

BIG BEES DO A BETTER JOB: INTRASPECIFIC SIZE VARIATION INFLUENCES POLLINATION EFFECTIVENESS

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Abstract—1. Bumblebees (*Bombus* spp.) are efficient pollinators of many flowering plants, yet the pollen deposition performance of individual bees has not been investigated. Worker bumblebees exhibit large intraspecific and intra-nest size variation, in contrast with other eusocial bees; and their size influences collection and deposition of pollen grains.

2. Laboratory studies with *B. terrestris* workers and *Vinca minor* flowers showed that pollination effectiveness PE, as measured from pollen grains deposited on stigmas in single visits (SVD), was significantly positively related to bee size; larger bees deposited more grains, while the smallest individuals, with proportionally shorter tongues, were unable to collect or deposit pollen in these flowers. Individuals did not increase their pollen deposition over time, so handling experience does not influence SVD in *Vinca minor*.

3. Field studies using *Geranium sanguineum* and *Echium vulgare*, and multiple visiting species, confirmed that individual size affects SVD. All bumblebee species showed positive SVD/size effects, though even the smallest individuals did deposit pollen. *Apis* with its limited size variation showed no such detectable effect when visiting *Geranium* flowers. Two abundant hoverfly species also showed size effects, particularly when feeding for nectar on *Echium*.

4. Mean size of foragers also varied diurnally, with larger individuals active earlier and later, so that pollination effectiveness varies through a day; flowers routinely pollinated by bees may best be served by early morning dehiscence and visits from larger individuals.

5. Thus, while there are well-documented species-level variations in pollination effectiveness, the fine-scale individual differences between foragers should also be taken into account when assessing the reproductive outputs of biotically-pollinated plants.

Keywords: Pollinator, Pollen deposition, Bumblebee, Body size, Intraspecific variation

INTRODUCTION

Bees are the most plentiful and successful of the pollinators, and depend entirely on flowering plants since they feed only on pollen and nectar throughout their lives. Eusocial bees are highly efficient pollen-gatherers, and bumblebees (*Bombus*) often collect significantly more pollen from anthers and deposit more pollen on stigmas than *Apis* honeybees (Willmer et al. 1994; Thomson & Goodell 2001; King, Ballantyne & Willmer 2013). *Bombus* have a substantial capacity to improve flower handling time by learning (e.g. Laverty 1994), and some species will learn from each other (Leadbeater & Chittka 2009; Dawson et al. 2013). However individual pollinator effectiveness (PE) variation within a species, whether for naïve foragers or after a learning period, has not been investigated.

Bumblebees show substantial inter-specific differences in worker size (Benton 2006), but unlike most other eusocial bees also demonstrate large intra-specific and intra-nest size variation (Plowright & Jay 1968; Peat et al. 2005); workers can exhibit a ten-fold size variation within a single nest

(Alford 1975) compared with less than two-fold variation in honeybees and stingless bees (Waddington et al. 1986; Roulston & Cane 2000). This is not genetically controlled as workers within a nest are normally full sisters, but instead probably stems from unequal larval feeding (Sutcliffe & Plowright 1988; Couvillon & Dornhaus 2009); Persson & Smith (2011) have shown that adult size of bumblebee workers is significantly related to the availability of floral resources. Intra-nest size variation dictates the polyethism found within the colony; larger bees are more likely to become foragers for the colony, and are more efficient as they can transfer more pollen and nectar to the colony (Morse 1978; Goulson et al. 2002). There is also a positive interspecific relation between worker size and foraging range in bees (Greenleaf et al. 2007) which may also hold within species and should mean that larger workers gain access to a wider range of floral resources. Larger individuals are also better able to broaden their diet when stored food supplies require it, when compared with smaller nest mates (Fontaine et al. 2008). However there has been little investigation of the pollen-depositing abilities of individuals, and hence the influence of visitor size variation on plant pollination.

Here we investigate whether individual flower foragers, especially bumblebees, vary in pollen deposition performance according to their body size, as well as aspects of their

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behaviour and their foraging experience. A variety of techniques have been employed to quantify pollinator success (or effectiveness, or efficiency; see Willmer 2011), from visit number, frequency or duration to pollen carried or deposited, or eventual seed-set. However the number of pollen grains deposited on a stigma from a single visit is the most robust measure of pollinator effectiveness (PE) (Ne'eman et al. 2010), for a particular plant species and visitor pairing. Therefore we measured Single Visit Deposition (SVD), recently demonstrated by King, Ballantyne & Willmer (2013) as a reliable and practical method of distinguishing true pollinators from mere visitors. In this study SVD is used to address the specific pollination ability of individual visitors within a species.

MATERIALS AND METHODS

A. Laboratory Studies

Bombus terrestris was used from a single commercial nest box (Syngenta Bioline, The Netherlands), containing 50-80 worker bees, whose activity out of the nest (in an arena with test flowers) could be controlled by doors. The nest was in a glasshouse with exposure to both natural sunlight and additional overhead lights, at a temperature of $22 \pm 3^\circ\text{C}$. During testing, bees had access into a wooden arena (100cm x 100 cm x 30 cm) with a plexiglass lid and side-access doors. In this enclosed space a worker could forage on the plants provided. The bees were fed artificial nectar in the nest, but could be isolated from this supply overnight to encourage foraging in the arena. No additional pollen pellets were provided in the nest (so avoiding heterospecific deposition onto flower stigmas), although withholding pollen does deprive workers of dietary protein and may decrease nest longevity (Smeets & Duchateau 2003). Bees that entered the arena were tagged with queen-marker discs on the dorsal thorax to distinguish individuals, and the thoracic inter-tegular width was recorded with digital calipers (LTL Linear Tools). Disc placement did not influence flight ability, and no bee was tested on the same day as a disc was applied, to reduce possible stress effects on behaviour.

Vinca minor (Apocynaceae) was the test flower, being native to temperate Europe (Fjell 1983) and pollinated by several insect genera, including *Bombus* spp. (Horwood 1919). Within the corolla the reproductive anatomy is unusual (Fig. 1), as first described by Darwin (1861). The pistil bears a horizontal stigmatic "wheel", with the hairy anthers above. Only the concave ventral wheel surface is receptive, and its sticky exterior rim prevents self-pollen from above reaching the stigma. Through this use of spatial herkogamy, the flower avoids self-fertilization and requires crossing by insect visitors (Fryxell 1957). Bumblebees can reliably accomplish this while probing for nectar, since pollen from other plants, borne on the tongue, reaches the underside of the stigma wheel as the tongue is withdrawn. The proboscis, now sticky with stigmatic secretions, then also collects the fresh dry pollen from the brush on top of the style, so that there can be a near-complete exchange of

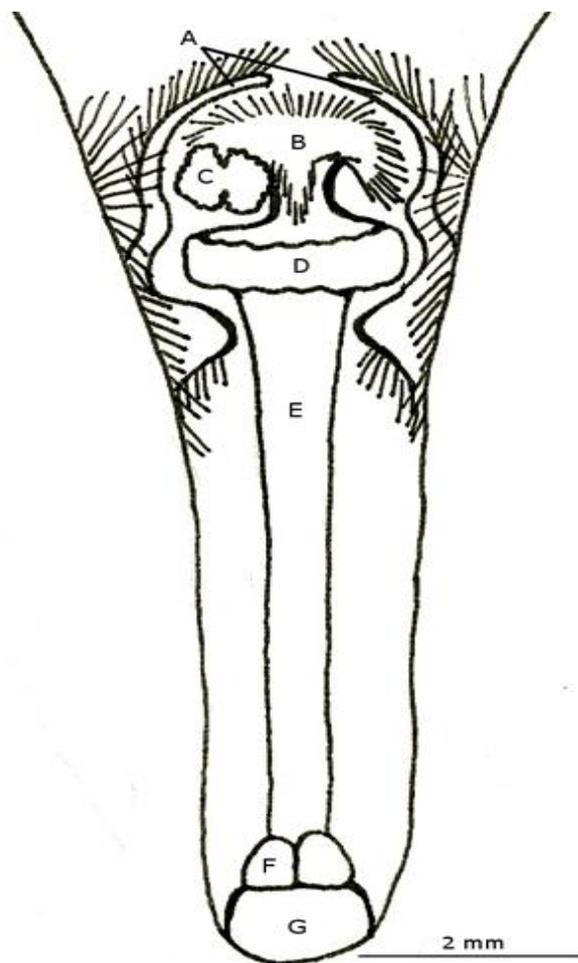


Figure 1. Internal anatomy of a *Vinca* flower. A - anthers; B - filament brush; C - pollen grains; D - stigmatic 'wheel'; E - style; F - ovaries; G - receptacle bearing nectaries.

pollen grains during nectar-feeding. Hence stigma wheels only reveal pollen sourced from another flower, and confounding variables from self-fertilization or the deposition of multiple flowers' pollen upon a single stigma are largely avoided.

Potted plants with unopened, virgin blossoms were purchased from local nurseries and kept in the closed greenhouse free from extraneous insect visitors, with two cultivars used interchangeably throughout the experiment (*Vinca minor* 'Atropurpurea' and *Vinca minor* 'Ralph Shugart'; similar in flower size and with identical reproductive anatomy). Each plant provided 30-50 flowers over 2-4 weeks.

The experiments took place in January-April 2013, from early morning to mid-afternoon, in accordance with maximum natural sunlight within the glasshouse and peak bee activity. Testing sessions lasted 1-4 hours, depending on the activity levels of the bees and their willingness to exit the hive to forage (varying mainly with outside weather and light levels).

Tagged bees were allowed free access for 24 hours into the arena containing one potted *Vinca minor* plant, as a

familiarisation period. Thereafter, a single *V. minor* plant with virgin flowers was placed in the arena, and the door to the hive opened until a single bee entered. If the bee did not visit a flower within ten minutes, it was recaptured, tagged if necessary, and returned to the nest, as longer times in the arena rarely led to any visitation, irrespective of bee size. When a bee did visit a flower, it was closely observed for tongue extension (indicating nectar-feeding) and for grooming behaviours (tongue-wiping after leaving a flower). Each bee was normally allowed to visit three flowers per trial, and each flower was removed after a single visit.

Flowers removed from the plant after a single visit (234 in total) were assessed for pollen deposition (Single Visit Deposition, SVD) as detailed in King, Ballantyne & Willmer (2013). Briefly, the stigma was removed with clean forceps and stored in a plastic cell-culture array (24 cells, TPP test plate) kept covered and cool. The number of adherent pollen grains on each stigma was assessed with a dissecting microscope (x40), counting only the grains located on the receptive underside of the stigma 'wheel'. Unvisited control flowers were taken periodically to ensure the flowers were not experiencing self-fertilization.

B. Field Studies

Two plants were chosen for field work, based on their availability at a field site in NE Fife, Scotland (NO 3719) and on the ease of recognition of their pollen. *Echium vulgare* has distinctive purple/blue pollen and is characterised as a bumblebee-pollinated flower (Rademaker et al. 1999). Flowers produced were entirely hermaphrodite (though some gynodioecy may occur at other sites (Klinkhamer et al. 1991), and strongly protandrous, with spatially separated anthers and style. They were pink when opening (usually before midday) but turned mauve/blue during day 1 and deep blue on day 2, before wilting by day 3. Day 1 flowers were functionally male, and individual bees visiting the youngest flowers could pick up thousands of pollen grains (~7,000 per visit, Rademaker et al. 1999), so that pollen was substantially depleted from anthers by midday of day 1 whenever weather conditions permitted

regular visitation. Day 2 blue flowers were female phase with receptive stigmas, and were used for this study. *Geranium sanguineum* grew in the same site, having large orange pollen grains with distinctive reticulate sculpturing. The population contained entirely hermaphrodite flowers, which were almost completely protandrous and lasted 3-5 days. They were male on day 1 and usually part of day 2, pollen dehiscing within 1-2 hours of bud opening and often available for 24-30 hours thereafter. The stigmatic lobes opened on day 2 (occasionally day 3) after virtually all the pollen had been shed from that flower, so avoiding within-flower selfing. Only flowers with open stigmatic lobes, on days 2 and 3, were used for SVD analysis. *Echium* observations occurred in June-August 2012 and 2013, and *Geranium* in July 2013, covering all daylight hours of suitable weather conditions (between 0620 and 2030 on different days).

Buds were enclosed in fine net the evening before they would open, and then exposed at varying times the following day (or on subsequent days for *G. sanguineum*). The first visitor was noted (identification, nectar and/or pollen feeding, visit duration); then the flower was removed and its stigma picked. Methodology for measuring SVD was as above, but conspecific pollen grains on the stigma were sufficiently distinctive to be scored with a 20x lens in the field. At intervals fresh flowers were exposed and their stigmas counted immediately, with no visitation, to act as controls for self-pollen moved during netting and handling.

All visitors were identified to species as far as possible in the field, with uncertain insects caught for later checking. Within a species, each was scored as large, medium or small by eye. A proportion of all visitors already assessed in each size category were captured through the season for accurate measurement of individual size (inter-tegular thorax breadth, using calipers as above). Table 1 shows the measured size ranges for the various bee species that had been assigned to each size class, and mean size for the species; only one case of overlap between size classes occurred (a 'medium' *B. terrestris* with a size actually in the small range), so that the 3-level size scoring was accepted as appropriate.

TABLE 1. Measured size ranges (as inter-tegular thoracic width, mm) of bees and hoverflies at the field site in each size class, with the 3 ranges tailored for each species; only one case of overlap (*) occurred between assigned size class and subsequently measured thorax width. (Numbers in brackets for *Apis* were each for only one individual).

	Large	Medium	Small	Mean
<i>Apis</i>	(4.7)	3.3-4.3	(3.0)	3.6
<i>B. terrestris</i>	6.5-7.3	4.2-6.3	3.5-4.4*	5.0
<i>B. lapidarius</i>	5.6-6.2	4.6-5.5	3.5-4.5	4.5
<i>B. lucorum</i>	5.5-6.0	4.2-5.2	<4.0	4.8
<i>B. pascuorum</i>	5.5-6.5	4.5-5.3	3.1-4.2	4.3
<i>B. pratorum</i>	5.0-5.8	4.2-4.9	3.3-4.0	4.1
<i>Episyrrhus balteatus</i>	2.6-2.9		2.2-2.5	
<i>Platycheirus albimanus</i>	2.2-2.4		1.9-2.1	

C. Statistical Analysis

Pollen grain counts and bee measurements were normally distributed. Pearson's correlations were therefore carried out to compare bee size and SVD, and t-tests to compare SVD following different behaviours. ANOVAs compared multiple species in relation to body size, and SVD/size effects within each species. Linear regression was used to test effects on SVD of learning over time. Tests were applied using either Minitab v.17 or SPSS v.21, the latter particularly for non-linear relations tested with simple and quadratic regression. Data are shown as means \pm 1 SE, and vertical bars on Figures also show \pm 1 SE.

RESULTS

A. Laboratory Studies with captive bumblebees.

In total 45 individual *B. terrestris* were tagged, with 26 participating in foraging activities. Thorax widths were 2.5-7.4 mm (mean 4.9 ± 0.2 mm); the range and mean are larger

than some reported values (del Castillo & Fairbairn 2012; Persson & Smith 2013), but Peat et al. (2005) did record larger bees of this species in Scotland relative to English colonies, and commercial bee nests may often produce a wider size range of foragers than wild nests.

Pollen grain deposition overall (for 234 visits) varied from 0 to 300 grains per flower, with zero deposition on the first flower visit and means of 39.8 ± 2.6 and 38.0 ± 4.5 grains (difference NS) on the second and third visits (after pollen had been acquired from the previous flower(s)) within a trial. Mean SVD was therefore calculated from flowers 2 and 3. Fourteen of the test bees emerged and foraged multiple times. For example bee 8, with a thorax width of 6.9 mm, engaged in 14 flower-visiting trials over 7 days, depositing 0-130 grains per visit, but it did not get significantly better or worse over time in the trials (Fig. 2; linear regression, $df = 1,12$, $F = 0.599$, $P = 0.45$). The same was true of 13 other bees that each made between 2

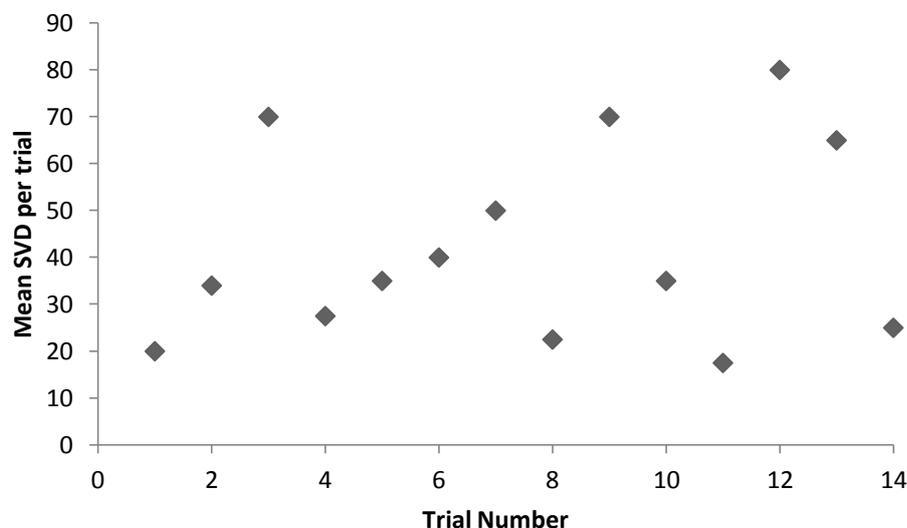


FIGURE 2. The performance of one bee on *Vinca* flowers (bee #8, thorax width 6.9 mm) for 14 trials across 7 days. Mean SVD range 20-80 grains, with no significant trend over time (linear regression, $df=1,12$, $F = 0.599$, $P = 0.45$).

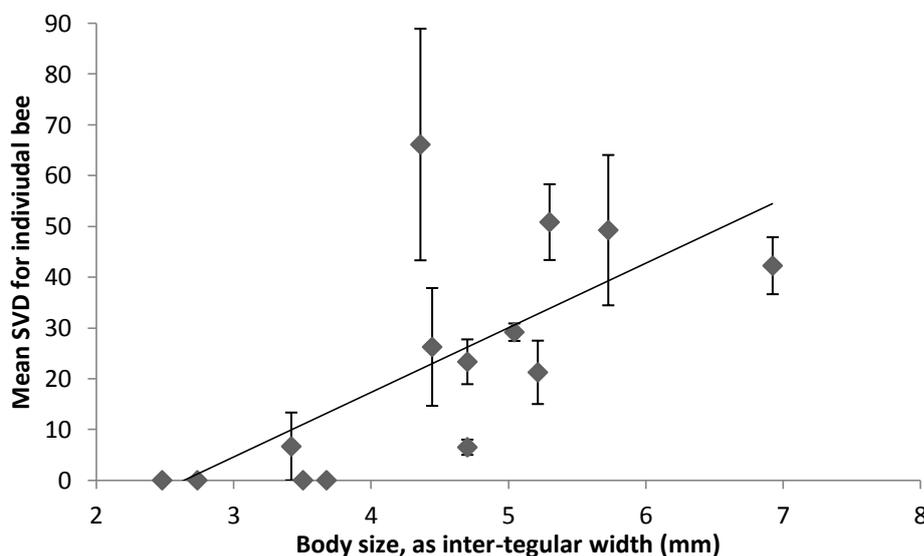


FIGURE 3. Mean SVD (\pm SE) in relation to body size for individual *Bombus terrestris* working *Vinca minor* flowers in laboratory conditions (for the 14 individuals where more than one flower visit was recorded). Pearson correlation line shown ($r = 0.692$, $P = 0.006$).

and 9 visits, thus there was thus no evidence of improved handling or learning over time with this flower, and time of trials was excluded from further analysis.

However larger bees generally deposited more pollen, and Fig. 3 shows the significant correlation between bee size (thorax width) and mean SVD ($r = 0.692$, $P = 0.006$). The smallest bees produced zero pollen deposition; these bees were commoner later in the trials (date versus thorax width, $r = -0.52$, $P < 0.0001$), perhaps because pollen in the nest was becoming depleted and the later-emerging brood were less well fed, and/or because smaller bumblebees are more resistant to starvation (Couvillon & Dornhaus 2010).

There was also a significant effect of individual grooming ('tongue-wiping', where some bees scraped pollen from their proboscis after withdrawal from the flowers) on pollen deposition and hence PE. When no wiping occurred, mean SVD was 93.3 ± 10.3 , whereas in bees that tongue-wiped mean SVD on the next flower visited was 39.8 ± 7.2 . The very small bees that deposited no pollen never had pollen on their faces and thus never showed tongue-wiping behaviour; when they were removed from the analysis grooming behaviour (above a threshold body size) did significantly reduce SVD ($t = 4.28$, $df = 1,61$, $P < 0.001$).

B. Field analyses with multiple visitors

1. *Echium vulgare*

SVD results for a range of visitors to *E. vulgare*, summed across all dates and times, are shown in Table 2 arranged by body size (mean control SVD = 0.4 ± 0.2 pollen grains, too low to merit subtraction from the experimental data). Deposition of grains per stigma was similar to the range of 1-10 previously recorded in studies using manipulated dead bees as carriers (Rademaker et al. 1997). The great majority of visits (94%) were made by *Bombus* species, all purely nectar-feeding. For all five species of bumblebee mean SVD was greater in larger individuals, with around 3- to 6-fold differences between the large and small size categories. The

effect was significant for 4 of the 5 species, and also strongly significant for all bumblebees combined (ANOVA, $df = 2,194$, $F = 27.15$, $P < 0.001$). Several genera of hoverflies were also occasional visitors, especially in autumn 2012, but nearly always foraged only for pollen, feeding at the protruding anthers and depositing no pollen on stigmas. However *Eristalis pertinax*, *Episyrphus balteatus* and *Platycheirus* spp. also made a few nectar-collecting visits, and SVD values for the latter two (commonest) hoverflies are also given in the Table, split into two size categories; although size differences were smaller than for bumblebees, there were still significantly greater SVD values for larger individuals for *Platycheirus*, and for all hoverfly species combined (see Table). The only other visitors observed were *Pieris rapae* butterflies on two occasions, taking nectar and depositing 3 and 10 pollen grains. But as predicted from previous studies (Rademaker et al. 1999) bumblebees were by far the most important pollen-depositing visitors.

SVD also showed variation through time, summed for all bee visitors (Fig. 4). Mean pollen deposition was greatest in early- to mid-morning (0800-1000), and fairly constant at all other times. Peak SVD coincided with the observed peak of anther dehiscence in the majority of newly-opened flowers. However there were also variations in the mean size of recorded visitors through a day (Fig. 5), as expected from known thermal effects on insects in relation to their size (Willmer 1983; Willmer & Stone 2005). Larger individuals were more likely to be active before 1100h and after 1700h, with a preponderance of individuals in the small size category between midday and 1600h giving a curvilinear relationship (simple regression, quadratic term significant $t_{195} = 2.19$, $P = 0.03$). Thus larger bumblebees were mainly responsible for visitation during the peak period of pollen presentation and deposition in this plant species. Inevitably SVD did not increase in the evening when larger bumblebees were active again, because by then the pollen was substantially depleted from flowers of *Echium*.

TABLE 2. Mean SVD for large, medium and small individual bumblebees (\pm SE, n in parentheses) visiting *Echium vulgare*, and for two size categories of the two commonest hoverflies. Details of ANOVA tests are also shown.

	Large	Medium	Small	All	F	P
Bees						
<i>B. terrestris</i>	13.8 \pm 4.7 (5)	8.0 \pm 1.7 (19)	4.0 \pm 2.5 (4)	8.5 (28)	2.05	ns
<i>B. lapidarius</i>	12.4 \pm 3.7 (5)	5.8 \pm 1.1 (14)	5.0 \pm 1.6 (4)	7.1 (23)	3.98	0.035
<i>B. lucorum</i>	15.8 \pm 4.4 (5)	5.4 \pm 1.6 (8)	2.7 \pm 1.1 (7)	6.8 (20)	4.83	0.022
<i>B. pascuorum</i>	7.9 \pm 3.0 (8)	5.2 \pm 0.7 (40)	2.4 \pm 0.7 (14)	4.9 (62)	3.67	0.031
<i>B. pratorum</i>	11.0 \pm 5.8 (2)	4.9 \pm 0.9 (25)	2.6 \pm 0.4 (35)	3.9 (62)	7.53	0.001
All <i>Bombus</i>	11.8 \pm 1.7 (25)	5.6 \pm 0.5 (106)	2.8 \pm 0.3 (64)		27.15	<0.001
Hoverflies						
	Larger		Smaller			
<i>Episyrphus balteatus</i>	6.4 \pm 1.6 (11)		1.2 \pm 0.7 (4)		2.21	ns
<i>Platycheirus albimanus</i>	9.0 \pm 6.2 (3)		1.2 \pm 0.4 (8)		4.84	0.055
All hoverflies	6.9 \pm 1.6 (14)		1.2 \pm 0.4 (12)		7.41	0.012

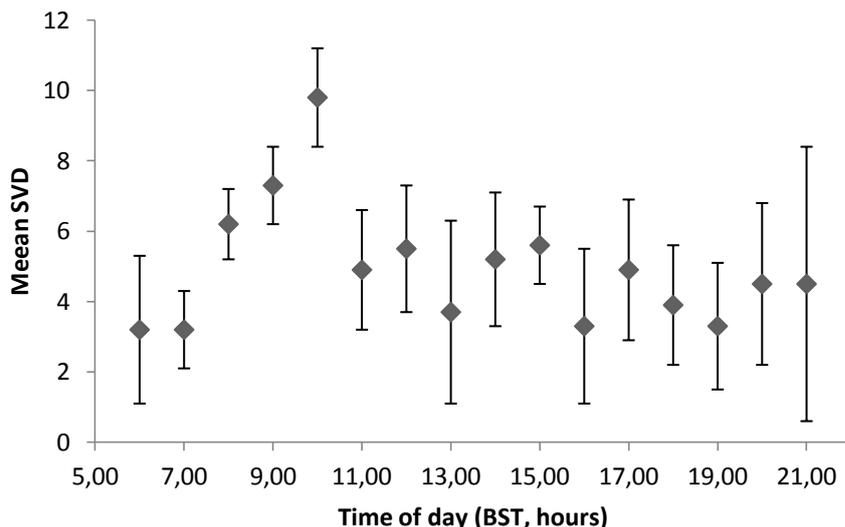


FIGURE 4. Mean SVD (\pm SE) for all visitor species to *Echium vulgare*, against time of day.

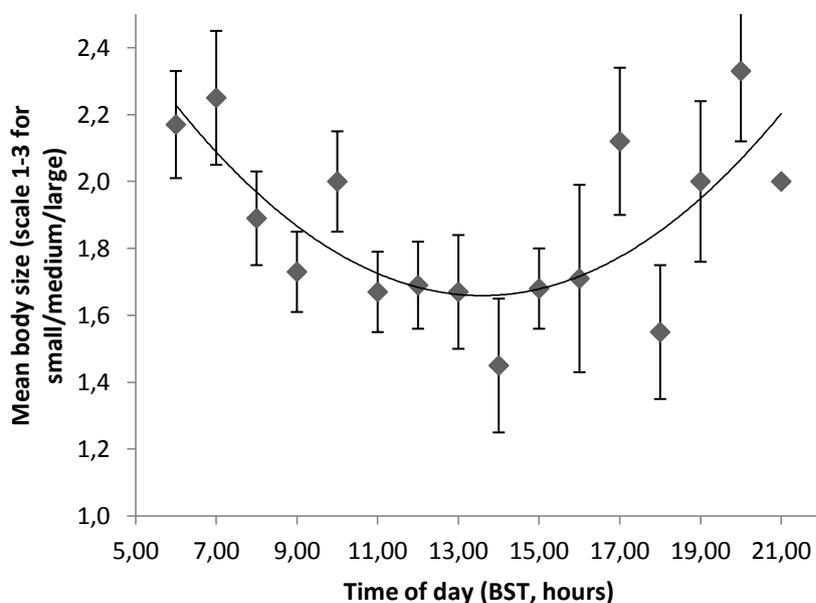


FIGURE 5. Mean body size (\pm SE) against time of day for all visitor species to *Echium vulgare*. (Best-fit polynomial is shown; simple regression, quadratic term significant $t_{195} = 2.19$, $P = 0.03$).

2. *Geranium sanguineum*

Data were only recorded at times when the flower was in the female phase, and are summed across flowers of age 2-5 days (predominantly day 2). Table 3 shows the mean SVD for all visitors, in three size categories. Rather few large individuals were recorded, but for *Bombus* larger bees still deposited more pollen than smaller ones, with the differences greatest for the abundant *B. terrestris*. For all *Bombus* combined size had a significant positive effect on SVD (ANOVA $P = 0.041$, see Table). Virtually no size variation occurred for the honeybees, and their mean SVD value was lower than the mean for *Bombus* species. Many hoverflies also visited the flowers but only for pollen when they were in the male phase, and occasional butterflies visited for nectar; but no records of SVD above the control level were recorded for visitors other than bees.

The diurnal pattern of SVD (Fig. 6) is low initially (10-30 grains per visit) until about 0800h, but then fairly constant (40-70 grains) through the remaining daylight hours, consistent with the observed presence of pollen in male-phase flowers over most of the daylight hours in the early life (day 1-2) of a flower. Fig. 7 shows the mean body size of visitors against time of day; here the pattern of larger visitors early and late is missing, with no significant trend (simple regression, quadratic term NS, $t_{184} = 1.86$, $P = 0.07$).

DISCUSSION

A) Effects of body size on SVD

Both in laboratory studies and in the field, intraspecific variation in body dimensions strongly influenced pollen

TABLE 3. Mean SVD for large, medium and small individual bees (\pm SE, n in parentheses) visiting *Geranium sanguineum*. Details of ANOVA tests are also shown.

	Large	Medium	Small	All	F	P
<i>Apis</i>				34.4 \pm 3.4 (46)		
<i>B. terrestris</i>	69.4 \pm 12.4 (5)	65.7 \pm 10.4 (22)	36.2 \pm 10.0 (19)	53.9 \pm 6.5 (19)	2.79	ns
<i>B. lapidarius</i>		44.0 (2)		44.0 (2)		
<i>B. lucorum</i>		48.9 \pm 7.8 (10)		48.9 \pm 7.8 (10)		
<i>B. pascuorum</i>		37.0 \pm 4.0 (30)	31.0 \pm 7.6 (7)	35.8 \pm 3.3 (37)	0.49	ns
<i>B. pratorum</i>		38.0 \pm 5.5 (11)	29.5 \pm 7.6 (8)	34.4 \pm 5.1 (19)	0.65	ns
All <i>Bombus</i>	69.4 \pm 12.4 (5)	47.4 \pm 4.2 (75)	33.5 \pm 3.9 (34)		3.29	0.041

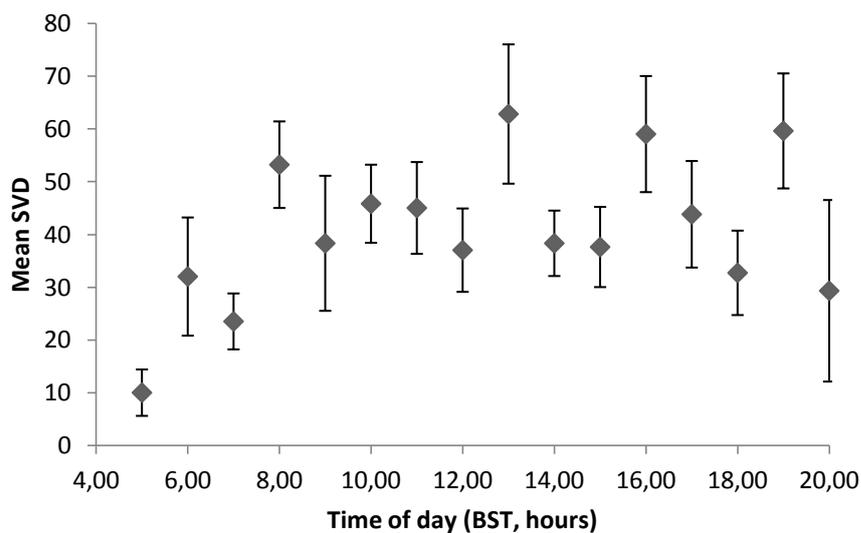


FIGURE 6. Mean SVD (\pm SE) for all visitor species to *Geranium sanguineum*, against time of day.

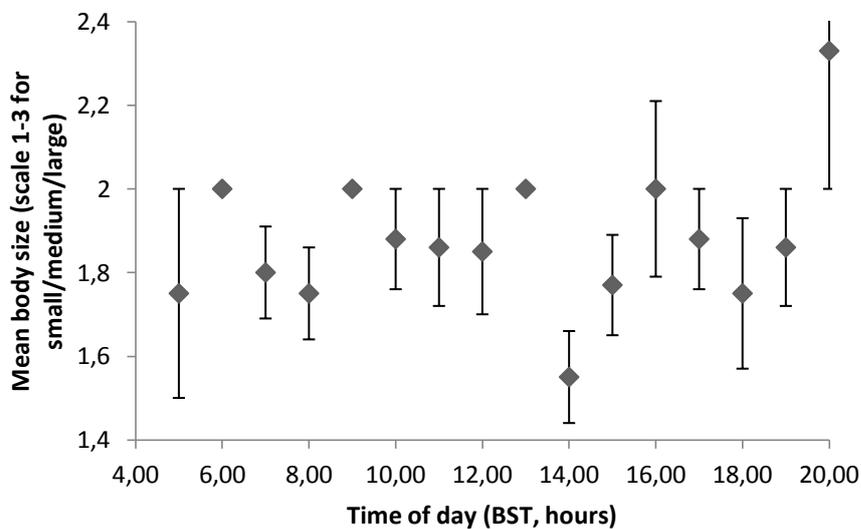


FIGURE 7. Mean body size (\pm SE) against time of day, for all visitor species to *Geranium sanguineum*, with no significant trend. (Simple regression, quadratic term not significant, $t_{184} = 1.86$, $P = 0.07$).

deposition by flower-visiting social bees, particularly for bumblebees where size variation is substantial. It is not surprising to find larger visitors being more efficient pollinators on reasonably open ('generalist') flower types, as documented by Sahlí & Connor (2007) for *Raphanus* when considering interspecific body-mass differences and seed-set. Better pollination by larger visitors has also been explicitly reported for other *Geranium* species (Kandori 2002). For more complex flowers such as keel-type Fabaceae large body size is generally reported as advantageous for 'tripping' the flowers, although Stout (2000) showed that small and medium bees tripped *Cytisus* flowers better than very large queen bumblebees. But an intraspecific difference in pollinator effectiveness for a flower offering reasonably open access to visitors has not previously been demonstrated.

In eusocial bees, the survival of the colony depends upon the division of tasks amongst workers (polyethism). In most genera it is progressive ageing that determines the tasks a worker performs (Seeley 1982), notably in honeybees and stingless bees where workers are of fairly uniform size. However bumblebees do not employ age-determined polyethism, and do exhibit large intraspecific and intra-nest worker size variation (Goulson 2010); this results in alloethism, the performance of different behaviours and tasks as dictated by size (Goulson et al. 2002). In *Bombus terrestris*, alloethism leads to the largest bees becoming foragers, and switching to foraging behaviours earlier than smaller counterparts (Pouvreau 1989). Larger bumblebee foragers have longer tongues, resulting in more nectar collection (Peat et al. 2005; Peat & Goulson 2005), larger more sensitive eyes and brains (Macuda et al. 2001; Mares et al. 2005) and greater antennal sensitivity (Spaethe et al. 2007), leading to better learning and memory (Worden et al. 2005). They also show better thermoregulatory efficiency (Bishop & Armbruster 1999), so being more efficient foragers in colder weather (Heinrich & Heinrich 1983; but see Peat & Goulson 2005), where their size also positively influences flight ability (Kapustjanskij et al. 2007); and they may have better competitive capacity associated with improved access to resources (Inoue & Yokoyama 2006). Overall, they contribute more nectar and pollen per unit time to the colony than smaller foragers (Spaethe & Weidenmüller 2002) consistent with the view that worker size is largely determined by foraging-related non-reproductive factors (whilst size in males and queens is more strongly linked to selection on reproductive functions (del Castillo & Fairbairn 2011)).

Goulson et al. (2002) observed a linear relationship between forage mass collected and thorax width in bumblebees. From our study, individual bees of greater size (Fig. 3, Tables 2 and 3) were capable of depositing a higher mean number of pollen grains, whether on the underside of the *Vinca* stigma wheel or on the simpler protruding stigmas of *Echium* and *Geranium* flowers. This was not due to any variation in visit duration, which was unrelated to bee size but did increase later in the day for *Echium* as both pollen and nectar became scarcer (pers. obs.). Instead it may be largely attributable to their larger surfaces (of ventral body, or of tongue length) so that they may contact more anthers and gather more pollen; larger bees can evidently carry larger

pollen loads in their scopa and ungroomed pollen loads on the rest of the body should be similarly greater. Larger individual foragers are therefore potentially beneficial to the plant, as long as their depositions are not exceeding the maximum pollen grains required per flower to fertilise all ovules and are therefore not causing 'pollen clogging'.

For *Vinca minor* flowers the smallest bees failed to deposit pollen at all. A visiting nectar-seeking bumblebee must have a tongue able to extend the length of the corolla (mean 9.3mm) to access the basal nectaries. Thus a *Vinca* flower must be co-adapted with relatively long-tongued visitors. Goulson et al. (2002) found a simple proportional relationship between overall body size and tongue length in bumblebees, whilst Morse (1977) and Harder (1982) also showed that bees with larger wings, linked to a larger body, had longer tongues. Thus larger foraging bees with longer tongues are advantageous to *Vinca minor*, being able to contact the critical points within the flower to acquire and deposit pollen grains. The plant thereby has a potentially increased success in cross-fertilization from greater pollen deposition.

While small bumblebees proved unsuccessful at pollinating *Vinca minor*, and less effective on *Echium* and *Geranium*, they may of course function as effective foragers at other species of flowers. Individual bees tend to specialize on certain flowers (Heinrich 1979; Cane & Sipes 2006) and may feed on flowers appropriate to their body size and tongue length, so that smaller bees do prefer to forage at flowers with shallower corollae where they may more easily access nectar (e.g. Peat, Tucker & Goulson 2005). Size variation within the nest thus contributes to exploitation of a wider resource range and can be advantageous to the bee colony as well as to the plants that they visit. A *Bombus* colony with a large size range of individuals may therefore be more successful in food-gathering overall. It would be interesting to explore influences on this size range, other than the obvious weather and food constraints; for example pesticide exposure can influence the size of *Bombus* workers (Baron et al. 2014), and competition from honeybees may also have an effect (Butz Hury 1997; Thomson 2004), both of these being potentially deleterious to colony success.

B) The influence of grooming behaviour

All Hymenoptera, including bumblebees, perform grooming behaviours to maintain their condition and remove foreign contaminants. The cleaning of the head, with the forelegs performing a scraping action, allows cleansing of mouthparts and antennae (Jander 1976). With *Vinca* a bee would occasionally perform 'tongue wiping' behaviour before moving on to another flower, which strikingly influenced pollen deposition and often reduced SVD to zero resulting in a total loss of cross-fertilization. Post-visit grooming is common in bees, with pollen being packaged into the scopa; but specific grooming behaviours between flower visits were observed only rarely for both *Echium* and *Geranium* flowers (less than one in 20 and one in 35 visits respectively) so in these cases did not affect mean SVD values.

C) The performance of foragers over time

Bumblebees normally show decreased handling time on flowers with increased practice (Goulson 2010), the effect increasing markedly with morphologically more complex flowers (Lavery 1994), although the direct effects may be mainly short-term (Durisko et al. 2011). They may take three times as many visits to learn the motor skills needed for effective pollen collection compared with learning how to feed on nectar (Raine & Chittka 2007). Hence a similar improvement in flower handling might be expected in our trials with individual bees, and would need to be taken into account in assessing effectiveness. However there was no significant increase in pollen deposition with prolonged exposure to *Vinca minor* flowers, presumably because of the unusual morphology involved. A visiting bee did not need to learn how to 'handle' the flower beyond the process of inserting its proboscis, and the inherent structure of the flower controlled pollen deposition onto the proboscis via its complex spatial arrangement of pollen and stigma.

Field trials did not involve individually marked bees, so no direct comparisons of SVD over time could be made. However for both flower species several visibly distinctive bees (based on wing-wear patterns and/or hair loss on dorsal thorax) did return multiple times to the flower patches within a day, but with no indication of improving SVD. This lack of effect is presumably attributable to the rather simple flower forms, where visitation for nectar extraction was a straightforward process of relatively constant and short duration (2.52 ± 0.06 s for *Echium* and 3.10 ± 0.11 s for *Geranium*). In more complex flowers handling time should indeed decrease, but whether this leads to an increase or a decrease in SVD is hard to predict: we previously found little or no relation between visit duration and SVD across 13 plant species (King, Ballantyne & Willmer 2013, though longer visits on particularly nectar-rich flowers may improve pollen deposition in some cases (e.g. Thomson & Plowright 1980). A bee that learnt to extract nectar more quickly but was not engaged in deliberate pollen collection might show decreasing SVD; one that was specifically gathering both nectar and pollen might become more efficient at pollen collection and show increased SVD even on shorter visits. Bumblebees are well known to specialise in either pollen or nectar trips on different flowers (Gonzalez et al. 1995; Goulson 2010), different days or different weather conditions (Peat & Goulson 2005). From the plant's point of view, then, the changes in pollinator performance could potentially be either positive or negative in effect.

D) Diurnal effects on size of visitors and resultant SVD

Visitor size at individual flowers varies on a diurnal basis, and there will also be seasonal effects and between-year effects at the level of individual flowers and between flowering communities (explicitly documented for *Geranium*, spp. by Kandori (2002)). Since visitor patterns vary in this way, we cannot accurately give a single SVD 'pollinator effectiveness' value for a given visitor to a given plant species, but must record the situation for a particular time and place.

This variation should also have significant effects for the plant's reproduction, and particular plants may be able to exploit it to improve their own cross-pollination. *Echium* flowers mainly dehisce in the early morning, and have pollen available primarily in the first half of their first day of opening; and across many plant species this pattern has been widely assumed to relate to attracting bees as pollinators (Shelly & Villalobos 2000; Castellanos et al. 2006; Willmer 2011) and so to be indicative of a bee-pollination syndrome. In practice, it may specifically be related to attracting the largest and most efficient bumblebees as pollinators. In contrast *Geranium* flowers are longer-lasting and in this study had pollen available for at least 24 hours, visitors collecting it with equal efficiency at any hour of the day; this pattern should be better suited to a more generalist flower, with open or bowl-shaped anatomy, targeted by many visitors (including those with shorter tongues), and able to achieve reproductive success from the services of many of them.

Conclusions

There is a significant positive correlation between body size and average pollen deposition for individual bees, and the smallest individuals may be unable to collect or deposit pollen onto the stigma of certain flowers. Individual body size, as well as specific behaviours on the flower and after leaving the flower (such as grooming), can result in significant variation in pollen deposition, so that it is inappropriate to give values of SVD for a particular visitor species on a particular flower without taking these factors into account. This is particularly true for visitors such as bumblebees, where intra-nest size variation is substantial at any one time and can be variable through a season (though with no consistent pattern; see Goulson & Sparrow 2009). This has numerous implications for colony and plant success. Bumblebee nests might benefit from producing larger workers, as these will be more efficient foragers and may visit a range of flowers inaccessible to smaller workers; however this must be offset against greater initial investment and the metabolic costs of larger individuals. Flowering plants also benefit from being visited by larger bumblebees, as more pollen grains may be collected during a single visit, and more may then be deposited at the next flower or next few flowers (useful so long as the number deposited does not exceed the number needed for full fertilisation of ovules). Potentially (as long as bees are moving between plants) this should improve cross-fertilisation for the plant. Small forager bees, however, may be less efficient for both the colony and flowering plants. They are unable to collect as much nectar or pollen (Goulson et al. 2002), and may be too small to pollinate certain flowers at all.

Our results have marked implications for pollination biology. Generally in the current literature visiting organisms are 'ranked' on a species-level (or even a generic or family level) for their suitability for a given flower or flower type, and their true effectiveness as pollinators is rarely assessed. King, Ballantyne & Willmer (2013) showed that pollination ability and efficiency varies more within functional group, family or genus than commonly supposed; the results here argue that we must additionally consider individual-level

variance in PE. This also accords with the findings of Tur et al. (2014) who showed that pollinator network structure can be affected by individual (within-species) variation associated with differing levels of generalization and specialization in flower visiting behaviours, although those authors did not link the effect to size differences.

From a plant's-eye-view, it is most beneficial to have visitors (even within species) that are appropriately size to maximise efficient pollen transfer. Flowers employ many methods to attract pollinators of the appropriate type or species (as described by pollination syndromes; see Willmer 2011) but may also modify their architecture and/or by the timing and pattern of reward presentation to draw in the 'best-fit' individuals. There is thus a particularly delicate relationship between an individual foraging bee and the flowers it visits; and the size of a bee determines not only its value to the nest as a forager, but also its PE value to the plants that it chooses to visit.

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REFERENCES

- Alford DV (1975) *Bumblebees*. Davis-Poynter, London.
- Baron GL, Raine NE & Brown MJF (2014) Impact of chronic exposure to a pyrethroid pesticide on bumblebees and interactions with a trypanosome parasite. *Journal of Applied Ecology* 51:460-469.
- Benton T (2006) *Bumblebees*. Harper Collins, London.
- Bishop JA & Armbruster WS (1999) Thermoregulatory abilities of Alaskan bees: effects of size, phylogeny and ecology. *Functional Ecology* 13:711-724.
- Butz Huryn VM (1997) Ecological impacts of introduced honeybees. *Quarterly Review of Biology* 72:275-297.
- Cane JH & Sipes S (2006) Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. In: Waser NM & Ollerton J (eds) *Plant pollinator interactions: from specialization to generalization*, Chicago University Press, Chicago, pp 99-112.
- Castellanos MD, Wilson P, Keller SJ, Wolfe AD & Thomson JD (2006) Anther evolution: pollen presentation strategies when pollinators differ. *American Naturalist* 167:288-296.
- Couvillon MJ & Dornhaus A (2009) Location, location, location: larvae position inside the nest is correlated with adult body size in worker bumble-bees (*Bombus impatiens*). *Proceedings of the Royal Society B* 276:2411-2418.
- Couvillon MJ & Dornhaus A (2010). Small worker bumblebees (*Bombus impatiens*) are hardier against starvation than their larger sisters. *Insectes Sociaux* 57:193-197.
- Darwin C. (1861) Fertilization of Vincas. *Gardeners' Chronicle* 552
- Dawson EH, Avargues-Weber A, Chittka L & Leadbeater E (2013) Learning by observation emerges from simple associations in an insect model. *Current Biology* 23:727-730.
- del Castillo RC & Fairbairn D (2011) Macroevolutionary patterns of bumblebee body size: detecting the interplay between natural and sexual selection. *Ecology & Evolution* 2:46-57.
- Durisko Z, Shipp L & Dukas R (2011) Effects of experience on short and long-term foraging performance in Bumblebees. *Ethology* 117:49-55.
- Fjell I (1983) Anatomy of the xeromorphic leaves of *Allamanda neruifolia*, *Thevetia peruviana* and *Vinca minor* (Apocynaceae). *Nordic Journal of Botany* 3:383-392.
- Fontaine C, Collin CL & Dajoz I (2008) Generalist foraging of pollinators: diet expansion at high density. *Journal of Ecology* 96:1002-1010.
- Fryxell P (1957) Mode of reproduction of higher plants. *Botanical Review* 23:135-233.
- Gonzalez A, Rowe CL, Weeks PJ, Whittle D, Gilbert FS & Barnard CJ (1995) Flower choice by honeybees (*Apis mellifera* L) – sex phase of flowers and preferences among nectar and pollen foragers. *Oecologia* 101:258-264.
- Goulson D (2010) *Bumblebees: behaviour, ecology, and conservation* (2nd ed.) Oxford University Press, Oxford.
- Goulson D, Peat J, Stout JC, Tucker J, Darvill B, Derwent LC & Hughes WOH (2002) Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Animal Behaviour* 64:123-130.
- Goulson D & Sparrow KR (2009) Evidence for competition between honeybees and bumblebees; effects on bumblebee worker size. *Journal of Insect Conservation* 13:177-181.
- Greenleaf S, Williams N, Winfree R & Kremen C (2007) Bee foraging ranges and their relationship to body size. *Oecologia* 153:589-596.
- Harder L (1982) Measurement and estimation of functional proboscis length in bumblebees (Hymenoptera: Apidae). *Canadian Journal of Zoology* 60:1073-1079.
- Heinrich B (1979) 'Majoring' and 'minoring' by foraging bumblebees, *Bombus vagans*; an experimental analysis. *Ecology* 60:245-255.
- Heinrich B & Heinrich MJE (1983) Size and caste in temperature regulation by bumblebees. *Physiological Zoology* 56:552-562.
- Horwood A (1919) *British Wild Flowers – In Their Natural Haunts*. Gresham Publ Co.
- Inoue MI & Yokoyama J (2006) Morphological variation in relation to flower use in bumblebees. *Entomological Science* 9:147-159.
- Jander R (1976) Grooming and pollen manipulation in bees (Apoidea): the nature and evolution of movements involving the foreleg. *Physiological Entomology* 1:179-194.
- Kandori I (2002) Diverse visitors with various pollinator importance and temporal change in the important pollinators of *Geranium thunbergii* (Geraniaceae). *Ecological Research* 17:283-294.
- Kapustjanskij A, Streinzer M, Paulus HF & Spaethe J (2007) Bigger is better: implications of body size for flight ability under different light conditions and the evolution of alloethism in bumblebees. *Functional Ecology* 21:1130-1136.
- King C, Ballantyne G & Willmer P (2013) Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology & Evolution* 4:811-818.
- Klinkhamer PGL, de Jong TJ & Wesselingh RA (1991) Implications of differences between hermaphrodite and female flowers for attractiveness to pollinators and seed production. *Netherlands Journal of Zoology* 41:130-143.

- Laverty TM (1994) Bumblebee learning and flower morphology. *Animal Behaviour* 47:531-545.
- Leadbeater E & Chittka L (2009) Bumble-bees learn the value of social cues through experience *Biology Letters* 5:310-312.
- Macuda T, Gegear RJ, Laverty TM & Timney B (2001) Behavioural assessment of visual acuity in bumblebees (*Bombus impatiens*). *Journal of Experimental Biology* 204:559-564.
- Mares S, Ash L & Gronenberg W (2005) Brain allometry in bumblebee and honey bee workers. *Brain Behaviour & Evolution* 66:50-61.
- Morse D (1977) Estimating proboscis length from wing length in bumblebees (*Bombus* spp.). *Annals of the Entomological Society of America* 70:311-315.
- Morse DH (1978) Size-related foraging differences of bumble-bee workers. *Ecological Entomology* 3:189-192.
- Ne'eman G, Jürgens A, Newstrom-Lloyd L, Potts S, & Dafni A (2010) A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Reviews* 85:435-451.
- Peat J, Darvill B, Ellis J & Goulson D (2005) Effects of climate on intra- and interspecific size variation in bumble-bees. *Functional Ecology* 19:145-151.
- Peat J & Goulson D (2005) Effects of experience and weather on foraging rate and pollen versus nectar collection in the bumblebee, *Bombus terrestris*. *Behavioral Ecology & Sociobiology* 58:152-156.
- Peat J, Tucker J & Goulson D (2005) Does intraspecific size variation in bumblebees allow colonies to efficiently exploit different flowers? *Ecological Entomology* 30:176-181.
- Persson AS & Smith HG (2011) Bumblebee colonies produce larger foragers in complex landscapes. *Basic & Applied Ecology* 12:695-702.
- Plowright RC & Jay SC (1968) Caste differentiation in bumblebees (*Bombus latr.*: Hym.) I. The determination of female size. *Insectes Sociaux* 2:171-192.
- Pouvreau A (1989) Contribution à l'étude du polyéthisme chez les bourdons, *Bombus Latr.* (Hymenoptera, Apidae). *Apidologie* 20:229-244.
- Rademaker MCJ, de Jong TJ & Klinkhamer PGL (1997) Pollen dynamics of bumble-bee visitation on *Echium vulgare*. *Functional Ecology* 11:554-563.
- Rademaker MCJ, de Jong TJ & van der Meijden E (1999) Selfing rates in natural populations of *Echium vulgare*: a combined empirical and model approach. *Functional Ecology* 13:828-837.
- Raine N & Chittka L (2007). Pollen foraging: learning a complex motor skill by bumblebees (*Bombus terrestris*). *Naturwissenschaften* 94:459-464.
- Roulston TH & Cane JH (2000) The effect of diet breadth and nesting ecology on body size variation in bees (Apiformes). *Journal of the Kansas Entomological Society* 73:129-142.
- Sahli HF & Conner JK (2007) Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany* 94: 203-209.
- Seeley TD (1982) Significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology & Sociobiology* 11:287-293.
- Shelly TE & Villalobos E (2000) Buzzing bees (Hymenoptera: Apidae, Halictidae) on *Solanum* (Solanaceae): floral choice and handling time track pollen availability. *Florida Entomologist* 83:180-187.
- Smeets P & Duchateau M (2003) Longevity of *Bombus terrestris* workers (Hymenoptera: Apidae) in relation to pollen availability, in the absence of foraging. *Apidologie* 34:333-337.
- Spaethe J, Brockmann A, Halbig C & Tautz J (2007) Size determines antennal sensitivity and behavioural threshold to odors in bumblebee workers. *Naturwissenschaften* 94:733-739.
- Spaethe J & Weidenmüller A (2002) Size variation and foraging rate in bumblebees (*Bombus terrestris*) *Insectes Sociaux* 49:142-146.
- Stout JC (2000) Does size matter? Bumblebee behaviour and the pollination of *Cytisus scoparius* L. (Fabaceae). *Apidologie* 31:129-139.
- Sutcliffe GH & Plowright RC (1988) The effects of food supply on adult size in the bumble bee *Bombus terricola* Kirby (Hymenoptera: Apidae). *The Canadian Entomologist* 120:1051-1058.
- Thomson D (2004) Competitive interactions between the invasive European honey bee and native bumble bees. *Ecology* 85: 458-470.
- Thomson J & Goodell K (2001) Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied Ecology* 38:1032-1044.
- Thomson JD & Plowright RC (1980) Pollen carryover, nectar rewards and pollinator behaviour with special reference to *Diervilla lonicera*. *Oecologia* 46:68-74.
- Tur C, Vigalondo B, Trojelsgaard K, Olesen J & Traveset A (2014) Downscaling pollen-transport networks to the level of individuals. *Journal of Animal Ecology* 83:306-317.
- Waddington KD, Herbst LH & Roubik DW (1986) Relationship between recruitment systems of stingless bees and within-nest worker size variation. *Journal of the Kansas Entomological Society* 59:95-102.
- Willmer PG (1983) Thermal constraints on activity patterns in nectar-feeding insects. *Ecological Entomology* 8:455-469.
- Willmer PG (2011) *Pollination and floral ecology*. Princeton University Press, Princeton.
- Willmer PG, Bataw AAM & Hughes JP (1994) The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. *Ecological Entomology* 19:271-284.
- Willmer PG & Stone GN (2005) Behavioural, ecological and physiological determinants of the activity patterns of bees. *Advances in the Study of Behavior* 34:347-466.
- Worden BD, Skemp AK, & Papaj DR (2005) Learning in two contexts: the effects of interference and body size in bumblebees. *Journal of Experimental Biology* 208:2045-2053.