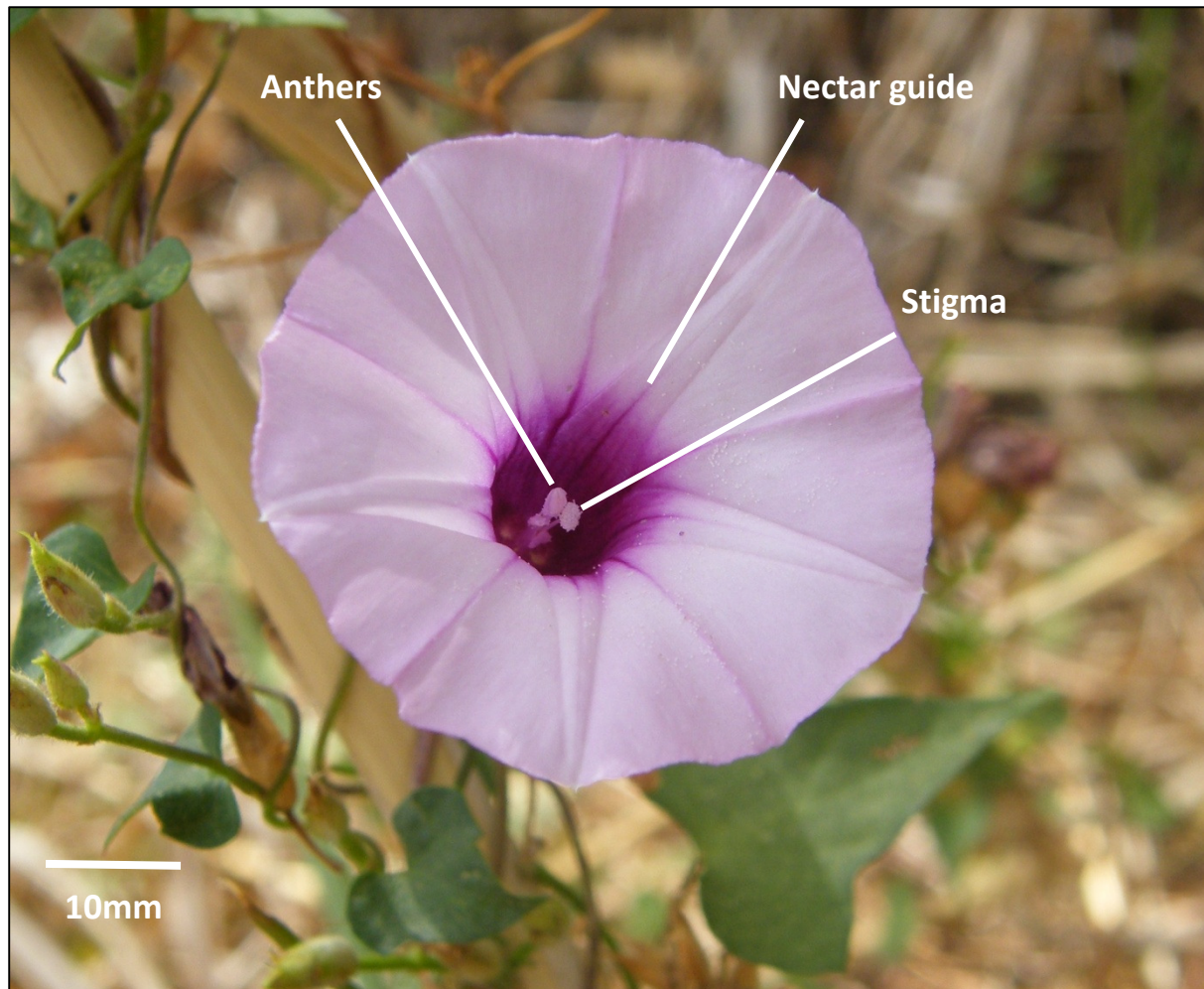


Fig. 1: Flower form of *Ipomoea trifida*. Anthers, stigma and nectar guides indicated.

Nectar

In addition to normal floral nectar produced in the corolla tube, the sepals of *Ipomoea trifida* possess crypt nectaries (Keeler and Kaul, 1984), which are deeply recessed cavities (Keeler and Kaul, 1979), and the petioles possess basin nectaries, open, slightly recessed depressions filled with secretory hairs (Keeler and Kaul, 1979). The proposed function of these extrafloral nectaries is as a defence against herbivores, attracting species such as ants to serve as protectors of the plant (Keeler, 1977; 1980; Beckman and Stucky, 1981; Koptur, 1992; Heil and McKey, 2003; Rico-Gray and Oliveira, 2007; Heil, 2008). Floral nectar production begins when flowers open, and continues until shortly before flowers wither and close after midday.

Timing

Previous studies on the timing of anther dehiscence and stigma receptivity in this species were not available; therefore measurements of such were recorded prior to the pollinator effectiveness study (see Results).

Heracleum sphondylium

Heracleum sphondylium, or hogweed, is a perennial, widespread throughout Britain and Europe, and growing from sea level to around 1000m in Scotland. Flowering period is highly variable, though the main flowering time is from June to September (Sheppard, 1991).

Structure

The species is erect, possessing 1 to 5 hollow stems, usually up to 2m tall. Flowers are arranged in umbel inflorescences, usually flat or slightly concave, and approximately 40-100mm across, with a mass of small central flowers surrounded by between 10 and 20 hairy, somewhat unequal (asymmetric) ray flowers with elongated corollas approximately 2-12mm long. Stems usually possess one apical umbel and a variable number of lateral umbels, usually between 1 and 9 (see Fig. 2).

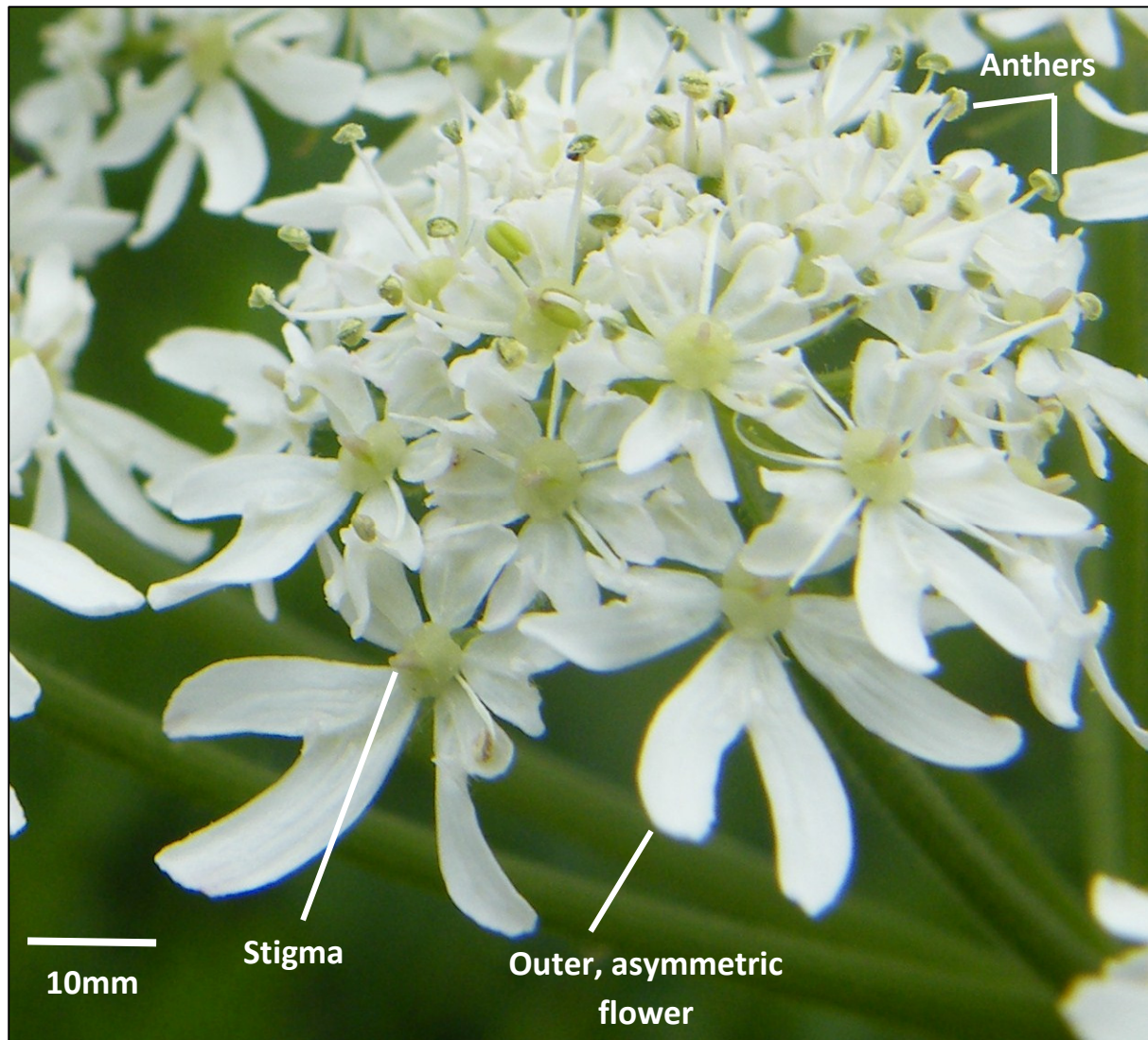


Fig. 2: Flower form and inflorescence structure of *Heracleum sphondylium*. Anthers, stigma and the outer, zygomorphic ray flower of the inflorescence indicated.

The flowers themselves are either male (i.e. flowers with aborted female parts) or hermaphroditic, though the percentage of male flowers is variable: in some populations not exceeding 40% (Wróblewska, 1992) while in others close to 100% (Zych, 2007). Central flowers have petals 2-4mm in length, white, greenish-white or rarely pink in colour. The filaments are 2-3mm long, and the anthers release oval-shaped, tricolpate pollen grains 38.4µm in diameter (e.g. Grace and Nelson, 1981). Styles have an enlarged base, forming the stylopodium. Fruit, of the two one-seeded mericarps, is approximately 7-8mm long. Styles on mature seed are approximately twice the length of the stylopodium, divergent or

slightly recurved, with capitate stigma (Sheppard, 1991). Flowers give off a slightly musky scent.

Nectar

Nectaries are exposed on an open, epigynous disc (with ovaries enclosed in a receptacle and stigmas and stamens above). Data on the volume and composition of the nectar are not available, probably due to the difficulty encountered when dealing with such small volumes of nectar in tiny flowers; but nectar is often visibly crystalline in warm weather (Willmer personal communication)

Timing

Flowering is phased within the umbel, the outer row of larger flowers on each umbellet opening first, maximising the visibility of the umbel to pollinators and prolonging the pollination period. Self-pollination can occur, but, as described above, the andromonoecious flowers are protandrous, which tends to limit this. Stigma receptivity follows anthesis after between 8 and 10 days, however the relatively long filament allows for geitonogamy to occur (Sheppard, 1991). It is thought that the pollination of neighbouring flowers may be advantageous to ensure fertilisation after a brief period of time if outcrossing does not occur (Bell, 1971). The earliest flowers to open, those on the primary umbel, are usually the most likely to set seed (Sheppard, 1991).

Rubus fruticosus

Rubus fruticosus, also known as bramble or blackberry, is widespread across the Northern Hemisphere and South America. In the British Isles, *Rubus fruticosus* is an aggregate of approximately 300 variants (Edees and Newton, 1988; Newton and Randall, 2004). Flowering is usually between June and September (Yeboah Gyan and Woodell, 1987a).

Structure

Rubus fruticosus is a deciduous shrub growing up to 3m by 3m in height and width. Flowers have a classic bowl-shape and are approximately 25-30mm in diameter. The colour of buds is rose-pink, but flowers become white to pale pink after the onset of anthesis. *Rubus fruticosus* is a self-compatible species and can reproduce asexually (Yeboah Gyan and Woodell, 1987a), but the arrangement of the anthers determines the extent to which the flowers self-pollinate (Nybom, 1985). A ring of many anthers (up to 80, personal observations) surrounds the multiple stigmas (up to 40, personal observations, see Fig. 3). The scent of flowers is slightly musky.

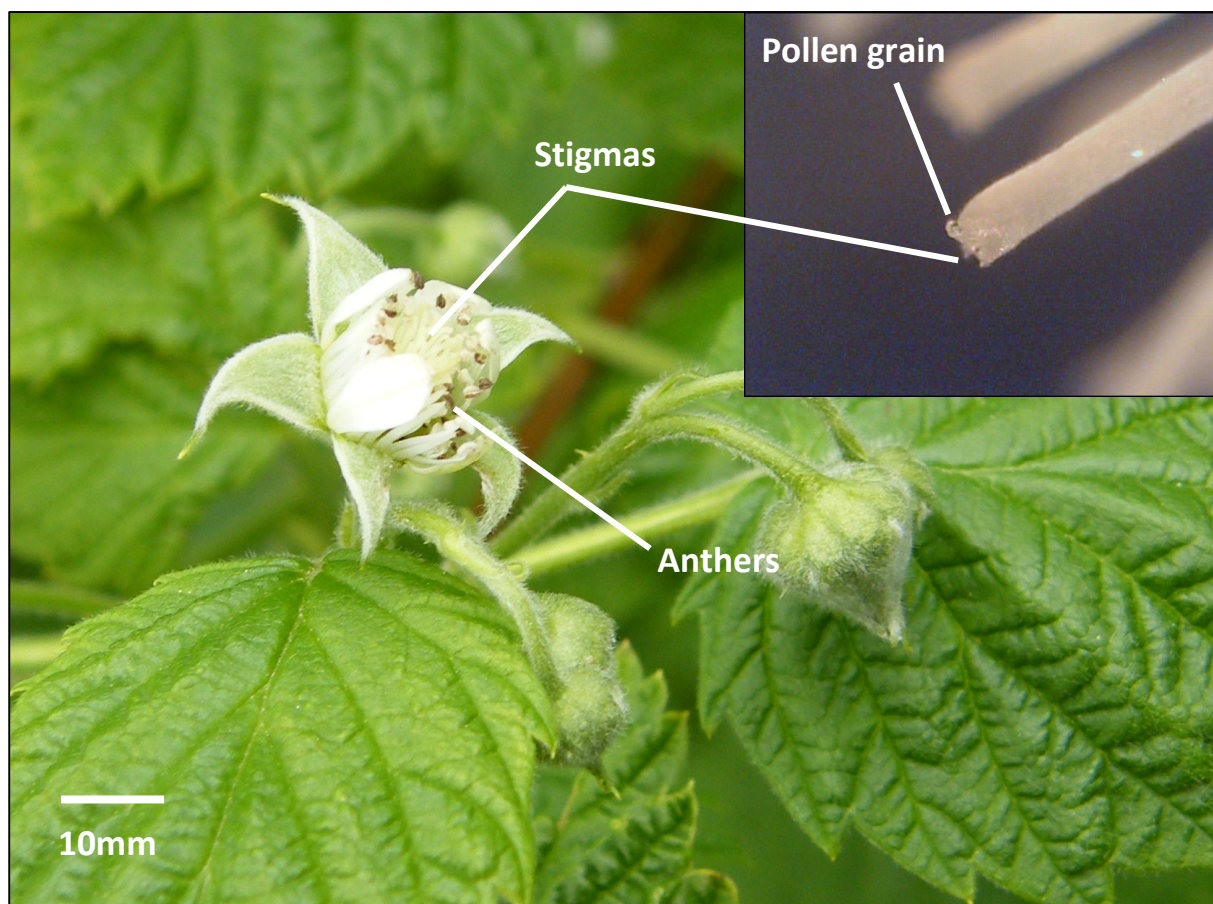


Fig. 3: Flower form of *Rubus fruticosus*. Stigmas and ring of anthers indicated. Insert shows Stigmas of *Rubus fruticosus* (x10 magnification, Watson Barnet Microsystem 70 Compound Light Microscope). Pollen grain indicated.

Nectar

Nectar secretion begins just shortly before the petals of flowers unfold, and continues until the petals have fallen and the filaments of the dehiscent stamens are beginning to incur over the carpal. *Rubus fruticosus* has a relatively shallow nectar cup, and changes in relative humidity can drastically affect the volume and concentration of nectar available (see Corbet *et al.*, 1979). The amount of sugar produced in nectar varies greatly between flowers, from approximately 3.7mg to 19.5mg, the mean total amount excreted over four days being around 15mg (Percival, 1946; Yeboah Gyan and Woodell, 1987b). Concentration is between 11 and 31% (Yeboah Gyan and Woodell, 1987b). In general, nectar secretion is maintained at a high level on the first day of flower opening, and remains steady over the second day, tailing off in the evening. On the third day, no nectar is found until a reflexion of the petals takes place, after which secretion is continuous throughout the day, ceasing as the stamens incur over the carpels (Percival, 1946). The nectar of *Rubus fruticosus* consists of fructose, sucrose, glucose and small quantities of maltose and melibiose at different times of the day (Wykes, 1952).

Timing

The terminal flowers of shoots open first, and are the longest-lived. Next to open are the terminal buds on each of the lateral branches of the inflorescence. When these flowers have finished blooming there is generally a lapse of 1-2 days before the remaining flowers of the shoot begin flowering. Pollen presentation is from 08:00 to 18:00 over a period of 1 to 2 days, with approximately 1.1mg of pollen presented per flower per day (Percival, 1955).

Sampling Period

Field research for *Heracleum sphondylium* and *Rubus fruticosus* at West Quarry Braes occurred during the spring and summer months of 2009 and 2010. Field research for

Rubus fruticosus occurred over a week long sampling period in June 2010. Sampling of *Ipomoea trifida* occurred between January and April of 2009.

Each sampling session was between 1 and 3 hours long depending on the frequency of visitations and how long it took all opened flowers to be visited. Sampling continued throughout the day, except in the case of *Ipomoea trifida*, where sampling occurred through the morning only, as flowers, whether visited or not, would wither and close by the afternoon.

Temperature and humidity readings were taken from a shaded area of the study site using a HM34 Vaisala Pocket Size Relative Humidity Meter every half hour during each sampling session, with the exception of readings for *Rubus fruticosus* at Loch Tay, where temperature readings only were collected continuously over a period of 4 days using two Tinytag TGP-4017 data loggers, which were placed in undergrowth beside the study site.

Results

Temperature and Humidity

Temperature and relative humidity readings for each of the study species are shown below (see Figs. 4-6). All species showed similar patterns of temperature and humidity. Mean temperatures were lowest early in the morning, and rose steadily throughout the study period to the peak at midday. Mean relative humidity was more variable, though showed a general decline over the morning to its lowest points at midday, then rising again in the afternoon and evening. While *Rubus fruticosus* was also studied during a week long period in June 2010 at a different location, the temperature data for this time period are shown in other chapters (Chapter 5 and 7) and are not included here as the vast majority of results were obtained from the West Quarry Braes study site, and there was very little variation between the mean temperatures of the two sites.

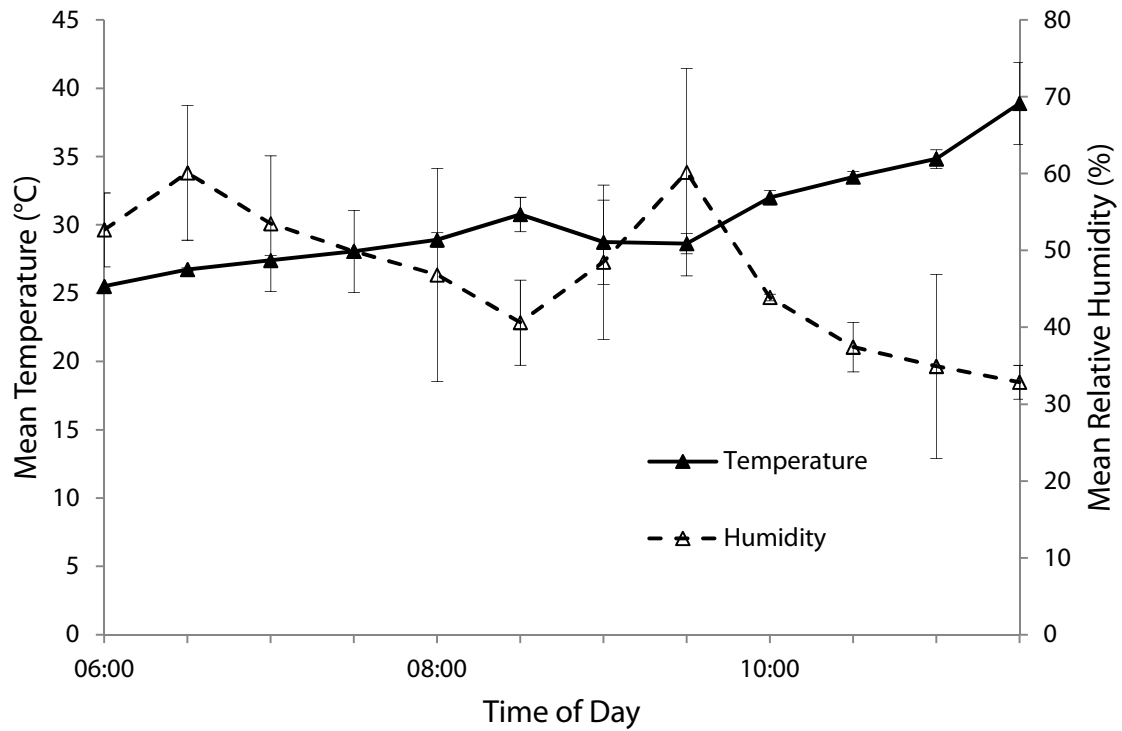


Fig. 4: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Ipomoea trifida*. Standard deviations are shown (N=4).

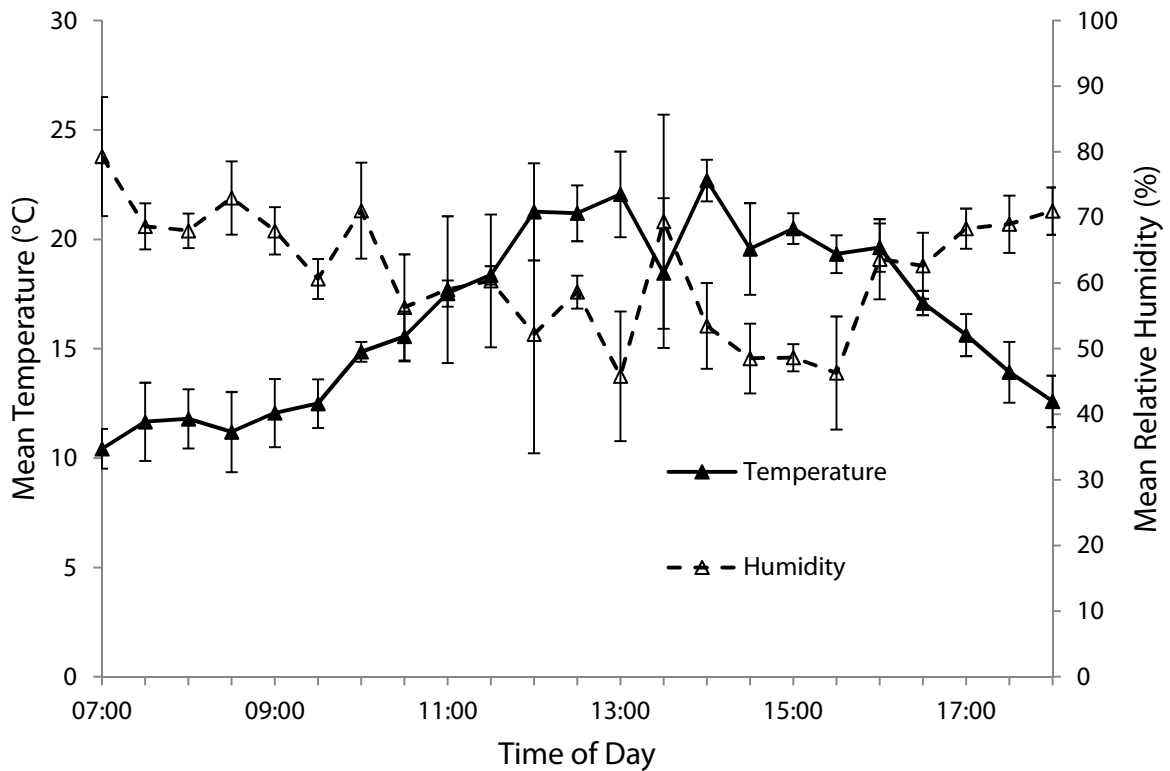


Fig. 5: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Heracleum sphondylium*. Standard deviations are shown (N=4).

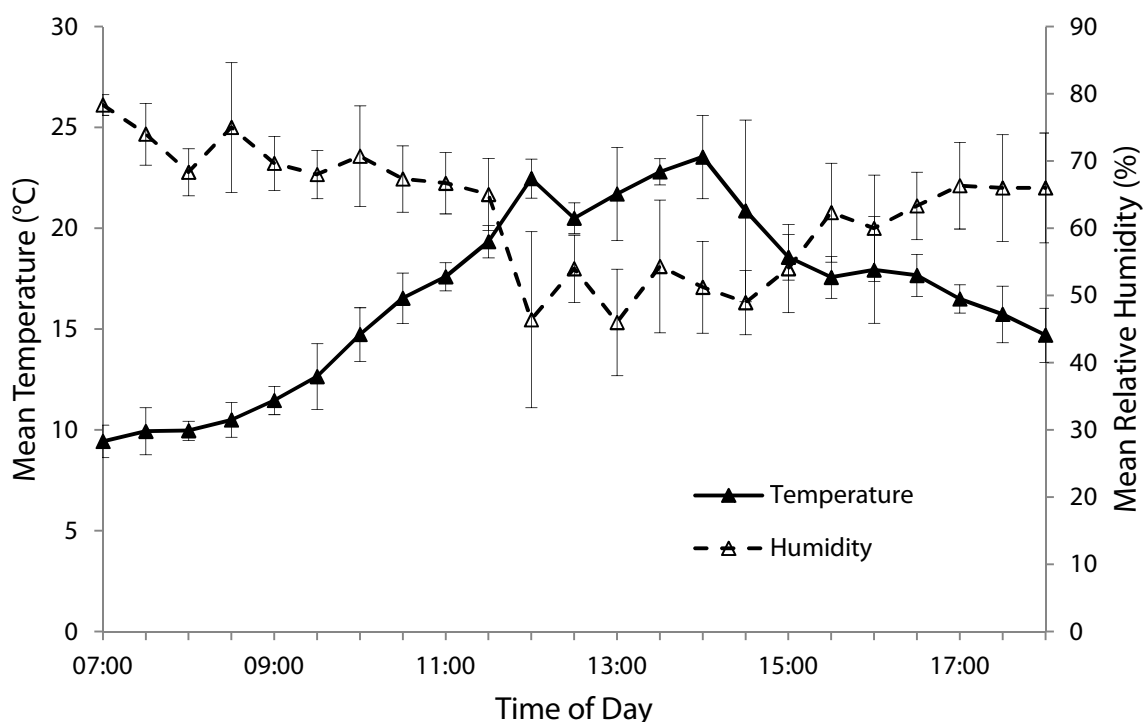


Fig. 6: Mean daily temperature (°C) and relative humidity (%) readings during the study period of *Ipomoea trifida*. Standard deviations are shown (N=4).

Partitioning of Visitors over Time

Visitors were first treated by functional groups (see Fig. 7, Fig. 9 and Fig. 11), and then by individual species (see Fig. 8, Fig. 10 and Fig. 12). As in other chapters, visits recorded do not provide a complete representation of the visitor assemblage of the plant species throughout the day, as only visits to targeted, previously-bagged flowers were recorded; however some patterns of visitation can be seen in the limited data available.

As information on the timing of flowering, dehiscence and stigma receptivity of *Ipomoea trifida* was not available, observations were made prior to the pollinator effectiveness study. Flowers remained open for a single day, and closed shortly after midday whether visited or unvisited, therefore the study period of this species was limited to 06:00-12:00. Anthers began dehiscence around dawn. No apparent separation of sexual phases occurs, as stigmas were observed to be glossy and apparently receptive at the same time as anthers presented pollen.

Ipomoea trifida

The earliest visitors to *Ipomoea trifida* were bees from 06:00, followed by ants and a few beetles from 06:30 (see Fig. 7). Visits by bees peaked at 07:30, but remained fairly constant throughout the morning. Ant visits declined after their earliest visits, though they were present on flowers until around 12:00. Butterflies were present on flowers until around 12:00. Butterflies were present at 08:30 and 09:30 only. One hoverfly species was observed at 09:00 only, and a single wasp visitor was observed at 11:00.

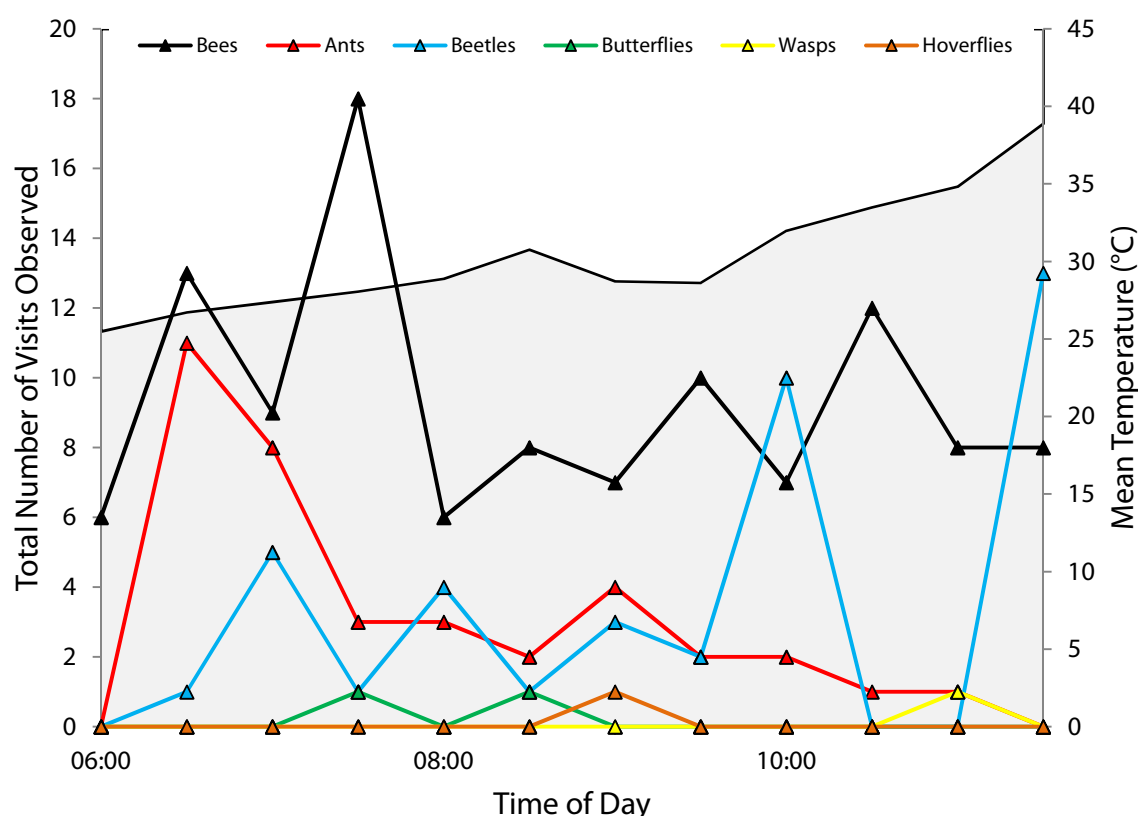


Fig. 7: Partitioning of flower visitor groups over time to flowers of *Ipomoea trifida*. Mean daily temperature (°C) is shown as an area plot.

When analysed by species, the earliest flower visitors were the bees *Agapostemon* sp., *Ceratina* sp. and *Tetragonisca angustula* at 06:00 (see Fig. 8). The bee *Trigona fulviventris*, the ant species *Camponotus novograndensis* and *Pseudomyrmex gracillis* and an unknown coleopteran visitor were next observed on flowers at 06:30. The beetle *Notoxus*

sp. arrived at flowers from 07:00, and the bees *Andrena* sp. and *Lasioglossum* sp. and an unknown lepidopteran visitor arrived at flowers from 07:30, though this was the only visit observed of the unknown lepidopteran. The latest bee species to visit was *Partamona musarum* at 09:30.

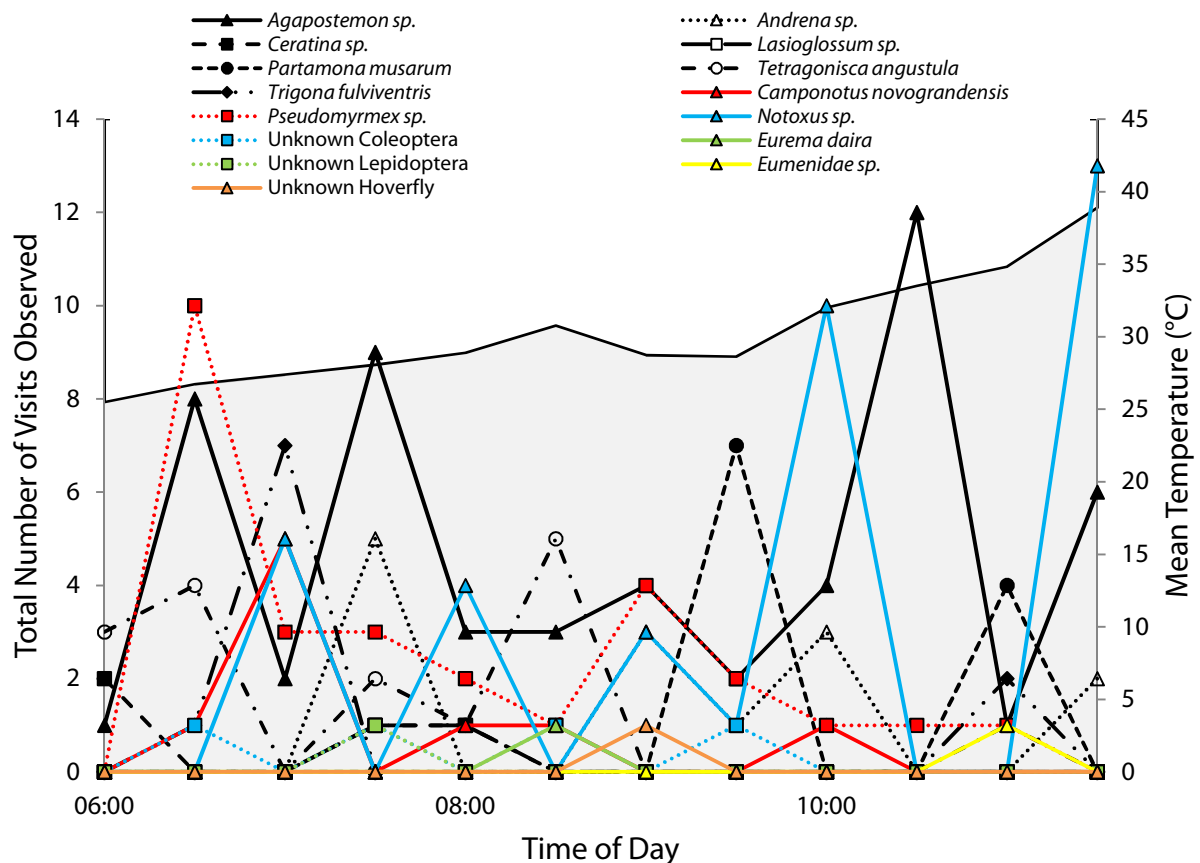


Fig. 8: Partitioning of flower visitor species over time to flowers of *Ipomoea trifida*. Mean daily temperature (°C) is shown as an area plot. Bees are indicated in black, ants in red, beetles in blue, butterflies in green, wasps in yellow and hoverflies in orange.

Heracleum sphondylium

Dipterans were the first visitor to *Heracleum sphondylium* at 07:00, followed by the hoverfly group at 08:00 (see Fig. 9). Neither taxon showed marked declines over midday, though hoverfly visitation showed a declining trend from 11:00 onwards, and dipterans declined after 15:00. Coleopterans were only present on flowers at 13:00. Both the dipteran

group and hoverfly group were observed on flowers until the end of the study period, at 18:00.

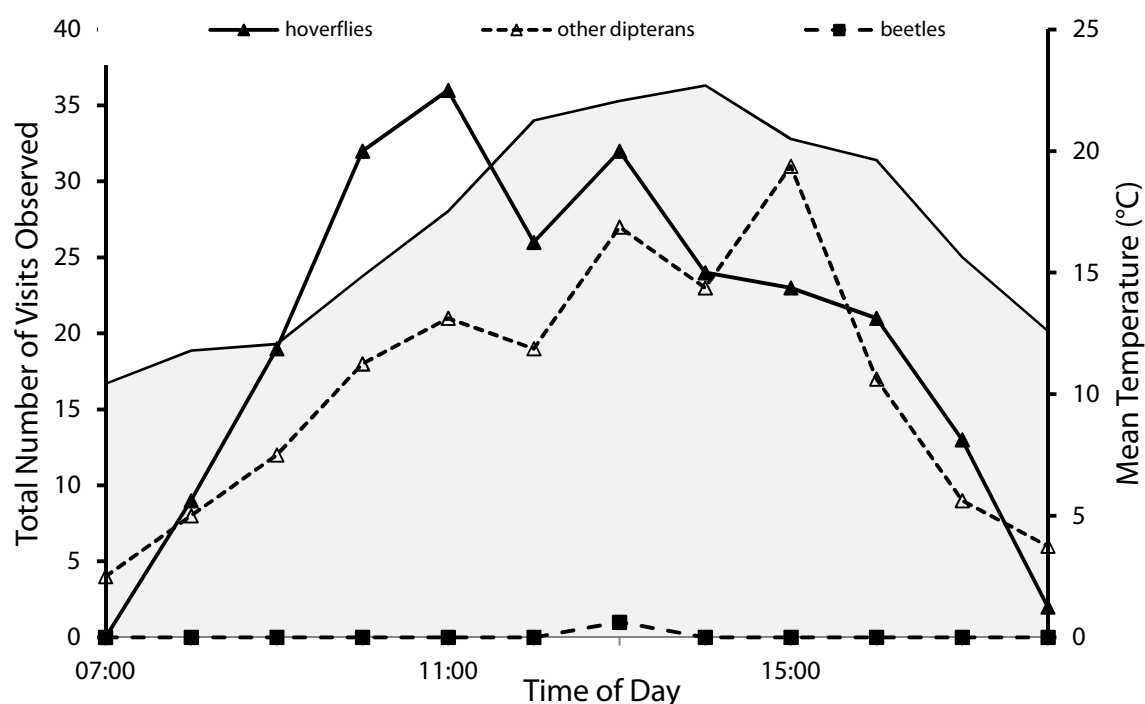


Fig. 9: Partitioning of flower visitor groups over time to flowers of *Heracleum sphondylium*. Mean daily temperature (°C) is shown as an area plot.

When analysed by species, the earliest flower visitors were the greenbottle fly *Lucilia sericata*, and the muscids *Phaonia subventa* and *Anthomyiidae* sp. at 07:00 (see Fig. 10). These were followed by the brachyceran fly *Platypezidae* sp. and the hoverflies *Epistrophe grossulariae*, *Episyrphus balteatus*, *Eupeodes corollae* and *Syrphus ribesii* at 08:00. The hoverflies *Eupeodes latifasciatus* and *Platycheirus albimanus* were only present on flowers between 09:00 and 12:00. The other hoverfly species were present on flowers throughout the day, and did not show marked declines in visitation over midday when temperatures were highest. The other dipteran species were also present throughout most of the day, though not as frequent over midday. The beetle *Chrysomelidae* sp. was observed on flowers at 13:00 only.

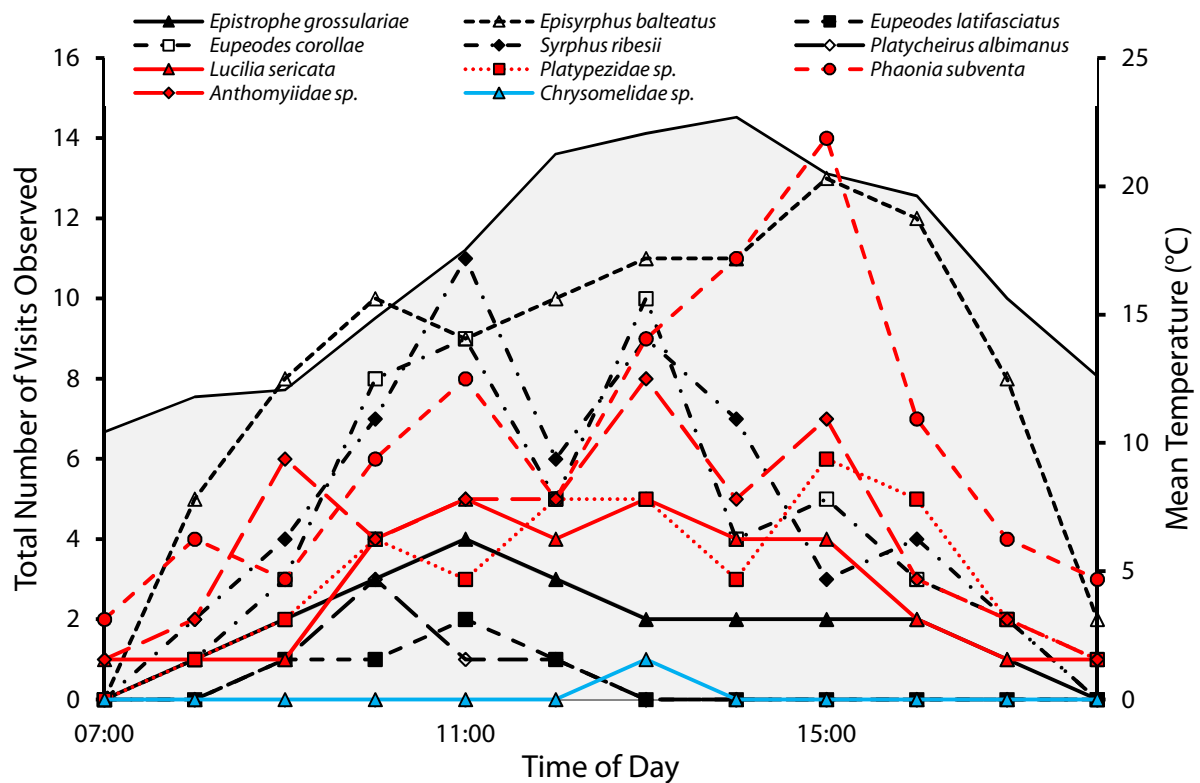


Fig. 10: Partitioning of flower visitor species over time to flowers of *Heracleum sphondylium*. Mean daily temperature (°C) is shown as an area plot. Hoverflies are indicated in black, other dipterans in red and beetles in blue.

Rubus fruticosus

Bees were the earliest visitors to *Rubus fruticosus*, present from 07:00, followed by hoverflies and muscids from 08:00, and finally the wasps, which were present on flowers from 10:00 until 13:00 (see Fig. 11). Muscids were present of flowers until around 11:00, and bees showed a marked decline in visitation between 12:00 and 15:00, while hoverflies were present throughout the day.

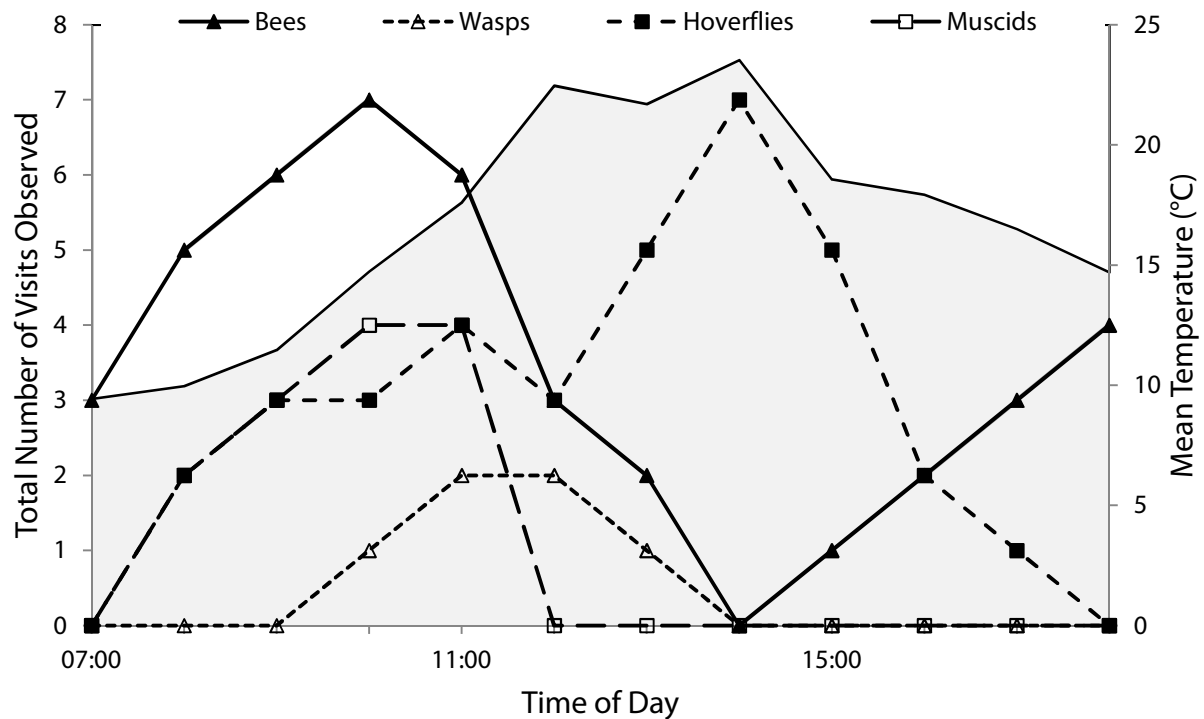
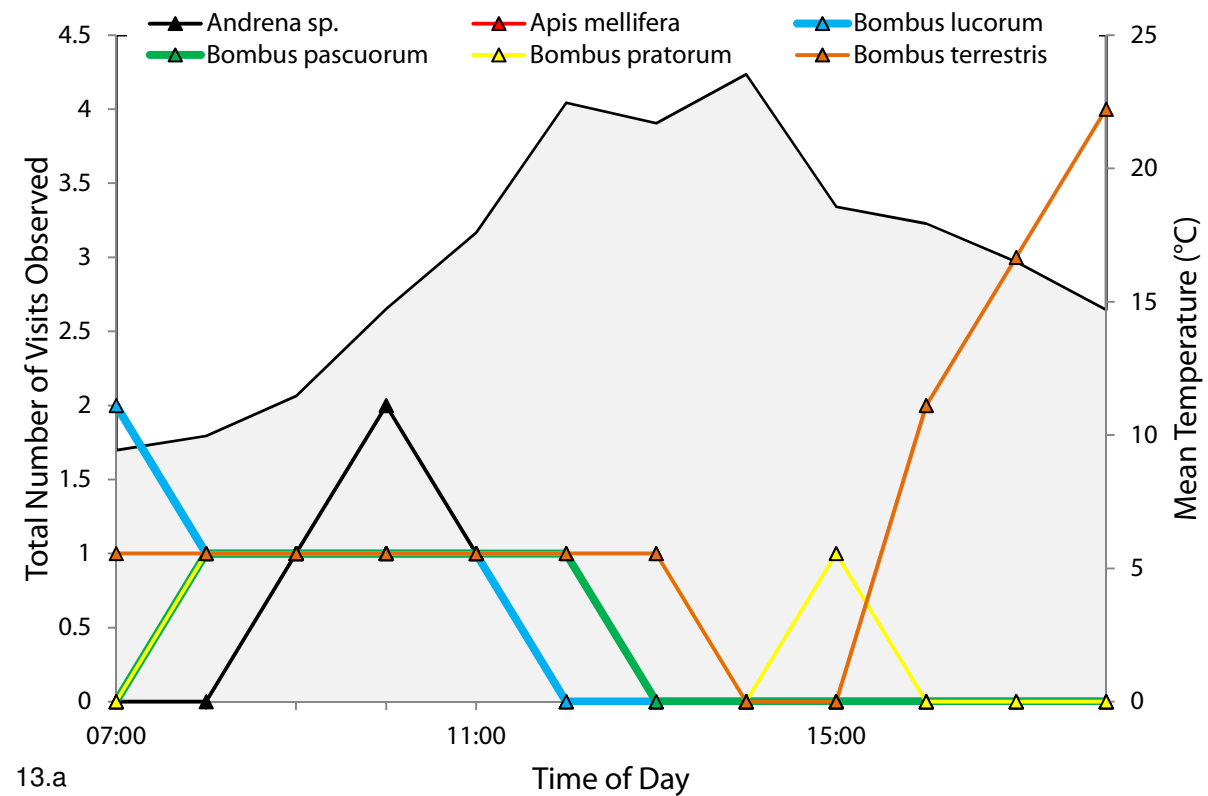
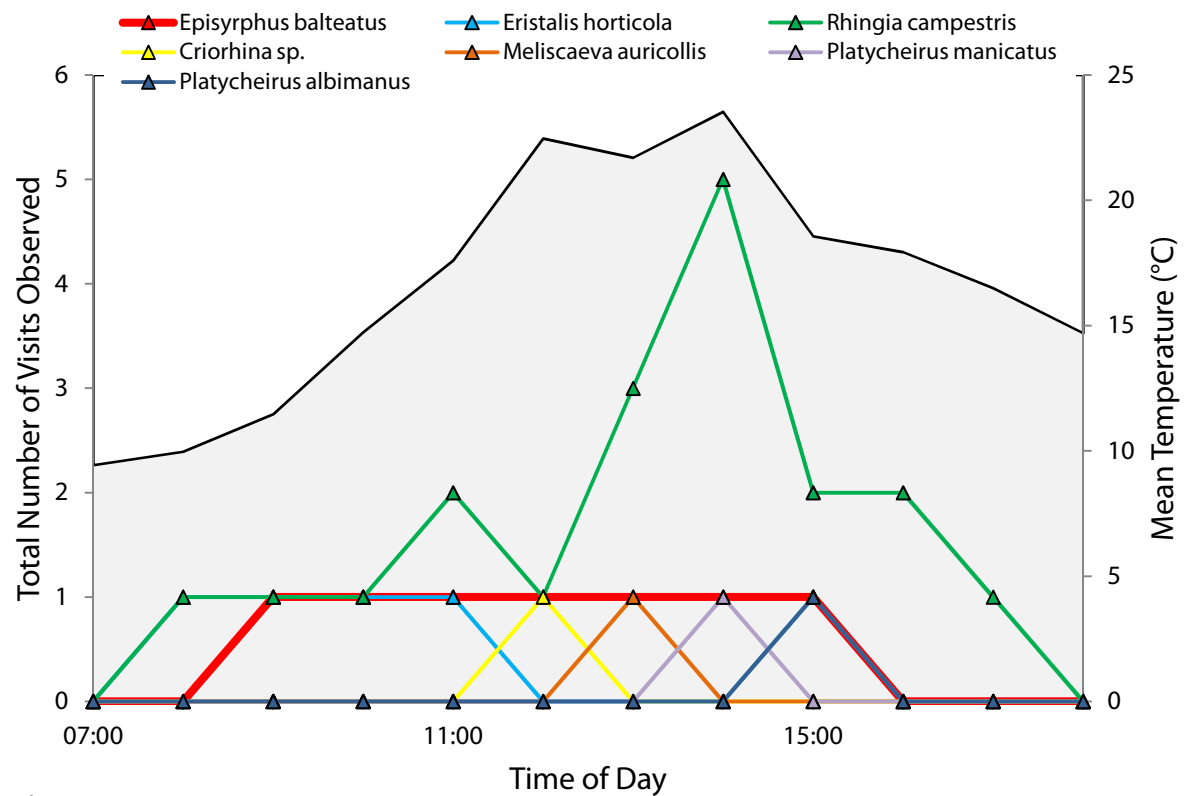


Fig. 12: Partitioning of flower visitor groups over time to flowers of *Rubus fruticosus*. Mean daily temperature (°C) is shown as an area plot.

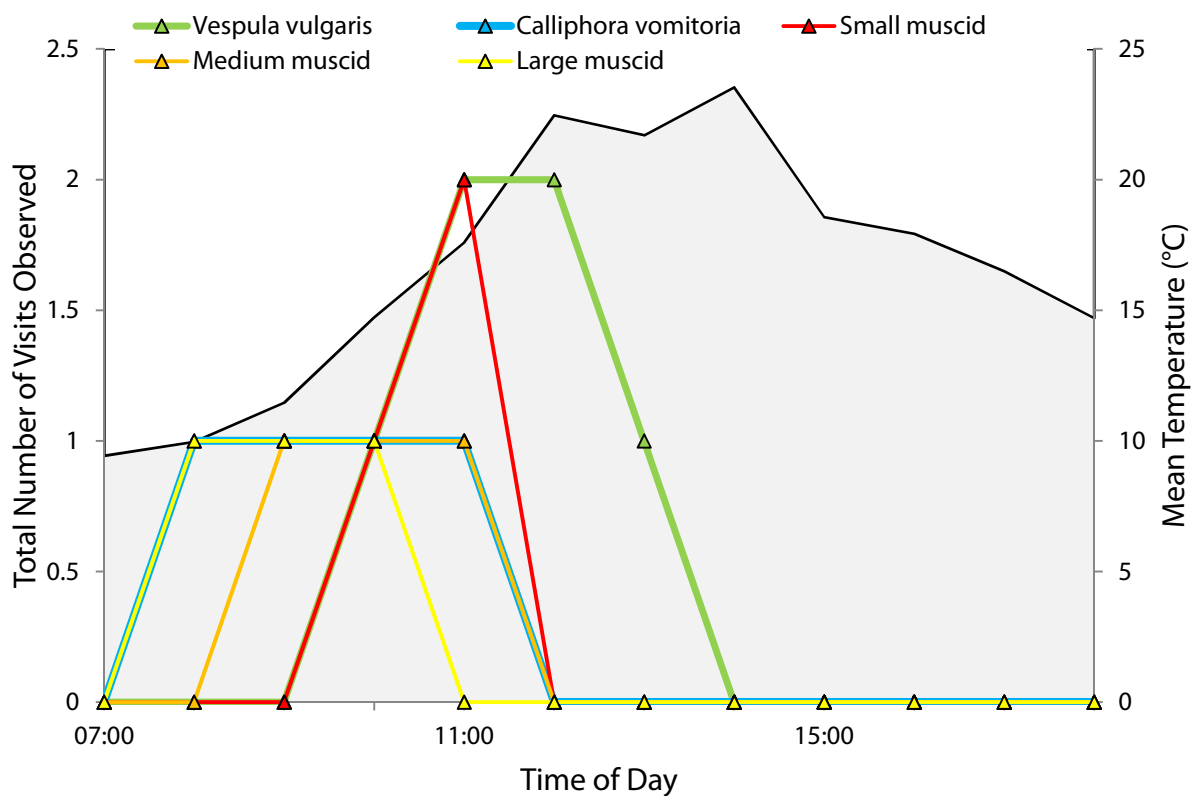
When analysed by species (see Fig. 13a-c), the earliest visitor species to *Rubus fruticosus* were the bumblebees *Bombus lucorum* and *Bombus terrestris* at 07:00. The bumblebee species *Bombus pascuorum* and *Bombus pratorum*, the honeybee *Apis mellifera*, the hoverflies *Eristalis horticola* and *Rhingia campestris*, the bluebottle *Calliphora vomitoria* and an unknown large muscid were present on flowers from 08:00. The solitary bee *Andrena* sp., the hoverfly species *Episyrphus balteatus* and an unknown medium-sized muscid arrived at flowers from 09:00. An unknown small muscid species and the wasp *Vespula vulgaris* were present from 10:00. The hoverfly *Criorhina* sp. was observed only once at 12:00. The hoverfly species *Meliscaeva auricollis*, *Platycheirus manicatus* and *Platycheirus albimanus* were observed at 13:00, 14:00 and 15:00 only respectively. The unknown large muscid, *Calliphora vomitoria*, a solitary bee *Andrena* sp., the honeybee *Apis mellifera* and the bumblebees *Bombus lucorum* and *Bombus pascuorum* were only present on flowers in the early morning. Of the species that were also present in the afternoon, *Bombus pratorum* was absent from flowers at 14:00, *Bombus terrestris* was absent from

14:00 to 16:00, *Episyrphus balteatus* was only present until 15:00, *Eristalis horticola* was absent between 12:00 and 15:00 and *Rhingia campestris* was observed throughout the day, showing no obvious patterns of decline at any time.





13.b



13.c

Fig. 13a-c: Partitioning of flower visitor species over time to flowers of *Rubus fruticosus*, split by functional group for clarity (a = bees; b = hoverflies; c = wasps and other dipterans). Mean daily temperature (°C) is shown as an area plot.

Pollen Deposition by Visitors

The mean number of pollen grains per stigma (MPS) of each flower species was first calculated by visitor functional group (Fig. 13, Fig. 15 and Fig. 17) and then by visitor species (Fig. 14, Fig. 16 and Fig. 18). As in other chapters, a pollinator was defined as a species that deposited a statistically significant amount of conspecific pollen on stigmas in comparison to the unvisited control stigmas.

Ipomoea trifida

Group	MPS	Sig. of MPS (in comparison to MPS of Controls)
Bees	108.2	$P < 0.001$
Beetles	93.5	$P = 0.001$
Butterflies	68.0	NA
Ants	65.0	$P = 0.235$
Hoverflies	23.0	NA
Wasps	14.0	NA

Table 2: Statistical analysis of visitor groups to *Ipomoea trifida*. Comparisons were calculated by Least Significant Differences Post Hoc Test. MPS of controls was 52.8.

When analysed by visitor group (see Fig. 13 and Table 2), the bee group had the highest MPS of the visitor groups to *Ipomoea trifida*. The wasp, hoverfly and butterfly groups were excluded from further analysis as the number of visits from each was too low for statistical analysis. Variation between groups was statistically significant. Of the groups included, only the bee and beetle group deposited significant MPS in comparison to controls, therefore these were the only groups classified as pollinators. Variation between the bees and beetles was not significant ($P = 0.328$), but variation between both the bees and ants ($P = < 0.001$) and the beetles and ants ($P = 0.029$) was significant.

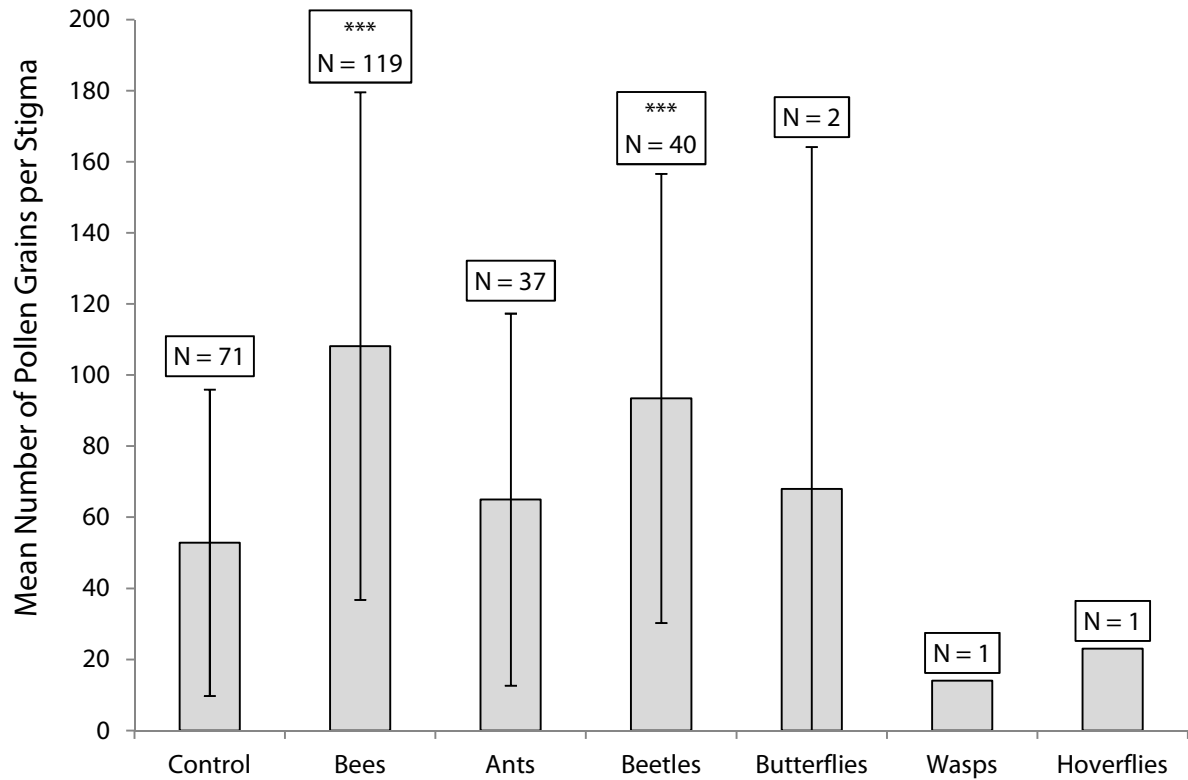


Fig. 13: Mean pollen deposition by visitor groups to *Ipomoea trifida*. N values and SD shown. Significance refers to the difference in pollen deposition between the groups and the unvisited control flowers. Variation between groups was statistically significant (One-Way ANOVA: $F = 6.117$; $df = 2$; $P = 0.003$).

Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
<i>Agapostemon</i> sp.	118.5	P < 0.001
<i>Andrena</i> sp.	155.7	P < 0.001
<i>Ceratina</i> sp.	93.8	P = 0.700
<i>Lasioglossum</i> sp.	106.5	NA
<i>Partamona musarum</i>	113.5	P < 0.001
<i>Tetragonisca angustula</i>	70.4	P = 0.148
<i>Trigona fulviventris</i>	35.8	P = 0.195
<i>Camponotus novograndensis</i>	52.6	P = 0.939
<i>Pseudomyrmex gracillis</i>	69.0	P = 0.155
<i>Notoxus</i> sp.	87.8	P < 0.001
Unknown coleopteran	144.5	P = 0.003
<i>Eurema daira</i>	0	NA
Unknown lepidopteran	136	NA
Family Eumenidae	14	NA
Unknown hoverfly	23	NA

Table 3: Statistical analysis of visitor species to *Ipomoea trifida*. Statistical analysis was performed by a LSD Post Hoc Test. MPS of controls was 52.8.

When analysed by species (see Fig. 13 and Table 3), the highest MPS was by the solitary bee *Andrena* sp. The butterfly *Eurema daira* did not deposit any pollen grains in its visit. The butterflies, wasp and hoverfly species were excluded from further analysis due to their low numbers of visits. Variation between species was statistically significant. Of the visitors that were included, *Andrena* sp., *Agapostemon* sp., *Partamona musarum*, the unknown coleopteran and *Notoxus* sp. were the only species to deposit a significantly higher

MPS than was found on the control stigmas, and were therefore the only species to be classified as pollinators.

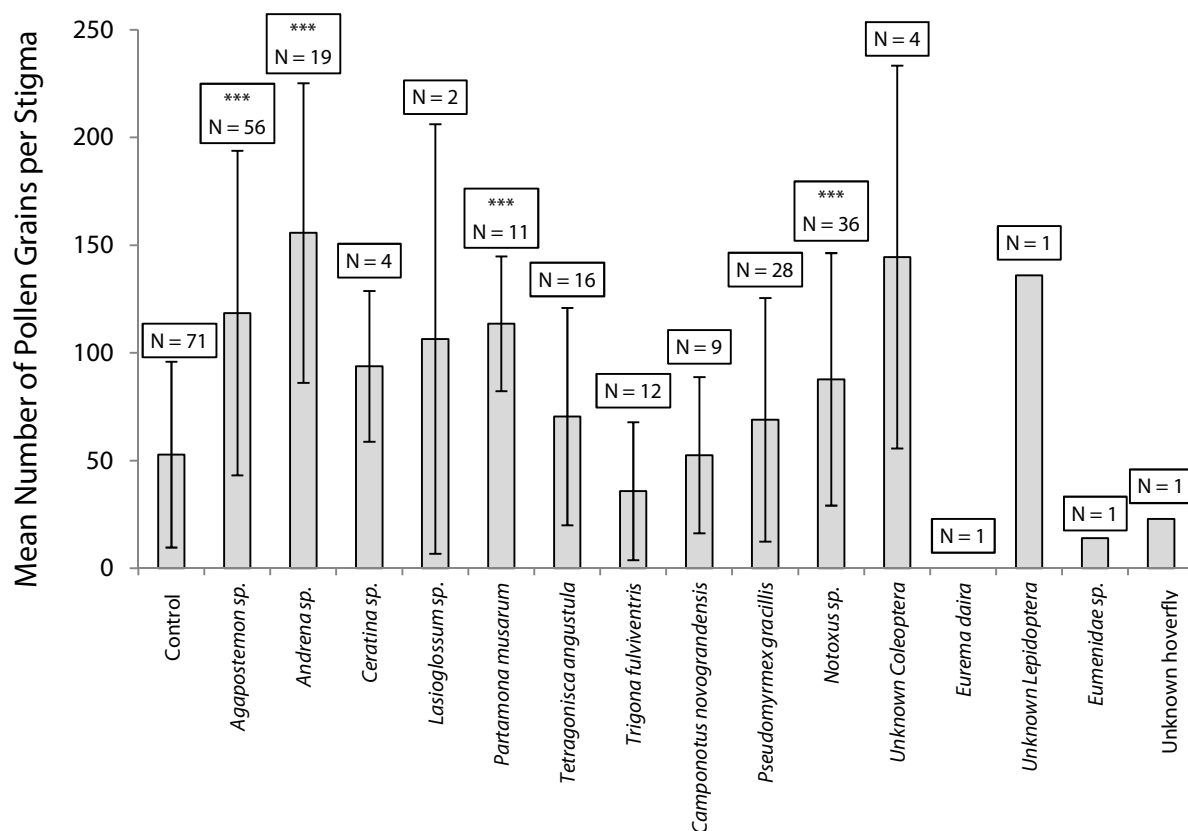


Fig. 14: Mean pollen deposition by visitor species to *Ipomoea trifida*. N values and SD shown. Significance refers to the difference in pollen deposition between the species and the unvisited control flowers. Variation between species was statistically significant (One-Way ANOVA: $F = 6.548$; $df = 9$; $P < 0.001$).

Heracleum sphondylium

Groups	MPS	Sig. of MPS (in comparison to MPS of Controls)
Hoverflies	43.7	$P < 0.001$
Other dipterans	80.5	$P < 0.001$
Beetles	27	NA

Table 4: Statistical analysis of visitor groups to *Heracleum sphondylium* Statistical analysis was performed by a Mann-Whitney U Test with Bonferroni correction. In this case, $\alpha = 0.025$. MPS of controls was 16.8.

Of the visitors to *Heracleum sphondylium* (see Fig. 15 and Table 4), the dipterans had the highest MPS, followed by the hoverflies and the beetles. As there was only a single visit by the beetle group, this group was excluded from further analysis. Variation between the other two groups was statistically significant. Both the hoverflies and other dipterans deposited a significant MPS in comparison to control stigmas, and were therefore classified as pollinators.

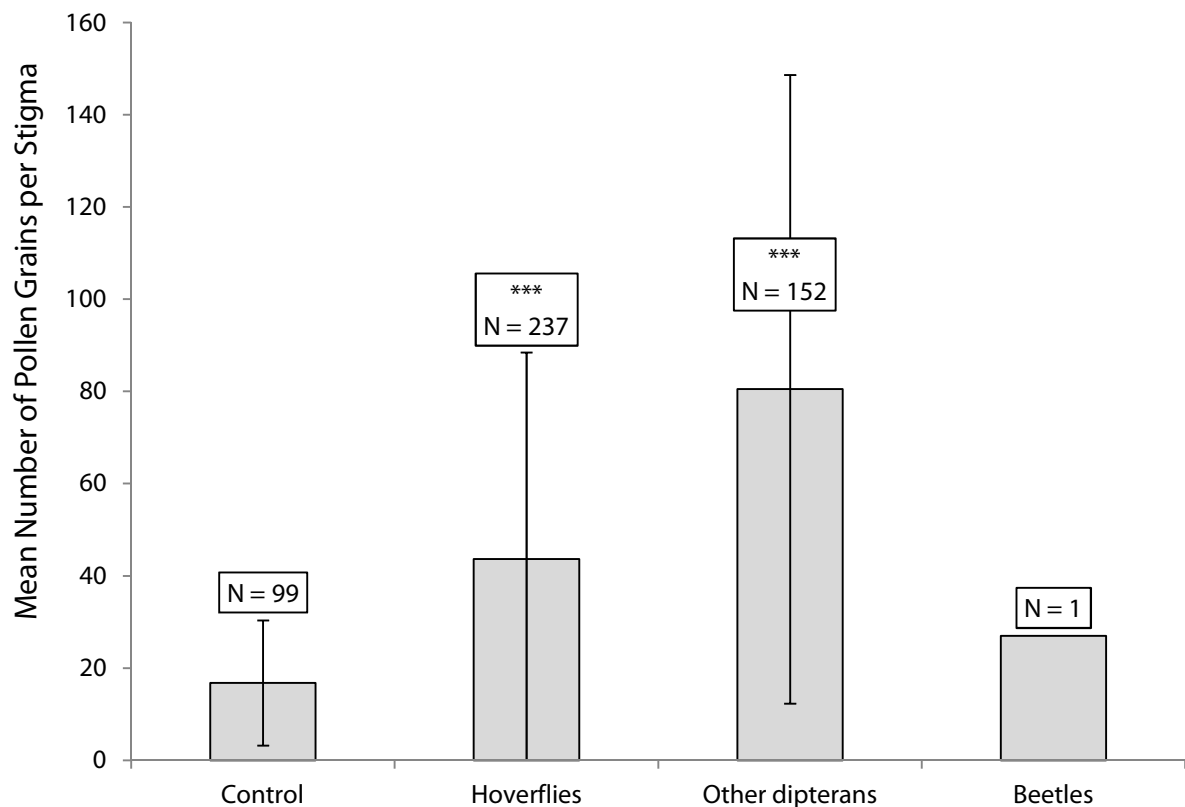


Fig. 15: Mean pollen deposition by visitor groups to *Heracleum sphondylium*. N values and SD shown. Significance refers to the difference in pollen deposition between the groups and the unvisited control flowers. Variation between groups was statistically significant (Kruskal-Wallis Non-Parametric Test: Chi-squared = 33.783; df = 1, $P < 0.001$)

Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
<i>Epistrophe grossulariae</i>	61.8	P < 0.001
<i>Episyrphus balteatus</i>	55.8	P < 0.001
<i>Eupeodes latifasciatus</i>	41.2	P = 0.026
<i>Eupeodes corollae</i>	22.5	P < 0.001
<i>Syrphus ribesii</i>	32.1	P < 0.001
<i>Platycheirus albimanus</i>	25.8	P = 0.994
<i>Lucilia sericata</i>	116.1	P < 0.001
<i>Paraplatypleza atra</i>	79.9	P < 0.001
Anthomyiidae sp.	62.8	P < 0.001
<i>Phaonia subventa</i>	67.4	P < 0.001
Chrysomelidae sp.	27.0	NA

Table 5: Statistical analysis of visitor groups to *Heracleum sphondylium*. Statistical analysis was performed by a Mann-Whitney U Test with Bonferroni correction. In this case, $\alpha = 0.025$. MPS of controls was 16.8.

When analysed by species (see Fig. 16 and Table 5), the highest MPS was by the greenbottle *Lucilia sericata*. There was only a single visit by the beetle Chrysomelidae sp., therefore it was excluded from further analysis. Variation between species was significant. Nearly all species deposited a significant MPS in comparison to controls and were classified as pollinators. The exception was *Platycheirus albimanus*.

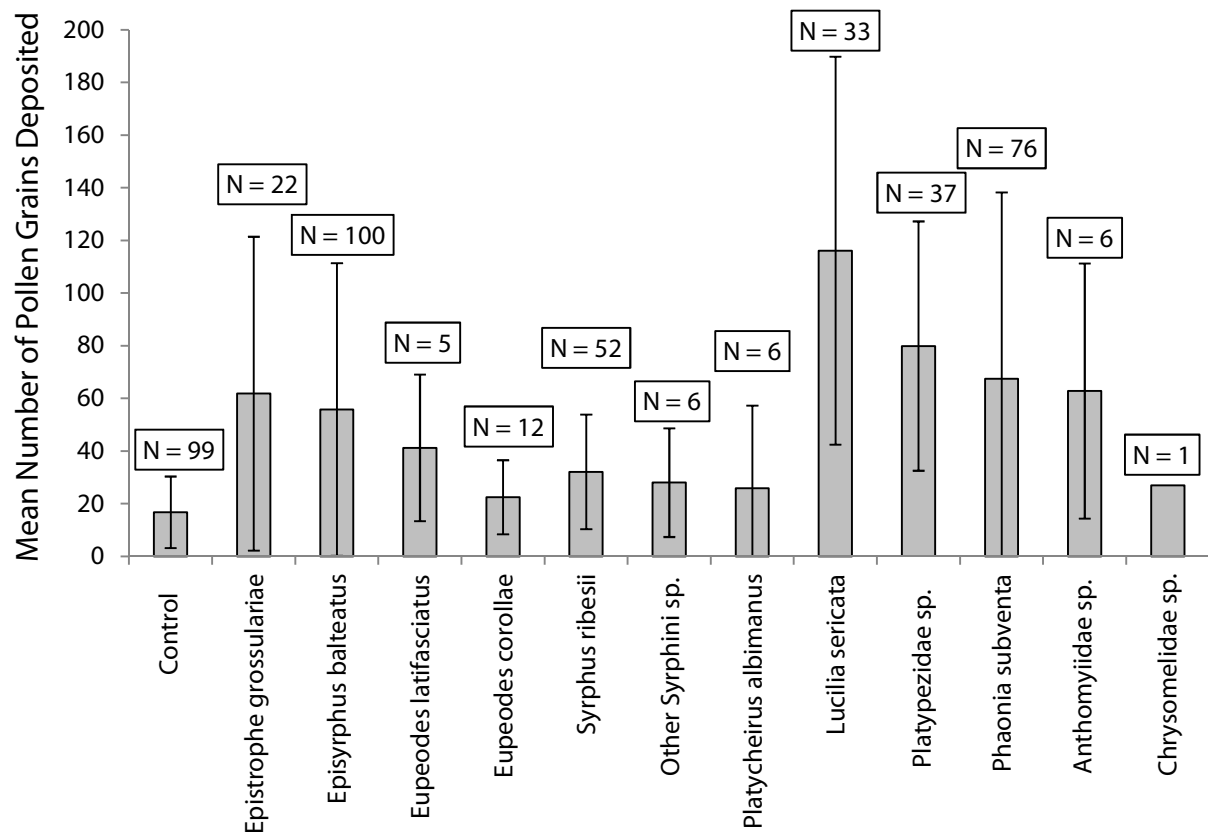


Fig. 16: Mean pollen deposition by visitor species to *Heracleum sphondylium*. N values and SD shown. Significance refers to the difference in pollen deposition between the species and the unvisited control flowers. Variation between species (Kruskal-Wallis Non-Parametric Test: Chi-squared = 70.733; df = 9, $P = < 0.001$).

Rubus fruticosus

Groups	MPS	Sig. of MPS (in comparison to MPS of Controls)
Bees	256.2	$P = 0.038$ (TMC)
Hoverflies	136.6	$P = 0.236$ (TMC)
Wasps	80.9	NA
Muscids	54.6	NA

Table 6: Statistical analysis of visitor groups to *Rubus fruticosus*. Statistical analysis was performed by a Tamhane's Multiple Comparisons Post Hoc Test. MPS of controls was 52.7.

Of the visitor groups to *Rubus fruticosus* (see Fig. 17 and Table 6), the highest MPS was by the bee group. Due to unusual variance the wasp and muscid groups were excluded from further analysis. Variation between the remaining groups was statistically significant. Only the bee group deposited a significant MPS in comparison to controls, and bees were therefore classified as pollinators. Hoverflies did not deposit significantly greater pollen amounts than controls.

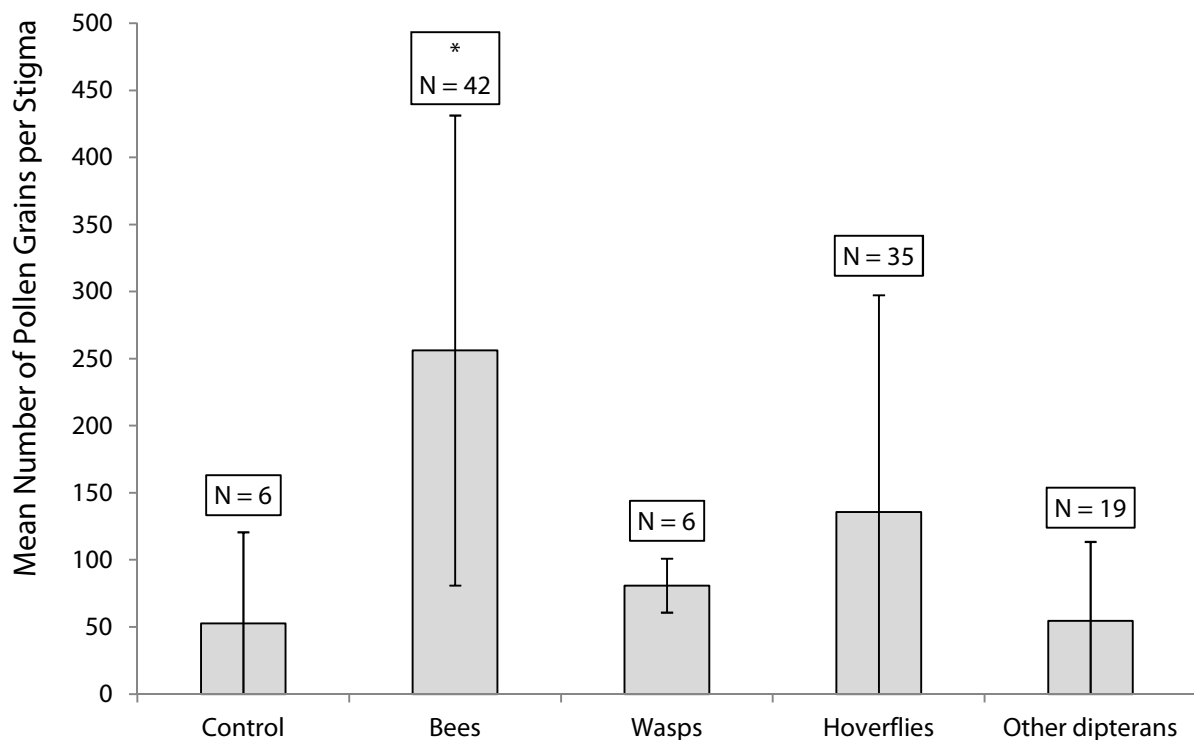


Fig. 18: Mean pollen deposition by visitor groups to *Rubus fruticosus*. N values and SD shown. Significance refers to the difference in pollen deposition between the groups and the unvisited control flowers. Variation between groups was statistically significant (Welch's Robust Test of Equality of Means: $F = 27.092$; $df = 2$; $P < 0.001$).

Species	MPS	Sig. of MPS (in comparison to MPS of Controls)
<i>Bombus lucorum</i>	343.3	P < 0.001
<i>Bombus terrestris</i>	295.5	P < 0.001
<i>Bombus pratorum</i>	223.0	P = 0.005
<i>Bombus pascuorum</i>	142	P = 0.019
<i>Apis mellifera</i>	270	P = 0.001
<i>Andrena</i> sp.	154.9	P = 0.026
<i>Platycheirus manicatus</i>	338	NA
<i>Rhingia campestris</i>	172.6	P = 0.009
<i>Eristalis horticola</i>	87	P = 0.241
<i>Episyrphus balteatus</i>	80	NA
<i>Meliscaeva auricollis</i>	80	NA
<i>Platycheirus albimanus</i>	45	NA
<i>Criorhina</i> sp.	9.5	NA
<i>Vespula vulgaris</i>	80.9	NA
<i>Calliphora vomitoria</i>	82.5	P = 0.481
Small muscid	52.3	NA
Medium muscid	42	NA
Large muscid	36.2	NA

Table 7: Statistical analysis of visitor species to *Rubus fruticosus*. Statistical analysis was performed by a LSD Post Hoc Test.

When analysed by visitor species (see Fig. 18 and Table 7), the highest MPS was by the bumblebee *Bombus lucorum*. The hoverfly species *Platycheirus albimanus*, *Platycheirus manicatus*, *Meliscaeva auricollis* and *Criorhina* sp. were excluded from statistical analysis as only a single visit was recorded for each. The wasp species *Vespula vulgaris*, the hoverfly

species *Episyrphus balteatus* and the unknown large, medium and small muscid species were also removed from the analysis as unusual levels of variance did not allow for them to be analysed statistically. Variation between species was statistically significant. *Andrena* sp., *Apis mellifera*, *Bombus lucorum*, *Bombus pascuorum*, *Bombus pratorum*, *Bombus terrestris* and *Rhingia campestris* deposited a statistically significant MPS in comparison to control flowers and were therefore classified as pollinators while *Eristalis horticola* and *Calliphora vomitoria* did not.

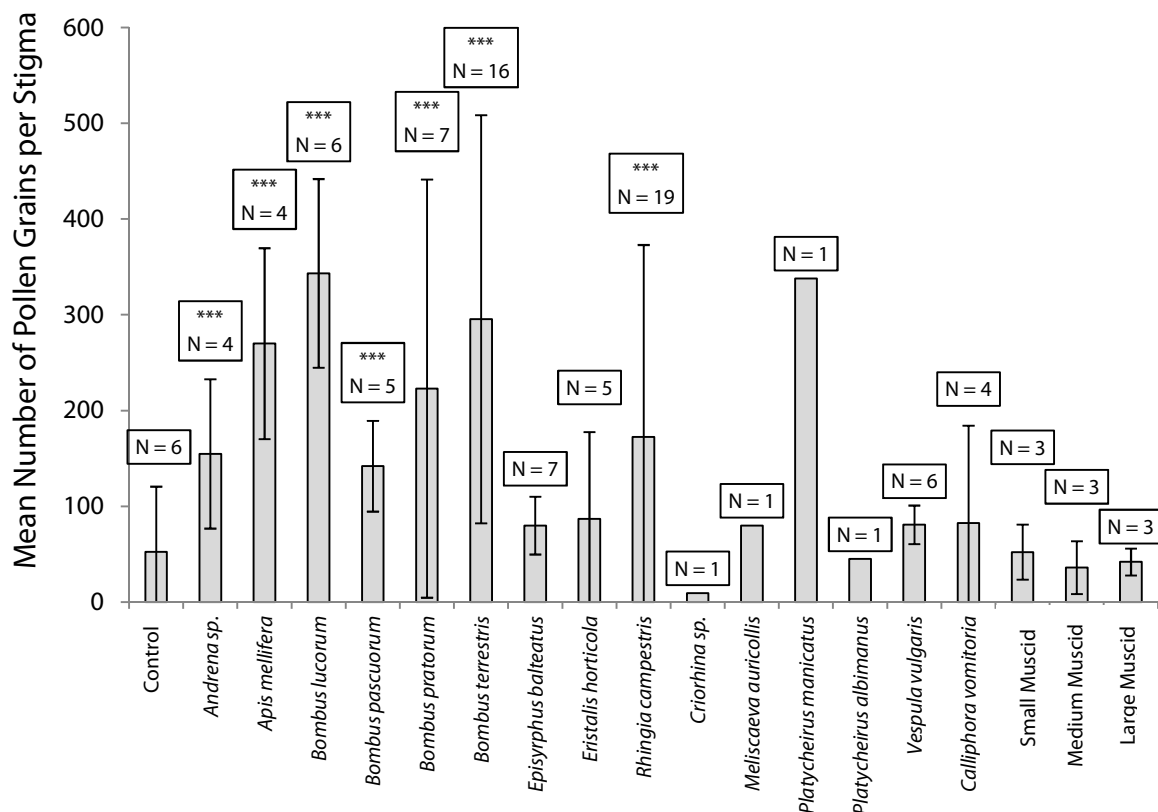


Fig. 18: Mean pollen deposition by visitor species to *Rubus fruticosus*. N values and SD shown. Significance refers to the difference in pollen deposition between the species and the unvisited control flowers. Variation between species was statistically significant (One-Way ANOVA: $F = 3.317$; $df = 8$; $P = 0.003$).

Visitor Assemblage

Ipomoea trifida

As would be expected from a flower showing traits indicative of a generalist pollination syndrome, insects from a variety of taxonomic and functional groups visited flowers of *Ipomoea trifida*; flowers were visited by several bee species, as well as multiple species of beetle, ant and butterfly (see Table 8). With the exception of the butterfly species, all visitors to *Ipomoea trifida* possessed relatively short tongues, though due to the limitations of equipment and preservation methods used it was not possible to accurately measure the tongue lengths of specimens caught.

All visitors, with the exception of the butterflies, entered the corolla to feed on the nectar or pollen of the flowers. When visitors exited flowers, their bodies and faces were coated in a fine dusting of pollen. Some, like the ants, groomed the majority of this pollen from their bodies before moving on to subsequent flowers; however most visitors remained dusted with pollen when they entered subsequent flowers, thereby transferring pollen grains to the stigmas of flowers as they arrived and becoming covered in pollen again as they left.

Group	Species	N	Mean Size (mm)
Bee	<i>Agapostemon</i> sp.	56	WL 7.0-7.5mm ¹
Bee	<i>Andrena</i> sp.	16	WL 8.0-8.5mm ¹
Bee	<i>Tetragonisca angustula</i>	16	WL 2.4-3.0mm ¹
Bee	<i>Trigona fulviventris</i>	12	WL 6.7-7.0mm ¹
Bee	<i>Partamona musarum</i>	11	WL 2.7-2.9mm ¹
Bee	<i>Ceratina</i> sp.	5	WL 7.8-8.5mm ¹
Bee	<i>Lasioglossum</i>	22	WL 6.7-7.3mm ¹
Beetle	<i>Notoxus</i> sp.	36	TW 2.0-2.5mm ¹
Beetle	Unknown coleopteran	4	NA
Ant	<i>Pseudomyrmex gracilis</i>	28	TW 2.3-2.5mm ¹
Ant	<i>Camponotus novograndensis</i>	9	TW 0.5-1.0mm ¹
Butterfly	<i>Eurema daira</i>	1	WL 25-35mm ¹
Butterfly	Unknown lepidopteran	1	WL 30-40mm ¹

Table 8: Visitor assemblage of *Ipomoea trifida*. WL refers to wing length. TW refers to thorax width.

¹ Personal measurements.

Heracleum sphondylium

The most frequent visitor to *Heracleum sphondylium* was the hoverfly *Episyrphus balteatus*. Flowers were also visited frequently by other hoverfly and dipteran species, and infrequently by a beetle species (see Table 9). Visitors fed on both the pollen and nectar of flowers, collecting pollen on the undersides of their bodies and transferring it to stigmas as they fed, and all species normally fed on more than one flower per inflorescence. Other species of beetle and some bumblebees were also observed on flowers, though not on targeted, previously-bagged flowers.

Group	Species	N	Mean Size (mm)	Mean Tongue Length (mm)
Hoverfly	<i>Episyrphus balteatus</i>	100	WL 6-10.25mm ¹	2.89mm ²
Hoverfly	<i>Syrphus ribesii</i>	52	WL 7.25-10.25mm ¹	2.99mm ²
Hoverfly	<i>Eupeodes corollae</i>	12	WL 5-8.25mm ¹	3.20mm ³
Hoverfly	<i>Epistrophe grossulariae</i>	22	WL 8-11.25mm ¹	2.9mm ³
Hoverfly	<i>Platycheirus albimanus</i>	6	WL 5-8mm ¹	3.4mm ³
Hoverfly	<i>Eupeodes latifasciatus</i>	5	6.75-7.75mm ¹	3.0mm ³
Other dipteran	<i>Phaonia subventa</i>	76	TW 3-4mm ³	2.2mm ³
Other dipteran	Anthomyiidae sp.	6	TW 2.5-3.5mm ³	2.1mm ³
Other dipteran	Platypozidae sp.	37	TW 0.7-1mm ³	0.8mm ³
Other dipteran	<i>Lucilia sericata</i>	33	TW 3.5-4.5mm ³	2.8mm ³
Coleopteran	Chrysomelidae sp.	1	TW 1-2mm ³	0.5mm ³

Table 9: Visitor assemblage of *Heracleum sphondylium*. WL refers to wing length. TW refers to thorax width. ¹ Stubbs and Falk, 2002; ² Gilbert *et al.*, 1985; ³ Personal measurements.

Rubus fruticosus

The most frequent visitor to *Rubus fruticosus* was the hoverfly *Rhingia campestris*. Flowers were also visited by other dipterans, bumblebees, other bees, and wasps (see Table 10). As species fed on the nectar of flowers, their upper bodies came into contact with the anthers and stigmas of the flowers. Species feeding on pollen also came into contact

with both anthers and stigmas as they fed, though it is likely that much of the pollen transferred by these species is self-pollen from the same flower.

Group	Species	N	Mean Size (mm)	Mean Tongue Length (mm)
Bumblebee	<i>Bombus terrestris</i>	16	TW 4.99mm ¹	7.6mm ²
Bumblebee	<i>Bombus lucorum</i>	6	TW 4.21mm ¹	7.3mm ²
Bumblebee	<i>Bombus pratorum</i>	7	TW 4.70mm ¹	7.5mm ²
Bumblebee	<i>Bombus pascuorum</i>	5	TW 4.5mm ¹	8.5mm ²
Bee	<i>Andrena</i> sp.	4	TW 2-3mm ⁷	4.0mm ⁷
Bee	<i>Apis mellifera</i>	4	3.7-4.0mm ¹	5.3-5.4mm ³
Hoverfly	<i>Rhingia campestris</i>	19	WL 6-9.5mm ⁴	11mm ⁵
Hoverfly	<i>Episyrphus balteatus</i>	7	WL 6-10.25mm ⁴	2.89mm ⁶
Hoverfly	<i>Eristalis horticola</i>	5	WL 7.75-11.25mm ⁴	6.2mm ⁶
Hoverfly	<i>Criorhina</i> sp.	1	WL 10-13mm ⁴	4.0mm ⁷
Hoverfly	<i>Meliscaeva auricollis</i>	1	WL 6-9.5mm ⁴	2.73mm ⁶
Hoverfly	<i>Platycheirus manicatus</i>	1	WL 6-7.25mm ⁴	2.7mm ⁷
Hoverfly	<i>Platycheirus albimanus</i>	1	WL 5-8mm ⁴	3.42mm ⁶
Wasp	<i>Vespula vulgaris</i>	6	TW 2.5-3.0mm ⁷	2.0mm ⁷
Other dipteran	<i>Calliphora vomitoria</i>	4	TW 2-3mm ⁷	1.3mm ⁷
Other dipteran	<i>Unknown small muscid</i>	3	NA	NA
Other dipteran	<i>Unknown medium muscid</i>	3	NA	NA
Other dipteran	<i>Unknown large muscid</i>	3	NA	NA

Table 10: Visitor assemblage of *Rubus fruticosus*. WL refers to wing length. TW refers to thorax width.

¹ Peat *et al.*, 2005; ² Goulson *et al.*, 2005; ³ Kato *et al.*, 1999; ⁴ Stubbs and Falk, 2002; ⁵ Ssymank, 1991; ⁶ Gilbert *et al.*, 1985; ⁷ Personal measurements.

Discussion

Floral Traits

Heracleum sphondylium showed many of the classical traits of a generalist plant. Flowers were small, bowl-shaped, white, grouped together in an inflorescence and with easily accessible nectar and pollen. Flowers of *Rubus fruticosus* were not as small, and while several were present on any one stem, they were not grouped into as large an inflorescence as flowers of *Heracleum sphondylium*. They were however, white in colour, bowl-shaped and with easily accessible nectar and pollen. At first glance, the flowers of *Ipomoea trifida* may not seem to be as obviously generalist in terms of structure, however the pink to lilac colouring of their corollas is extremely pale, especially in comparison to the other brightly coloured flowers present in the same environment. The flowers are trumpet-shaped rather than flat or bowl-shaped; however the corolla is rather wide, making nectar easily accessible to all but the largest of visitor species. The nectar itself is highly concentrated (my own single measurement gave 57%), and the anthers and stigma are situated in such a way that any insect crawling into the corolla will become covered in pollen, even the very small ones (see Fig. 19), and will then transfer that pollen to subsequently visited flowers. If we consider the generalist syndrome as a “small and short-tongued” syndrome, *Ipomoea trifida* fits well with this. Large-bodied insects are unable to enter the corolla without damaging the flower, and long-tongued insects who visit to feed on nectar will not normally come into contact with the anthers and stigma, therefore failing to pollinate the flowers effectively. Some pollen could be transferred via the tongue, though in my study this was not apparent.



Fig. 19: *Notoxus* sp. dusted with pollen grains after visiting a flower of *Ipomoea trifida*.

Partitioning of Visitors over Time

Visitors to all plant species largely followed the daily partitioning shown in earlier chapters and previous studies (see Willmer and Stone, 2004). Larger species (see Tables 8-10) were active from earlier in the morning, when temperatures were lowest, and showed a decline over midday when temperatures were at their highest (see for example *Agapostemon* sp. on *Ipomoea trifida*, *Lucilia sericata* on *Heracleum spondylium* and *Bombus lucorum* on *Rubus fruticosus*). Smaller species were active later in the morning, though they were able to withstand the higher temperatures of midday for longer than larger species (see for example *Tetragonisca angustula* on *Ipomoea trifida*; *Episyrphus balteatus* on both *Heracelum sphondylium* and *Rubus fruticosus*).

In addition, because of the heat generated by flight, flying insects were more susceptible to overheating during high ambient temperatures than non-flying insects such as beetles and ants (Willmer *et al.*, 2000), and the activity of diurnal ant species in the tropics (the habitat of *Ipomoea trifida*) is known to increase in the hottest hours of the day, with reduction in periods of high humidity and during rains (Del-Claro and Oliveira, 1999; Oliveira *et al.*, 1999; Cogni and Freitas, 2002; Yamamoto and Del-Claro, 2008).

Body size is not the only factor to affect temperature regulation however, and not all bee species are equal in their thermoregulatory abilities. Darker coloured bees, like *Agapostemon* sp. and *Ceratina* sp., are better at absorbing radiation and are generally more active early in the day, while lighter coloured, reflective or smaller species are more common later in the morning and throughout the afternoon (Willmer, 1983; Schmida and Dukas, 1990; Potts *et al.*, 2003a,b). Mean temperature at 06:00 for *Ipomoea trifida* was still relatively high (25.5°C) in comparison to those for *Rubus fruticosus* and *Heracleum sphondylium*. Therefore, temperature constraints on flight may not be as apparent in this species at this time as they would be earlier in the morning, or in more temperate environments as, due to the higher ambient temperature, bees do not need to raise their thoracic temperature by as much as species in colder climes (Casey *et al.*, 1985; Stone and Willmer, 1989).

The flight activity of butterflies is also constrained by temperature and their foraging is limited to higher ambient temperatures, when they can bask in sunlight to gain heat, (Heinrich 1986; Dennis 1993; Watt 2003), which explains the observations of butterflies later in the morning than other visitor species

Visitations in the afternoon to all species were low in general, possibly more an effect of reduction in flower reward as flowers are visited frequently and drained of nectar (or emptied of pollen), though some visits to bagged flowers continued as these flowers had not yet been visited and therefore still offered full rewards.

Given the level of variation in factors such as body size in the species as described above however, it is again clear that analysing the visitors by visitor group could not give a true picture of their daily partitioning. Therefore, to gain a better idea of the factors underlying the different timing of activity on flowers, visitors should be analysed by individual species rather than functional groups.

Pollinator Effectiveness

Ipomoea trifida

Previous studies have indicated this species is pollinated by species from the beetle, fly, bee, butterfly, moth, ant, cricket, hummingbird and bat groups (McDonald, 1991; Chemás-Jaramillo and Bullock, 2002; Galetto and Bernardello, 2004; Price *et al.*, 2005; Wolfe and Sowell, 2006; McMullen, 2009; Pick and Schlindwein, 2011), though these studies are of the genus *Ipomoea* in general, and are based on visitation alone, and no studies of *Ipomoea trifida* specifically, or of the effectiveness of its visitors, has yet been carried out.

This population of *Ipomoea trifida* was visited by species of bee, ant, beetle, butterfly, hoverfly and wasp; however the visits of butterflies, hoverflies and wasps were too infrequent to allow for statistical analysis. A more intensive study of *Ipomoea trifida* may allow for more visits by these species and therefore an opportunity for their effectiveness as pollinators to be determined.

The visitors were first analysed by visitor functional group (see Fig. 13). The most effective pollinator was defined as that with the highest MPS, in this case the bee group. Only the bee and beetle groups deposited a significant MPS and were therefore the only groups classified as pollinators.

As described above, there is much variation in characters such as body size and tongue length within a functional group, and therefore we would expect variation in pollinator

effectiveness within functional groups. Pollinator effectiveness was therefore analysed by individual visitor species (see Fig. 14). When analysed in this way, we see that not all members of the bee and beetle groups are effective pollinators of *Ipomoea trifida*. Of the bee species, only *Agapostemon* sp., *Andrena* sp. and *Partamona musarum* were classified as effective pollinators, and only *Notoxus* sp. of the beetle group was an effective pollinator of *Ipomoea trifida*. The most effective pollinator was *Andrena* sp. (MPS = 155.68).

Ants are known to be generally poor pollinators, for many reasons described above (and see Beattie *et al.*, 1984; Hull and Beattie, 1998; Galen and Butchart, 2003; Willmer, 2011), and this is shown in *Ipomoea trifida*. The ants groomed themselves thoroughly after visiting flowers before visiting subsequent flowers, therefore limiting the amount of pollen they transport between flowers and reducing their pollinator effectiveness.

Given the floral traits of *Ipomoea trifida*, we would expect effective pollinators to be fairly small to medium in size, so as to fit inside to corolla tubes of flowers without damaging the flower itself, and thereby coming into contact with the anthers and stigma as they enter and exit the flower to feed. Long-tongued visitors would not be expected to be effective visitors as they may be able to feed on the nectar of flowers without entering the corolla tube, and therefore not coming into contact with the reproductive structures.

The floral visitors of this species are varied and from a range of taxa, however the most effective species share the traits of being relatively small and possessing short tongues. These visitors were seen to enter the flowers to feed in the manner described above, thereby effectively pollinating the flowers (see Fig. 21). Those visitors with long tongues which did not enter flowers to feed, such as the butterfly species, were not effective pollinators of *Ipomoea trifida*. While this plant was visited by many different insect types, as would be expected for a generalist flower, the visitors were not all effective pollinators, and those that were varied significantly in their pollen deposition. This plant therefore, is perhaps not as broadly generalist as initial observations of its visitor assemblage would suggest. In

that respect, its only partial match with the shapes and colours of a true generalist flower type could be said to indirectly reinforce the reality of a ‘generalist syndrome’.



Fig. 20: *Trigona fulviventris* dusted with pollen grains after visiting a flower of *Ipomoea trifida*.

Heracleum sphondylium

Previous studies have indicated the pollinators of *Heracleum sphondylium* flowers to include members of the orders Ephemeroptera, Dermaptera, Hemiptera, Thysanoptera, Neuroptera, Mecoptera, Lepidoptera, Diptera, Hymenoptera and Coleoptera (e.g. Willmer 1983; Sheppard, 1991), and the species is often touted as an exemplary generalist, being visited by at least 40 insect taxa. Studies into the effectiveness of such visitors, however, have indicated that not all visitor species carry significant amounts of pollen (Zych, 2002),

and that the only consistently effective pollinators were syrphid flies and greenbottles (Zych, 2007).

The population of *Heracleum sphondylium* I studied was visited by species of hoverfly, other dipterans and beetles. As described in other chapters, bee populations at the time of the study were unusually low, and while bees were observed to make occasional visits to flowers of *Heracleum sphondylium*, these visits were exceptionally rare and they did not visit any of the target flowers.

The pollinator effectiveness of flower visitors was first analysed by visitor functional group (see Fig. 15), in this case hoverflies, other dipterans and beetles. The most effective pollinator group for *H. sphondylium* was the other dipteran group. Only the other dipteran and hoverfly groups were identified as effective pollinators.

Again, when pollinator effectiveness was analysed by individual visitor species (see Fig. 16), the most effective visitor could be identified. This was the calliphorid *Lucilia sericata* (MPS = 116.09). Of the other dipterans, only *Lucilia sericata*, Platypezidae sp., and Anthomyiidae sp. were pollinators; and of the hoverflies, *Epistrophe grossulariae*, *Episyrphus balteatus*, *Eupeodes latifasciatus* and *Syrphus ribesii* were the only species to be identified as pollinators. The beetle Chrysomelidae sp. was only observed in a single visit, and was therefore excluded from statistical analysis.

Given the floral structure of *Heracleum sphondylium*, we would expect effective pollinators to have relatively small bodies and short tongues, so that they must get close to flowers to feed upon them and therefore come into contact with the anthers and stigmas. Longer-tongued insects would be able to feed on flowers without substantially coming into contact with the reproductive structures of the flowers and would therefore not be expected to elicit effective pollination. Some pollen may be carried on feet or on long tongues, though in my study this was not evident. The flowers are also particularly suited to less mobile insects such as beetles, which can move across the umbel inflorescence with ease. While

beetles were not frequent visitors to *Heracleum sphondylium*, a more intensive study may allow for a measure of their effectiveness as pollinators.



Fig. 21: Hoverfly *Syrphini* sp. feeding upon flowers of *Heracleum sphondylium*. Due to the short tongue of the species, it must get close to the nectar source in order to feed, and in doing so makes contact with the anthers and stigma of the flower.

The effective pollinators of *Heracleum sphondylium* all fit the profile suggested by the floral structure, and all were observed to feed upon flowers in the “correct” manner (see Fig. 21). Long-tongued or large-bodied visitors did not visit flowers during the study period. The floral rewards of this species may not have been attractive to such visitors, given the high cost of floral handling time at such small flowers for a large, long-tongued insect. While the visitor assemblage of *Heracleum sphondylium* was varied, the visitors could be considered part of a functional group of small, short-tongued visitors. This plant species, therefore, could be considered as being “specialised” towards “generalist” flower visitors. It should be noted,

however, that not all visitors were equal in their pollen deposition, and therefore their pollinator effectiveness was significantly variable. Not all visitors should be treated as equally efficient, therefore, and their true pollinator effectiveness should be determined via methods such as those above.

Rubus fruticosus

The flowers of *Rubus fruticosus* have been indicated in previous studies to be pollinated by bees and hoverflies (e.g. Yeboah Gyan and Woodell, 1987a). As before, such studies have not involved any measure of pollinator effectiveness, and have relied on visitation records and observations only, therefore the identification of species as pollinators is not necessarily accurate.

My population of *Rubus fruticosus* was visited by species of bee, wasp, hoverfly and other dipterans. Pollinator effectiveness was first analysed by visitor functional group (see Fig. 17), and the most effective visitor group was the bee group. The wasp and other dipteran groups were excluded from analysis due to unusual variance. Only the bee group deposited a significant MPS in comparison to control stigmas and bees were therefore the only group identified as effective pollinators.

When analysed by visitor species (see Fig. 18), the variation in traits such as body size and tongue length within visitor groups led, once again, to variation in MPS between species. Several visitor species had to be excluded from analysis due to low visitation rates or unusual variance (see above). Of the species analysed, the most effective pollinator was *Bombus lucorum*. The bees *Bombus lucorum*, *Bombus pratorum*, *Bombus terrestris*, *Bombus pascuorum*, *Andrena* sp., *Apis mellifera* and the hoverfly *Rhingia campestris* were all identified as effective pollinators.

Given the results from the other generalist flowers studied above, this pollinator assemblage may seem unusual. It must be remembered, however, that several visitor

species were excluded from analysis for various reasons which were probably linked to the limits of the study. A more extensive study of the species may yield more data for a more accurate analysis, eliminating issues which caused certain visitors to be excluded from analysis (such as low N values or unequal variance) and allowing for effective analysis of their pollinator effectiveness. The flowers of *Rubus fruticosus*, while fitting with the generalist syndrome, differ from the two other studied species in ways which may allow larger, longer-tongued insects to effectively pollinate the flowers. The flowers are larger than those of *Heracleum sphondylium*, the ring of anthers possess relatively long filaments, and the ring is rather wide, meaning that even longer-tongued insects feeding from the edge of the flower will still make contact and pick up pollen grains (as smaller insects would do when crawling through the anthers to reach nectar). Larger bodies would also make contact with the extensive anther display in this way. Though their tongues are longer than those of the hoverfly and dipteran visitors, the bees observed are all considered relatively short-tongued species in comparison to other bees (see Chapters 5 and 7). The same applies to the long-tongued hoverfly *Rhingia campestris* which, despite its long proboscis, was still observed to contact anthers when feeding on flowers.

Conclusion

Flowers which share traits such as flower shape, colour or reward are often considered to be part of the same flower syndrome. A flower within a given syndrome has certain characteristics that attract particular visitors, excluding others. Certain flower visitors which are suited to these floral traits will be able to effectively pollinate flowers, while those who do not fit the traits may be ineffective pollinators. While the generalist syndrome is often not included alongside other typical pollination syndromes, and is perhaps not considered a “true” syndrome, I would contend from my results that this particular flower form deserves to be considered as a syndrome in its own right.

The flowers studied here share traits such as flower colour and shape (though not size), and attract similar broad visitor assemblages. These visitor assemblages were highly varied across taxonomic groups, however the basic feature of short tongues was prevalent, and in most cases body size was small. While generalist flowers attract a broad visitor assemblage, the placement of reproductive structures can limit effective pollination to only certain visitors of a particular size or body shape, for example the small *Lasioglossum* sp. and *Ceratina* sp. bee visitors of *Cypripedium plectrochilum*, part of a diverse assemblage of visitors including large bees, small bees, ants, flies and butterflies but being the only species to make contact with the reproductive structures in such a way as to effect pollination (Li *et al.*, 2008). The placement of reproductive structures in the flower studied here is such that, when pollen deposition on stigmas was measured, they were shown not to be effectively pollinated by long-tongued species which can feed on nectar without substantially contacting anthers or stigmas. Conversely, short-tongued insects must get close to the nectar source in order to feed, and therefore come into contact with the reproductive structures and effectively pollinate the flowers. Small visitor body size is generally also associated with these flowers, most obviously in *Ipomoea trifida*, where the width of the corolla tube excludes larger-bodied visitors and forces small visitors to crawl into the flower for access to nectar, thereby contacting the anthers and stigma and pollinating the flower.

As with all syndromes however, there are flowers which vary slightly from the general traits. *Ipomoea* flowers have an unusually attractive visual display, and are rather large and rather elongate compared to other generalists; some apparent visitors are not in fact good pollinators. *Rubus fruticosus* flowers are also larger in size and they provide a large enough nectar reward for bigger-bodied species to visit; but these visitors still fit with the short-tongued requirement, and must still get relatively close to the nectar source and therefore to the reproductive structures of the flower. Given the convergence of floral traits, the attraction and increased effectiveness of certain flower visitors, and the exclusion of other visitor types, it is reasonable to consider flowers of both these species as part of a broadly defined

generalist flower syndrome. However, flowers such as *Rubus fruticosus* could perhaps be considered a subdivision of the generalist syndrome, having a higher nectar reward and attracting a greater visitor diversity, including some long-tongued bees. This subdivision was termed a 'cornucopia generalist syndrome' by Willmer (2011), and could possibly include other more morphologically specialised flowers such as *Cirsium* and *Centaurea* (Ellis and Ellis-Adam, 1993; Corbet, 2006), where nectar production is so high that the corolla tube is filled to a level where short-tongued visitors can effectively feed (see Chapter 7).

This leads us to another issue with the definition of pollinator syndromes. Pollinator syndromes are usually applied to flowers which are pollinated by particular taxonomic groups, such as bees, flies, beetles or birds. As has been shown, in this and other chapters, the variation within these taxonomic groups in factors which will affect pollination (such as body size, tongue length and other traits) is high, and some of these traits overlap across taxonomic groups. While generalist flowers appear to be pollinated by a range of visitors from different taxonomic groups, these visitors share characteristics of being usually small-bodied, and possessing short tongues. If we consider the definitions of pollinator syndromes from the point of view of visitor functional groups rather than taxonomic groups, the range of visitors to generalist flowers is not as broad as would at first appear, rather, it comprises a smaller subset of pollinators across functional groups but possessing similar traits. Can such a syndrome, therefore, be accurately described as a generalist according to current definitions of the term?

In a review of generalist pollination systems, Ollerton *et al.* (2007) highlighted the concepts of "apparent generalisation" and "fundamental generalisation". The first refers to the number of flower visitors which interact with its flowers, whether pollinators or not, and is moderated by the phenotypic influences of the flower, the set of morphological (size, shape, colour) and biochemical (odour, reward) traits, and the behavioural responses of the flower visitors, which determine the "fit" between the flower and visitors. The latter relates to the number of potentially effective pollinators with which it could interact (Vásquez and Aizen,

2006). The moderating influence between this and the “realised generalisation” of a given plant species is therefore the setting for the interaction, i.e. the “community context in which the plant finds itself (Vásquez and Aizen, 2006). They offered five different case studies of generalist flowers to illustrate this point:

- Example A: Open access flowers in which all flower visitors provide more or less equally good pollination services (ecological and functional generalisation).
- Example B: Open access, apparently classical generalist flowers that attract only a very narrow spectrum of pollinators (functional, and possibly ecological, specialisation).
- Example C: Flowers that attract a wide range of visitors, but which are pollinated mainly by a narrow subset of those flower visitors (functional specialisation)
- Example D: Flowers that appear to be phenotypically specialised, implying a functionally specialised pollination system, but which are in fact pollinated by whatever flower visitors are a suitable size and shape, and have appropriate behaviour (functional and ecological generalists).
- Example E: Plant species which are pollinated by different animal species in different parts of their range, and therefore are more ecologically or functionally generalised at a species level than is apparent at a population level.

Example D could perhaps describe the pollination syndrome of *Ipomoea trifida*. While this species may not be specialised in the sense of some other plant species seen in earlier chapters, however its floral traits do not fit completely with those of a classical generalist syndrome either. It is however pollinated by a rather wide range of different insect species (though perhaps not equally effectively by all) with a suitable size and shape for effective pollination. *Ipomoea trifida* can therefore be defined as a functional and ecological generalist.

Rubus fruticosus and *Heracleum sphondylium* on the other hand perhaps fit better with the traits of example C, where they attract a wide range of flower visitors but are effectively pollinated by only a subset of these visitors, in this case those which are small and short-tongued. These plant species can therefore be termed as functional specialists rather than the broad generalists they would at first appear without data on pollinator effectiveness.

My study of these 3 plant species was limited however, and the effectiveness of several visitor species was not analysed due to low visitations. Further intensive studies of these species may show other species to be effective pollinators of *Ipomoea trifida*, *Heracleum sphondylium* and *Rubus fruticosus*. What is clear from this study however is that these flower species converge in floral traits, and share visitors from a functional group of generally small-bodied species with short tongues, which belong to a variety of taxonomic groups. This convergence suggests that the flowers share a pollinator syndrome, in this case termed the generalist flower syndrome, though perhaps more accurately the short-tongued insect-pollinated syndrome. Long-tongued visitors were not effective pollinators of these species when pollen deposition on stigmas was used as a measure of effective pollination, as they did not make substantial contact with the reproductive structures of the flowers when visiting. The most effective pollinator species was not always the most frequent flower visitors; therefore measures such as visit frequency should not be used as a determinant of effective pollination. The inclusion of measures of pollinator effectiveness, in this case single-visit pollen deposition, allows us to not only define the effective pollinators of a given species, but also to measure their relative effectiveness and therefore their real importance to the flower species.

Chapter 9:

Scaling up Single-Visit Pollen Deposition

Introduction

While records of visitation frequencies are not an efficient means of assessing pollinator importance on their own, when combined with values of single-visit pollen deposition (see Chapters 3-8) they can be used to scale up pollinator performance to a per-day or per-hour pollinator performance value (Ne'eman *et al.*, 2010). This chapter takes single-visit pollen deposition values for the visitor assemblage of a population of *Agrimonia eupatoria* (see Chapter 4) and combines it with a visitation survey of the same population to gain a better understanding of the performance of pollinators over a given period of time.

Visitation Frequency Surveys

The study of behavioural patterns of foragers on flowers is key to any investigation in pollination biology, and, while it is seldom the primary goal, it is a crucial element related to the study of pollen deposition, dispersal and carry-over; pollination efficiency; pollination energetics; resource utilisation by foragers; advertisement and visitation frequency; pollinator community composition; and activity related to weather. The protocols in this chapter follow the guidelines of Dafni *et al.* (2005), described further below.

As has been shown in earlier chapters, not all visitors are pollinators of a given plant species. To show conclusively that a flower visitor is indeed a pollinator, the following statements must all be true:

- The agent visits the flower
- It carries conspecific and viable pollen
- Sufficient pollen is deposited on the correct receptive stigma at the right time

The single-visit pollination effectiveness protocols of earlier chapters address the latter two conditions. While it was not possible to determine the viability of pollen deposited, I did determine if pollen was conspecific, and grains which were not were discounted from analyses. Observations of the plant species investigated in previous chapters, and notations of the behaviour of visitors to these flowers, addressed the first condition. As these observations only applied to the first visit of an agent to previously bagged flowers however, they do not give us an accurate representation of the overall visitation frequencies of the visitor assemblage of these plants, especially if agents make multiple visits to flowers. To gain an accurate representation of a visitor assemblage of a given plant species, incorporating visitation frequency as well as pollinator effectiveness, Dafni *et al.* (2005) offered the following recommendations:

a) Sampling units should be standardised. The number of flowers or inflorescences, and the age distribution of flowers within the observation unit, should be recorded. Reward availability greatly influences the activity of visitors, therefore it is advisable to determine the quantity and type of floral rewards available, as well as phenological events in single flowers prior to the main study. An observation unit of at least 10 to 20 minutes per hour through the pollinator's activity period (depending on the frequency of visitors) was suggested to be a fair representative sample.

b) The behaviour of many flower visitors, and in particular insects, is related to temperature (e.g. Herrera, 1995; Willmer and Stone, 2004; Hegland *et al.*, 2009). Flight activity and the rate of nectar consumption are related to the temperature and water balance of the pollinator, which is in turn influenced by relative humidity, wind velocity and solar radiation

(Willmer, 1985). An effective visitation survey, therefore, should consider the possible effects of such variables and record them in conjunction with the study.

c) Visitors should be categorised appropriately. While it is agreed that pooling of data of individual species into broader categories such as genera or functional group may ease recordings of observations and statistical analysis, it may risk masking differences among species in their visitation and pollination effectiveness. Where possible, visitors should be categorised by individual species, or to as high a level as possible, as in this thesis.

d) Observing visitor behaviour in the field requires that the sampling unit be defined, for example a focal plant or transect. The most common units for sampling are a single flowering plant, a group of small plants or part of a large flowering individual. This method has the advantage that the same area of habitat can be repeatedly sampled, for example throughout a day, and is well suited to the study of a single flowering species when detailed data on the foraging behaviour of visiting fauna are required. The size of the study area should be small enough that the observer is able to see the whole area, yet large enough that visitation rates are high enough for sufficient data to be collected. Making a sketch-map of relative flower positions, recording the colour and age reward status of each then assigning them numbers, may facilitate rapid collection of data. The observer should be located as close as possible, without interfering with visitor behaviour. The behaviour of the visitor on the plant should be recorded, for example nectar feeding, pollen collection or thievery.

Knowing the number of visits made to a plant by each species throughout the day allows for calculation of a number of useful parameters, such as the absolute number of flowers visited per day in a focal patch. Combining this with information on the mean number of pollen grains deposited per flower visit allows the absolute pollination effectiveness of a particular pollinator on a particular flower to be estimated (e.g. Potts *et al.*, 2001). Once

these data are collected they can be analysed appropriately in conjunction with complementary studies to allow:

- Determination of relative visit frequencies of various flower visitors
- Identification of the effectiveness of pollinators
- Examination of the rewards collected by visitors
- Differentiation between thieves and legitimate visitors
- Determination of foraging behaviour in relation to weather
- Measurement of pollen movement

The above recommendations were used to construct an effective protocol for recording visitation to *Agrimonia eupatoria* and combining it with single-visit pollinator effectiveness for a per hour or per day pollination effectiveness value of the visitor assemblage of this plant at this site.

Methods and Materials

Study Site

Ten inflorescences of a population of *Agrimonia eupatoria* were investigated at West Quarry Braes, a Scottish National Heritage Site in Fife (NO 597 088), as described in previous chapters.

Visitation Survey

Each inflorescence was assigned a letter as a means of identification, and each individual flower on each inflorescence was assigned a number. Flowers were observed from a sufficient distance where inflorescences and flowers were visible, but visitor activity was not disturbed, for twelve 45 minute intervals from 06:45 until 18:30 on the 30th of July

2009. Temperature and relative humidity records were taken at the start of each observation period using a HM34 Vaisala Pocket Size Relative Humidity Meter.

As each visitor (always a hoverfly) arrived it was photographed, and the flower number and inflorescence letter was noted. To avoid inaccurate results by removing visitors before a foraging bout was completed; visitors were not captured for identification and were allowed to visit subsequent flowers. The sequences of these subsequent visits were also recorded. Species were identified as accurately as possible from photographs using keys from Stubbs and Falk (2002), though for some it was only possible to identify to Tribe.

The behaviour of visitors on the flowers was also recorded, though, as *Agrimonia eupatoria* provided only pollen as a floral reward, all visitors fed only upon the pollen of flowers.

Observations continued until no pollinator activity had been observed for 30 minutes, allowing for a full visitation record for the assemblage of *Agrimonia eupatoria* throughout the day.

Scaling up Pollinator Performance

Single-visit pollen deposition values for the pollinator assemblage of *Agrimonia eupatoria* (see Chapter 4: Testing Pollinator Syndromes: The Hoverfly-Pollinated Syndrome) were combined with visitor frequencies to generate a per hour and per day pollinator performance value using the formula below (equation 1; Ne'eman *et al.*, 2010; see Chapter 1: Introduction).

$$D_t = D_v fr \quad \text{Equation 1}$$

Here, D_t refers to pollen deposition effectiveness at the per hour (or per day) level, D_v refers to pollen deposition effectiveness at the single visit level, f is the visitation frequency per hour (or per day) and r is the proportion of flowers with receptive stigmas within the

observed patch. As only flowers with receptive stigmas (at least as inferred from inspection; see Dafni *et al.*, 2005) were observed during both the visitation and pollinator effectiveness studies, the value for r is taken as 1.

To calculate visitation frequency per hour for each visitor, the total number of visits across the study period was divided by the length of the observation period, in this case 12 hours. Visitation frequency across the day was defined as the total number of visits during the observation period as visitors were assumed to be active during daylight hours only (observations showed visitation to end after the sun went down, and flowers were depleted of pollen by early evening), and the length of observation period coincided with daylight hours at this time of year, as evidenced by a complete lack of visitation after approximately 18:00.

As it was not always possible to identify visitors to species level during the visitation survey in the same way as for the pollination effectiveness study, I could not match the two studies completely. Visitors of the Tribes Bacchini and Syrphini were grouped together, and a mean value of MPS was calculated across these species. Species and functional groups which were observed during the visitation survey, but not during the pollinator effectiveness study, were excluded from analysis.

Results

Flower Visitation

The number of individual flowers visited, the visitors, and the visitor species variation were recorded throughout the day (see Fig. 1). All variables peaked between 09:45 and 11:30, and all showed a marked dip between 13:45 and 14:30, coinciding with the highest temperature and relative humidity of the day (see Fig. 2). All three variables declined again through the afternoon as temperatures decreased and floral resources depleted. Figures 3a

and b show the partitioning of visitor species throughout the day, with the appropriate temperature for each observation period.

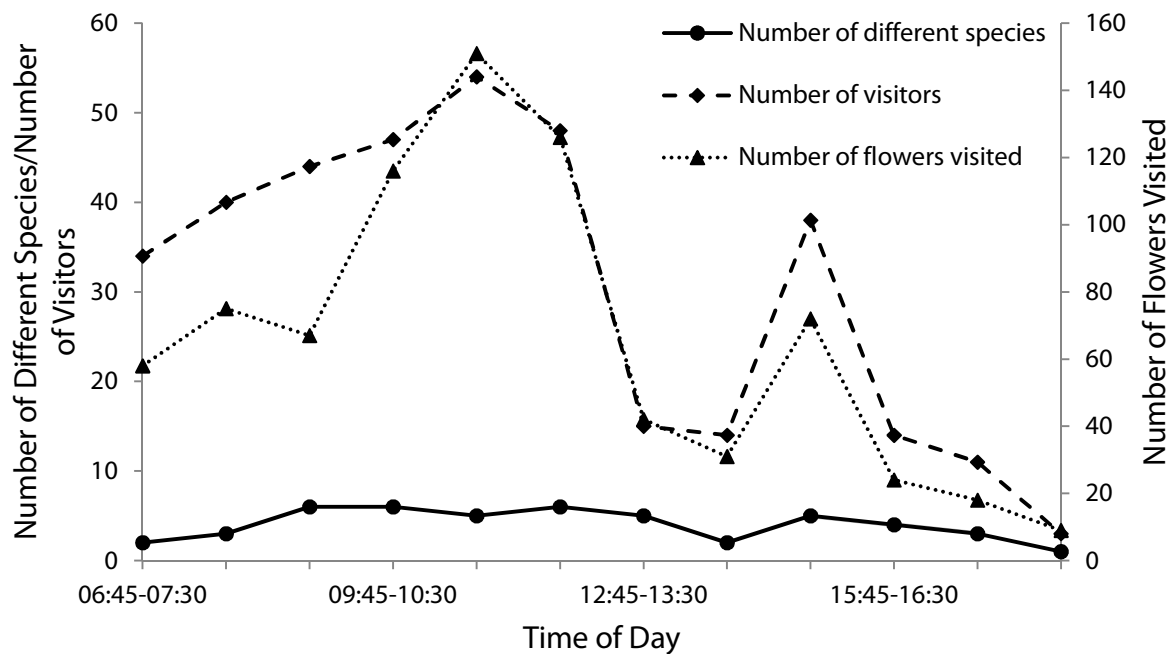


Fig. 1: Number of different visitor species, total number of visitors and total number of flowers visited during each of the 12 time periods.

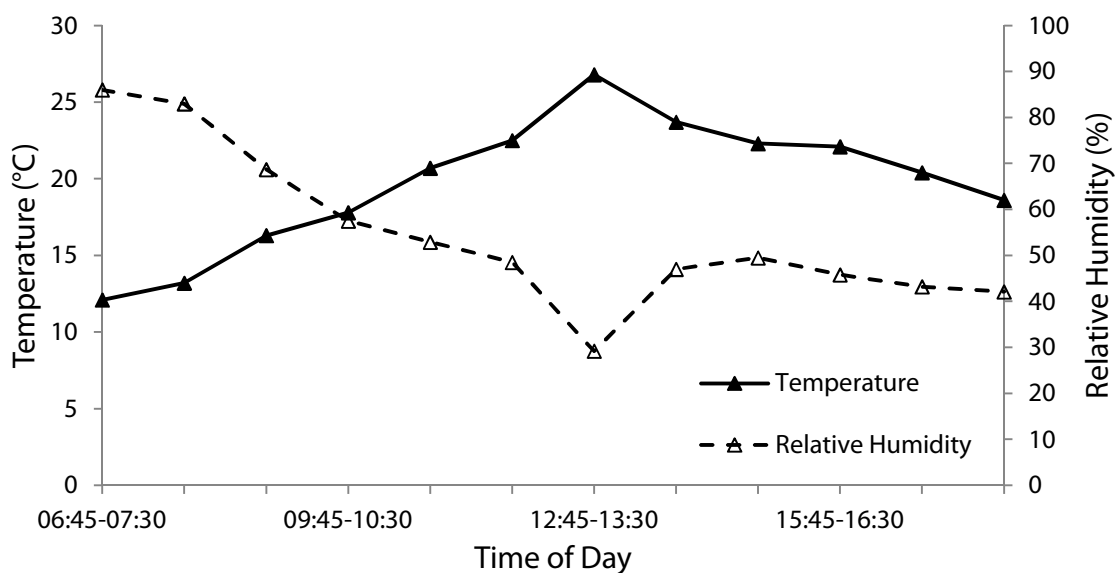


Fig. 2: Temperature (°C) and relative humidity (%) readings during each of the 12 observation periods of *Agrimonia eupatoria*.

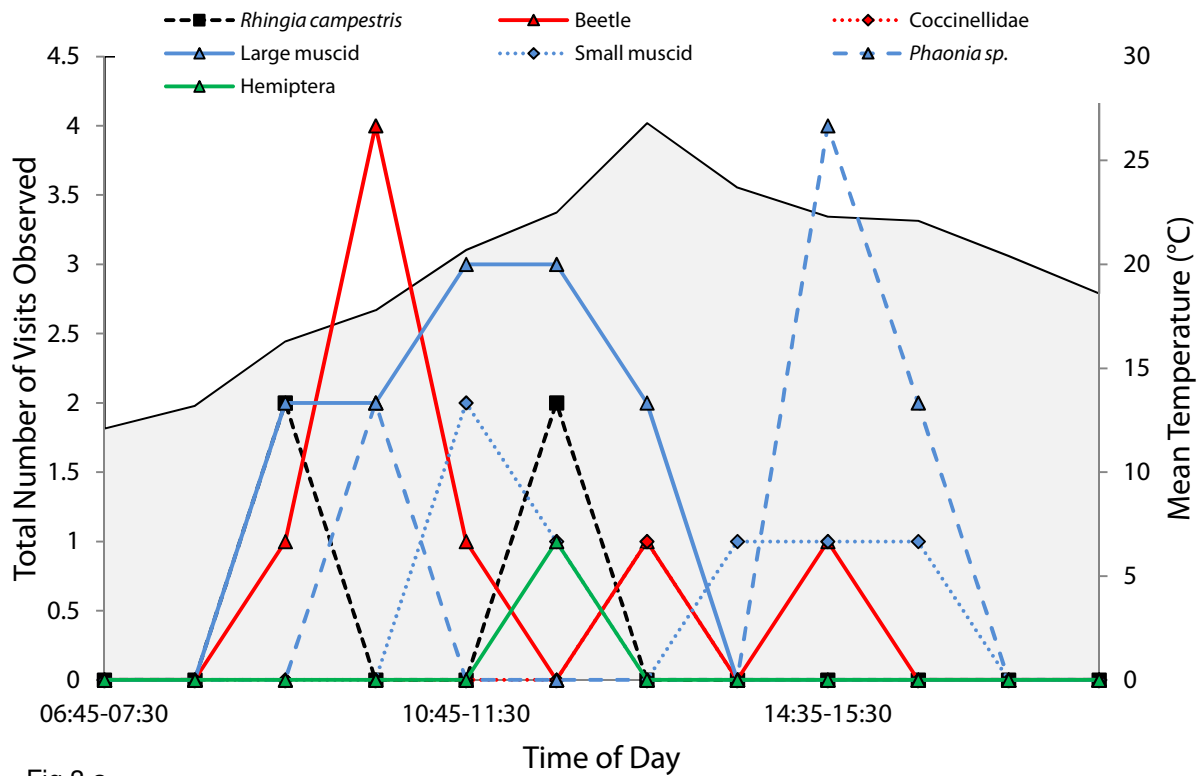


Fig 3.a

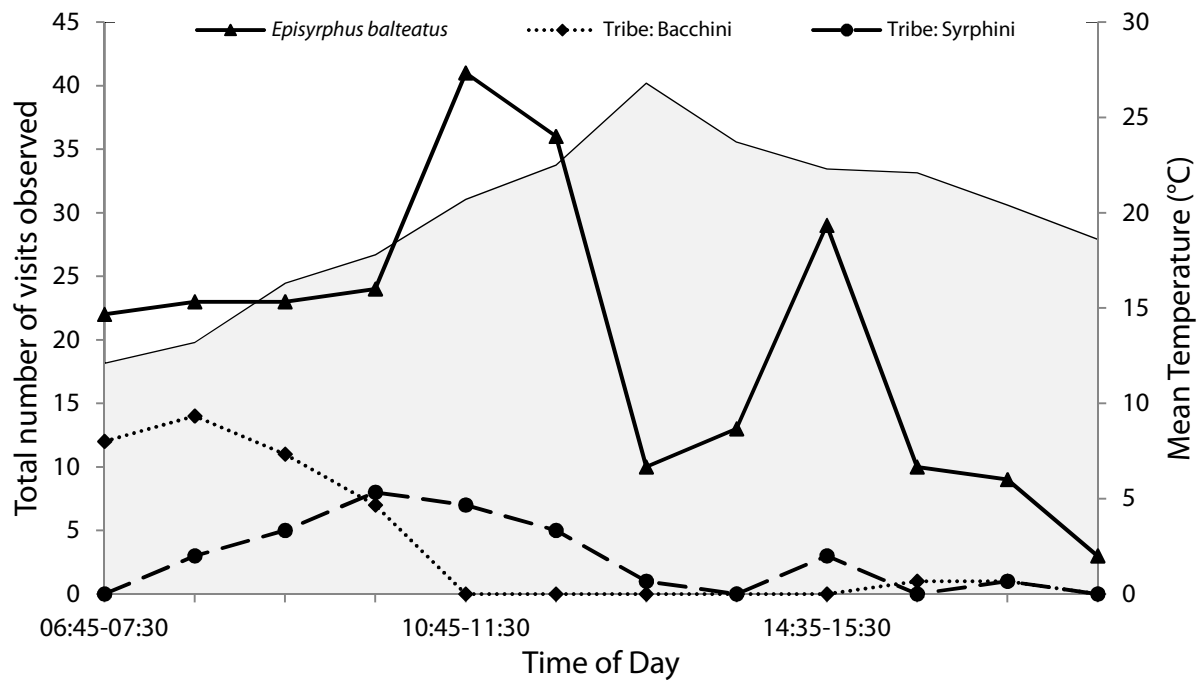


Fig 3.b

Fig. 3 a and b: Partitioning of flower visitors over time to flowers of *Agrimonia eupatoria*. Hoverfly species are indicated in black, beetles in red, other dipterans in blue and hemipterans in green. For clarity, the most abundant visitors (hoverflies *Episyrphus balteatus*, Tribe: Bacchini and Tribe: Syrphini) are shown separately in Fig 3b; note the different y axis. Mean daily temperature (°C) is shown as an area plot.

The number of visits each individual flower received was also recorded (see Fig. 4). Generally, the youngest flowers (at the top of the inflorescence) were visited least frequently, followed by the oldest flowers (at the bottom of the inflorescence). The mid-age flowers (at the middle of the inflorescence) were the most frequently visited. Each flower received a mean of 8.5 visits in a single day. Inflorescences with more flowers (i.e. A, F, G, H) received more visits in total than inflorescences with fewer flowers (i.e. B, C, D, E, I, J), however individual flowers on smaller inflorescences received significantly more visits than individual flowers in large inflorescences (Mann-Whitney U test: $U = -2.649$; $N = 92$; $P = 0.008$).

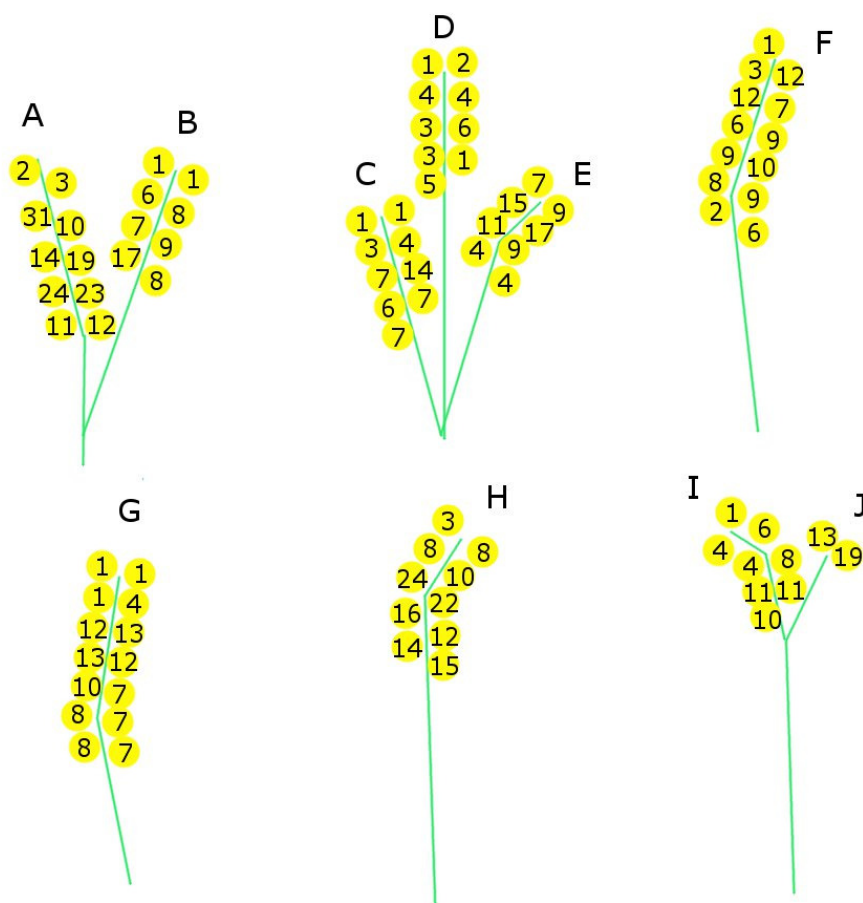


Fig. 4: Formation of inflorescences observed. Total number of visits to each individual flower indicated. Each flower received a mean of 8.53 visits over the course of the day ($N = 93$; $SD = 6.02$). 793 flower visits in total were observed.

Some flower visitors showed particular patterns in their foraging bouts (see Table 1). All visitors, with the exception of Coccinellidae, made at least some 'directional' visits between flowers on inflorescences, the majority of which involved upward movements. All visitors made more upward movements than downward movements (with the exception of the hemipteran, which made only one movement in each direction). *Episyrphus balteatus* made the highest total upwards movements, and in particular the highest sequential upward movements. More than half of the upward movements by *Episyrphus balteatus* were to the next sequential upward flower. *Rhingia campestris* made few directional visits, however those it did make were to sequentially upward flowers. Beetles and other dipterans also made consistent sequentially upwards movements.

Of the hoverfly visitors, those from the tribe Bacchini visited the highest mean number of flowers per inflorescence. Beetles visited the highest mean number of flowers per inflorescence overall and other dipterans such as the muscids and *Phaonia* sp. visited on average only 2 flowers per inflorescence.

As shown in Fig. 4, the most frequently visited flowers on an inflorescence were those positioned in the middle of the inflorescence.

Visitor	Total No. flower visits recorded	Mean No. flowers visited per inflorescence	Mean position of 1 st flower	Intra-spike movements					
				No. overall upward movements	Percentage of total visits which are upward	No. overall downward movements	Percentage of total visits which are downward	No. movements to next upward flower	No. movements to next downward flower
<i>Episyrphus balteatus</i>	459	2.21	4.22	219	48%	32	7%	162	14
<i>Rhingia campestris</i>	9	2.25	2.50	4	44%	0	0%	4	0
Tribe: Bacchini	104	3.10	4.18	55	53%	9	9%	53	5
Tribe: Syrphini	78	2.47	3.13	41	53%	5	6%	37	4
Beetle	36	4.75	1.13	30	83%	0	0%	30	0
Coccinellidae	3	3.00	4.00	2	67%	0	0%	2	0
<i>Phaonia</i> sp.	19	2.00	4.25	8	42%	0	0%	7	0
Large muscid	27	2.33	3.75	14	52%	0	0%	14	0
Small muscid	13	2.17	2.83	6	46%	1	8%	5	0
Hemiptera	3	3.00	4.00	1	33%	1	33%	1	0

Table 1: Summary of the feeding behaviour of flower visitors to inflorescences of *Agrimonia eupatoria*. Columns relating to upwards and downward movements refer to movements made on the same spike inflorescence. Flowers on each inflorescence were numbered from the base of the inflorescence up. The mean midpoint of the inflorescences was flower number 4.7.

Per Hour and Per Day Pollinator Performance

Visitor	MPS	Total No. Visits	Visits/Hour	MPS/Hour	MPS/Day
<i>Episyrphus balteatus</i>	27.6	209	23.2	640.9	7691.2
Tribe: Syrphini	41.4	32	3.6	147.2	1766.4
Tribe: Bacchini	50.2	33	3.7	184.1	2208.8
<i>Rhingia campestris</i>	55.2	4	0.4	24.5	294.4

Table 2: Summary of the MPS values at the single visit scale for each of the four main visitor categories, with the relevant scaled up MPS values at the per hour and per day level.

Pollinator performance per hour was calculated for each visitor (correcting for the fact that flower spikes were observed for only 45 minutes out of each hour during the study period by multiplying by 4/3; see Fig. 6). *Episyrphus balteatus* had the lowest MPS at the single visit scale, however because of its very high visitation rate (Fig. 4) it had the highest MPS at the per hour scale. Conversely, *Rhingia campestris* had the highest MPS at the single visit scale, but the lowest MPS at the per hour scale. Visitors from the tribe Syrphini and the tribe Bacchini both showed a moderately higher MPS at the per hour scale. It should be noted, however, that visitors from the tribe Bacchini were more active earlier in the morning, while those of the tribe Syrphini were more active later in the day. This may mean that those of the tribe Bacchini are more effective pollinators as, while both tribes deposited a similar MPS, the Bacchini visitors will deposit pollen on stigmas first, and may effectively pollinate flowers before any Syrphini visit, meaning subsequent pollen deposited by Syrphini visitors could be essentially wasted.

Pollen deposition was then scaled up to the per day level (see Fig. 6), though in this case, as pollinators were only active during daylight hours, this was limited to a 12 hour period, corresponding with daylight hours at the time of the study. *Episyrphus balteatus*

remained the most effective pollinator in terms of pollen deposition over the day, while *Rhingia campestris* was the least effective visitor.

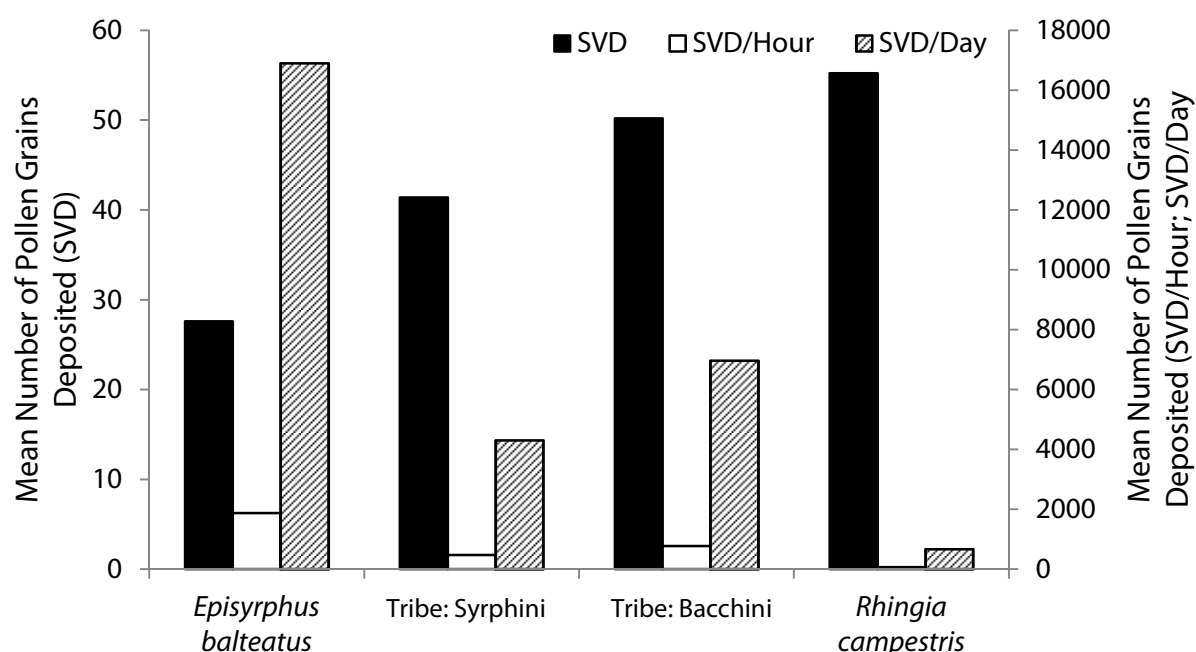


Fig. 6: Corrected MPS values at the single visit, per hour and per day level for each of the four main visitor groups.

Discussion

Flower Visitation

While it was possible to capture and identify visitors of *Agrimonia eupatoria* when measuring single visit stigmatic pollen deposition, to do so when recording frequency of visitation would have altered results dramatically, by not allowing visitors to continue with subsequent flower visits. As a result, not all species recorded could be identified to species level. While there is much variation within tribes and even genera, there is also some level of uniformity and common traits, therefore variation is not as extreme as within the functional

group of “all hoverflies”. It is thus reasonable to group visitors into their relevant tribe or genus where identification to individual species is not possible.

Mid-age flowers at the middle of the inflorescence were more frequently visited than the younger flowers at the top or the older flowers at the bottom (see Fig. 5). This is most likely due to floral reward availability. Flowers are typically viable for up to two days, stigmas becoming receptive after than anthers have dehisced (see Chapter 4), and the anthers and stigmas withering on the third day, though the flowers retain their yellow corolla as a visual attractant for visitors. Flowers at the bottom of the inflorescence are therefore almost completely depleted of pollen, and not as attractive to visitors, while younger flowers at the top of the inflorescence have possibly not fully opened or dehisced. No nectar was visible on young, mid-aged or old flowers at any time. Inflorescences with fewer flowers received fewer visits in relation to those with more flowers, as in other studies (e.g. Thompson, 1988; Harder *et al.*, 2004; Makino and Sakai, 2007; Ishii *et al.*, 2008). Individual flowers of small inflorescences received statistically more visits than did individual flowers of large inflorescences as has been seen in some plant species and modelled as the optimum response for the plant (e.g. Iwasa *et al.*, 1995).

Almost all flower visitors (hoverflies, beetles and other dipterans) made consistent sequential visits to flowers, as has been shown in other species such as bees and some wasps (Pyke, 1978; Corbet *et al.*, 1981; Jordan and Harder, 2006; Ishii *et al.*, 2008; see also Chapter 5). This behaviour, however, has not previously been recorded in hoverflies, other dipterans or beetles. Sequentially upward flower visiting would be particularly beneficial in terms of promoting out-crossing in *Agrimonia eupatoria* due to the separation of sexual phases in this species. As shown in Table 1, visitors usually ignored the lower flowers of the inflorescence, which retain their yellow corolla but have withered anthers and stigmas, landing first on mid-age flowers which have receptive stigmas and either fully or mostly dehisced anthers. Pollen picked up from previously visited inflorescences (i.e. outcrossed pollen) is deposited on these stigmas, while pollen is picked up from the anthers. The visitor

then moves upwards to the next flowers, which are typically younger, with dehiscing anthers (and sometimes with receptive stigmas, meaning that self-pollen may be deposited) where more pollen is picked up. These younger flowers are still viable on the second day, when the stigma usually becomes receptive, where they stand a higher likelihood of receiving outcrossed pollen as above. When the insect leaves the inflorescence, usually after 2-4 flowers, it can then carry pollen to the mid-age flowers of the next inflorescence. While this does not guarantee the receipt of outcrossed pollen, it does increase the likelihood of this happening. As younger, higher up flowers are less likely to have opened, and therefore to have pollen, which is in this case the only reward produced, visitors are more likely to leave the inflorescence in search of rewarding flowers, again reducing the likelihood of self-pollen being deposited.

Scaling up Pollinator Performance

Visitors which may be poor pollinators at a single visit level may increase their pollination performance at the per hour, or per day, scale by making frequent flower visits. Conversely, species with high single visit pollinator effectiveness may have a poor pollination performance at a per hour, or per day, scale if they are infrequent flower visitors.

Episyrphus balteatus was an example of the former. At a single-visit level it was one of the poorer pollinator species to visit *Agrimonia eupatoria*, however it had one of the highest visitation frequencies (see Fig. 3). As a result, when the pollination effectiveness of *Episyrphus balteatus* was scaled up to the per hour level, and the per day scale, the species had by far the highest pollen deposition of the visitors studied. It should be noted though that *E. balteatus* individuals visited several flowers on each inflorescence, therefore it is likely that much of the pollen deposited is self-pollen rather than outcrossed pollen.

Rhingia campestris provided an example of the opposite effect. It was the highest performing pollinator at the single-visit scale, though visits by this species were infrequent (Fig. 3), therefore it became one of the apparently poorer performing pollinators at the per

hour or per day scale. As Table 1 shows, however, it visited fewer flowers per inflorescence than other syrphids, as well as visiting many fewer flowers in total, so would have moved more outcrossed pollen onto stigmas, enhancing its beneficial effect for the plant. *Rhingia campestris* is also one of several hoverfly species known to show good floral constancy (Gilbert, 1981; 1985; Haslett, 1989a; Hickman *et al.*, 1995; Gilbert and Jervis, 1998), and if this is also true for its behaviour on *Agrimonia eupatoria*, the low visitation to this particular population of *Agrimonia eupatoria* may mean that other populations are being visited; therefore again *Rhingia campestris* could be depositing high levels of outcrossed pollen rather than self-pollen. Further studies of the foraging behaviour of *Rhingia campestris* on *Agrimonia eupatoria* could prove the quality of pollen deposition by *R. campestris*. A significant proportion of the visits *Rhingia campestris* made, and in particular all the flower visits where it visited more than one flower per inflorescence, involved sequentially upward movements, which, as described above, would again increase its pollination effectiveness.

Conclusion

Foraging behaviour of insect flower-visitors is dependent on a combination of intrinsic and extrinsic factors. Intrinsic factors may be dimensional, behavioural and physiological, as described in earlier chapters. Abiotic factors such as temperature, wind velocity and solar radiation, and biotic factors such as competition for floral resources and predation fall under the category of extrinsic factors (e.g. Boyle-Makowski and Philogène, 1985; Gilbert, 1985; Stone *et al.*, 1988; Herrera, 1995; Hegland *et al.*, 2009). The visitor assemblage of *Agrimonia eupatoria* was also related to temperature and humidity. Visitation frequency, species variation and number of visitors all increased throughout the morning as temperatures rose, but also declined when temperatures became too high. The decline in visitors throughout the afternoon and evening was also probably due to a decrease in floral rewards as pollen was depleted by earlier visitations.

Visitors showed a preference for certain flowers on an inflorescence, as has been seen in many other species (e.g. Karron *et al.*, 2009; Nishikawa, 2009). Mid-aged flowers were visited more frequently than young or old flowers, again likely due to the amount of floral reward available. Hoverfly and beetle visitors frequently visited several flowers on an inflorescence, moving from bottom to top along a spiral path in a manner that would decrease the likelihood of self pollen deposition, although this behaviour was less apparent in *Rhingia campestris* (see Table 1).

Visitation frequency data allowed calculation of the pollinator performance of the visitor assemblage of *Agrimonia eupatoria* at a per hour or per day level, showing the true pollinating potential of the given visitors. A “good” pollinator performance study, therefore, should include at the very least:

- a) a measure of per-visit effectiveness, and
- b) visitation frequency data

to accurately define the performance of the pollinator over time. Ideally, such a study should also include some measure of pollen quality to determine the outcrossing potential of the pollinator, especially in the case of self-compatible plants (De Jong *et al.*, 1993; Snow *et al.*, 1996); however at present this factor is fairly time-consuming and expensive to incorporate (see Sage *et al.*, 2005).

Chapter 10:

Testing Proxies for Pollinator Effectiveness

Introduction

Previous chapters have tested the effectiveness of single-visit stigmatic pollen deposition as a measure of pollinator performance. This method is relatively simple to incorporate into pollination studies, and gives an accurate value for the pollinating performance of visitors at the single-visit level, which can then be combined with visitation records to generate a value of pollinating performance over a given time, e.g. per hour, per day or per season, however until fairly recently it has rarely been included in pollination studies.

Stigmatic pollen deposition is a direct measure of pollinator effectiveness, as defined by Ne'eman *et al.* (2010). In practice, this method is often substituted with other parameters such as pollinator behaviour within the flower (for example feeding type, number of stigma touches, or duration of visit, e.g. Tepedino, 1981; Montalvo and Ackerman, 1986; Dafni *et al.*, 1987; Kaiser-Bunbury *et al.*, 2010), or visitation frequency or pollinator abundance (e.g. Richards, 1987; Olsen, 1997; Calzoni and Speranza, 1998), and it is therefore worth discussing the pros and cons of some of these proxy measures.

An often quoted plant-pollinator network study by Forup and Memmot (2005), for example, used absolute abundance values for each insect and insect-pollinated plant along with the frequency of interactions between them to generate a quantitative visitation web, and suggested that qualitative measurements of pollination could be calculated from analysis of pollen loads on the bodies of visitors. A later study (Forup *et al.*, 2008) included this apparent measure of pollinator effectiveness, identifying pollinators according to pollen present on visitor bodies, and defining their importance according to pollen fidelity (the

average proportion of individual pollen loads on the pollinator species that originate from the given plant species) and pollinator abundance. Since then, many authors have used such studies as justification for the use of proxies such as visitor abundance or pollen load composition as an alternative to direct measurements of pollinator effectiveness (e.g. Hopwood, 2008; Petanidou *et al.*, 2008; Bosch *et al.*, 2009; Heleno *et al.*, 2009; Kaiser-Bunbury *et al.*, 2010). As covered in previous chapters, visitor frequency, while an important component of pollinator studies, is not a measure of pollination effectiveness on its own. While a direct positive link has been found between visitation rate and pollen deposition, only 36% of the variation in pollen deposition was explained by this relationship (Engel and Irwin, 2003). At best, the results of such a study merely indicate that for one particular species, in that case *Ipomopsis aggregata*, there is a positive link between visit frequency and pollen deposition, and this relationship is by no means present in all plant-pollinator relationships.

Pollen present on the bodies of visitors is also not necessarily a representation of the pollination potential of the visitor. Pollen may be lost through deposition on an incompatible flower species or deposition on non-receptive stigmas for example, or it may also be lost before reaching the next flower, either by being eaten, discarded or being lost passively (Inouye *et al.*, 1994); therefore not all the pollen present on the bodies of visitors is available for pollination, even when, as in Forup *et al.*, (2008), pollen groomed into pollen baskets is ignored. Despite studies showing the lack of correlation between pollen present on visitor bodies and pollen deposited on conspecific stigmas (e.g. Adler and Irwin, 2006), this measure is still frequently used as a proxy for pollinator effectiveness. Another frequently used measure is the number of stigma touches observed (e.g. Carthew, 1993), though it is entirely possible that a visitor with a large load of conspecific pollen on its body in the “correct” place may deposit a higher number of pollen grains in just one or a few stigma touches. Conversely, a visitor with few pollen grains (removed by grooming, eaten, or merely not picked up effectively in the first place if the visitor is particularly smooth or does not make contact with anthers effectively), a pollen load containing a large proportion of pollen grains from a different plant species, or even a large proportion of conspecific pollen grains placed

on the body in the “wrong” place for effective deposition, may deposit relatively few pollen grains even when making a large number of contacts with the stigma.

Measurements of pollen removal (e.g. Young and Stanton, 1990; Conner *et al.*, 1995, Ivey *et al.*, 2003) pose similar problems to those of pollen load analyses described above, namely that pollen can be “lost” or become unavailable for pollination in a variety of ways (see Inouye *et al.*, 1994), and the pollinator which is the most effective at pollen removal may not necessarily be the most effective at pollen deposition (Thomson and Thomson, 1992; Aigner, 2001).

Duration of visit is also a frequently used proxy for pollinator effectiveness (e.g. Fishbein and Venable, 1996; Pellmyr and Thompson, 1996, Ivey *et al.*, 2003), though, as with measures of stigma contact, a longer duration does not mean the given visitor is doing something worthwhile in the flower during this time; it may be excessively grooming, or eating pollen, or may be making lots of contact with the reproductive structures but not effectively depositing pollen due to some other factor affecting its pollen deposition effectiveness, such as its physical fit with flowers.

Referring to measures such as those above as “proxies” for pollinator effectiveness is misleading anyway, as there is no explicitly proven relationship between these indirect measurements and direct measurements of pollinator effectiveness, such as stigmatic pollen deposition, therefore their real value as proxies is unknown. It is necessary, therefore, to first determine that such a relationship exists, and what the nature of this relationship is. For example does a longer visit duration correlate with a high or low pollen deposition, or does a high pollinator abundance mean a high or low pollinator effectiveness? To include such measures in pollination studies in place of more time-consuming or costly direct methods we must first determine their true value as proxies.

This chapter investigates the relationship between two such proxies for pollinator effectiveness, visit duration and visitor feeding behaviour on flowers (whether the visitor is

feeding on pollen, nectar or both), and measured stigmatic pollen deposition. This should give some insight into whether these measures are accurate alternatives to a direct measure of pollination effectiveness.

Methods and Materials

During the single visit stigmatic pollen deposition investigations of previous chapters (see Chapter 2: Assessing Single-Visit Pollen Deposition, for protocols), in addition to recording the identification of each visitor and the number of pollen grains deposited on stigmas in a single visit, I also noted whether the given visitor was feeding upon nectar, pollen or both, and timed the duration of visit with a stopwatch where possible, though in most cases duration was rounded up to the nearest 10 second interval. For the hummingbird species, the total time spent in and around a single flower was timed, as was the number of probes. The time spent feeding on flowers was then calculated using the number of probes and the average duration of probes (determined from photographic footage of the birds feeding).

To test the effectiveness of these indirect measurements as proxies for the direct measurement of stigmatic pollen deposition, the relationships between all of these measurements were analysed statistically for each of the 13 plant species investigated in earlier chapters: *Malvaviscus arboreus*, *Helicteres guazumifolia*, *Cirsium arvense*, *Knautia arvensis*, *Trifolium pratense*, *Centaurea nigra*, *Digitalis purpurea*, *Geranium pratense*, *Byrsonima crassifolia*, *Agrimonia eupatoria*, *Heracleum sphondylium*, *Ipomoea trifida* and *Rubus fruticosus*.

Firstly, the relationship between visit duration and single-visit stigmatic pollen deposition was tested with a Spearman Rank Correlation. The relationships between pollen deposition and visitor species, visit duration and visitor feeding behaviour on flowers were then analysed using a General Linear Model to determine which measurement or measurements explained the variation in pollen deposition (with the exception of *Trifolium*

pratense and *Digitalis purpurea*, as feeding type was not noted during the study of these species). Both statistical tests were carried out using SPSS 18.

Results

Correlation between Visit Duration and Pollen Deposition

A summary of the Spearman Rank Correlation results between visit duration and pollen deposition for each of the study plant species (for all visitors combined) is shown below (Table 1 and Fig. 1).

Plant Species	Correlation Coefficient	Significance	N
<i>Malvaviscus arboreus</i>	-0.640	< 0.001	76
<i>Helicteres guazumifolia</i>	-0.412	< 0.001	127
<i>Cirsium arvense</i>	-0.224	0.021	106
<i>Knautia arvensis</i>	-0.106	0.016	516
<i>Trifolium pratense</i>	-0.037	0.472	389
<i>Centaurea nigra</i>	-0.245	< 0.001	240
<i>Digitalis purpurea</i>	-0.154	0.362	37
<i>Geranium pratense</i>	0.190	0.103	75
<i>Byrsonima crassifolia</i>	-0.142	0.202	82
<i>Agrimonia eupatoria</i>	0.114	0.177	141
<i>Heracleum sphondylium</i>	-0.038	0.449	390
<i>Ipomoea trifida</i>	-0.143	0.047	194
<i>Rubus fruticosus</i>	0.084	0.428	92

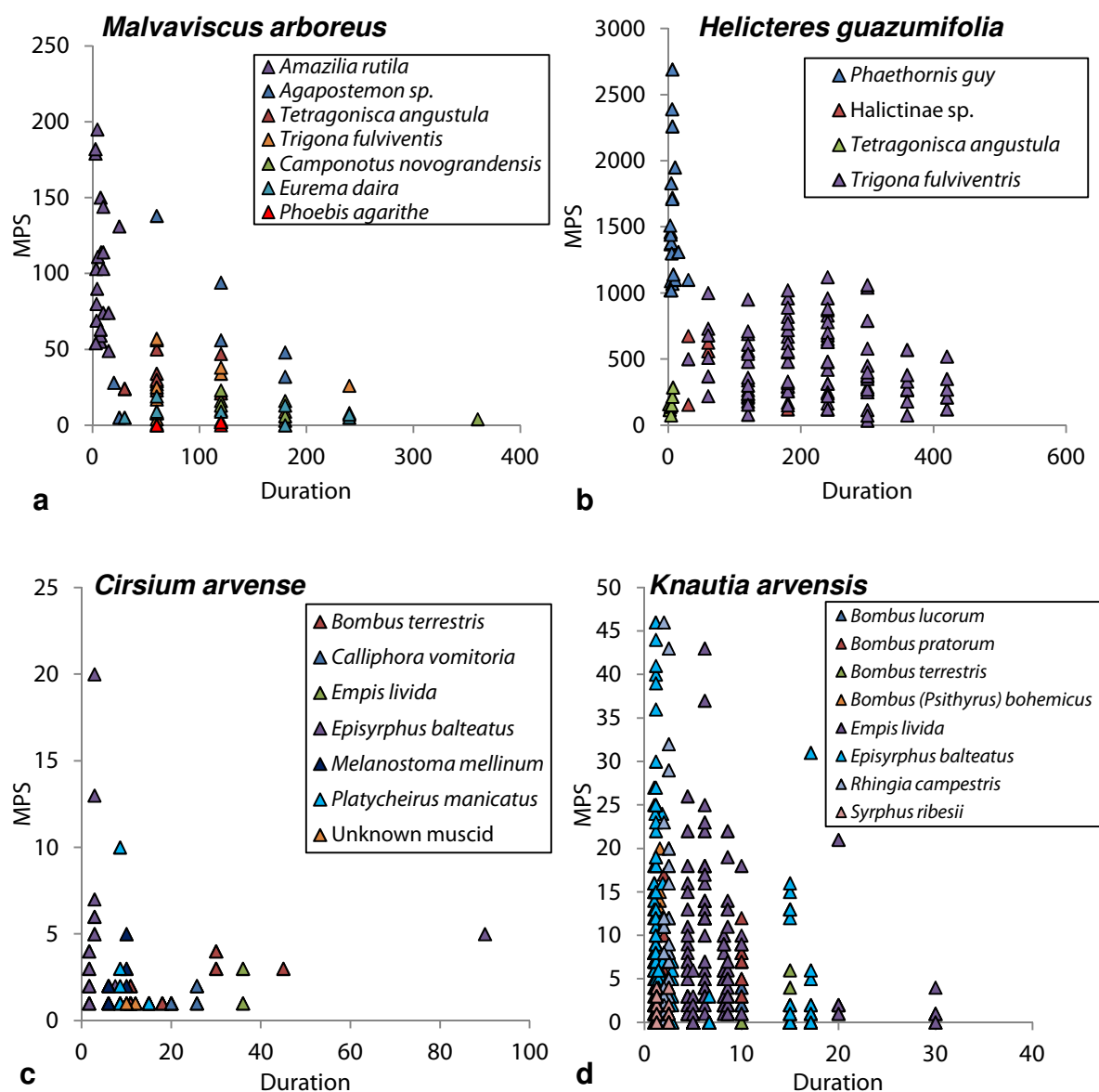
Table 1: Summary of Spearman Rank Correlation between single-visit stigmatic pollen deposition and visit duration in each of the plant species studied. Significant correlations are highlighted in bold.

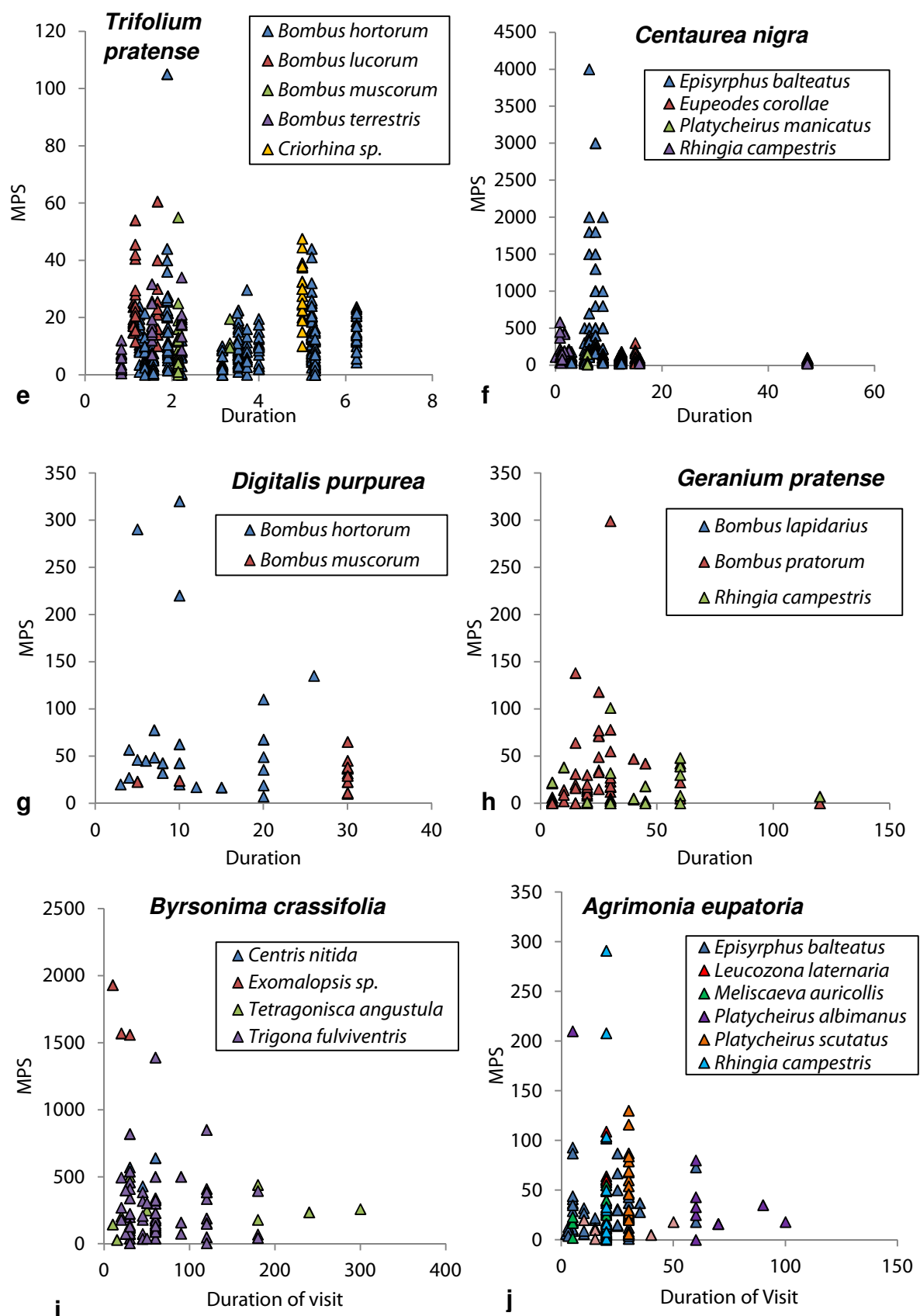
As can be seen, of the 13 plant species studied, 7 showed no significant correlation between visit duration and single-visit stigmatic pollen deposition while 6 showed a significant negative correlation between the two (*Malvaviscus arboreus*, *Helicteres guazumifolia*, *Cirsium arvense*, *Trifolium pratense*, *Centaurea nigra* and *Ipomoea trifida*).

When visitor species is considered however, several interesting patterns emerge.

- The significant negative correlations of visit duration and pollen deposition in *Malvaviscus arboreus* and *Helicteres guazumifolia* are no longer evident (see Fig. 1a and b respectively). This is largely because the hummingbird visitors were responsible for all the very short visits.
- In *Cirsium arvense* and *Centaurea nigra*, where a significant negative correlation was shown for all visitors combined, the only individual visitors to show a significant correlation were *Calliphora vomitoria*, *Bombus terrestris* and *Melanostoma mellinum* on *Cirsium arvense*, and this correlation was negative ($r = 0.535$; $P = 0.040$; $R = 0.635$, $P = 0.001$; $R = 0.770$, $P < 0.001$ respectively; see Fig. 1c), while *Rhingia campestris* was the only visitor to show a significant negative correlation on *Centaurea nigra* ($r = -0.598$, $P < 0.001$, see Fig. 1f).
- There was also a significant negative correlation overall for *Ipomoea trifida*, though when analysed by species *Pseudomyrmex gracillis* was the only visitor to show this negative correlation ($r = -0.477$, $P = 0.010$, see Fig. 1l).
- The significant negative correlations for *Cirsium arvense*, *Trifolium pratense* and *Geranium pratense* largely disappeared when split by visitor species, and in fact there was a significant positive correlation between visit duration and pollen deposition for *Calliphora vomitoria*, *Bombus terrestris* and *Melanostoma mellinum* on *Cirsium arvense* ($r = 0.535$; $P = 0.040$; $R = 0.635$, $P = 0.001$; $R = 0.770$, $P < 0.001$ respectively; see Fig. 1c); *Bombus terrestris* on *Trifolium pratense* ($r = 0.746$, $P < 0.001$; see Fig. 1e) and for *Bombus pratorum* on *Geranium pratense* ($r = 0.323$; $P = 0.019$; Fig. 1h).
- In *Knautia arvensis*, where no significant correlation was previously found, *Empis livida* showed a significant positive correlation ($r = 0.639$; $P < 0.001$) while *Episyrphus balteatus* showed a significant negative correlation ($r = -0.415$; $P < 0.001$; see Fig. 1d).

- *Heracleum sphondylium* and *Rubus fruticosus* also showed no significant overall correlation, but there was a significant positive correlation for *Episyrphus balteatus* and Platyzetidae sp. ($r = 0.333$; $P = 0.001$; $r = 0.539$, $P = 0.001$ respectively; Fig. 1k), and a significant negative correlation for *Lucilia sericata* ($r = -0.403$, $P = 0.022$) on *Heracleum sphondylium* and for *Bombus terrestris* on *Rubus fruticosus* ($r = 0.569$; $P = 0.021$; see Fig. 1m).
- In *Digitalis purpurea* (Fig. 1g), *Byrsonima crassifolia* (Fig. 1i) and *Agrimonia eupatoria* (Fig. 1j) a lack of any significant correlation remained even after splitting the data by visitor species.





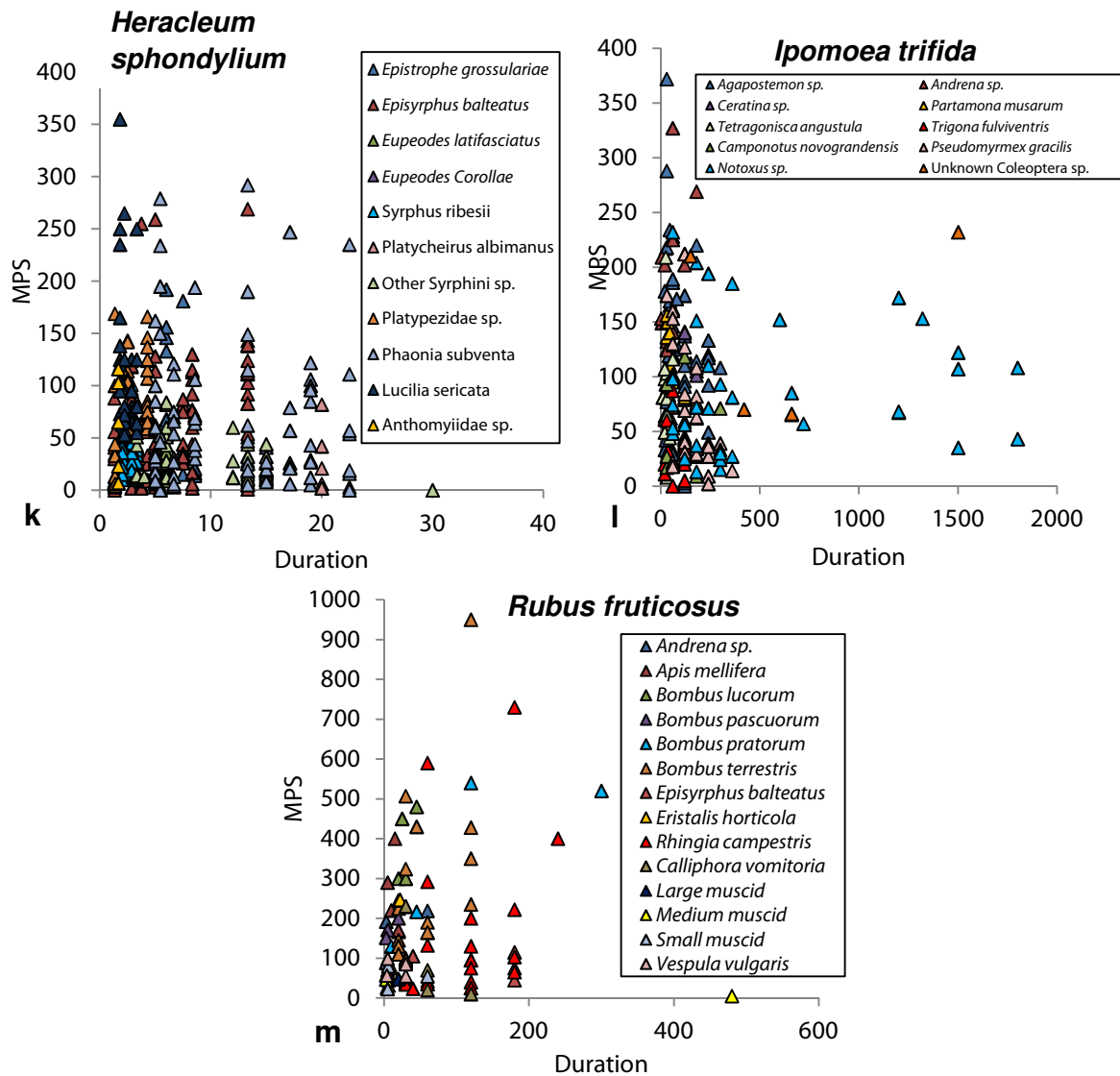


Fig. 1 (a-m): Scatter plots of single-visit stigmatic pollen deposition and duration of visit (s) for each of the plants investigated, split by visitor species.

The mean visit duration of the visitor assemblage of each plant species is summarised in Table 2. The hummingbird visitors showed very short visit durations in general, as did the hoverflies and other dipteran visitors, while bees and butterflies had longer visit durations in general; however visit durations were variable between different plant species. It is clear, therefore, that visit duration is very commonly related to visitor species, which may explain the unusual correlation results shown above.

Plant Species	Visitor Species	Mean Visit Duration	N
<i>Malva viscus arboreus</i>	<i>Amazilia rutila</i>	6.1 (±1.2)	21
	<i>Agapostemon</i> sp.	91.9 (±23.8)	8
	<i>Tetragonisca angustula</i>	75.0 (±8.2)	14
	<i>Trigona fulviventris</i>	110.8 (±17.8)	13
	<i>Camponotus novograndensis</i>	180.0 (±29.9)	8
	<i>Eurema daira</i>	138.8 (±29.4)	8
	<i>Phoebis agarithe</i>	90.0 (±17.3)	4
<i>Helicteres guazumifolia</i>	<i>Phaethornis guy</i>	1.73 (±0.4)	21
	<i>Agapostemon</i> sp.	80.0 (±24.1)	6
	<i>Tetragonisca angustula</i>	68.6 (±14.2)	7
	<i>Trigona fulviventris</i>	232.5 (±10.8)	92
<i>Cirsium arvense</i>	<i>Bombus terrestris</i>	19.1 (±2.4)	22
	<i>Episyrphus balteatus</i>	8.7 (±3.5)	26
	<i>Melanostoma mellinum</i>	10.9 (±0.3)	16
	<i>Platycheirus manicatus</i>	7.5 (±0.5)	11
	<i>Calliphora vomitoria</i>	22.7 (±0.8)	15
	<i>Empis livida</i>	36 (±0)	5
	Unknown muscid	10.9 (±1.0)	11
<i>Knautia arvensis</i>	<i>Bombus lucorum</i>	10.0 (±0)	12
	<i>Bombus pratorum</i>	4.3 (±0.8)	21
	<i>Bombus terrestris</i>	14.3 (±0.5)	14
	<i>Bombus (Psithyrus) bohemicus</i>	1.6 (±0)	19
	<i>Episyrphus balteatus</i>	3.6 (±0.3)	203
	<i>Eupeodes corollae</i>	10.0 (±0)	4
	<i>Rhingia campestris</i>	2.2 (±0.1)	54
	<i>Syrphus ribesii</i>	1.8 (±0.1)	42
<i>Trifolium pratense</i>	<i>Empis livida</i>	7.9 (±0.5)	147
	<i>Bombus hortorum</i>	3.7 (±0.1)	275
	<i>Bombus lucorum</i>	1.3 (±0.1)	31
	<i>Bombus muscorum</i>	2.3 (±0.1)	31
	<i>Bombus terrestris</i>	1.5 (±0.1)	34
<i>Centaurea nigra</i>	<i>Criorhina</i> sp.	5.0 (±0)	18
	<i>Episyrphus balteatus</i>	8.2 (±0.2)	158
	<i>Eupeodes corollae</i>	15.0 (±0)	12
	<i>Platycheirus manicatus</i>	6.0 (±0)	5
<i>Digitalis purpurea</i>	<i>Rhingia campestris</i>	18.6 (±2.4)	65
	<i>Bombus hortorum</i>	11.4 (±1.3)	25
	<i>Bombus muscorum</i>	26.3 (±2.6)	12
	<i>Bombus terrestris</i>	10.0 (±0)	1
<i>Geranium pratense</i>	<i>Bombus lapidarius</i>	5.0 (±0)	4
	<i>Bombus pratorum</i>	25.2 (±2.6)	44
	<i>Melanostoma mellinum</i>	120.0 (±0)	1
	<i>Meliscaeva auricollis</i>	20.0 (±0)	1
	<i>Platycheirus occultus</i>	25.0 (±5.0)	2
	<i>Rhingia campestris</i>	42.6 (±5.8)	19
<i>Byrsonima crassifolia</i>	<i>Centris nitida</i>	45.0 (±5.5)	6
	<i>Exomalopsis</i> sp.	20.0 (±5.8)	3
	<i>Tetragonisca angustula</i>	92.5 (±29.7)	12
	<i>Trigona fulviventris</i>	64.9 (±5.3)	61
<i>Agrimonia eupatoria</i>	<i>Episyrphus balteatus</i>	19.9 (±1.6)	63
	<i>Leucozona laternaria</i>	20.0 (±0)	12
	<i>Meliscaeva auricollis</i>	16.5 (±1.3)	13
	<i>Platycheirus albimanus</i>	63.5 (±7.9)	2
	<i>Platycheirus scutatus</i>	30.0 (±0)	10
	<i>Rhingia campestris</i>	20.0 (±0)	15
<i>Heracleum sphondylium</i>	<i>Epistrophe grossulariae</i>	7.1 (±0.3)	22
	<i>Episyrphus balteatus</i>	7.8 (±0.4)	99
	<i>Eupeodes latifasciatus</i>	6.0 (±0)	5
	<i>Eupeodes corollae</i>	10.0 (±1.5)	12
	<i>Platycheirus albimanus</i>	20.0 (±0)	6
	<i>Syrphus ribesii</i>	2.7 (±0.1)	38
	<i>Phaenicia subventa</i>	9.1 (±0.7)	76
	<i>Anthomyiidae</i> sp.	1.7 (±0)	6
	<i>Lucilia sericata</i>	20.0 (±0)	1

<i>Ipomoea trifida</i>	<i>Andrena</i> sp.	44.3 (±9.8)	19
	<i>Notoxus</i> sp.	566.7 (±92.1)	36
	<i>Camponotus novograndensis</i>	123.3 (±29.8)	9
	<i>Agapostemon</i> sp.	81.4 (±9.8)	56
	<i>Partamona musarum</i>	50.9 (±7.6)	11
	<i>Pseudomyrmex gracillis</i>	148.9 (±15.5)	28
	<i>Tetragonisca angustula</i>	30.7 (±3.4)	16
	<i>Trigona fulviventris</i>	85.0 (±18.2)	12
	<i>Lasioglossum</i> sp.	81.4 (±30.3)	2
<i>Rubus fruticosus</i>	<i>Andrena</i> sp.	35.8 (±14.4)	4
	<i>Apis mellifera</i>	12.5 (±3.2)	4
	<i>Bombus lucorum</i>	30.0 (±3.4)	6
	<i>Bombus pascuorum</i>	12.2 (±5.4)	5
	<i>Bombus pratorum</i>	77.1 (±39.8)	7
	<i>Bombus terrestris</i>	55.3 (±10.3)	16
	<i>Episyrphus balteatus</i>	112.9 (±26.0)	7
	<i>Eristalis horticola</i>	14.8 (±4.8)	5
	<i>Meliscaeva auricollis</i>	120.0 (±0)	1
	<i>Platycheirus manicatus</i>	7.0 (±0)	1
	<i>Platycheirus albimanus</i>	30.0 (±0)	1
	<i>Rhingia campestris</i>	111.3 (±14.3)	19
	<i>Calliphora vomitoria</i>	67.5 (±18.9)	4
	<i>Criorhina</i> sp.	360.0 (±0)	1
	Large muscid	18.0 (±7.6)	3
	Medium muscid	162.0 (±158.9)	3
	Small muscid	23.7 (±18.2)	3
	<i>Vespula vulgaris</i>	21.5 (±5.4)	6

Table 2: Mean visit durations for visitor assemblage of each of the 13 plant species studied. Standard errors are shown.

Visit Duration and Feeding Type as Proxies for Pollination Effectiveness

A summary of the General Linear Model tests of the effectiveness of visit duration and type of feeding (nectar, pollen, both; or in the case of *Byrsonima crassifolia*, oil, pollen or both) as proxies for pollen deposition in each of the 13 species is shown below in Table 3 (with the exception of *Trifolium pratense* and *Digitalis purpurea*, as explained above). Visitor species was also included in the model, as variation in pollen deposition between species has been shown in earlier chapters.

Plant Species		DF	F	Significance	Estimate of Effect Size
<i>Malvaviscus arboreus</i>	Corrected Model	11	11.349	P < 0.001	66.1%
	Visitor Species	6	10.840	P < 0.001	50.4%
	Duration of Visit	1	0.022	P = 0.883	0.00%
	Feeding Type	2	0.474	P = 0.625	1.5%
<i>Helicteres guazumifolia</i>	Corrected Model	5	43.844	P < 0.001	64.6%
	Visitor Species	2	3.886	P < 0.023	6.1%
	Duration of Visit	1	2.517	P = 0.115	2.1%
	Feeding Type	2	0.071	P = 0.790	0.1%
<i>Cirsium arvense</i>	Corrected Model	8	4.458	P < 0.001	26.9%
	Visitor Species	6	0.964	P = 0.454	5.6%
	Duration of Visit	1	5.558	P = 0.020	5.4%
	Feeding Type	1	15.817	P < 0.001	14.0%
<i>Knautia arvensis</i>	Corrected Model	11	6.082	P < 0.001	11.7%
	Visitor Species	8	3.677	P < 0.001	5.5%
	Duration of Visit	1	4.046	P = 0.045	0.8%
	Feeding Type	2	17.690	P < 0.001	6.6%
<i>Trifolium pratense</i>	Corrected Model	5	21.429	P < 0.001	21.9%
	Visitor Species	4	26.721	P < 0.001	21.8%
	Duration of Visit	1	1.012	P = 0.315	4.0%
	Feeding Type	1	0.117	P = 0.732	0.1%
<i>Centaurea nigra</i>	Corrected Model	5	1.948	P = 0.087	5.8%
	Visitor Species	3	1.099	P = 0.350	1.4%
	Duration of Visit	1	0.448	P = 0.504	0.2%
	Feeding Type	1	0.117	P = 0.732	0.1%
<i>Digitalis purpurea</i>	Corrected Model	2	1.453	P = 0.248	7.9%
	Visitor Species	1	1.399	P = 0.245	4.0%
	Duration of Visit	1	0.004	P = 0.950	0.0%
	Feeding Type	1	0.117	P = 0.732	0.1%
<i>Geranium pratense</i>	Corrected Model	5	1.007	P = 0.420	6.8%
	Visitor Species	2	1.190	P = 0.310	3.3%
	Duration of Visit	1	0.148	P = 0.702	0.2%
	Feeding Type	2	1.191	P = 0.310	3.3%
<i>Byrsonima crassifolia</i>	Corrected Model	5	24.505	P < 0.001	61.7%
	Visitor Species	2	34.005	P < 0.001	47.2%
	Duration of Visit	1	1.194	P = 0.278	1.5%
	Feeding Type	1	2.361	P = 0.129	3.0%
<i>Agrimonia eupatoria</i>	Corrected Model	7	2.545	P = 0.017	12.1%
	Visitor Species	6	2.963	P = 0.010	12.1%
	Duration of Visit	1	3.501	P = 0.064	2.6%
	Feeding Type	0	NA	NA	0.0%
<i>Heracleum sphondylium</i>	Corrected Model	11	7.676	P < 0.001	18.3%
	Visitor Species	9	5.274	P < 0.001	11.2%
	Duration of Visit	1	1.345	P = 0.247	0.4%
	Feeding Type	0	NA	NA	0.0%
<i>Ipomoea trifida</i>	Corrected Model	12	4.596	P < 0.001	23.4%
	Visitor Species	9	4.447	P < 0.001	18.1%
	Duration of Visit	1	0.003	P = 0.956	0.0%
	Feeding Type	1	2.046	P = 0.154	1.1%
<i>Rubus fruticosus</i>	Corrected Model	16	2.088	P = 0.031	43.2%
	Visitor Species	8	2.387	P < 0.001	30.3%
	Duration of Visit	1	0.444	P = 0.508	1.0%
	Feeding Type	2	2.386	P = 0.104	9.8%

Table 3: Summary of results of General Linear Models for each of the 13 plant species, considering the relationship between visitor species, duration of visit and feeding type and the variation in single-visit stigmatic pollen deposition. "Corrected model" refers to the variation in pollen deposition effected by visitor species, duration of visit and feeding type combined, after being corrected for the mean. Significant results are highlighted in bold.

In 8 of the 13 plant species analysed (*Malvaviscus arboreus*, *Helicteres guazumifolia*, *Trifolium pratense*, *Byrsonima crassifolia*, *Agrimonia eupatoria*, *Heracleum sphondylium*, *Ipomoea trifida* and *Rubus fruticosus*), the only factor with a significant relationship to pollen deposition was visitor species, as shown in previous chapters (see Table 3). In three species, *Centaurea nigra*, *Digitalis purpurea* and *Geranium pratense*, none of the factors investigated explained a significant percentage of the variation in pollen deposition, nor did the overall corrected model (though it should be noted that in the case of *Agrimonia eupatoria* all visitors were observed feeding upon pollen only, therefore feeding type could not be a contributing variable). Visit duration explained a significant part of pollen deposition variation, along with visitor species, in *Helicteres guazumifolia* and *Cirsium arvense* (along with visitor species and feeding type respectively). All three measures accounted for a significant percentage of the variation in pollen deposition in *Knautia arvensis* alone.

Discussion

Correlation of Visit Duration and Pollen Deposition

Of the 13 plant species studied, 7 showed no significant correlation between visit duration and single-visit stigmatic pollen deposition for all visitors combined, while 6 showed a significant negative correlation between the two. Therefore, for the species *Malvaviscus arboreus*, *Helicteres guazumifolia*, *Cirsium arvense*, *Trifolium pratense*, *Centaurea nigra* and *Ipomoea trifida*, single-visit stigmatic pollen deposition decreases as the length of visit increases. This is at first sight surprising, as it could be argued that a longer duration of visit would increase chances of the visitor making contact with the reproductive structures of the flower, and/or of transferring more pollen to the stigma. It is possible, however, that a longer visit means the visitor is consuming more pollen directly from anthers, or is grooming and removing adhered pollen for direct consumption or for deposition in structures such as pollen baskets for later consumption, and therefore leaving less pollen on its body for later

deposition. If this behaviour is repeated on all flowers visited, pollen deposition will be lower than for a visitor species which does not groom to remove pollen.

When we take visitor species into account, some of the potentially anomalous relationships are explained, because the nature of these correlations can change. In some species (*Malvaviscus arboreus*, *Helicteres guazumifolia*), the negative correlation disappears. This is because individuals within a species may have rather similar visit durations, and therefore the overall correlation does not imply causation, rather that pollen deposition is related to visitor species, which is in turn related to visit duration. In this case, for example, the hummingbird visitors of these flowers all had very short visit durations with little variation (see Table 3) and deposited the highest number of pollen grains in a single visit (see Chapter 3), while other visitors spent longer on flowers but were not as effective at depositing pollen due to their poor physical fit with the flowers.

In other cases (*Centaurea nigra* and *Ipomoea trifida*), the negative correlation is only apparent in one or a few visitor species (*Rhingia campestris* and *Pseudomyrmex gracillis* respectively). It is therefore possible (and in the case of *Pseudomyrmex gracillis* observed, see Fig. 2) that this species was a more extensive groomer or pollen eater in comparison to other visitors, resulting in the strong negative correlation. *Rhingia campestris* had the longest visit duration of visitors to *Centaurea nigra*, but one of the lowest pollen depositions. In this case it is perhaps more likely that pollen deposition is related to visitor species (see Chapter 7 for further descriptions of the morphological features and single-visit pollinator effectiveness of these species). The apparent correlation with visit duration is thus a coincidental effect of visitor species rather than a cause of variation in pollen deposition.



Fig. 2

Fig. 2: *Pseudomyrmex gracillis* feeding on flower of *Ipomoea trifida*. The coating of pollen visible is extensively groomed and eaten by the ant before moving to subsequent flowers.

Two of the plant species (*Trifolium pratense* and *Geranium pratense*) had a significant negative correlation initially, but when split by species, certain bumblebee visitors (*Bombus terrestris* and *Bombus pratorum* respectively) showed a significant positive correlation between visit duration and pollen deposition. In this case then, the longer the bumblebee visitors spent on flowers, the higher the number of pollen grains deposited. However we can see from Table 2 that these species both had relatively low mean visit durations in relation to the other flower visitors, and both were relatively poor pollinator species in terms of pollen deposition (see Chapters 5 and 7). This relationship was masked by the lack of relationship between pollen deposition and visit duration in the other visitor species present on flowers. It is possible therefore that in these species, longer visit duration means more opportunity for stigma and anther contact, and therefore increased pollen deposition, and conversely short visit durations mean less opportunities for contact with the reproductive structures and therefore less pollen deposited. In addition, *Trifolium pratense* is a flower species possessing long corolla tubes and concealed nectar (see Chapter 7). Short-tongued visitors are able to visit flowers but they cannot fully empty them. It is therefore possible that the relatively short-tongued bumblebee *Bombus terrestris* is spending less time

on flowers as its tongue is not sufficiently long to fully empty flowers, and once it can no longer reach the nectar it ends foraging earlier than other longer-tongued species..

Conversely, some plant species which initially showed no significant correlations between visit duration and pollen deposition showed significant correlations when visitor species was considered. *Rubus fruticosus* showed a strong positive correlation for *Bombus terrestris*, again probably explained by increased likelihood of contact with reproductive structures in a longer visit, or lower likelihood of such contact with shorter visits, and masked by other flower visitors with a wide range of visit durations and pollen depositions. *Heracleum sphondylium* showed a significant positive relationship for *Episyrphus balteatus* and *Platypeza* sp. and a significant negative correlation for *Lucila sericata*, and *Knautia arvensis* also showed a significant positive correlation for one species (*Empis livida*) while another had a significant negative correlation (*Episyrphus balteatus*). In these cases, two species show differing interactions between visit duration and pollen deposition (increased grooming/eating and increased likelihood of contact with reproductive structures) which are masked when visitor species is not considered in the analysis.

Three plant species (*Digitalis purpurea*, *Byrsonima crassifolia* and *Agrimonia eupatoria*) did not have significant correlations either when visitors were lumped together, or when the data were split by visitor species.

As can be seen from all these comparisons, one visitor species is of particular interest. *Episyrphus balteatus* showed both positive and negative correlations between visit duration and pollen deposition in different plant species, though the mean visit duration did not differ greatly between these plant species (see Table 2). This suggests that the variation comes from the behaviour and pollinating effectiveness of this species on different plant species rather than from visit duration alone, and perhaps in certain species it is a more extensive groomer or pollen eater, while in others it is a more effective pollinator and makes more contact with reproductive structures. Again this helps to underline not just that it is inappropriate to use visit duration as a proxy in its own right, but that it cannot be assumed

that there is a particular kind of relation between visit duration and pollen deposited, either for a visitor group or even for a single visitor species.

Visit Duration and Feeding Type as Proxies for Pollination Effectiveness

Table 3 shows that in 8 of the 13 plant species analysed, the only factor having a significant relationship with pollen deposition was visitor species (as in previous chapters), even in cases where visit duration was correlated with pollen deposition. This apparent correlation, however, was shown above to be a result of the relationship between visitor species and pollen deposition in most cases, rather than a direct correlation between visit duration and pollen deposition.

In three species, *Centaurea nigra*, *Digitalis purpurea* and *Geranium pratense*, none of the factors investigated explained a significant percentage of the variation in pollen deposition, nor did the overall corrected model.

It should be noted that even in those plant species where visit duration and feeding explained some of the variation in pollen deposition, these factors only accounted for a small percentage of the variation in each case, and by far the largest proportion of variation in pollen deposition was explained by visitor species.

Conclusion

The aim of this chapter was to test the value of visit frequency and feeding behaviour as proxies for direct measures of pollination effectiveness. In some of the plant species investigated, initial analyses showed that visit duration was correlated negatively with pollen deposition; however in the majority of cases studied it was not. It is possible that the apparent significant correlations recorded are a result of some other unknown factor related to pollen deposition, most obviously visitor species, as we would expect visitors of the same

species to have similar feeding behaviours. Hence the further analysis with GLM, which incorporated visitor species, may be more meaningful. When visitor species was taken into account, the significant negative correlations in some plant species were no longer apparent, and where still present were restricted to one (or in rare cases, two) visitor species. In some cases, a positive correlation was found, and most tellingly one flower visitor, *Episyrphus balteatus*, showed both positive and negative correlations between visit duration and pollen deposition on different plant species.

The results of the general linear models, combined with the evidence that correlations between pollen deposition and visit duration can vary in strength and direction between plant species, indicate that, in the majority of cases, variation in pollen deposition is not significantly explained by either visit duration or feeding behaviour. Therefore these factors are not accurate replacements for a direct measurement of pollination effectiveness, such as single-visit stigmatic pollen deposition. In almost all cases the largest percentage of variation in pollen deposition was explained by visitor species. This follows the results of previous chapters, which indicate that in most of the plant species studied, there is significant variation in pollen deposition between visitor species.

This chapter reinforces the concept that single-visit stigmatic pollen deposition is a valuable direct means of measuring pollinator effectiveness. Indirect methods are not effective proxies for direct methods such as this, and the only factor to show a consistent relationship with pollen deposition is visitor species.

To ensure the accuracy of pollinator performance studies, therefore, it is necessary that a direct measurement of single-visit pollination effectiveness be taken, for example single-visit stigmatic pollen deposition as in previous chapters, rather than an indirect proxy such as visit duration or pollinator feeding behaviour.

Chapter 11: Conclusions and Future Directions

The main focus of this thesis was to determine both the feasibility and desirability of including measures of pollinator performance in plant-pollinator interaction studies, though in the course of this study several other interesting and important conclusions were reached. Some of these findings are extensions of previous studies, applying previously known phenomena to new species, while others may serve as arguments against the conclusions drawn by earlier studies. The results of this thesis not only offer some clarification of current key issues in the field of pollination biology, but also some insight as to how future studies can be improved with the inclusion of certain protocols.

Single-Visit Stigmatic Pollen Deposition

Single-visit stigmatic pollen deposition was tested as a measure of pollinator performance as proposed by earlier authors, and reviewed by Ne'eman *et al.* (2010, and references within), and was found to be both relatively practical and inexpensive to implement into studies (see Chapters 3-8). Previous measures such as insect pollen loads (e.g. Schlindwein and Wittman, 1995; Ashman and Stanton, 1991; Galloni *et al.*, 2008; Bosch *et al.*, 2009), removal of pollen from anthers (Suzuki *et al.*, 2002), percentage of flower visits with a stigma touch in a foraging bout (Dafni *et al.*, 1987; Kaiser-Bunbury *et al.*, 2010), visit frequency (Calzoni and Speranza, 1998; Lopes and Buzato, 2007), time taken for a certain number of flower visits (Richards, 1987), the fraction of a visitor's pollen load deposited on a stigma (Kearns and Inouye, 1993; Lau and Galloway, 2004), the relative pollen load contributed by a specific pollinator (Inouye *et al.*, 1994), probability of the removed pollen reaching the target stigma (Galen and Stanton, 1989), proportion of visited flowers that receive pollen (Herrera, 1987), behaviour of visitors within flowers (Yanagizawa and Maimoni-Rodella, 2007), visit duration (Escaravage and Wagner, 2004), and many others (see Table 1.1 of Chapter 1) are inaccurate measures of pollinator performance, as pollen may be lost from a pollination system in a variety of ways, and even pollen on the

bodies of insects does not always reach the stigma of a conspecific flower to participate in effective pollination (see Inouye *et al.*, 1994, summarised in Fig. 1.4 of Chapter 1). By analysing only the conspecific pollen which reaches the receptive stigma of a given flower species, a more accurate measure of the given flower visitor's pollination performance can be calculated.

My results also show that certain measures proposed as proxies for pollinator effectiveness are inaccurate, and in most cases show no significant, or indeed consistent, relationship to pollen deposition (see Chapter 10). Observations of behaviour on flowers (whether the visitor was feeding on pollen, nectar (or oil), or both) had no consistent relationship with pollen deposition. Duration of visit, shown previously to have no significant relationship to seed set (Blair and Williamson, 2008), was sometimes shown to have either a positive or negative relationship to pollen deposition; but this parameter was primarily related to visitor species. In fact, in almost all cases the factor which explained the greatest proportion of variation in pollen deposition was visitor species.

Visitation frequency, proposed by authors such as Waser and Price (1990), Calzoni and Speranza, (1998), Smith-Ramírez *et al.* (2005), Traveset and Sáez (1997), Engel and Irwin (2003), Vázquez *et al.* (2005), Wiggam and Ferguson (2005) and Lopes and Buzato (2007) as a proxy for pollinator effectiveness, was also not an effective indicator of pollinator performance on its own, as shown previously (Blair and Williamson, 2008; Hoen *et al.*, 2008), but when this was combined with single-visit pollen deposition it was possible to calculate a pollinator performance value at a given time scale, for example per-hour or per-day (see Chapter 9). In this way, visitors which were not effective, or not highly effective, pollinators at a single-visit level could be effective over a given period of time if visitation rates were high, as shown in other studies (Sahli and Conner, 2006; Rader *et al.*, 2009; Zheng *et al.*, 2011).

A further important conclusion drawn during the course of the study was that analysis of visitors by functional groups, as proposed by several authors (e.g. Escaravage and

Wagner, 2004; Fajardo *et al.*, 2008; Galloni *et al.*, 2008; Fleming *et al.*, 2009), often masked important relationships, or attributed significant relationships such as effective pollination to large groups where, in practice, not all members showed significant pollen deposition. Given the vast variation in characters which will affect pollinator performance, such as size, shape, hairiness and feeding and flower-visiting behaviour, visitors should be treated in terms of species wherever possible, or even at an individual level (e.g. Adler and Irwin, 2005; Dupont *et al.*, 2011).

Testing Pollination Syndromes

Single-visit stigmatic pollen deposition was used to test the apparent pollination syndromes of 13 plant species showing a variety of traits indicative of certain pollination syndromes. By determining firstly which visitors are indeed pollinators as opposed to visitors, and secondly which of the visitors is the most effective pollinator, I could determine the pollination syndrome appropriate to each species and establish whether this matched with the syndrome indicated by floral traits (see Table 1).

Not all flower visitors were effective pollinators of each given plant species, regardless of factors such as visit frequency or feeding behaviour, as described above. Hence, many plant species which would appear to be rather generalist given their varied and wide visitor assemblage may in fact be more specialised when pollinator performance is taken into account, and visitors which are ineffective as pollinators are no longer considered. Even those plant species with a flower form associated with a generalist pollination syndrome (once considered a “primitive” flower form, though more recently recognised as an evolved pollination syndrome in its own right (Weberling, 2007, Zheng *et al.*, 2011)), are not pollinated equally and effectively by all flower visitors, and are therefore not as broadly generalist as might at first appear. Rather they are effectively pollinated by a smaller subset

Plant species	Syndrome based on traits	No. functional groups visiting	No. species visiting	Functional groups that are effective pollinators	Species that are effective pollinators	Syndrome based on pollinator effectiveness
<i>Malvaviscus arboreus</i>	Hummingbird	4	7	Hummingbirds and bees	<i>Amazilia rutila</i> , <i>Tetragonisca angustula</i> and <i>Trigona fulviventris</i>	Hummingbird (bee backup)
<i>Helicteres guazumbifolia</i>	Hummingbird	3	5	Hummingbirds and bees	<i>Phaethornis guyi</i> , <i>Trigona fulviventris</i> and <i>Agapostemon</i> sp.	Hummingbird (bee backup)
<i>Agrimonia eupatoria</i>	Hoverfly	2	9	Hoverflies	<i>Episyphus balteatus</i> , <i>Leucozona lateraria</i> , <i>Platycheirus albimanus</i> , <i>Platycheirus scutellatus</i> , <i>Rhingia campestris</i> and <i>Meliscaeva auricollis</i>	Hoverfly
<i>Geranium pratense</i>	Short-Medium tongued bee	3	8	NA	NA	NA
<i>Digitalis purpurea</i>	Medium-long tongued bee	1	3	Bees	<i>Bombus hortorum</i>	Bee
<i>Byrrsonima crassifolia</i>	Oil-collecting bee	1	4	Bees	<i>Exomalopsis</i> sp., <i>Centris nitida</i> , <i>Trigona fulviventris</i> and <i>Tetragonisca angustula</i>	Oil-bee (pollen-eating bee backup)
<i>Cirsium arvense</i>	Long-tongued insect	3	7	Hoverflies, bees and other dipterans	<i>Episyphus balteatus</i> , <i>Melanostoma melinum</i> , <i>Calliphora vomitoria</i> and <i>Bombus terrestris</i>	Long-tongued insect (short-tongued backup)
<i>Knaulia arvensis</i>	Long-tongued insect	3	8	Hoverflies, other dipterans, bumblebees and cuckoo bumblebees	<i>Rhingia campestris</i> , <i>Episyphus balteatus</i> , <i>Syrphus ribesii</i> , <i>Empis livida</i> , <i>Bombus (Psithyrus) bohemicus</i> , <i>Bombus pratorum</i> , <i>Bombus terrestris</i> and <i>Bombus lucorum</i>	Long-tongued insect (short-tongued backup)
<i>Centaurea nigra</i>	Long-tongued insect	1	4	Hoverflies	<i>Rhingia campestris</i> , <i>Episyphus balteatus</i> and <i>Eupredes corollae</i>	Long-tongued insect (short-tongued backup)
<i>Trifolium pratense</i>	Long-tongued insect	2	5	Bees and hoverflies	<i>Cichorhina</i> sp., <i>Bombus lucorum</i> , <i>Bombus hortorum</i> , <i>Bombus terrestris</i> and <i>Bombus muscorum</i>	Long-tongued insect (short-tongued backup)
<i>Ipomoea trifida</i>	Generalist/bee	6	14	Bees and beetles	<i>Andrena</i> sp., <i>Agapostemon</i> sp., <i>Partamona musarum</i> , <i>Notoxus</i> sp. and unknown coleopteran	Generalist/small short-tongued insect
<i>Heracleum sphondylium</i>	Generalist	3	11	Hoverflies and other dipterans	<i>Epistrophe grossulariae</i> , <i>Episyphus balteatus</i> , <i>Eupredes latiasciatus</i> , <i>Syrphus ribesii</i> , <i>Lucilia sericata</i> , <i>Paraplatyplezia atra</i> , <i>Anthomyiidae</i> sp. and <i>Phaonia subventa</i>	Generalist/ small, short-tongued insect
<i>Rubus fruticosus</i>	Generalist	4	18	Bees	<i>Bombus hortorum</i> , <i>Bombus terrestris</i> , <i>Bombus pascuorum</i> , <i>Bombus pratorum</i> , <i>Apis mellifera</i> , <i>Andrena</i> sp. and <i>Rhingia campestris</i>	Generalist/ large-bodied insect

Table 1: Summary of the apparent and observed pollination syndromes, visitor assemblages and pollinator assemblages of the 13 plant species studied. Effective pollinators were defined as those which deposited a significant number of pollen grains in comparison to controls.

of visitors only, in the case of the species studied here either those of a functional group of small-bodied, short-tongued insect visitors, or relatively large-bodied insect visitors. Effective pollination is known to be influenced by how closely the morphology of flower visitors “fits” with the morphology of a given flower species, for example in terms of placement of reproductive structures in the case of flowers, and in terms of traits such as body size or tongue length in their animal visitors (e.g. Suzuki *et al.*, 2007; Bloch and Erhardt, 2008; Armbruster *et al.*, 2011). This is in direct contrast with earlier studies which suggested plant-pollinator interactions may be more generalised than previously thought (see Waser and Ollerton, 2006; and references within). Effectiveness of visitors should therefore always be experimentally tested, and can never be assumed. Indeed, from the opposite point of view, as even obviously nectar-robbing visitors may be effective pollinators (e.g. Fumero-Cabán and Meléndez-Ackerman, 2007; Zhu *et al.*, 2010). In addition, pollinator assemblage is known to vary both spatially and temporally (e.g. Price *et al.*, 2005; Wiggam and Ferguson, 2005; Jürgens *et al.*, 2009), in particular for generalist flower species (e.g. Fleming *et al.*, 2001; Herrera, 2005; Davila and Wardle, 2008; Lázaro *et al.*, 2008), and pollinator assemblages that have been defined experimentally for a given plant species in a given location cannot be assumed for all populations of that plant species.

Another important conclusion drawn from this study is the existence of “backup” pollinators, also referred to in the literature as bi-modal pollination syndromes or mixed pollination syndromes. Many of the plant species studied appeared to have one main pollinator which was the most effective and had a good fit with floral traits, but they could also benefit from visits by other, less effective flower visitors which in many cases moved either small amounts of pollen or transferred self-pollen in self-compatible plant species, and would therefore allow for effective pollination should the main pollinator become rare (or extinct). In this way a plant can remain specialised, while protecting itself from the risk of such a strategy should visitor extinctions occur (locally or globally), rather than resorting to the more ecologically stable strategy of generalisation (Buchmann and Nabhan, 1996; Waser *et al.*, 1996; Renner, 1998; Vásquez and Simberloff, 2002; Memmot *et al.*, 2004;

Gomez and Zamora, 2005). Plant species which are able to show plasticity in their pollination interactions should be able to persist by responding quickly to environmental changes, even though the identity of their mutualistic partners may change (Burkle and Alarcón, 2011). Flowers of the long-tongued insect pollination syndrome in particular showed clear adaptations towards long-tongued visitors, which were often the most effective flower visitors, yet short-tongued visitors were also able to feed on nectar from full corollas and move small amounts of pollen. This additional pollination service clearly proved useful in the absence of many long-tongued bee and butterfly pollinators from local populations during my study period as indicated in previous studies (Yanagizawa and Maimoni-Rodella, 2007).

Many plant species are known to rely upon self-pollination or wind-pollination as a back-up should visits by effective pollinators be scarce or non-existent (e.g. Consiglio and Bourne, 2001; Lázaro and Traveset, 2005; Ladd, 2006; Yu and Huang, 2006; Fenster and Martén-Rodríguez, 2007; Mitchell and Ashman, 2008; Steiner, 2010). However, some are also known to rely on alternative, less effective animal pollinators in such conditions. This strategy can be beneficial for plant reproductive success in numerous different ecological situations.

For example, many plant species showing traits indicative of being pollinated at night or dusk are also visited by either less effective (Aigner and Scott, 2002; Brunet and Sweet, 2006; Muchhala *et al.*, 2008; Brunet and Holmquist, 2009, Maruyama *et al.*, 2010; Martinell *et al.*, 2010; Walter, 2010), or equally effective (Barthell and Knops, 1997; Valdiva and Niemeyer, 2006; Gimenez-Benavides *et al.*, 2007; Morinaga *et al.*, 2009; McMullen, 2011), diurnal pollinators, and vice versa (Fleming *et al.*, 1996; Dar *et al.*, 2006).

In a similar way to the hummingbird-pollinated species studied here (*Malvaviscus arboreus* and *Helicteres guazumifolia*), visits to *Ipomopsis aggregata* by bumblebees allowed for backup pollination should hummingbirds become scarce (Pleasants and Waser, 1985; Mayfield *et al.*, 2001), and many other hummingbird or perching-bird pollinated plant species are also pollinated to some extent by insect visitors such as bees, lepidopterans and

flies (Diaz and Cocucci, 2002; Aigner, 2005; Devoto *et al.*, 2006; Freitas *et al.*, 2006; Fumero-Cabán and Meléndez-Ackerman, 2007; Navarro *et al.*, 2007; Dalsgaard *et al.*, 2009; Schmidt-Adam *et al.*, 2009; Symes *et al.*, 2009; Schmid *et al.*, 2011). Hummingbirds themselves may provide a backup pollination service for bat-pollinated flowers (Wolf and Stiles, 1989). Many bee-pollinated plant species are also pollinated to a similar or lesser extent by fly species (Wolff *et al.*, 2008; Zheng *et al.*, 2011), as shown in Chapter 5, and the reverse is also true (de Merxem *et al.*, 2009). Wasps are considered backup pollinators in some pollination networks (Shuttleworth and Johnson, 2007; Mello *et al.*, 2011). More unusual pollination syndromes such as shelter-pollination, where flowers offer protection and a shelter for insect visitors as a reward (Faegri and van der Pijl, 1979), are also pollinated to some extent by typical flower foraging insects (e.g. Monty *et al.*, 2006).

There are numerous explanations for the existence of such mixed or backup syndromes. For example, some may represent an ancestral pollination syndrome which has been replaced by a more generalist pollination syndrome, but without any necessary floral adaptations required (e.g. Li and Huang, 2009), or conversely a change in floral traits from the ancestral condition which still allows for effective pollination by the original mutualistic partner (e.g. Devoto *et al.*, 2006). Others may correspond to an intermediate between two differently adapted species within a genus, for example between hummingbird and bee pollinated *Penstemon* spp. (Lara and Ornelas, 2008). Others again may have occurred (or be occurring in contemporary time) as a response to climatic effects on pollinator assemblages (e.g. Epindola *et al.*, 2011). In one particularly unusual case, the hawkmoth-pollinated flower species *Nicotiana attenuata* can shift the composition of its odour secretion, thereby attracting day active hummingbirds instead of its usual pollinators as a means of escaping herbivory by the hawkmoth larva when damage is excessive (e.g. Kessler *et al.*, 2010).

The concept of back up pollinators is more plausible when we consider that adaptation towards a particular pollinator does not necessarily mean other, morphologically

different, pollinators are excluded from flowers. Such a trade-off is assumed in a model of floral evolution which suggests that quality and quantity components of pollinator effectiveness would evolve to be positively correlated (Waser *et al.*, 1996), and this assumption is implicit in discussions of floral specialisation. However, if we consider that trade-offs may in fact be weak, and that floral traits can allow a flower to become adapted towards a particular pollinator without losing the ability to use others (Robinson and Wilson, 1998; Aigner, 2001), or if there is no evidence of conflicting selection or non-additive selection in the presence of multiple pollinators (Sahli and Conner, 2010) then the existence of backup pollinators to which the flower does not appear adapted, or of mixed pollination syndromes, is clearly possible.

In many of the plant species studied here, the observed visitor assemblage does not appear to fit with the pollination syndrome suggested by floral traits, as in other studies (Waser *et al.*, 1996; Ollerton, 1998; Ollerton and Watts, 2000; Ollerton *et al.*, 2009). When we consider only the effective pollinator species or functional groups, however, the relationship between floral traits and pollination syndromes is clearer. It is also evident that there is a large overlap between bee-pollinated and hoverfly-pollinated flowers, and in particular flowers visited by long-tongued bees and long-tongued hoverflies are difficult to differentiate between (Gilbert *et al.*, 1985; Dicks *et al.*, 2002; Pontin *et al.*, 2006; Willmer, 2011). The long-tongued insect pollination syndrome is perhaps a better term for such flowers, especially given the morphological similarities and similar pollinator effectiveness of long-tongued bees and long-tongued hoverflies as described in previous chapters.

Flower-Visitor Behaviour

Over the course of the study, it was clear that timing of visitation was strongly linked to temperature and humidity, with visitor size and colouration also important factors. Temperature and humidity values showed similar patterns over the study of all 13 species, as would be expected, with temperatures rising throughout the morning to a peak around

midday and declining again in the afternoon, while humidity values started high and declined over midday, rising again over the afternoon. In general, large-bodied visitors were active earlier in the morning than small-bodied visitors, and dark-coloured species were active earlier than light-coloured or iridescent species. When temperatures were at their highest, smaller, lighter coloured or reflective species were less likely to overheat, and larger species were often absent during such temperature peaks. Non-flying visitors were also better able to withstand high temperatures than flying visitors. These patterns are in line with previous thermal studies on flower visitors (Willmer, 1983; Willmer *et al.*, 2000; Potts *et al.*, 2003a,b; Willmer and Stone, 2004).

An interesting form of flower-visiting behaviour noted during this study was the directional flower visiting shown by hoverflies, other dipteran species and beetles on spike inflorescences of *Agrimonia eupatoria*. Not only did these insects make directional flower visits, in most cases up the inflorescence, but also in many cases these visits were sequential. This visiting behaviour on spike inflorescences has been shown previously in bee species and some wasps (e.g. Corbet *et al.*, 1981; Jordon and Harder, 2006; Ishii *et al.*, 2008), but until now has not been reported for other visitor species. As described in Chapter 9, this behaviour will promote out-crossing in *Agrimonia eupatoria* and increases the effectiveness of these visitors as pollinators.

Limitations of Study

It is important to note several limitations of this study, which could be expanded upon in future studies. Firstly, effective pollinators were defined as those which deposited a significant amount of pollen in comparison to that found on control stigmas. A more effective cut-off point for effective pollination, however, could be determined by calculating the minimum number of pollen grains required per stigma for full or optimum seed set to occur. Secondly, while Chapter 9 illustrated that visitation frequency could be combined with single-visit stigmatic pollen deposition, a visitation frequency survey was only carried out for one

plant species, and more such studies are needed. Implementing both these measures would allow a much more accurate pollinator performance value to be calculated for the visitor assemblage of a particular plant species.

Not all suggested pollinator syndromes were studied here, and in particular no apparent perching-bird, bat, beetle, butterfly, moth, non-flying mammal or carrion fly-pollinated plant species were investigated. It was also not possible within the scope of this study to determine the quality of pollen deposited by visitor species, and considerations of whether pollen is outcrossed or viable are important when comparing the pollinating performance of different pollinator species (Rader *et al.*, 2011). In addition, while the effects of other factors such as herbivory (e.g. Söber *et al.*, 2010; Willmer, 2011) and predation (e.g. Louda, 1982; Lima, 1991; Willmer and Stone, 1997; Dukas and Morse, 2003) on plant-pollinator interactions and floral selection are known, it was not possible to account for these factors within the scope of this study.

A further limitation of this study is one which is not so easy to resolve, but which should be considered when viewing the results of this thesis. Weather conditions during the three years of fieldwork in Scotland were exceptionally poor in comparison to expected conditions for the summer. As a result, there were prominent declines and even absences of particular flower visitors from the assemblages of the study sites. Low populations of bees and butterflies were the most notable issues, and in particular the honeybee *Apis mellifera* was almost entirely absent from the study, being found on only one plant species at Loch Tay. Local honeybee hives surrounding the field site at West Quarry Braes in Fife suffered extreme losses during the winter of 2007, prior to the commencement of fieldwork, from which they did not recover over the course of the study (Jim Cobb, personal communication), and similar pollinator declines are being reported worldwide (e.g. Ricketts *et al.*, 2008; Winfree *et al.*, 2009). The assessments of visitor assemblages for the UK plant species studied here, therefore, do not necessarily represent the full visitor assemblages of each species and care should be taken when interpreting them. A similar study carried out in

more favourable weather conditions, with a “complete” visitor assemblage, may therefore yield different results to those reported here, though the principles would still remain.

Future Directions

This thesis opens up a variety of avenues for future studies and offers some further guidelines to protocols that should ideally be included in future plant-pollinator interaction studies. The key message of this thesis, well known to pollination biologist in principle, but too often disregarded in practice, though with some notable exceptions (Primack and Silander, 1975; Motten *et al.*, 1981; Herrera, 1990; Dieringer, 1992; Kearns and Inouye, 1993; Inouye *et al.*, 1994; Osorio-Beristain *et al.*, 1997; Rodet *et al.*, 1998, Falque *et al.*, 1996; Mitchell, 1997; Waser and Price, 1990; Ashman and Stanton, 1991; Pettersson, 1991; Willmott and Burquez, 1996; Cane and Schiffhauer, 2001; 2003; Hiei and Suzuki, 2001; Mayfield *et al.*, 2001; Suzuki *et al.*, 2002; Lau and Galloway, 2004; Bloch *et al.*, 2006; Reynolds and Fenster, 2008), is that not all flower visitors are effective pollinators; hence it must not be assumed that any flower visitor is effective based on factors such as visitation frequency, behaviour within the flower, size, shape or species. The only accurate means of distinguishing between pollinators and visitors is to establish that effective pollination has occurred using a measure of single-visit stigmatic pollen deposition. Records of visitation frequency should also be taken, to allow calculations of pollinator performance over a larger time-scale. It is also important to treat flower visitors as individual species rather than functional groups, or risk missing some of the more interesting and significant interactions present (or conversely identifying all members of a functional group as effective pollinators when certain individual species may be ineffective).

Observing the visitor assemblage of a given plant species therefore does not necessarily show the true pollinator assemblage of the species, and it is not feasible to confirm or deny the existence of pollination syndromes based on flower visitors which may taking floral resources without effectively pollinating the species. While concepts such as

“backup” pollinators may muddy the waters, it is important to consider that, while we would expect a plant species to evolve adaptations towards its most effective pollinator, such adaptations do not prevent the flower from utilising other species as backups in the event of short-term pollinator declines or long-term local or global extinctions. In such cases, specialisation and the existence of a pollination syndrome are still clear, though the risks of such a strategy have been minimised by the tolerance of the plant to other, less effective yet still pollinating, flower-visiting species. Given the issues raised by this thesis, recent “pollination” networks and webs, which are based upon records of visitation frequency or other, supposedly qualitative, measurements (e.g. observations of visitor behaviour (Petanidou and Potts, 2006) those including pollen load analyses (Forup *et al.*, 2008) or observed contact with reproductive structures (Kaiser-Bunbury *et al.*, 2010)) rather than pollinator performance, should perhaps be treated with more caution.

In conclusion, single-visit stigmatic pollen deposition as a measure of pollinator performance is easily implemented in pollination studies. In my work, it has helped to confirm the existence of pollination syndromes in the plant species studied, and allowed for distinctions to be made between pollinators, “backup” pollinators and mere flower visitors or thieves.

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