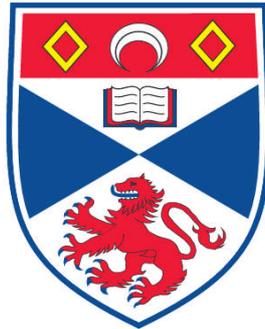


**SPERM COMPETITION AND MALE FORCEPS DIMORPHISM IN  
THE EUROPEAN EARWIG FORFICULA AURICULARIA  
(DERMAPTERA: FORFICULINA)**

**Gordon S. Brown**

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



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**Sperm competition and male forceps dimorphism  
in the European earwig**

***Forficula auricularia* (Dermaptera: Forficulina)**

Gordon S. Brown

A thesis submitted to the University of St. Andrews in application for the  
degree of

Doctor of Philosophy

Submitted: 6 October 2006

Supervisor: Dr. Joseph L. Tomkins

Co-supervisor: Prof. Michael G. Ritchie

## Declaration

I, Gordon S. Brown, hereby certify that this thesis, which is approximately 25000 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.

Date ..... signature of candidate .....

I was admitted as a research student in August 2002 and as a candidate for the degree of PhD in September 2003; the higher study for which this is a record was carried out in the University of St Andrews between 2002 and 2006.

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Date ..... signature of supervisor .....

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*The hornie gollach is an awesome beast*

*soople and scaly, it has twa horns*

*and a hantle o feet and a forkie tailie*

(Burnett 1937)

## Acknowledgements

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Finally, I would like to thank NERC for the studentship I received from them and my examiners Chris Todd and Matt Gage for giving up their time to examine my thesis.

## **Abstract**

The European earwig exhibits a remarkable male-dimorphism in forceps morphology that is associated with alternative reproductive tactics under the control of a conditional evolutionarily stable strategy. Populations on the small, rocky islands of the Farnes off the Northumberland coast are known to sustain populations with dramatically higher morph ratios than observed on the UK mainland. A survey conducted of island and mainland sites around the UK showed that the dimorphic populations of the Farnes are similar to other islands and that mainland populations generally exhibit low morph ratios. Additionally, a correlation between morph ratio and population density was found lending support to the hypothesis that the ESS thresholds that define the morph ratios have diverged through local adaptation.

A set of seven microsatellite markers are presented that were developed from a Farne island population of *F. auricularia* with one additional, previously published locus. These eight markers exhibit genetic variability within and between populations and as such can potentially be applied at a range of scales, from broad-scale phylogeography to within population parentage studies. A phylogeographic study of the UK populations using these markers suggests a single postglacial colonisation from mainland Europe and give further support to the local adaptation hypothesis of ESS threshold evolution.

A study of ejaculate size in *F. auricularia* showed that the males transfer free sperm at a steady rate and that the morphs do not differ in the number of sperm per ejaculate. Measurements of change in body-mass were found to be ineffective measures of ejaculate size, but that macrolabic males lost more weight during copula than brachylabic males. This may be the result of differential investment in accessory ejaculate components between the morphs, as a result of the differing risk of sperm competition.

**Chapter 1: Alternative reproductive strategies,  
male dimorphism and the European earwig:  
An Introduction**

Traits that convey an advantage over conspecifics in relation to mating success are said to be under sexual selection (Andersson 1994). The work presented in this thesis investigates the evolution of the dimorphic male forceps of the earwig *Forficula auricularia* in relation to phylogeography, demography and sperm competition.

## **Sexual Selection**

Darwin first described sexual selection after observing species with conspicuous traits that he could not explain within the remit of natural selection (Darwin 1859). Sexual selection often results in traits that are opposed by natural selection, but are maintained by the advantage gained in reproduction (Andersson 1994). For example, a large unwieldy ornamental trait such as the long tail of a bird like the peacock, increases the male's attractiveness (Petrie *et al.* 1991; Petrie & Williams 1993) but may slow the bearer down making it more susceptible to predation (Petrie 1994), despite the increase in its mating success. However, as long as the possession of the enhanced trait does not convey a decrease in survival, so severe that the lifetime number of offspring is ultimately fewer, individuals with the trait will have greater overall fitness.

Males are more susceptible to extreme sexual selection than females due to anisogamy, the disparity in the size of the gametes (Andersson 1994). In general, females maximise their reproductive success through the provision of nutrients to relatively few large gametes. As a result, females are constrained to reproduce at the rate at which they can synthesise these more costly gametes. In contrast, males produce gametes with no nutrient provision for the embryo, and hence can produce smaller and more numerous gametes (Bateman 1948). This asymmetry in the cost of gametes regularly selects males to mate with many females as their reproductive success increases with the number of matings (Andersson 1994). However, anisogamy constrains female reproductive success to increase more slowly with the number of matings, and therefore selects females that are discriminating of the quality of the males with which they mate. Under these contrasting strategies, females rarely need to compete with other females for access to individual males. Males, however, are likely to compete directly with other males for access to individual females. The differing levels of competition cause more intense sexual selection on males than females and this is reflected in the observation that weapons and ornaments are typically borne by males.

Sexual selection operates in two modes: intrasexual selection that results from competition between individuals within a sex and intersexual selection between individuals of different sex (Halliday 1983). Sexual selection acts prior to mating, intrasexually during male-male competition (contests, scrambles or endurance rivalry) and intersexually through females' mate-choice (Andersson 1994). Sexual selection also acts following mating, intrasexually as a result of competition between the sperm of different males (Birkhead & Moller 1998; Simmons 2001) and intersexually through female sperm selection (Birkhead 1998; Simmons 2001). This thesis is primarily concerned with the study of intrasexual selection through Sperm Competition.

## **The Evolutionary Importance of Sperm Competition**

"Sperm competition is widely recognised as a pervasive force in evolution" (Simmons et al. 1999b).

Competition between the sperm of different males within the reproductive tract of a female has given rise to adaptations in male gametes (e.g. motile sperm) and gamete production (e.g. large testes), primary reproductive traits (e.g. genitalic spines found in dragonflies, seed beetles and cats) and behaviours (e.g. mate guarding and mating rate observed in many vertebrates and invertebrates), (Simmons 2001). Sperm competition must therefore exert a keen selective force in order to instigate changes in such varied and critical sexual characteristics and across such a diversity of taxa.

The evolution of testis size in many species has been tightly linked to the prevalence of sperm competition (Gage et al. 1995; Simmons et al. 1999b). The role that testis size plays in sperm competition appears to be simply that larger testes produce larger ejaculates. Theoretical models show that when sperm compete numerically, either externally or in the female tract, males that inseminate more sperm tend to fertilise more of the female's eggs (Gage *et al.* 1995; Parker 1982) exerting selection for larger ejaculates. A number of comparative studies of testis size across species show that where sperm competition is likely to be most intense, testis sizes are relatively large (Gage 1994; Harcourt et al. 1981; Simmons 2001; Simmons et al. 1999b; Stockley et al. 1997).

## Alternative Reproductive Tactics and Male Dimorphism

Genetic and environmental variation among the males of a population will give rise to individuals that are more capable than others of producing the sexually selected traits required to exploit a particular reproductive tactic. Males of high competitive status will therefore win access to females through direct rivalry with other males by fighting (Le Boeuf 1974) or endurance (Clutton-Brock & Albon 1979). Those males that are out-classed in this direct mode of competition will be under intense selection to achieve reproductive success by avoiding competition (Gross 1996). The consequence of intense selection to be competitive is thought to have given rise to the alternative male reproductive tactics observed within a sex in many species, since uncompetitive males are under strong selection to gain reproductive success by using an alternative tactic to avoid competition (Gross 1996; Maynard Smith 1982).

Where alternative male reproductive behaviours are favoured, disruptive selection on male morphology can arise (Hunt & Simmons 2001). For example, selection may favour larger, stronger individuals that employ a fighting tactic whilst, for a sneak-mating tactic, smaller and less conspicuous individuals may be favoured. Therefore, disruptive sexual selection can act in divergent directions on phenotypes within a sex and lead to the evolution of a male dimorphism, the existence of specialised morphological groups within a sex (Gadgil 1972). These dimorphisms include extreme cases of phenotypic plasticity (West-Eberhard 2003).

Well studied examples of species that show male dimorphism are the *Onthophagus sp.* dung beetles (Emlen 1997; Hunt & Simmons 2001; Kotiaho & Tomkins 2001). The males of *Onthophagus taurus* occur in two morphs; large bodied major males which have large cephalic horns, and small bodied minor males that possess, at most, only small vestiges of horns (Kotiaho & Tomkins 2001). The reproductive behaviour of these males is typical of the genus in that the major males control access to females by guarding whilst the minor males sneak matings with already guarded females (Simmons et al. 1999b). Female *O. taurus* excavate tunnels beneath a dung pad and bury compacted balls of dung. A single egg is laid in each brood ball, which provides the sole food source for the developing larva. Major males guard these tunnels and, if other males attempt to enter, they will engage them in contests of strength (Moczek & Emlen 2000). The horns are projected forward during these bouts to afford purchase on the

opponent. The contest ends when the grip of the weaker male is broken and it is displaced from the tunnel (Moczek & Emlen 2000).

Male dimorphism is a phenomenon that has been studied across many taxa of dioecious animals (Gross 1996). Other than in dung beetles (Scarabaeidae), male dimorphisms have been documented in numerous arthropod taxa including; staphylinid beetles (Forsyth & Alcock 1990; Hanley 2001), Dermaptera (see Table 1), Hymenoptera (Cook et al. 1997; Cremer & Heinze 2003; Hamilton 1979), spiders (Clark & Uetz 1992), acarid mites (Radwan 1995; Radwan 2001; Timms et al. 1980; Woodring 1969) and Crustacea (Haq 1972). Amongst these arthropods most of the observed male dimorphisms tend to reflect alternative tactics for optimising reproductive success. One morph tends to have an advantage in direct competition with other males whilst the other morph has evolved as a means to avoid this competition. The advantage of the competitive or fighter morphs may relate in part to greater body size and the greater weight or strength that it confers. This advantage has been increased by the evolution of disproportionately large weapons that enhance fighting ability. In dung beetles these accentuated weapons take the form of cephalic horns (Emlen 1997), in the staphylinid beetle *Leistotrophus versicolor* they are enlarged mandibles (Forsyth & Alcock 1990), whilst in the acarid bulb mites of the *Sancassania*, *Rhizoglyphus* and *Schwiebia* genera the weapons are reinforced, pointed walking limbs (Woodring 1969).

Morphs that avoid direct male-male competition either tend to be less conspicuous by lacking the enlarged weapons or by behavioural or morphological adaptations such as female-mimicry and typically employ some form of sneak-mating. This avoidance is achieved in the *Cardiocondyla* ants (Cremer & Heinze 2003) and the *Philotrypesis* fig wasps (Cook et al. 1997) by dispersal effected by morph-specific wing development. The close genetic relatedness of ants from a single colony makes it difficult to relate the simpler scenario of morph-specific fitness functions found in other male-dimorphic arthropods, however the similarities remain.

The male dimorphism in the copepod *Euterpina acutifrons* differs from these trends. This dimorphism exploits the breeding advantage of early maturation when the breeding season, which is constrained by seasonal water temperature, is short (Haq 1972). The consequence of this early maturation is a significant reduction in body size; Males that develop early are small but mate readily, whilst males that develop slower are markedly

larger. Male-male competition appears to be secondary to environmental constraints with respect to this dimorphism, which may reflect mobility of this species on ocean currents and its need to adjust to a variable environment.

Male dimorphisms have also been documented amongst vertebrates. In the teleost fish *Porichthys notatus* there are two male morphs; a singing/nesting morph, which courts acoustically, excavates a nest and egg-guards, and a sneak-mating morph that parasitizes the nests of the other morph (Brantley et al. 1993). Other teleosts exhibit similar dimorphisms (Modesto & Canario 2003; Ryan et al. 1992); these resemble the fighting/avoiding dimorphisms described in the arthropods. Reptiles show similar dimorphisms (Mason & Crews 1985; Sinervo & Lively 1996) and common side-blotched lizards (*Uta stansburiana*), in particular, have further evolved three male colour morphs with antagonistic behavioural tactics (Sinervo & Lively 1996). Orange males usurp territory from blue males that mate-guard against sneak-mating by yellow males; the yellow males are most successful in sneaking matings from females in territories usurped by orange males (Sinervo & Clobert 2003).

Like numerous birds the Ruff, *Philomachus pugnax*, breeds by lekking. Males control territories on a confined lekking ground and perform courtship displays to the females, which congregate there. In this species a satellite male morph occurs, which has brown, less conspicuous plumage and that attempts to sneak matings with females in the territories guarded by elaborately-plumed lekking males (Lank et al. 1995). This dimorphism, again, reflects the antagonism between direct male-male competition and its avoidance. A similar dimorphism exists in Dawson's burrowing bee, *Amegilla dawsoni*, which breeds as the adult females emerge from their subterranean brood-chambers (Alcock 1997). Larger male bees control access to the emergence sites whilst smaller satellite males sneak matings and search for departing females.

Male dimorphisms are not common amongst mammals although discrete behavioural phenotypes are exhibited by males of some species of litter-bearing rodent (Clark & Galef 1995; Gross 1996). For example, one behavioural class of male of the Mongolian gerbil (*Meriones unguiculatus*) exhibit greater aggression and are more successful at copulating than other males (Clark & Galef 1995). These behavioural morphs correspond to discrete testosterone levels, which arise due the position at which the male develops in the uterus. Specifically, males that develop in the uterus between

other male siblings sequester high levels of testosterone; Males that develop amongst females have much lower testosterone levels and correspondingly less aggressive behaviour. The need to produce females will counter the production of male-biased litters, such that this system may, to some extent, mirror the discrete morphs and their discrete fitness functions described in the other vertebrate and invertebrate taxa.

In the red deer (*Cervus elaphus*) antler-less 'hummel' males occur in natural populations (Gadgil 1972). Despite the lack of these conspicuous sexual traits, hummels have been observed to successfully defend harems of females and reproduce. Anecdotal evidence suggests that the male offspring of hummel stags tend to develop normal antlers, hence there does not appear to be a strong genetic basis for this phenomenon. Furthermore, the antlers of hummel males can be induced to grow normally by surgically interference of the antler pedicle tissues, showing that such individuals have the potential to grow antlers but do not (Lincoln & Fletcher 1976). Hummel males may be simply the result of stochastic developmental defects in the antler pedicles. However, if a suitable large number of hummel males can be studied, an alternative reproductive tactic may be found to be associated with this morphological phenomenon.

The 'game theory models' by which alternative reproductive tactics arise and are maintained were reviewed by Gross (1996). He postulated three models based around the concept of evolutionarily stable strategies (ESS); *alternative strategies*, the *mixed strategy* and the *conditional strategy*. A strategy is the genetically determined decision rule employed by an individual to determine which tactic it will exploit. A reproductive tactic is the 'phenotype that results from a strategy'; more specifically, it is the morphology, physiology or behaviour that effects a particular means of reproducing (e.g. being inconspicuous and sneak-mating).

Alternative strategy and mixed strategy models rely on negative frequency dependent selection. Hence if a tactic becomes relatively more abundant its fitness will decrease; the fitness of the other tactic will thus increase as it becomes more rare. This frequency dependence is critical because it prevents either tactic from driving the other to extinction. A consequence of frequency dependent selection is that the frequencies of alternative tactics tend to return to a point of equilibrium. The tactics are maintained at this 'evolutionarily stable state' frequency where their fitness is equal (Gross 1996).

The *alternative strategies* model requires that there are several genetically distinct strategies. Each strategy is genetically determined, thus in the simplest scenario each strategy would exploit a single tactic. The genotypes would be maintained under Hardy-Weinberg equilibrium where the fitness of the associated tactics is equal (Gross 1996)

The *mixed strategy* model implies a single strategy with no genetic distinction within the population relative to the tactics they exploit. Instead this single strategy encodes a decision rule governed by the ESS frequency, the outcome of which is to exploit one of several possible tactics. The tactics are adopted in a probabilistic manner at the equilibrium frequency, at which the population achieves the highest overall fitness (Gross 1996). There are no robust examples of the mixed strategy, probably because selection strongly favours individuals that can modify their tactic expression to suit variation in environmental or social conditions.

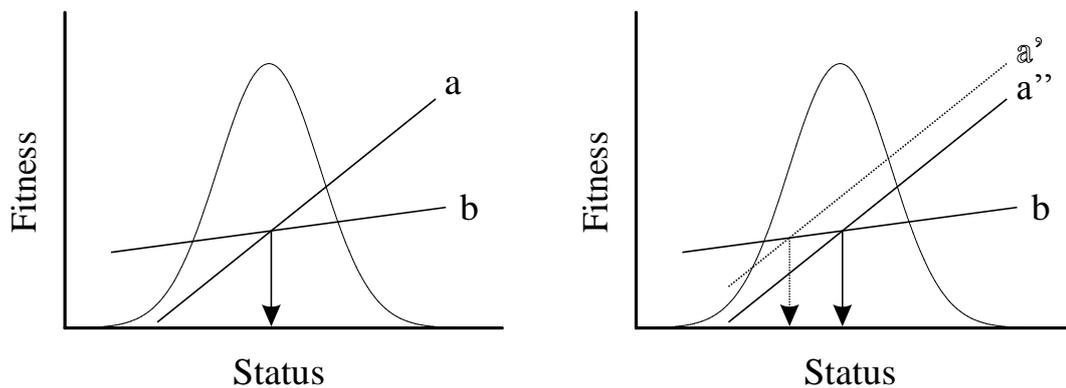
The third ESS postulated by Gross (1996) is the *conditional strategy*, which in the context of reproductive competition between males, determines the tactic to be exploited based on the competitive status of the individual. This strategy is to use a particular tactic on the *condition* that certain intrinsic (e.g. body size) or environmental (e.g. season) criteria are satisfied.

Status is the social rank conferred by the underlying competitive prowess or 'state' of an individual relative to the rest of the population. This will vary between individuals in a population due to genetic variation, environmental heterogeneity (e.g. through varying degrees of parasitism and food availability) or inequality in the stage of development (differences in time of oviposition) (Gross 1996).

The conditional strategy is common in the behavioural decision-making exhibited by animals, however in the context of male dimorphisms the intrinsic link to male status means that this model is also referred to as 'status-dependent'. Gross suggests that the majority of male dimorphisms documented are either best explained by the conditional strategy mechanism or are ambiguous due to lack conclusive data.

The conditional strategy can only exist where the fitness benefit for a high status individual is to employ a different tactic than a lower status individual. Unlike frequency dependent models, the average fitnesses of the tactics are not expected to be

equal but depend on the frequency of the male tactics and the slopes and elevations of the fitness functions. The intersection of the fitness functions of the alternative tactics, relating status to fitness, defines a switch-point in status (Gross 1996; Hazel *et al.* 1990). At this switch-point the tactics will achieve equal fitness; either side of the switch-point one tactic conveys a greater fitness advantage and is favoured over the other (Figure 1).



**Figure 1.** The principle of the status-dependent conditional strategy illustrated with tactics (a & b) with linear fitness functions. The normal frequency distribution of status in the population has been overlaid. Where the fitness functions intersect, the tactics are of equal fitness and the Evolutionarily Stable Strategy (ESS) switch-point exists. The right-hand diagram illustrates how the change in the fitness function of a tactic (in this case tactic a) will give rise to an evolutionary change in the ESS switch-point (the marked on the X axis by the arrow).

Within a population, a particular switch-point is selected for by stabilising selection; individuals switching away from the switch-point, either too early or too late with respect to their status, will have sub-optimal fitness. The heritable element of the conditional strategy is, therefore, the switch-point (Hazel *et al.* 1990). Genetic variation within the population allows this switch-point to adapt to changes in the environment e.g. the ecology or demography of the population. Where the switch-point varies between similar, adjacent populations, the ratio of the morphs associated with the tactics will differ (Tomkins 1999). Morph ratios can therefore be used as indicators of divergence between populations or species in the sexual selection pressures that determine the switch-points (Simmons *et al.* 1999b).

## **Sperm Competition in Male-Dimorphic Species**

Several comparative studies have examined the evolutionary effects of sperm competition on ejaculate characteristics across species (e.g. for testis size; (Gage 1994; Harcourt *et al.* 1981; Stockley *et al.* 1997; Stockley & Purvis 1993). However, in male dimorphic systems the evolutionary divergence in adaptations to sperm competition can be captured within a species (Gage *et al.* 1995; Simmons *et al.* 1999b; Stockley *et al.* 1993; Tomkins & Simmons 2002). The benefit of intraspecific studies is that phylogenetic effects, i.e. similarity of morphology through phylogenetic inertia rather than in response to sexual selection under sperm competition, are better controlled for.

Hence species that exhibit male dimorphisms make ideal model systems for studying sperm competition (SC) because they represent natural experiments in the effects of different sperm competition regimens on evolved phenotypes. This notion is based on the generality that the morphs themselves reflect reproductive tactics that will differ in their inherent risk of SC. The level of sperm competition experienced by a sneak-mating male will always tend to be higher relative to a guarding male because the sperm of the sneak will always have to compete with the sperm of the guard upon which it is sneaking. Nevertheless, the magnitude of the asymmetry in sperm competition risk comes down to population variation in the relative abundance of the reproductive tactics. The level of sperm competition experienced by guarding males will depend on the relative abundance of males that are attempting to sneak-mate with the females that they guard (Parker 1990a; Simmons *et al.* 1999b). When the sneaking morph is abundant, males of the guarding morph will experience high levels of sperm competition, similar to the norm for the sneaking morph (Parker 1990a). In contrast, when the sneaking morph is rare, guards will experience lower sperm competition, since they are rarely sneaked on, and will invest fewer resources into adaptations to sperm competition (e.g. testis size), whereas sneak males still always mate in competition with another male. Therefore, the morph ratio can be used as an indicator of the level of SC risk faced by the alternative tactics in a population (Simmons *et al.* 1999b).

### **The Study System: the European Earwig**

To summarise, this thesis is primarily concerned with how traits can evolve through intrasexual selection as a result of sperm competition. The study system employed to investigate this is the European earwig (*Forficula auricularia*), which exhibits a male

dimorphism controlled by a conditional strategy that is believed to be the result of sperm competition (Tomkins & Simmons 1996; Tomkins & Simmons 2002).

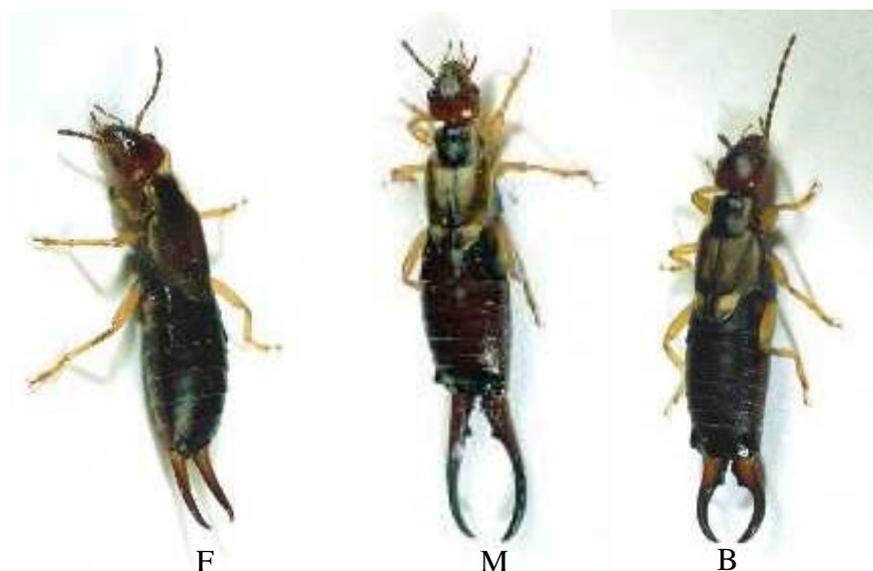
The earwigs or Dermaptera constitute a small but remarkable order of insects with a wide geographic range through temperate and tropical terrestrial environments (Chinery 1993; Eberhard & Gutierrez 1991). The European Earwig, *Forficula auricularia* L. (Forficulina; Forficulidae) is widespread throughout the Palaearctic (Chinery 1993) and now occurs in various places outside its native range such as North America (Guillet et al. 2000b; Lamb 1976b) and Australia (Tomkins pers. comm.). Aside from the success of the Dermaptera as colonists they are also unusual in that they exhibit maternal care through egg guarding and food-provisioning of nymphs (Crumb *et al.* 1941).

The Dermaptera are characterised by the paired abdominal cerci that are specialised to serve as forceps. In most species the morphology of the forceps exhibit a pronounced sexual dimorphism (Giles 1963; Ollason 1972; Popham 1965). The forceps of the male are curved whilst those of the female are more akin to shears, meeting along the medial edges when held together (Figure 2). In addition to this sexual dimorphism there are also male dimorphisms known in several species of earwig (Table 1 and Figure 2). The male dimorphism is characterised by a large-bodied ‘macrolabic’ morph with long forceps and a smaller ‘brachylabic’ morph with short forceps.

**Table 1. Species of earwig of the suborder Forficulina that exhibit male forceps dimorphism**

Species	Region	
<i>Forficula auricularia</i>	UK	(Bateson & Brindley 1892)
<i>Eluanon bipartitus</i>	Australia	(Tomkins & Simmons 1996)
<i>Proreus ludekingi</i>	Sumatra	(Tomkins & Simmons 1996)
<i>Timomenus aeris</i>	Taiwan	(Tomkins & Simmons 1996)
<i>Spongovostox assiniensis</i>	W&C Africa	(Tomkins & Simmons 1996)
<i>Oreasiobias stoliczkae</i>	India	(Tomkins & Simmons 1996)
<i>Metrasura ruficeps</i>	Costa Rica	(Eberhard & Gutierrez 1991)
<i>Doru taeniatus</i>	Costa Rica	(Eberhard & Gutierrez 1991)

The difference between the male morphs is so striking that the larger macrolablic morph was originally misclassified as a separate species (*F. forcipata*). The dimorphism was first correctly described in the UK from specimens collected in the Farne islands off the coast of Northumberland (Bateson & Brindley 1892). An aggregate sample was collected from three of the adjacent rocky islands of the inner Farnes; Knoxes Reef, West Wideopen and East Wideopen (this collection resides at the University of Cambridge). The frequency distribution of the forceps length that emerged was clearly bimodal representing the two distinct morphs, the macrolablic morph having a mode of 7 mm compared to the 3.5 mm of the brachylablic morph (Figure 3). Fifty-five percent of the males in Bateson and Brindley's sample had forceps greater than 5 mm long and 42 percent had forceps shorter than 4.5 mm.

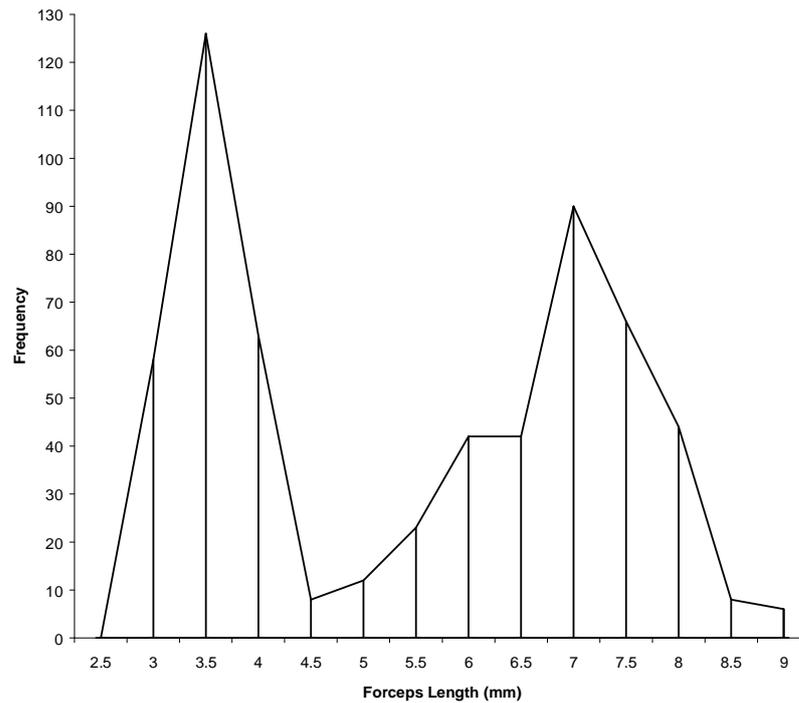


**Figure 2. The forceps morphology in *F. auricularia* exhibits both a sexual dimorphism and a male dimorphism (F = female, M = macrolablic male & B = brachylablic male).**

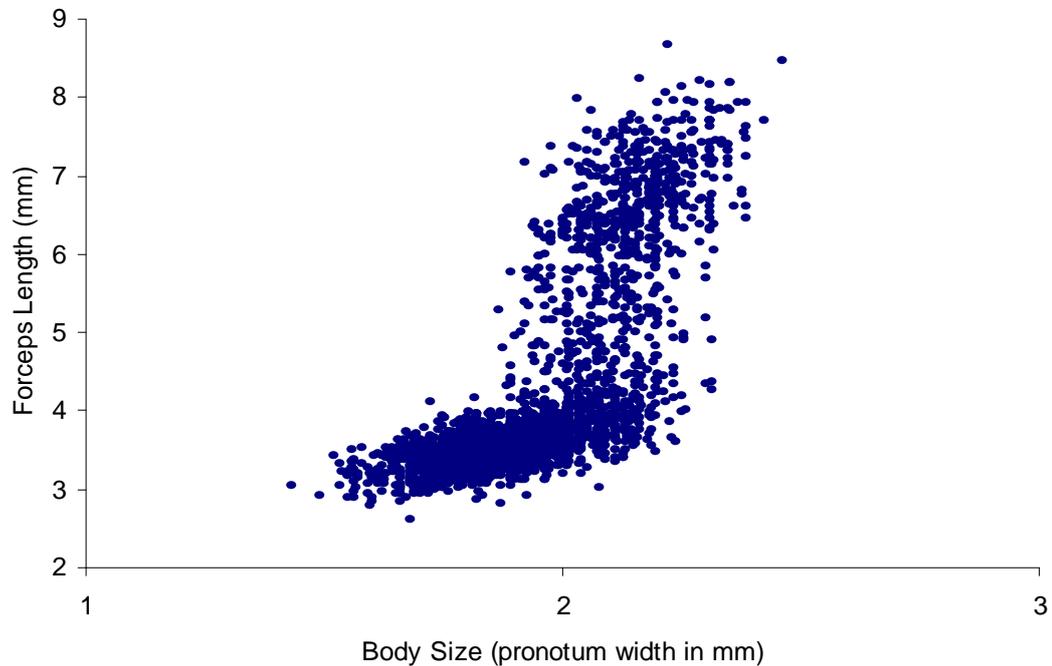
Bateson and Brindley noted that the dimorphism was present on the adjacent mainland, at Bamburgh, where "the high males [macrolablic] were...in fair quantity, though not so abundant as on the Farnes" (Bateson & Brindley 1892). They also recorded a sample from Cambridge where they considered only 3 percent of 163 males to be macrolablic. Sampling in 2001 from Waren Mill near Bamburgh found not one male from a sample of 91 to be macrolablic (J.L. Tomkins pers. comm.). The morphs are clearly distinguished by the relationship between the forceps length and the underlying body

size (Figure 4). Males increase in forceps length in an essentially linearly fashion with increasing body size, the slope being more shallow for the smaller brachylabic morph with body sizes smaller than the switch-point. Above the switch-point males of the macrolabic morph have much longer forceps relative to their body size. The data from the largest males in the distribution show some tendency for levelling off, suggesting that there is some constraint on forceps length. This pattern of intersecting slopes and reaching an asymptote is very similar to an equivalent relationship documented in horned beetle *Onthophagus taurus* (Kotiaho & Tomkins 2001). Interestingly, some males appear to have remained on the brachylabic trajectory even though their body size is greater than the switch-point (Figure 4). These are represented by the small number of data points dispersed to the right of the dense cloud of predictably brachylabic males, having pronotum widths of around 2.2 mm yet having forceps of less than 4.0 mm in length. These males are likely to have higher switch-points than that which defines the inflection between the two morphs in general and simply did not reach a size large enough to cross the threshold. These males may reflect underlying genetic variation within these populations that has enabled variation in switch-point to evolve among the populations. Genetic variability in switch-point is common in mites (Unrug et al. 2004) and this may be a common feature of species that exhibit male dimorphisms (Unrug et al. 2004).

Recent ‘common-garden’ rearing experiments show that, within populations, the dimorphism in *F. auricularia* is largely dependent on the nutrition of the developing nymph, but between populations the morph ratio depends on the location of the threshold with respect to the population’s mean body size (Tomkins 1999). It is probable that the rarity of the macrolabic morph in mainland populations accounts for the lack of other records of this dimorphism in this otherwise well-known and widespread insect.



**Figure 3.** The bimodal distribution of forceps length from earwig populations in the Farne islands (Northumberland, UK); figure reconstructed from Bateson & Brindley (1892).



**Figure 4.** Morphological measurements of 2576 individuals of *F. auricularia* from 22 UK populations (data presented in Chapter 2). The conspicuous increase in the slope of the relationship between underlying body size and forceps length distinguishes the two male morphs.

## The Function of Earwig Forceps

Several hypotheses have been suggested to explain the function of earwig forceps that has driven the evolution of their morphology (Moore & Wilson 1993). Earwigs will readily raise their forceps when attacked (Moore & Wilson 1993), suggesting that they may be of use in deterring predation or in defence of eggs/nymphs during maternal care. Furthermore, fights involving forceps between individuals over access to food have been observed in the earwig *Labia minor* (Fulton 1924). It has been suggested that forceps are used during the folding of the wings after flight. Fulton (1924) showed that normal flight behaviour of *F. auricularia* was not impeded by surgically removing the forceps. Furthermore, when the wings of *F. auricularia* individuals were experimentally unfolded, the subsequent wing-folding behaviour of the earwigs did not involve the use of forceps (Fulton 1924). Therefore, it is unlikely that the use of forceps in wing-folding is important to their evolution.

Some dermapterans are known to be predatory and a few of these species have been observed using their forceps during prey capture (Brindley 1918; Lucas 1920; Steidle & Dettner 1995). Many species of Dermaptera, including *F. auricularia*, are not predatory and the rarity of observations of forceps being used in this manner allows us to disregard this hypothesis here. The most extensive uses of forceps are associated with reproduction (Briceño & Eberhard 1995; Fulton 1924; Moore & Wilson 1993; Radesäter & Halldórsdóttir 1993b; Tomkins & Simmons 1998; Walker & Fell 2001).

Laboratory observations of male *F. auricularia* after having their forceps surgically removed showed that they failed to copulate (Kuhl 1928; Walker & Fell 2001). Male *F. auricularia* of both morphs use their forceps in courtship (Tomkins & Simmons 1998) and fighting (Forslund 2000; Forslund 2003; Radesäter & Halldórsdóttir 1993b).

The female's forceps are also involved in many of the categories of courtship behaviour that have been reported, especially in resisting the advances of the male during early courtship (Moore & Wilson 1993). The female's forceps may also have a sensory function important for courtship or may act as a physical guide during the coupling of genitals at the start of copulation. Indeed, male *F. auricularia* with one of the forceps experimentally shortened were found to be less successful in making genital contact before copulation (Kuhl 1928).

In addition to reproduction, female earwigs also readily fight with other females using their forceps, although these interactions are typically much shorter in duration and are not thought to establish dominance as between males (Moore & Wilson 1993). It is likely that females will benefit from mutually repelling other females to maintain a distance between nests; cannibalism is common in dense laboratory rearings (pers. obs.) and females are less likely to lose eggs through cannibalism the further their nest chamber is from other females. Mate-guarding and high mortality of males shortly after oviposition, make it likely that cannibalism of eggs would be committed chiefly by other females.

## **Earwig Reproductive Behaviour**

Populations of *F. auricularia* in the UK are univoltine such that copulation occurs in late summer, followed by the excavation of a nest chamber in the soil by the female and oviposition by early winter (Lamb 1976a). Populations of *F. auricularia* in more southerly parts of mainland Europe (Wirth et al. 1998) and in invaded ranges of North America (Guillet et al. 2000b) are bivoltine; this is discussed in chapter 4.

Male earwigs compete for access to females through aggressive fights where the forceps are used as weapons. Such fights result in dominance where the subordinate male ceases fighting and attempts to flee the attention of the dominant male. In laboratory trials using the earwig *Vostox apicedentatus*, where two males were pitted against each other for access to a female, the dominant male was observed to control complete access to the female (Moore & Wilson 1993). The dominance observed in these laboratory trials are likely to reflect natural behaviour, however, the simple Petri-dish arenas used may not reflect the natural environment such that sneak-mating may be easier in the wild. Furthermore the trials were for a finite period of time, beyond which the continuation of absolute control by the dominant male is not known. Nevertheless, these observations point to a significant mating advantage to dominant males.

Once male dominance has been established, the dominant male begins to court the female. From observations under laboratory conditions, earwig courtship has been described as complex, involving up to twenty-four behavioural categories (Moore & Wilson 1993; Walker & Fell 2001). Not all of these categories of behaviour were seen in all the pairs observed, indicating that courtship is non-stereotyped (Walker & Fell 2001). However, courtship was consistently found to be composed of two stages

(Moore & Wilson 1993). Early courtship is characterised by advances by the male using his forceps to display to the female and as a tactile stimulus. These advances are initially resisted by the female. Late courtship is characterised by the female becoming quiescent and accepting or soliciting copulation by the male.

In laboratory trials, male *F. auricularia* that had longer and wider forceps were more likely to win dominance in male-male competition and, furthermore, no effect of body size was found (Styrsky & Van Rhein 1999). However, males that had their forceps surgically shortened as adults did not show any reduction in the likelihood of their dominating over similar sized males that had not been manipulated. This suggests that male dominance in earwigs may be at least partially determined by a behavioural correlate of forceps size.

Forslund (2000) showed that male forceps and body size did not influence the total length of copula, the time to first copula or the number of copulations. Whether these metrics actually reflect reproductive success is not known. Indeed, females may be able to control fertilisation independently of copulation; Females may favour non-fertilizing copulation over male harassment that may result from early termination of copula. Furthermore, Forslund's study did not consider male morph as a factor, apparently because the male dimorphism was not very pronounced in the population of *F. auricularia* used. From the 118 pairs from which 'copulation success' could be assessed, 40 of the males (15%) were macrolabic versus the remaining 236 that were brachylabic. It is perhaps not surprising that no influence of forceps and body size on copulatory behaviour was found when most males observed were brachylabic. Whilst these males might be expected to show an advantage and did not, Forslund (2000) did find that heavier males in these trials were more successful at displacing other males during copulation and spent more time copulating in these trials overall. Additionally, in a similar study where 26% of the males used were macrolabic, macrolabic males were found to achieve both a larger proportion of the copulations and significantly longer copulations (Radesäter & Halldórsdóttir 1993b). Males with symmetrical forceps, which were shown to have increased condition in terms of higher weight per body length, were observed to copulate more times than asymmetrical males when in direct competition for access to a female (Radesäter & Halldórsdóttir 1993a). The male

forceps were also more symmetrical with greater forceps length suggesting that larger males tend to have better condition.

Several of these laboratory studies, where males were observed competing for access to a female, reported that females did not preferentially copulate with males based on forceps size or symmetry (Radesäter & Halldórsdóttir 1993a; Radesäter & Halldórsdóttir 1993b). However, females permitted males with longer forceps to copulate after a shorter duration of courtship than those with shorter forceps (Tomkins & Simmons 1998). Furthermore, experimentally shortening male forceps increased the duration of courtship required for a female to permit copulation (Tomkins & Simmons 1998). These two pieces of evidence show that female choice does exist in *F. auricularia*, conferring a selective advantage to males with longer forceps. Additionally, Radesäter & Halldórsdóttir (1993a) did find that forceps symmetry in males correlated with a greater number of copulations. However, a later study where an F1 generation were reared in the laboratory from measured sires showed that only forceps length and not forceps symmetry was heritable (Tomkins & Simmons 1999). This suggests that it is unlikely that females can be assessing male genetic quality through forceps symmetry.

In summary, larger-forceped males, which also tend to have more symmetrical forceps, will be more successful when in direct male-male competition for access to females and will benefit from female choice. Therefore, there will be a clear fitness advantage for the macrolabic morph. Indeed, data quantifying sexual selection in the field show that females are more likely to be found guarded by a macrolabic male than a brachylabic male (J. Tomkins unpubl.).

Although it is likely that all male earwigs will guard females when they can, the laboratory observations show that copulations are often interrupted by other males (Forsslund 2000; Radesäter & Halldórsdóttir 1993b). Therefore, it is highly unlikely that females in wild populations will encounter and mate with just one male given that earwigs tend to aggregate in close proximity in the day-time refuges where they nest. Thus, the mating system in *F. auricularia* is, in broad terms, polyandrous. It is not known if a single male will attempt to guard more than one female in wild populations. The persistence of the smaller brachylabic morph in the presence of the macrolabic morph supports the idea that the control of access to females is by no means absolute

and that sneak mating is likely to be an important means of reproductive success. This body of evidence is consistent with the hypothesis that in dimorphic populations macrolabic males tend to guard females and brachylabic males are forced to sneak copulations.

## **Defining a Morph: Morphometric Analysis of Forceps Size and Shape**

Over the last 30 years various attempts have been made to objectively characterise the male dimorphism in *F. auricularia*. Bateson and Brindley's (1892) original description of the male dimorphism in *F. auricularia* was based on a qualitatively bimodal frequency distribution of the forceps length (Figure 3). Lamb (1976b) saw that it was difficult to statistically test for bimodality and attempted to use a graphical method to distinguish the morph of individuals. However, Lamb concluded from his results that there were up to four morphs, a finding at odds with there being only two behavioural tactics known (mate-guarding and sneak-mating).

Most recently, a quadratic model incorporating a switch-point was developed to describe the relationship between dimorphic trait size (forceps length) and body size (Eberhard & Gutierrez 1991). The model permits testing for a change in slope and allows the position of the calculated switch-point to be tested statistically. This model was revised to consider the switch-point in terms of the dimorphic trait itself, rather than as a body-size threshold, for the more precise discrimination of individuals between morphs (Kotiaho & Tomkins 2001). Whilst body size clearly correlates with the status that determines the morph, the relationship is not necessarily exact. Genetic and environmental factors will cause some individuals to switch at higher or lower body size than the calculated point. Eberhard and Gutierrez's (1991) model does not account for this dispersion and misclassifies these individuals with respect to morph. The revised model (Equation 1) avoids this problem by assuming a morph can be defined by its trait size and not simply body size. Once all the individuals have been assigned to one morph or the other the population's switch-point can be determined by fitting a cubic spline to the dichotomous data for 'morph' against the continuous variable of body size. The mean switch-point being defined as the body size at which an individual has equal probability of being a macrolabic or brachylabic male (this is one of the methods used in chapter 2).

Ollason analysed the average shape of male and female forceps from *F. auricularia* (1972). However, this method did not use biologically meaningful landmarks, instead using arbitrary measurements taken at regular intervals along the forceps. Adopting morphometric methods that use biologically functional landmarks is more likely to reveal useful information from the shape of the forceps.

$$X = \alpha + \beta_1 Y + \beta_2 (Y - Y_D) D + \beta_3 D + \epsilon$$

**Equation 1.** Kotiaho & Tomkins' revised model from (Kotiaho & Tomkins 2001).  $Y$  is the natural log of the dimorphic trait,  $X$  is the natural log of body size,  $Y_D$  is the switch-point,  $\beta_i$  are regression coefficients,  $D$  is the presence or absence of a switch-point,  $\alpha$  is a constant and  $\epsilon$  is the random component (error) around the assumed normal distribution with a mean of zero and common variance.

### **Establishing *F. auricularia* as a Model**

With quantitative methods for objectively discriminating between the male morphs, accurate estimates of the ESS switch-point have been obtainable from populations of *F. auricularia*. In studies of populations of *F. auricularia* in the Farne islands, where Bateson and Brindley first described this species' male dimorphism a century before, Tomkins demonstrated that there is population variation in switch-point (Tomkins 1997).

This population variation could be the result of differences in the distribution of body size between the islands, local environmental factors influencing the development of the forceps or through genetic divergence between the populations. However, population variation in both mean body size and mean switch-point was found to be maintained even in 'common garden' laboratory rearing experiments and therefore these traits are in some part genetically determined (Tomkins 1999). It was found that under increased nutritional availability population size distributions shift upwards and a larger proportion of individuals of the macrolabic morph occur (Tomkins 1999). However, some monomorphic mainland populations will not produce macrolabic males even when reared on the high protein diets that produce macrolabic males in dimorphic populations (Tomkins 1999). This points to a significant genetic divergence in the

potential to be dimorphic between populations that are monomorphic and populations that are dimorphic. Such divergence is consistent with large-scale shifts in switch-point. Furthermore experimental manipulations in rearing density did not have any direct effect on the morph ratio, indicating that the strategy governing the dimorphism is not density dependent per se (i.e. the manipulation of density did not facultatively change the position of the mean switch-point within the population) and supports the hypothesis that it is a conditional strategy dependent on status (Tomkins 1999).

Within its native range, *F. auricularia* occurs at the northernmost reaches of the British Isles, regions that were glaciated during the Pleistocene. Genetic divergence may have arisen through random drift in these isolated populations (vicariance), be an artefact of heterogeneous phylogeographic origins (the *relict hypothesis*) or be the result of adaptation in the switch-point to local conditions (the *rapid evolution hypothesis*). In order to conduct a cross population study of sperm competition it is important to establish the nature of the divergence between male dimorphic and male monomorphic populations. Simply, in relating the divergence in morph ratio with the divergence in sperm competition traits, we need to establish whether we are observing the genetic signature of the postglacial recolonisation or numerous local adaptations. If we do not do this, the differences in morph ratio that we attribute to sperm competition may in fact be the result of distinct, or common, phylogenetic origins (similarity through phylogenetic inertia). In reality it is likely to be a case of removing a component of the variation in morph ratio due to phylogenetic effects in order to focus on the relationship between morph ratio and sperm competition.

In summary, the UK mainland hosts populations of earwig that are mostly monomorphic (for the smaller brachylabic morph) but on the Farne islands the populations have significant numbers of the macrolabic morph (Bateson & Brindley 1892; Tomkins & Simmons 1996). Dimorphic populations are known to occur on the European mainland (Forslund 2000; Kuhl 1928; Tomkins & Simmons 1999) but only in the Farne islands and adjacent mainland has such a striking difference in morph ratio been observed across such a short geographical distance.

The second chapter of this thesis presents work that quantifies the population variation in morph ratio from an extensive survey of UK populations of *F. auricularia* and investigates local ecological factors associated with the dimorphism.

The development of versatile molecular tools is presented in chapter 3. These microsatellite markers are suitable for investigating the phylogeographical origins of earwig populations and, at a finer scale, enable reproductive success of individuals to be traced through parentage analysis. The phylogeographical application of these molecular tools to the UK populations of *F. auricularia* is presented in chapter 4.

Finally a study is presented in chapter 5 of variation in traits associated with the risk of sperm competition, inferred from the male dimorphism quantified in chapters 2 and 4. The extreme variation in morph ratio in *F. auricularia* is used to test the theoretical predictions made by sperm competition models, in particular (Parker 1990a).

## **Chapter 2: Geographic variation of the male dimorphism in UK populations of the European earwig: Threshold evolution driven by population density**

The data presented in this chapter were collected and analysed in cooperation between G. Brown and J.L. Tomkins. These data constitute the dataset published in *Nature* (Tomkins & Brown 2004) and this chapter is a revised version of this manuscript edited and reformatted by the candidate (the original publication is enclosed as appendix C). Figures presented in this chapter are reproduced from Tomkins & Brown (2004).

## Introduction

Subtle between-population variation in morphological threshold has been documented in a number of dimorphic species reared under common-garden conditions, including Farne island populations of *F. auricularia* (Tomkins 1999), the mite *Sancassania berlesei* (Tomkins *et al.* 2004a) and the dung beetle *Onthophagus taurus* (Moczek *et al.* 2002). These studies suggest that differences between populations represent the genetic divergence of the ESS switch-points between populations. Artificial selection experiments support the notion that thresholds can harbour large amounts of genetic variation (Emlen 1996; Roff 1997; Unrug *et al.* 2004), fuelling population divergence.

In this chapter I present data that show threshold evolution in the forceps dimorphism of the European earwig *Forficula auricularia* and document the transition from completely monomorphic to classic male-dimorphic populations over only 40km. Variation in morph ratio is due to a change in position of the dimorphic threshold relative to the population mean body size. Hence morph ratio variation can either be due to threshold evolution independent of mean body size, or to changes in mean body size around a static threshold, or both. The former (Chapter 1, Figure 1) is consistent with ESS theory under which the threshold is determined by population-specific variation in fitness functions (Gross 1996), the latter is likely when a single population is reared across an environmental gradient (Tomkins 1999).

The superior fighting ability of the dominant morph (Radesäter & Halldórsdóttir 1993b) will be more frequently rewarded at high encounter rates, thus population density is likely to be a key determinant of the relative fitness of the alternative tactics and consequently the threshold. In this chapter I show that, as predicted, population density correlates strongly with the shift in threshold and that this factor drives the local evolution of the male dimorphism in these island populations. These data provide evidence for the origin of phenotypic diversity within populations (West-Eberhard 1986; West-Eberhard 1989; West-Eberhard 2003) through the evolution of a switch-point in a conditional strategy that has responded to local population density. In addition to population density, the variation in morph ratio is also compared to three possible factors that may explain the atypically high densities in island populations; the biomass of nesting birds, the island area and its proximity to the mainland. Earwigs eat both guano and the carcasses of dead seabirds, thus the biomass of these birds nesting

on the islands should be a good estimator of this nutrient resource. Island area is likely to directly affect the nutrient availability from other resources that are distributed more homogeneously. The proximity of an island to the mainland may reflect the populations' isolation from predators and parasites that do not disperse long distances over water.

## Methods

Traps were used to collect specimens of *Forficula auricularia* from focal island populations in the Firth of Forth and Farne Islands, where the male dimorphism is most evident due to the relative abundance of the macrolabic morph. Earwigs aggregate in crevices during the day and traps were designed to exploit this behaviour. Traps were composed of 150ml cylindrical plastic vials (diameter 45 mm length 102 mm) containing a roll of (380 x 95mm) standard corrugated cardboard (corrugation height 3mm, width 0.79mm). The vials were spray-painted silver to prevent solar heating and were each attached to a cane inserted into the ground. Traps were placed with the open end of the vial facing down, in contact with the ground and with the surrounding vegetation pulled over the vial. Traps were arranged in groups of four, one trap at each corner of a 1m quadrat. The quadrats of traps were located haphazardly on each of the islands and left *in situ* for three weeks, as a period of time in excess of that required for earwigs to encounter the traps. At the end of the trapping period the traps were collected with screw-fit caps securing the earwigs residing inside.

In addition, earwig samples from mainland and island sites on the west coast of Britain were collected from beneath driftwood and stones or from the hollow stems of hogweed *Heracleum sphondylium* (Umbelliferae). Samples collected by both methods were stored at  $-20^{\circ}\text{C}$ .

Morph ratio was estimated from all sites sampled, both trapped and hand-collected. Morph ratio was assessed by visually assigning the morph of each male. These data were used in the analysis of geographic variation in morph ratio, which does not require precise knowledge of the morphological switch-point, such that the morph can be assigned simply by eye.

Sex ratio was recorded from the specimens trapped from the focal populations and the males measured under a LEICA MZ5 binocular microscope. Measurements were made

using an eyepiece graticule or from captured digital images using SCION IMAGE (NIH) software. Male pronotum width and right forceps length were measured for estimation of the population switch-point or threshold in body size. The number of individuals collected per trap was used to estimate the earwig population density for each of the focal sites.

The biomass of nesting birds was estimated using breeding records from the Farne Islands made by the National Trust (1924-2003) and from the Firth of Forth using data from the Seabird Monitoring Program (1969-2003). The number of nesting pairs of the predominant species of ground-nesting bird was calculated for each island of interest. The biomass of nesting birds per island was calculated as the product of these census totals and the published body weight data of each species (Snow & Perrins 1998). The species surveyed were gannet *Morus bassanus*, lesser black backed *Larus fuscus* and herring gull *L. argentatus*, puffin *Fratercula arctica*, sandwich tern *Sterna sandvicensis*, common tern *S. hirundo*, arctic tern *S. paradisaea*, roseate tern *S. dougallii* and eider *Somateria mollissima*. With the exception of eiders all the birds have altricial young and therefore produce terrestrial organic matter during chick rearing. Cliff-nesting species were not included in the data set as storms wash organic matter from the cliffs each winter.

Island area and distance from the nearest point on the mainland was calculated from 1 : 25,000 maps obtained from the Ordnance Survey website and saved as JPEG files. Area was defined as the region within the mean high-water contour and was measured using SCION IMAGE.

### Switch-point / threshold estimation

The term ‘threshold’ has been used to describe the morphological transition between morphs (Roff 1996), the threshold estimates the position of the ESS ‘switch-point’ (Gross 1996). Thresholds were estimated using the method of Eberhard & Gutierrez (1991). A program written in SPLUS 2000 by K. Wilson (unpubl.) was used for this procedure, in which 100 possible thresholds could be tested per population. The threshold was chosen at which the  $r^2$  and the significance of the  $\beta_3$  inflexion were maximised. Using the Eberhard & Gutierrez (1991) method the proportions of the population lying each side of the morphological threshold was calculated as the proportion of a normal curve (Zar 1984). This estimate of morph ratio was used to

compare populations independently of among population variation in body size. Thus the Eberhard & Gutierrez (1991) method was used to estimate the morphological threshold and population morph ratio. In addition, Kotiaho and Tomkins' modification of Eberhard & Gutierrez' model was used to assign earwigs to different morphs based on their forceps length (Kotiaho & Tomkins 2001). These data were used in logistic regression analyses of population variation in threshold. The Kotiaho and Tomkins (2001) method was also used to summarise the relationship between forceps length and pronotum width using cubic splines (Figure 7a-c). Output from the cubic spline program GLMSWIN1.0 (Schluter 1988) was used to interpolate the body size at which males were macrolabic with a 50% probability. The Kotiaho & Tomkins (2001) threshold is strongly correlated with the Eberhard & Gutierrez (1991) threshold ( $r_{s,22} = 0.88$ ,  $P < 0.001$ ).

## Results

Twenty-two focal island sites were trap-sampled, 11 from the Firth of Forth and 11 from the Farne Islands (Table 2). Trap sampling of the Farne Islands was carried out in August 2001 and the Firth of Forth in September 2002 with an average of  $22 \pm 2$  traps set per island, yielding 16977 earwigs. From this collection measurements were taken from 2674 males with an average sample size of  $121 \pm 11$  individuals per island (Table 2). ANOVA with population as a random effect revealed that there was significant variance between islands in trap catches ( $F_{21,502} = 9.57$ ,  $P = 0.001$ ).

A further 3713 individuals were collected by hand from 11 mainland and 13 west-coast island sites during August and October 2002 yielding an average sample size of  $72 \pm 9$  males collected per site (range = 13 - 148) (see Appendix A).

**Table 2. The number of males measured from the 22 focal island sites where the incidence of macrolabic males is known to be high.**

Firth of Forth			Farne Islands		
1.	Inchcolm	145	Lindisfarne	73	
2.	Inch Garvie	100	Inner Farne	82	
3..	Cramond	37	Knoxes Reef	192	
4.	Inchmickery	92	West Wideopen	227	
5.	Inchkeith	145	East Wideopen	194	
6.	Eyebroughy	100	Staple	224	
7.	Lamb	71	Brownsman	176	
8.	Fidra	100	South Wamses	135	
9.	Craigleith	100	North Wamses	123	
10.	Bass Rock	105	Big Harcar	57	
11.	Isle of May	138	Longstone End	58	
	total	1133	total	1541	

British island populations of *F. auricularia* have a significantly higher proportion of the macrolabic male morph than do mainland populations (Figure 5). This phenomenon is equally true in the northeast of the British Isles as in the southwest, suggesting that the variation between islands and the mainland is not simply due to gross variation in climate or to milder microclimatic conditions on islands (air temperature at Boulmer in Northumberland, is on average 1.5°C cooler than St Mawgan in Cornwall; Met office data). To understand why island populations of earwigs are male-dimorphic we concentrated on population variation among North Sea islands in the Firth of Forth and the Farne Islands. This minimised differences in the climate, geological history, habitat and the ecology and diversity of interacting species. Only these islands are considered in further analyses in this chapter. Island earwig populations varied considerably in the observed ratio of macrolabic to brachylabic males, ranging from 0 - 20% in the Firth of Forth and from 8 - 45% in the Farnes group (Figure 5 & Figure 6).

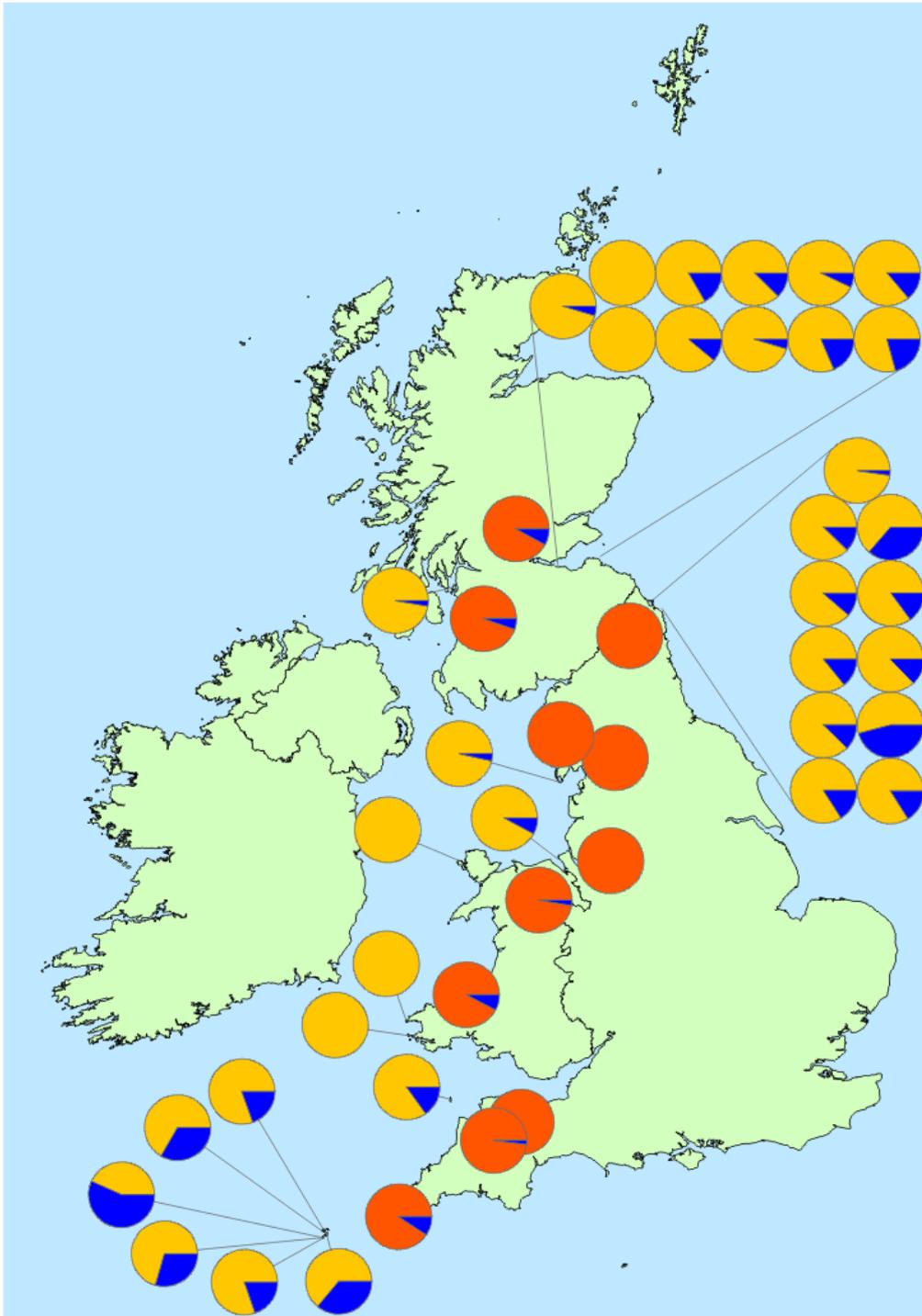
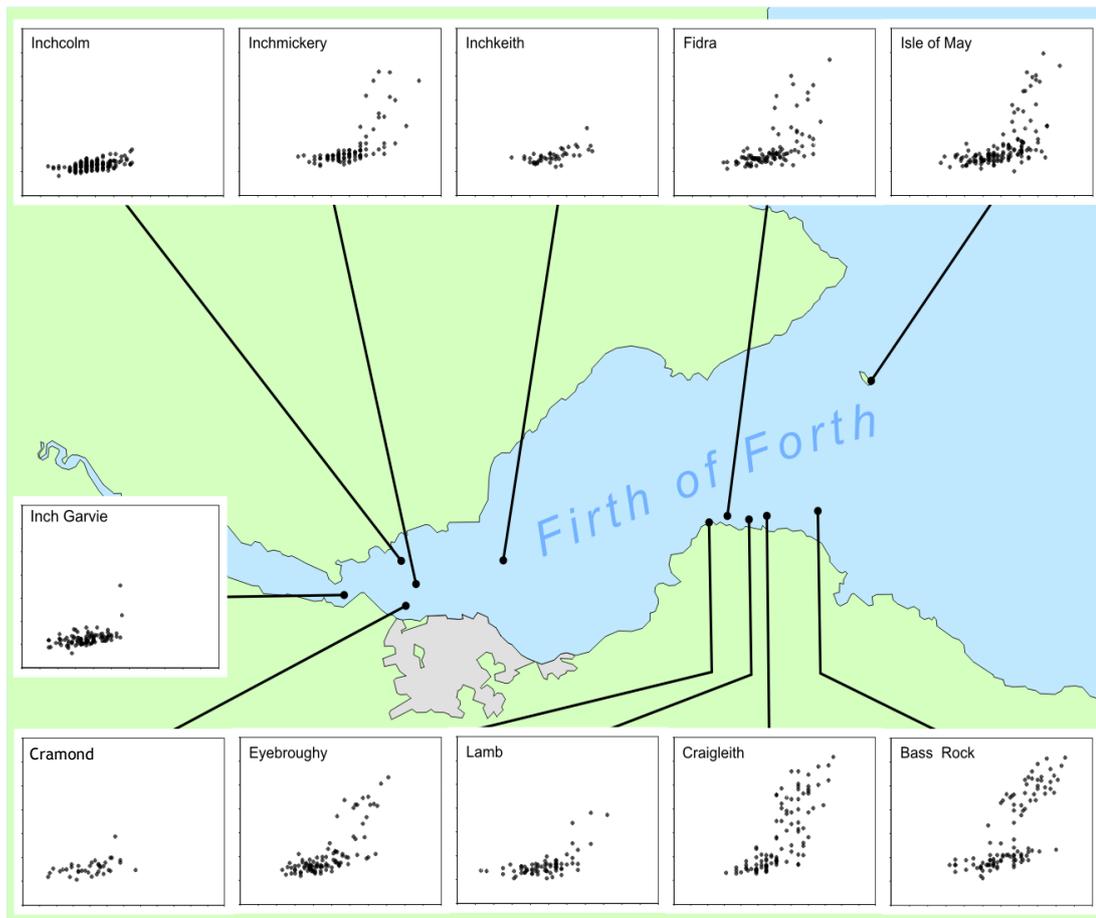


Figure 5. A map of Britain and Ireland with pie charts showing the proportion of male morphs in island and mainland populations of the European earwig *F. auricularia*. Macrolabic males represent a greater proportion of males in island populations than mainland populations (t-test on arcsine square-root transformed proportion macrolabic,  $t_{44} = 3.17$ ,  $P = 0.003$ ). Macrolabic males ●; brachylabic males in mainland populations ● and in island populations ●.



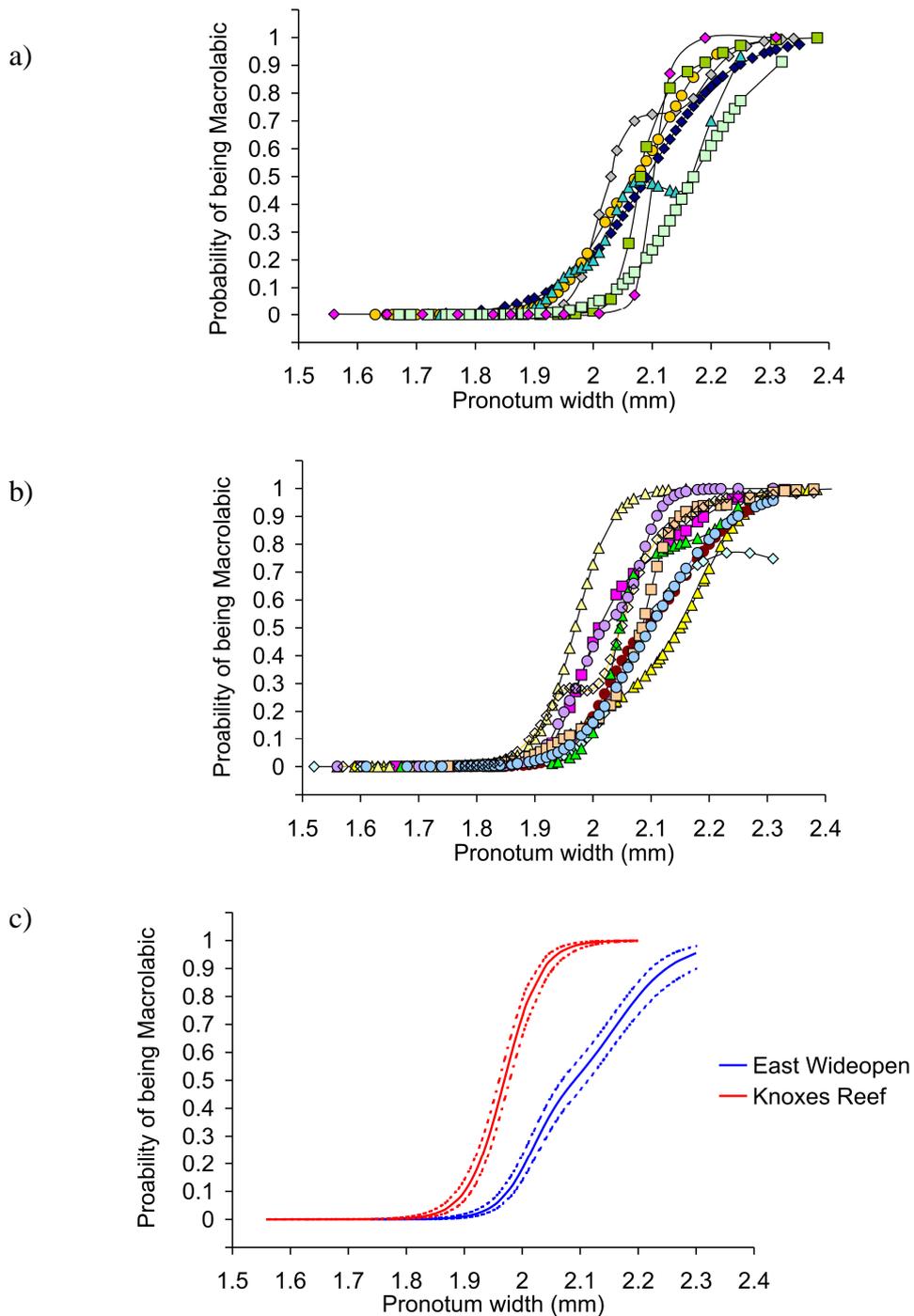
**Figure 6.** Variation in forceps length amongst island populations in the Firth of Forth. Inset scatter plots show the relationship between forceps length (y axis all graphs 2.0 - 9.0mm) and pronotum width (x axis, all graphs 1.4 - 2.5mm).

To determine whether populations varied significantly in the position of the body size threshold, males were categorised as macrolabic or brachylabic (see methods), then a logistic regression performed with morph as the dependent variable, pronotum width as a covariate and island as a factor (including all island populations with at least one macrolabic male). The whole model was significant ( $\chi^2_{21} = 1456$ ,  $P < 0.001$ ), as was the effect of pronotum width, indicating, as expected, that larger individuals are more likely to be macrolabic ( $\chi^2_1 = 1134$ ,  $P < 0.001$ ): the population term was also significant ( $\chi^2_{20} = 54$ ,  $P < 0.001$ ), demonstrating that populations differ in the absolute position of the morphological threshold (Figure 7a & b).

Further evidence that the position of the threshold is not fixed comes from the positive correlation between a population's mean pronotum width and the absolute position of

the morphological threshold in the population ( $r_s = 0.77$ ,  $n = 21$ ,  $P = 0.001$ ); the ESS switch-point apparently having evolved to some extent in parallel with increasing body size.

A logistic regression model with pronotum width standardised within each population to have a mean of zero and SD of one revealed that populations also differed in the position of the threshold relative to the population mean (population:  $\chi^2_{20} = 508$ ,  $P < 0.001$ ). Hence, not only does the absolute body size threshold show variation between populations, but so too does the relative position of the threshold: the parameter that determines male morph ratio. Population variation in the morphological threshold and body size has occurred on an extremely small scale. For example, the islands of East Wideopen (EWO) and Knoxes Reef (KR) in the inner group of Farne islands are separated by just 400m of sea and both are connected to an intermediate island (West Wideopen, WWO) by tidal causeways. Nonetheless, populations of earwigs on these adjacent islands differ significantly in body size (pronotum width mean  $\pm$  SE, EWO =  $2.05 \pm 0.01$ , KR =  $1.88 \pm 0.01$ ;  $t_{384} = 13.5$ ,  $P < 0.001$ ). Furthermore, these two populations differ in both the absolute position of the threshold (Final model,  $\chi^2_2 = 209$ ,  $P < 0.001$ ; pronotum width  $\chi^2_1 = 196$ ,  $P < 0.001$ ; Island,  $\chi^2_1 = 26.7$ ,  $P < 0.001$ ) and the relative position of the threshold (using standardised pronotum width, Island =  $\chi^2_1 = 19.1$ ,  $P < 0.001$ ) (Figure 7c).



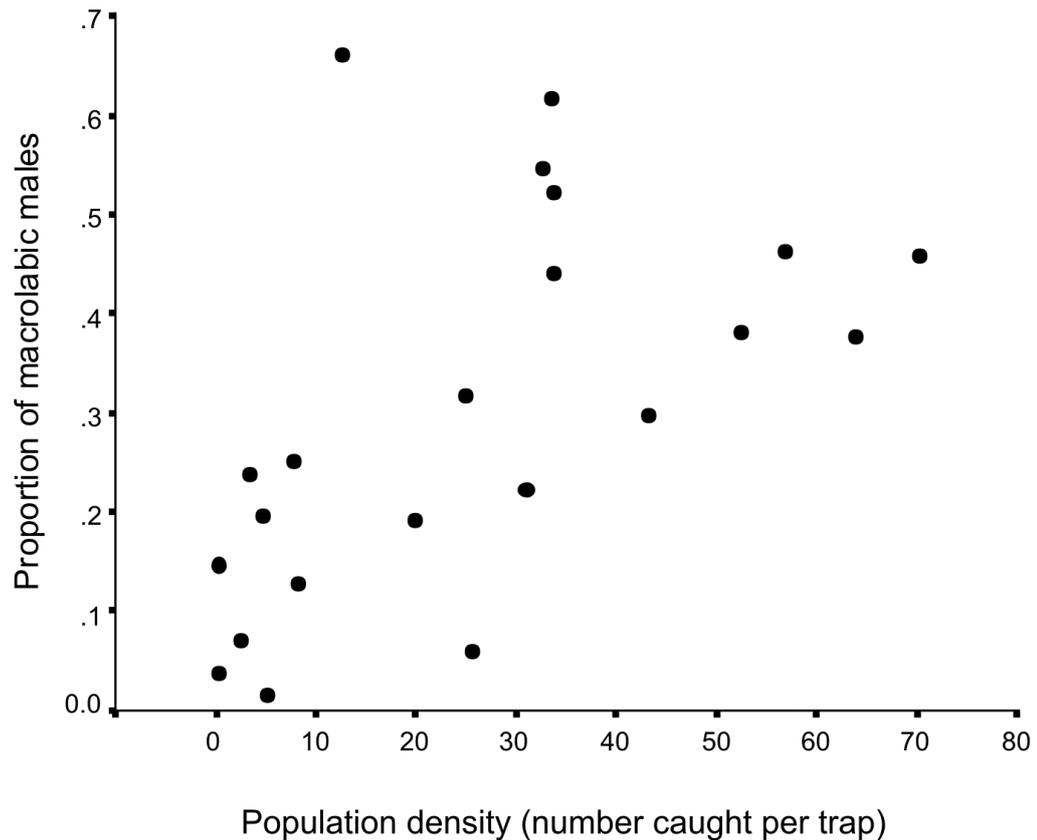
**Figure 7**

**Panel (a) & (b).** Cubic splines summarizing the threshold variation in the reaction norm of forceps length on pronotum width for populations of *F. auricularia* in (a) the Firth of Forth and (b) the Farne Islands.

**Panel (c).** Population variation in threshold on a very small geographic scale: Knoxes Reef and East Wideopen are islands in the Farnes group and are ~400m apart. Dashed lines are 95% CI for the splines.

Density data per island were left-skewed and on the verge of non-normality (Shapiro-Wilk = 0.91, d.f. = 21,  $P = 0.05$ ) and as log transformation did not normalise the data, nonparametric statistics were used. Across the North Sea island populations, there was a significant relationship between the overall density of the earwigs and the proportion of macrolabic males in the population ( $r_{s,22} = 0.66$ ,  $P = 0.001$ , Figure 8).

Operational sex ratio (OSR) is an important factor determining the intensity of sexual selection (Emlen & Oring 1977). Although not the confirmed OSR, the observed sex ratio did become more male-biased as population densities increased across islands. Thus, female earwig density was a decreasing function of male density across islands: the reduced major axis slope of log number of females on log number of males was 0.742 (CI = 0.63 - 0.88), significantly less than unity ( $F_{1,21} = 13.82$ ,  $P = 0.001$ ). To distinguish between the effects of density and increasing male bias in the sex ratio with density, the deviance in female density from a 1:1 ratio was calculated. Deviance in sex ratio was partialled from its correlation with population density ( $r_{22} = 0.83$ ,  $P < 0.001$ ) using a bivariate principal components analysis (PCA): PC 1 is the variance in the major axis, i.e. density, and PC 2 is the variance in the perpendicular, minor axis, i.e. deviations in sex ratio independent of density. The relationship between the proportion of macrolabic males with the population density (PC1) and the deviance from a 1:1 sex ratio (PC2), only density was significant ( $F_{2,19} = 4.64$ ,  $P = 0.023$ ; density (PC1),  $t = 2.85$ ,  $P = 0.010$ ; sex ratio (PC2),  $t = 1.06$ ,  $P = 0.302$ ).



**Figure 8.** The relationship between density, estimated as the average number of earwigs caught in standard traps, and the proportion of macrolablic males in a population, for populations of *F. auricularia* from islands in the Firth of Forth and Farnes.

The input of organic material on which earwigs feed may also be an important determinant of population density on small islands with sparse vegetation. Nevertheless, the biomass of ground-nesting birds per m<sup>2</sup> on each island did not correlate with earwig density ( $r_{s, 21} = 0.02$ ,  $P = 0.915$ ). The average number of survey years was  $10.6 \pm 1.6$  per species, per island, and the average number of annual surveys of any species was  $65.1 \pm 11.9$  per island. Lindisfarne and Cramond are connected to mainland by causeways and are large enough to support terrestrial predators. These islands have fewer ground-nesting birds than expected for their area; Cramond is not surveyed by ornithological groups for this reason. Bass Rock is a gannet colony, consequently the huge numbers (~39,000) of these heavy birds means the island has a much greater biomass of nesting birds than expected for its area. Excluding Cramond, Lindisfarne and Bass Rock from the analysis, a strong isometric relationship exists

between log island area and the log biomass of ground nesting birds ( $F_{1,17} = 48.52$ ,  $P < 0.001$ ,  $r^2 = 0.74$ , predicted log biomass =  $-1.214 + \log \text{ area} \times 1.01$ ) suggesting that the biomass estimates do generally correlate with stable habitat features. In order to calculate the biomass of birds relative to island area, we used the latter regression equation to obtain residuals from what is the principle across-island relationship.

There was a significant increase in earwig density with increasing distance of the island from the mainland ( $r_{s,22} = 0.616$ ,  $P = 0.002$ ), but no increase in density with decreasing island area ( $r_{s,22} = -0.05$ ,  $P = 0.836$ ). Despite the increase in density with distance to the mainland, there was no increase in the proportion of macrolabic males with distance to the mainland ( $r_{s,22} = 0.36$ ,  $P = 0.101$ ). The islands are small; 17 out of the 22 have an area above the mean high tide level equivalent to a circle with a diameter of less than 270m. This area will be an overestimate of the land habitable to earwigs because of spray, spring tides and storms. For the most part, the islands are rock with a cap of shallow soil or peat, which is restricted to rock fissures on Longstone End, Eyebroughy and Big Harcar. Knoxes reef is a shingle bank that has built up behind a reef of rock. The vegetation on all of the islands is dominated by grasses and annual herbs; only Cramond and Lindisfarne support trees.

## Discussion

The data I have presented in this chapter show that threshold evolution can occur on an extremely small geographic scale in a pattern consistent with local variation in demographic parameters theoretically important to variation in the intensity of sexual selection (Shuster & Wade 2003). Male-dimorphic threshold traits have been shown to respond readily to selection (Emlen 1996; Unrug *et al.* 2004), and although population variation consistent with change in density has been proposed for dung beetles the results were inconclusive (Moczek 2003). Unlike previous studies, these data demonstrate that threshold evolution can tailor populations to fine-scale variation in demography. This is expected in threshold traits that reflect status-dependent alternative reproductive tactics, because any differences in the slopes or elevations of the fitness functions of alternative tactics affect the position of the ESS switch-point (Chapter 1, Figure 1) thereby making it particularly sensitive to changes in parameters that alter relative fitness.

Theoretically, the increase in the frequency of macrolabic male earwigs in island populations occurs because the fitness function of macrolabic males is elevated relative to that of brachylabic males (Gross 1996; Hazel *et al.* 1990). Male earwigs guard females in chambers under stones and driftwood; following oviposition the female will not re-mate and expels the male. In *F. auricularia* macrolabic males have an advantage over brachylabic males when competing for females (Tomkins unpubl. data & (Radesäter & Halldórsdóttir 1993b). Nevertheless, adaptations for mate guarding, such as elongate forceps, are likely to be advantageous only where challenges occur regularly. The intensity of sexual selection is predicted to vary with certain demographic and ecological parameters such as density, the spatial distribution of resources and the operational sex ratio (Emlen & Oring 1977; Shuster & Wade 2003). In *F. auricularia* the probability of being challenged by an unpaired male, or conversely of successfully usurping a guarding male, depends on the probability of encountering another burrow. Across the very similar island habitats studied here, the predominant factor determining encounter rate is likely to be population density. Where earwigs occur at high densities, challenges over guarded females are likely to be frequent, elevating the fitness of males with adaptations for fighting for, or defending females. Extremely high population densities of earwigs are a conspicuous feature of the ecology of the islands of the Forth and Farnes.

The cause and significance of the increasingly male biased sex ratio with population density amongst the islands is intriguing. However, laboratory-reared clutches from two Farne island populations show no sex ratio bias at eclosion (Tomkins 1997), suggesting that the bias is environmental rather than genetic in origin.

Threshold evolution in earwigs on these North Sea islands has taken place in similar climatic conditions and similar habitats. The extraordinarily high population densities that appear to be driving threshold evolution may arise due to the escape from parasitic species or competitors present on the mainland. This hypothesis predicts increasingly high densities of earwigs as islands become more difficult for other species to colonise. This may well explain the increased population density observed on the more remote islands. The lack of a correlation between remoteness and morph ratio, through density, may be an indication that the relationship between remoteness and density is merely an artefact of the geography of the islands. In particular, the Farne islands are aggregated into two groups at different distances from the mainland.

This chapter documents a micro-evolutionary transition within a single species, over less than 40km from populations in which the ESS is for a single tactic expressed by all individuals, through to a classic conditional strategy in which alternative tactics are played in a status-dependent manner. These populations provide us with snap-shots in the evolution of a male dimorphism and support the game theoretic premise that evolutionarily stable strategies are population specific (Gross 1996; Maynard Smith 1982). The work presented in this chapter reveals a framework for the study of dimorphic variation in *F. auricularia*. This framework can be used to examine the extent to which gene flow between populations influences threshold variation (see Chapter 4).

## **Chapter 3: Development of multi-purpose molecular genetic markers for the European earwig**

The following chapter detailing the microsatellite loci developed as part of this thesis was submitted for review as a primer note to *Molecular Ecology Notes*. Preparation of the microsatellite enriched genomic DNA library and the subsequent cloning and sequencing of candidate fragments were performed by Bioprofiles Ltd. The design of primers, optimisation, wide-scale DNA extraction and genotyping were performed by G. Brown. This primer note manuscript was prepared by G. Brown and was edited in light of comments from the other authors (J. L. Tomkins and M. L. Hale).

## Introduction

The European earwig (*Forficula auricularia* L., Forficulidae, Dermaptera) exhibits a male dimorphism associated with alternative reproductive tactics (Chapter 1). The dimorphism is thought to represent an example of a conditional evolutionarily stable strategy; the alternative male phenotypes being separated by a body size threshold. The position of the threshold and the resulting male morph ratio constitute a heritable parameter of the population (Tomkins 1999). Populations differing in the ratio of the male morphs represent discrete arenas of sperm competition; as the proportion of sneak-mating males increases, so the risk of sperm competition increases for mate-guarding males (Parker 1990b).

Variation in the ratio of the two male morphs of *F. auricularia* among UK island populations correlates with population density (Tomkins & Brown 2004). In contrast, mainland UK populations generally only support earwig populations at low density and the major male (macrolabic) morph is rarely seen. This natural gradient in morph ratio provides the arena for natural experiments comparing these populations to study the impact of sneak male frequency on adaptations to sperm competition.

Microsatellites with variability both within and between populations can be used to establish a phylogeographic framework to correct for phylogenetic inertia when comparing populations. Furthermore, these markers can be applied to studies of reproductive success of individuals through parentage assignment. There are currently only four microsatellites published for this species, two of which are very closely linked (Guillet & Deunff 2000). *F. auricularia* has a global range and is considered an invasive economic pest in Australia and North America. These new microsatellite markers, being informative over shorter geographic distances, have the potential to compliment existing mtDNA markers that have been used to investigate these invasions (Guillet *et al.* 2000b).

## Methods

Genomic DNA was extracted from 45 male *Forficula auricularia* using a standard salt extraction method (Sunnucks & Hales 1996). One individual specimen collected from Brownsman (Farne Islands, Northumberland, UK) was used to construct a DNA library enriched for dinucleotide microsatellites (GA and CA repeats) using the double-

enrichment method described in Hale *et al.* (2002). Approximately 17ng of the enriched DNA was ligated into 100 ng BAP (dephosphorylated) *Bam*HI-digested 'ready-to-go' pUC18 vector (Amersham), following the manufacturer's instructions. Two microlitres of the enriched library was then transformed into 50µl JM109 competent cells (Promega) and plated onto Luria-Bertani agar plates containing 50µg/mL ampicillin. Plates were incubated at 37°C overnight, and then plasmids from 40 individual colonies were isolated using a QIAprep® Spin Miniprep Kit (Qiagen). Plasmid inserts were sequenced using BigDye Terminator Cycle Sequencing chemistry (Applied Biosystems) and sequences were detected on an ABI Prism® 310 automated sequencer. From the 40 clones that were sequenced, 14 unique microsatellite sequences were obtained (GenBank accession numbers DQ821414- DQ821427) and from these 7 sets of primers were successfully designed and optimised. Primers were designed using PRIMER3 (<http://frodo.wi.mit.edu/>) (Rozen & Skaletsky 2000) and appraised by simulating PCR amplification and primer dimerisation using AMPLIFY v1.2 (Engels 1993).

The loci were amplified in 10µL reactions [1.0 µL 10× NH<sub>4</sub> reaction buffer (Bioline), 1.5 mM MgCl<sub>2</sub>, 0.32 mM of the reaction of each dNTP, 0.3 µM of each primer, 0.25 U BIOTAQ DNA polymerase (Bioline) and 5-500 ng of template DNA] under the following conditions: An initial denaturation at 92°C for 3 min followed by 30 cycles of 92°C for 45 s, T<sub>a</sub> for 45 s (Table 3), 72°C for 1 min and a final elongation at 72°C for 5 min. The forward primer of each locus was fluorescently labelled using the WellRED dye system and genotypes were scored using a Beckman Coulter CEQ™ 8000 capillary sequencer.

Amplification and polymorphism was appraised using the DNA extracts from the remaining 44 individuals. The specimens were collected from 3 UK populations; Falmouth (mainland, 50°09.667'N, 05°03.367'W), Cramond (mainland, 55°58.773'N, 03°18.076'W) and Inch Garvie (island, 56°00.059'N, 03°23.133'W). Genetic diversity found in these 44 individuals was calculated using GENETIX (Belkhir 2000). Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were tested using GENEPOP (<http://wbiodem.curtin.edu.au/genepop/>) (Raymond & Rousset 1995).

## Results

The genetic diversity found in the three UK populations sampled for these seven new microsatellite loci is summarised in Table 3. None of the loci in any of the three populations were found to deviate from Hardy-Weinberg equilibrium after Bonferroni correction and no linkage was detected between any pairs of loci. All seven loci amplified consistently in individuals from northern and southern parts of the UK and all showed intra-population polymorphism in at least two of the populations sampled.

## Discussion

The European earwig presents an exciting model with which to study the evolutionary implications of sperm competition in a species with alternative reproductive tactics. The seven newly developed loci presented here add to the small number (3) of existing independent microsatellite loci previously available for this species (Guillet & Deunff 2000). The adherence to Hardy-Weinberg equilibrium frequencies of the alleles identified at these new loci shows no indication of null alleles, which would reduce the efficacy of these markers.

These markers are likely to be valuable for use in genetic studies between populations of *F. auricularia* within the UK given that they were successfully amplified with the reported method in specimens from disparate parts of this geographical region. The variability found in the populations sampled suggests that these loci can be used to relate populations in studies of phylogeography over tens or hundreds of kilometres.

The presence of variability within populations extends their application to studies on a much smaller geographical scale, such as for assigning parentage to individuals collected from natural populations or reared in laboratory experiments. Paternity assignment using molecular markers can provide a powerful approach to assessing reproductive success than other methods. Single-male ablation, where one male of each experimental pair is irradiated to render that male infertile, allows the paternity of the two males of the pair to be assessed from the proportion of eggs in the clutch that do not develop. Using molecular markers, paternity of more than two males can be assessed in one clutch of eggs. All offspring can, theoretically, be assigned successfully using molecular markers, whereas undeveloped eggs may be simply unfertilised or unviable

and indistinguishable from eggs fertilized by the sperm of the ablated male. Furthermore, the molecular approach is less invasive, where a technique such as single-male ablation may artificially influence the reproductive success of the altered male.

*Forficula auricularia* has a cosmopolitan distribution through anthropogenic translocation and is an economic pest outside its native range (Guillet et al. 2000b). These markers may also prove useful for study of these *F. auricularia* populations in this invaded range by lending finer resolution relative to Guillet and colleagues (2000b) previous work, which employed more slowly evolving mitochondrial markers.

**Table 3 Primer sequences and genetic diversity of 7 microsatellite loci developed from UK populations of *F. auricularia*.**

Locus name / GenBank Accession no.	Repeat in clone	Primer sequence (5'-3')	T <sub>A</sub>	Product size (bp)	No. alleles	Falmouth			Cramond			Inch Garvie		
						n	H <sub>E</sub>	H <sub>O</sub>	n	H <sub>E</sub>	H <sub>O</sub>	n	H <sub>E</sub>	H <sub>O</sub>
EW1 / DQ821414	[GA] <sub>11</sub>	F: CCCTTTACATTGGGAAGC R: TGCACACTGTTGGATTCTGT	60.5 °C	204-220	5	17	0.166	0.059	20	0.146	0.150	7	0.143	0.143
EW2 / DQ821415	[AC] <sub>11</sub>	F: CGTTTGGCCTCATTAAACAAA R: CGTTAATTTGAGCAAAGTCTCCA	52 °C	146-156	5	17	Mono	0.000	20	0.190	0.200	7	0.440	0.000
EW12 / DQ821421	[GA] <sub>12</sub>	F: AAGAGCTTGAGGTGGAATGC R: TATAGCGATTTGGCCTGGAG	54 °C	196-201	4	17	0.642	0.824	20	0.786	0.800	7	0.868	0.857
EW15 / DQ821422	[TC] <sub>14</sub>	F: CGTTGCAGACCTGGGTTATT R: AAACAAGATGTTGAGAGTAAGTCTTGA	54 °C	274-310	12	17	0.544	0.353	20	0.512	0.400	7	0.582	0.143
EW25 / DQ821424	[TC] <sub>13</sub>	F: AAACCAAGCACACCTGTAAATG R: ATCCCTTGCCTTGTCTCTT	54 °C	197-207	5	17	0.667	0.765	20	0.641	0.700	7	0.802	0.571
EW35 / DQ821426	[GT] <sub>10</sub>	F: TTCGAGAACCAGTGTGGTG R: GCCGGAGTTTCGAGAATG	54 °C	150-154	2	17	0.059	0.059	20	0.050	0.050	7	Mono	0.000
EW40 / DQ821427	[GA] <sub>11</sub>	F: TTTTCACTCTCATTTGTTGAGTTG R: CTGGCTTGAATGGCTGATG	54 °C	101-115	5	17	0.266	0.177	20	0.389	0.450	7	0.484	0.286

Genetic diversity estimates are given for two UK mainland and one dimorphic island site. Allele size ranges and counts include all alleles found in these populations. n = number of individuals genotyped, H<sub>E</sub> = heterozygosity expected under HWE with adjustment for sample size, H<sub>O</sub> = observed heterozygosity, Mono = only one allele found for that locus in that population.

## **Chapter 4: Phylogeography of the European earwig in the UK**

## Introduction

In the preceding chapters of this thesis I have shown how *F. auricularia* in the UK exhibits extreme plasticity in male forceps morphology. Population variation in the heritable threshold that defines this male dimorphism causes populations to differ in the ratio of the male morphs. Asymmetries in sperm competition risk will arise from this variation in morph ratio (Parker 1990b) and this makes *F. auricularia* in ideal model to investigate adaptations to sperm competition and the evolution of male dimorphisms. However although the observed population variation in morph ratio may be the result of adaptation to the local environment and the evolution of male dimorphic populations on islands it may also be that dimorphic populations have not evolved independently on islands but instead represent relicts with a single evolutionary origin.

This chapter reports a phylogeographic study of UK populations of *F. auricularia*. The objective here was to establish whether populations on islands can be viewed as independently evolving male dimorphisms and differences in morph ratio, and therefore whether these populations can be treated as independent replicates when it comes to investigating adaptations to sperm competition risk. Microsatellite markers reported in Chapter 3 are used to draw comparisons between populations on the Farne Islands, the Forth Islands, the Scilly Isles, the Pembrokeshire islands and Lundy Island relative to the UK mainland. The phylogenetic reconstruction presented is compared to corresponding geographic distances.

### The *F. auricularia* species complex

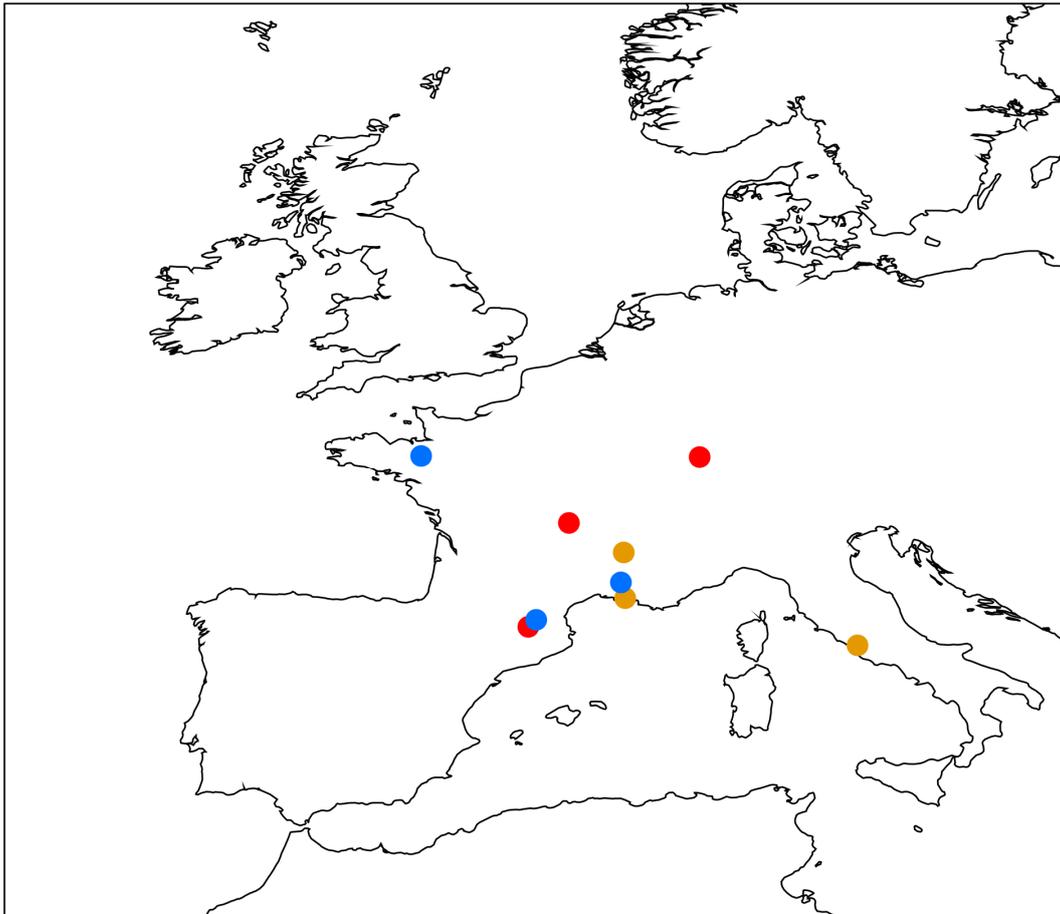
The genus *Forficula* comprises around 100 species worldwide (Sakai 1982) of which two are recorded as present in the British Isles, the European earwig (*F. auricularia*) and Lesne's earwig (*F. lesnei*) (Marshall 1988). There have been few genetic studies including this genus, however one important aspect of their evolutionary history, the existence of 'cryptic' sister species (Wirth 1998), has been revealed.

The morphological taxon first classified by Linnaeus (1758) as *F. auricularia* has recently been shown to constitute a pair of genetically, ecologically and ontogenically distinct sister species. Morphologically similar populations from sites in the native range (western Europe) and non-native range (North America) were found to inhabit a range of coastal to alpine habitats (Wirth *et al.* 1998). Two sister taxa were inferred

from a 627bp region of the mitochondrial genome encompassing the cytochrome oxidase genes COI and COII. Although these taxa overlap geographically, attempts to breed individuals from crosses of these mitochondrial variants produced markedly fewer offspring than intra-population crosses under the laboratory conditions.

The first taxon, *F. auricularia* subspecies A was found in highland and climatically cooler continental regions, whilst subspecies B was found only in the lower-lying and milder oceanic regions (Figure 9). Two strategies were identified from discrete variation in the number of reproductive events in a given year (uni- and bivoltine populations). The highland-continental populations (subspecies A) were univoltine except for those closer to the Mediterranean coast, which were bivoltine. All lowland-oceanic populations (subspecies B) were bivoltine.

The populations in Wirth *et al.*'s study that were closest to the English Channel and North Sea coast were characterised as subspecies B and bivoltine (Figure 9). However, their sampling in northern mainland Europe was limited and from the distribution of those locations it seems plausible that both subspecies may exist in northern parts of Europe. A subsequent study of a Pyrenean contact zone between the subspecies reported a cline (Guillet *et al.* 2000a), which suggests that, in some places, they naturally occur at short distances from each other and remain genetically distinct. The cline investigated by Guillet *et al.* (2000a) correlated with altitude in an alpine habitat, so the subspecies may be separated by an ecological barrier in addition to reproductive incompatibility suggested by Wirth *et al.* (1998).



**Figure 9.** Locations of European *F. auricularia* populations sampled by Wirth *et al.* (1998). Sample locations are marked by coloured circles according to clade and voltinism; ● = univoltine subspecies A, ● = bivoltine subspecies A and ● = bivoltine subspecies B.

All populations of subspecies A identified by Wirth *et al.* were univoltine except for those furthest away from the British Isles. No bivoltine reproduction has been observed in either mainland or island *F. auricularia* populations from the UK. Therefore, it seems likely that the UK was colonised by the univoltine subspecies A.

Interestingly, some adult male *F. auricularia* have been found alive in early spring from north of the Firth of Forth (J. Tomkins pers. comm.); adult males typically die in late November or December after mating and mate-guarding. However, male mate-guarding in spring will not increase male reproductive success unless these individuals survive until the following autumn, the likelihood of which is unknown. Most observations suggest that metabolic resources are invested such that male survival through the winter south of the Forth is almost nil. This anecdotal variation in male

survival suggests that the greater seasonal change in day length may have altered the phenology of *F. auricularia* in the British Isles. If subspecies B is capable of reducing its voltinism to a single reproductive event a year, unseen in Wirth *et al.*'s study, perhaps in response to the seasonality and climate of the UK then it may be present in the UK instead of or in addition to subspecies A. Given the sparsity of Wirth's data in northern Europe and the lack of definitive voltinism data from UK populations, the most practical resolution to this matter is by gathering genetic data from the UK.

### Hypotheses for the geographic variation of the male dimorphism in *F. auricularia*

There are two alternative hypotheses for the presence of male dimorphic populations on islands and monomorphic populations on the mainland. These are the *relict* hypothesis and the *local adaptation* hypothesis.

The relict hypothesis predicts that the distinct morphology of island and mainland populations results from the existence of two parapatric races or sister species. Moreover this hypothesis requires that the UK was colonised by two waves of invasion by *F. auricularia*; the first colonisation by a male dimorphic clade and the second by a monomorphic clade that displaced the first except in island refugia where the male dimorphic populations persist as relicts of the more ancient invasion.

Most of Britain was ice-covered during the last glaciation (Cox & Moore 1994). The recolonisation of northern Britain, including the regions of the Forth and Farne Islands, may even have been delayed until the end of the Younger Dryas, an interglacial 'cold snap' that ended abruptly around 10 000 years ago (Cox & Moore 1994). The current male dimorphic populations, whether a distinct clade from the monomorphic populations or not, are unlikely to have colonised as far north as the Firth of Forth earlier than this date.

If the discrete pattern of the dimorphism does result from populations being of heterogeneous genetic origins, the reason why both dimorphic and monomorphic clades are not present at all sites in the UK needs to be accounted for. The relict hypothesis offers two potential explanations for this.

Firstly, monomorphic and dimorphic clades that invaded the UK may each have been specialised to different ecological niches. Under this scenario, monomorphic invaders

might have been excluded from the islands by being competitively inferior to a male-dimorphic clade or clades, in the island environment. On the mainland the dimorphic clades could have likewise been displaced by the monomorphic clades through competition. This hypothesis suggests that dimorphic clades would have colonised the UK mainland prior to the monomorphic clade(s), for it to have established itself on the geographically disparate islands where it persists today. This may have been due to the glacial refuge of the dimorphic clade being closer to the UK, giving it a geographical advantage over the monomorphic clade at the time of invasion. Alternatively, successional changes in habitat of the deglaciating territory might have given the dimorphic clade a competitive advantage in colonising the post-glacial UK.

A recent study of perhaps an analogous situation, the invasion of the UK by the American grey squirrel (*Sciurus carolinensis*), suggests that such competitive refugia can exist (Lurz *et al.* 1995). Here the native European red squirrel (*S. vulgaris*) has been displaced in most of England, Wales and southern Scotland by *S. carolinensis*. Lurz *et al.* (1995) have shown that *S. carolinensis* is more competitive than *S. vulgaris* in habitats where oak acorns are available due to resistance to the plants' chemical defences. *S. vulgaris* persists in areas of coniferous woodland in Northumberland and most of northern Scotland where it is thought that it has an advantage over *S. carolinensis*. If such a situation of competitive refugia exists in *F. auricularia*, the competitive advantage could either be conveyed by the dimorphism itself or be incidentally correlated to it.

Sea plantain (*Plantago maritima*) is a further example of a species in which range restriction has occurred in a once widespread colonist of the UK. Sea plantain is currently restricted to relict alpine and coastal populations as a result of its early postglacial colonisation being followed by forestation (Cox & Moore 1994). Sea plantain was competitively displaced by the forest cover and, in the last 10 000 years, has subsequently diverged into distinct races in the different habitat types.

The hypothesis of competitive exclusion relies on there having been two or more invading *F. auricularia* clades, distinct with regard to the male dimorphism and each excluding the other through ecological specialisation. If this hypothesis is true, it seems likely that the specialisation would relate to population density given the observed association with the dimorphism (Chapter 2).

The alternative to the ecological specialisation argument is that the distribution of dimorphic earwig populations on UK islands reflects the existence of relicts arising from a more ancient invasion of dimorphic clades; the mainland being stocked by a more recent invasion by monomorphic clades. The temporal gap between the invasions of the different clades would have to be sufficiently long to have allowed physical barriers to migration (e.g. sea level rise) to arise in the interim, effectively marooning the dimorphic populations on the disjoining islands.

This phenomenon is shown in two pairs of closely related mammals, the common and pygmy shrews (*Sorex minutus* and *S. araneus*) and the stoat (*Mustela erminea*) and the weasel (*Mustela nivalis*) (Cox & Moore 1994). All four mammals are present in the UK but only *S. minutus* and *M. erminea* are also present in Ireland, to the exclusion of their respective congeners. The stoat is considered to be more of a generalist predator compared to the weasel which, in the UK is thought to prey largely on field and bank voles (*Microtus agrestis* & *Clethrionomys glareolus*) (King 1980). The generalist habits of the stoat may have permitted it to colonise as far as Ireland prior to the inundation of the Irish Sea and in advance of the weasel which is likely to have been dependent on established populations of voles (Jaarola & Searle 2002).

A second precedent supporting the plausibility of this temporal isolation hypothesis is the 'Lusitanian flora'. This is a term given to highlight a diverse group of plants that colonised the Atlantic seaboard of Europe after the last glaciation and that are typical of the Iberian Peninsula (Cox & Moore 1994). The Iberian Peninsula is thought to have been a separate refuge to more eastern areas of Europe during the peak of the last glaciation. The Lusitanian flora are thought to have colonised northern parts of Europe via milder coastal regions that were habitable before routes from central European regions became available. The incidence of this phenomenon is difficult to gauge because later eastern European colonists are not always readily distinguishable. Recent application of molecular tools has revealed a clade of Oak (*Quercus spp.*) of Iberian origin in the British Isles that is not present in central Europe (Ferris *et al.* 1998).

Given the affinities to discrete habitat types reported for *F. auricularia* (Wirth *et al.* 1998), a similar coastal expansion route may have resulted in the UK being colonised at different times from different regions of mainland Europe.

Through the field collections of earwigs made in the Scilly Isles in the course of this work, I found *F. lesnei* to be present on Samson Island but found no evidence for the presence of *F. auricularia* at all. This observation is corroborated by previous collections made from that island (J. Tomkins, pers. comm.). Unlike *F. auricularia*, *F. lesnei* is restricted to southern regions of the UK, but in this limited range the two species seem to coexist. This isolated island population is a fortuitously contiguous example of how distinct clades can become segregated on adjacent islands.

The second hypothesis for the divergence between mainland and island populations of *F. auricularia* is one of *local adaptation*. This hypothesis proposes that island populations may have evolved from the current local mainland populations, and therefore that the male dimorphism reflects a local adaptation. If this hypothesis is supported the male dimorphic island populations represent the multiple evolution of similar reaction norms in response to local optima.

### Testing the alternative hypotheses for island dimorphisms in *F. auricularia*

The alternative hypotheses that explain the population variation in the dimorphism can be tested by studying the phylogeography of the overall UK population. This approach is encapsulated by the following questions.

Was there a single (or several homogeneous) colonisation event with the current geographic pattern in the male dimorphism being a product of local adaptation? The prediction arising from this hypothesis is that populations should have the closest genetic affinity to the populations nearest to them, irrespective of male morph ratio. The genetic divergence between islands and other populations is likely to be greater than between mainland populations as the physical barriers to gene flow are greater. The islands populations, though more divergent, should still fall within the monophyletic radiation reflected in the inter-population genetic distances.

Alternatively if there was more than one colonisation event during which different clades with distinct morph ratios ended up being geographically isolated as relicts, the dimorphic island groups should be more closely related to other dimorphic island groups than to closer mainland populations.

In addition to evidence that populations from island groups are either distinct or derived from adjacent mainland populations, the fine-scale pattern in morph ratio can be examined within island groups for the signal of gene flow between the islands. Quite simply, does genetic distance between island populations within a group like the Farnes or the Firth of Forth correlate with the observed shifts in threshold variation reported in chapter 2?

There are various ways of determining the genetic similarity or affinity between populations. Firstly, distinct alleles or haplotypes that are common to particular populations and not to others (synapomorphies) would indicate a common origin. In addition to distinct differences between populations, more subtle differences in the genetic properties of populations can be used to reconstruct their phylogenetic relationships. Conventionally, this is done by calculating the genetic distance between populations; an analysis of the variance in gene frequencies seen within populations compared to variance between populations (Avice 1994). Distance-based phylogeographic reconstructions are possible using data from sequence based markers or simple Mendelian markers, such as microsatellites. The robustness of such analyses can be improved by establishing concordance across a range of distance measures that have differing inherent assumptions.

Hyper-variable population genetic markers such as microsatellites offer the advantage of high levels of inter- and intra-population variability that can be difficult to find through sequence analysis. Distance measures have been developed that apply marker-specific models of evolution to infer genetic distance from observed genetic variation. A simulation study has advocated the use of two distance measures for phylogenetic reconstructions based on microsatellite data (Takezaki & Nei 1996); Nei *et al.*'s  $D_A$  (1983) and Cavalli-Sforza & Edwards' Chord distance,  $D_C$  (1967).

## Methods

### Collection of specimens

*F. auricularia* adults and nymphs were collected by hand from under surface debris and from within the dry, hollow stems of dead hogweed (*Heracleum sphondylium* L.). Collections were made in August and October 2002 and a small number of supplementary collections were made in 2005 (see Figure 10 and appendix A). These collections were made from 58 sites across the UK of which 35 were islands, 21 were on the UK mainland and 2 were outgroups from continental Europe (Figure 10).

Collection sites were either islands that are isolated by the tide for most days of the year and most time of the day or mainland sites separated by at least 5 km (mean = 338 km, S.D. = 216 km and minimum = 7 km between mainland UK sites). Sites are hereafter considered as distinct populations for the purpose of this analysis.

The specimens were confined in perforated plastic containers at 16°C with a maintained air moisture level and an *ad libitum* protein-rich food source. Following eclosion into the final instar, adult earwigs were removed from the rearing containers and stored at -20°C awaiting DNA extraction.

### DNA extraction and microsatellite genotyping

DNA was extracted from 1390 individuals and amplification attempted for the 7 microsatellite loci reported in chapter 3 and the FA2 locus, published by Guillet & Deunff (2000). PCR conditions were as specified in chapter 3, with FA2 being re-optimised with an annealing temperature of 52°C (higher than the published 47°C).

Genotypes at one or more of these 8 loci were obtained from 735 individuals across 58 populations (Appendix F). The low success rate of genotyping was due to variation in the quality of DNA yielded by the frozen specimens (see appendix D). Later work showed that extraction from live specimens yielded a rate close to 100% successful amplifications per extraction and scoring of all 8 loci (see appendix D).

### Analysis

Data were tested for Hardy-Weinberg equilibrium and linkage disequilibrium using GENEPOP (Raymond & Rousset 1995) and GENETIX (Belkhir 2000).

*Genetic distance*

Data files were prepared following the suggestions in the documentation provided for the CONVERT utility (Glaubitz 2004). Infinite Alleles Model (IAM) distance measures were calculated using all 7 loci with the alleles defined nominally from the fragment lengths. Stepwise Mutation Model (SMM) distance measures were calculated with the alleles defined ordinally by the inferred number of repeats. Where loci exhibited non-standard alleles (or microvariants as they are referred to in Glaubitz's documentation) where allele size is out of phase with the frame predicted by the original sequence and motif length, these alleles were conservatively rounded down to the nearest complete number of repeats. Matrices for five distance measures were generated using MICROSAT v1.5d (Minch 1997);  $\delta\mu^2$  (Goldstein *et al.* 1995),  $R_{ST}$  (Slatkin 1995), the chord distance,  $D_c$  (Cavalli-Sforza & Edwards 1967), the proportion of shared alleles,  $D_{ps}$  (Bowcock *et al.* 1994) and  $F_{ST}$  (Reynolds *et al.* 1983). Neighbour joining trees based on these matrices were built using the program NEIGHBOR in Felsenstein's (2005) phylogeny inference package (PHYLIP) and where possible bootstrap values were calculated using POPULATIONS v 1.2.28 (Langella).

*Isolation by distance*

Mantel testing with 100 permutations was performed to test for isolation by distance using Genepop v3.4 (Raymond & Rousset 1995). Genetic isolation was estimated using both  $F_{ST}$  and the  $\rho_{st}$  ("Rho"<sub>st</sub>) estimator of  $R_{ST}$  according to Rousset (1996).

Geographical distance was calculated according to the haversine method as recommended by Sinnott (1984) and recently demonstrated by Ramachandran *et al.* (2005).

It was not possible to test for isolation by distance in the working data set as a whole as there were insufficient data to calculate  $F_{ST}$  for some pair-wise comparisons of populations. Isolation by distance was calculated using data from the 24 populations where 10 or more individuals were genotyped for at least one locus.

*Analysis of molecular variance*

Analysis of molecular variance (AMOVA) was performed on two versions of the dataset using ARLEQUIN v3.01 (Excoffier *et al.* 2005). A project file was built using data from 689 individuals in 56 populations, from all 7 accepted loci using

CONVERT v1.31 (Glaubitz 2004). Two of these loci, EW12 and EW25 were found to have several instances of non-standard alleles. Therefore, this project file was defined as containing 'standard' data and was analysed assuming the loci have evolved according to the infinite allele model (IAM).

A second project file was created without these two loci with non-standard alleles. The data from the five remaining loci was composed of 683 individuals in 56 populations and was analysed assuming the stepwise mutation model (SMM) of microsatellite evolution.

## Results

### *Microsatellite variability*

The eight microsatellite loci were found to be polymorphic across the 58 mainland and island sites sampled from around the UK with a mean allelic diversity of 13.25 alleles per locus. A summary of the variability of the markers is given in Table 4. Two of the 8 loci, EW12 and EW25, exhibit non-stepwise alleles, i.e. alleles whose lengths differ from the sequenced allele by an incomplete number of tandem repeats.

The sample collected from Longniddry Bents (LND, Firth of Forth) was found to deviate from Hardy-Weinberg equilibrium (Fisher's exact test  $\chi^2_{16} = \infty$ ,  $P < 0.0005$ ). Two collections were made from this site, in 2002 and in 2005. If these collections are not representative of a single population, pooling them creates a Wahlund effect. Analysing them separately controls this but the deviation from equilibrium remains in the 2005 sample, where more complete data were obtained due to these DNA extractions being made from tissue that had not been frozen (Fisher's exact test 2002,  $\chi^2_8 = 4.00$ ,  $P = 0.8572$ ; 2005,  $\chi^2_{16} = \infty$ ,  $P < 0.0005$ ). Therefore, this site was removed from the data set and has not been considered in any subsequent analyses.

Across the remaining sites, Locus EW2 was found to deviate significantly from HWE (Fisher's exact test  $\chi^2_{76} = 110.98$ ,  $P = 0.0055$ ) and was significantly linked with locus FA2 (Fisher's Test  $\chi^2_{440} = \infty$ ,  $P = 0.0004$ ). A one-tailed exact test indicated that EW2 had significantly fewer heterozygotes than expected at equilibrium ( $P = 0.0111$ , S.E. = 0.0014). With the removal of EW2 from the data set, there was no significant deviation from HWE at any of the seven remaining loci when only the UK mainland and European outgroups were considered. Following the removal of locus EW2, no evidence was found of linkage disequilibrium between any loci. Therefore, all subsequent analyses were performed without data from Longniddry Bents and locus EW2.

**Table 4. Summary of genetic variability in 8 microsatellite loci among the 735 scorable *F. auricularia* individuals collected from 58 mainland and island sites around the UK.**

Locus name	Original sequence motif	Originally sequenced allele (bp)	Product size (bp)	No. alleles	58 sites	
					n	H <sub>O</sub>
EW1	[GA] <sub>11</sub>	218	204-230	13	349	0.284
EW2	[AC] <sub>11</sub>	154	142-164	12	541	0.512
EW12	[GA] <sub>12</sub>	203	189-216	22	320	0.219
EW15	[TC] <sub>14</sub>	286	274-318	19	281	0.666
EW25	[TC] <sub>13</sub>	205	197-215	16	347	0.680
EW35	[GT] <sub>10</sub>	160	140-162	8	466	0.152
EW40	[GA] <sub>11</sub>	113	101-115	8	637	0.319
FA2	[CAA] <sub>9</sub>	147	132-153	8	453	0.243

**n = number of individuals scored for that locus.**

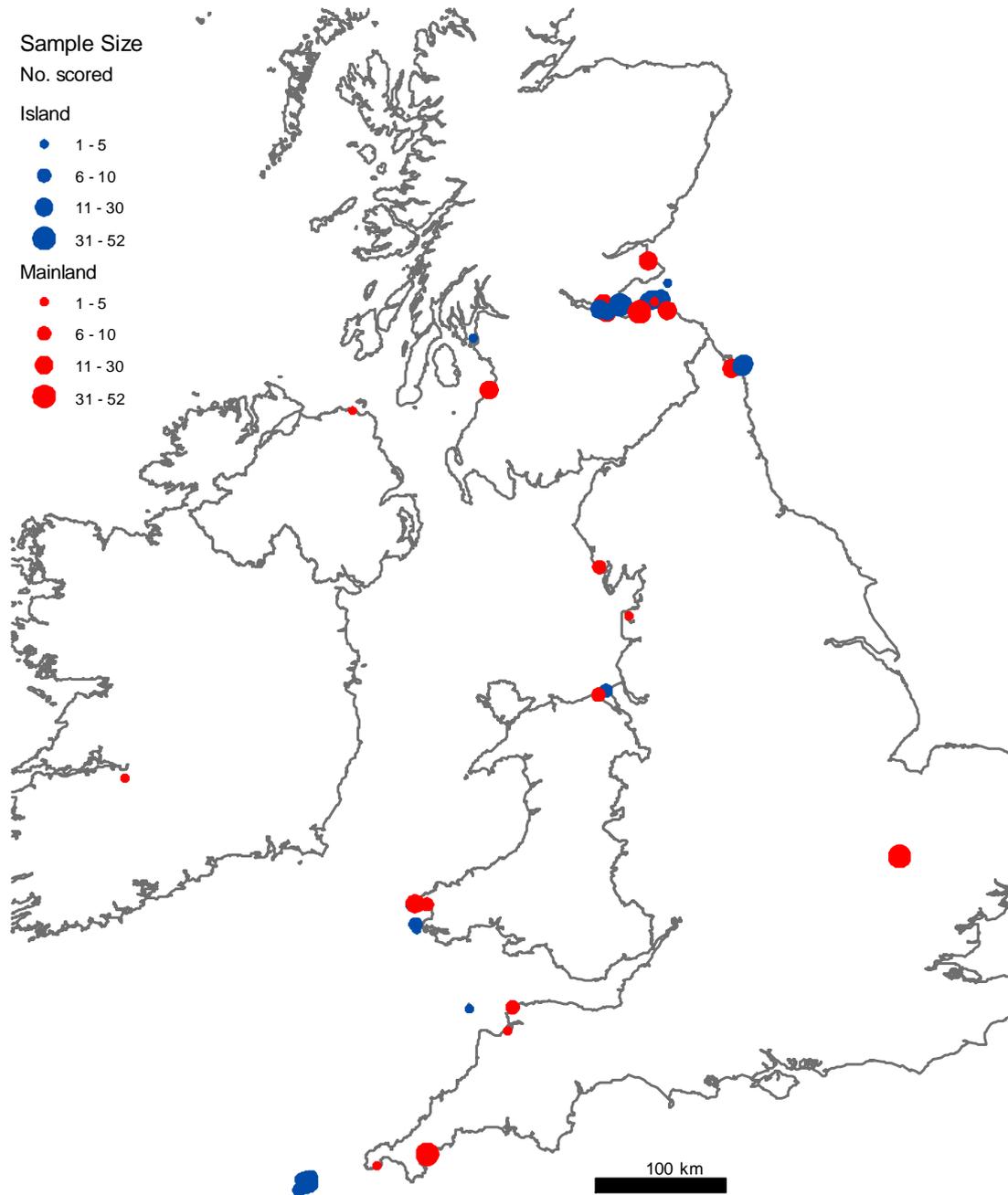
**H<sub>O</sub> = Observed heterozygosity**

### *Sample Size*

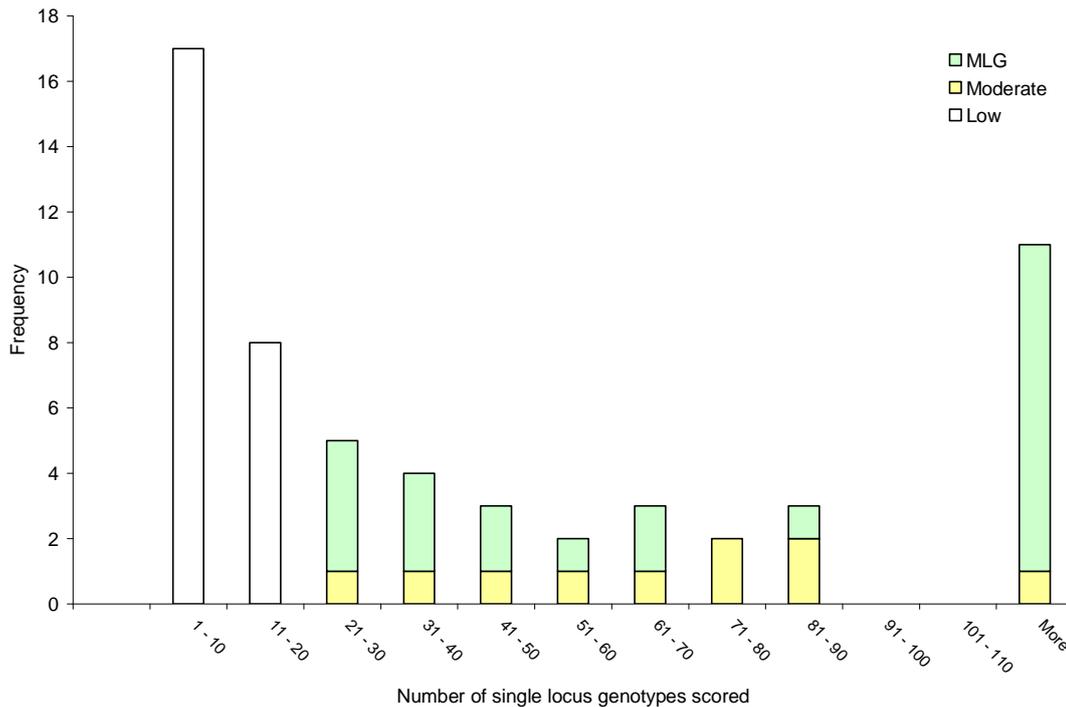
Many of the populations sampled yielded few genotypes; 34 of the 58 populations have fewer than 10 individuals scored at 1 or more locus. The remaining 24 populations account for 592 individuals (81% of the full dataset). Additionally, most individuals could not be genotyped for all loci; out of the 735 individuals that were scored at all, only 155 scored for all eight loci.

Two indices of sample size, and thus DNA quality, were used to appraise the data. Firstly, the number of individuals for which complete multi-locus genotypes (MLG) for the seven loci was counted. It is not possible to calculate bootstraps where there are gaps in the data and so the number of complete MLGs is a useful parameter as it identifies those populations that can be included in tree-building and have bootstraps generated to assess the robustness of the tree topology. The second index used was the product of the number of single-locus genotypes and the number of loci scored in a population. This index reflects the overall amount of data, irrespective of the distribution amongst the loci, weighted by the number of loci scored. Where two

individuals in a population are scored for all 7 loci this product will be 98. In practice, all populations where at least one individual was scored for all 7 loci also achieved values of this second index of well over 100 and furthermore had more than 20 single locus genotypes scored. These two observations, although arbitrary, concur in defining a further division in the data; those populations that scored below these threshold values were considered 'low' in sample size and those above but without any individuals scored for all seven loci were considered 'moderate' in sample size. Compared to the acceptance of only populations with complete multi-locus genotypes, which drastically reduced the overall data set, this arbitrary distinction provided a simple means for removing only populations with very small sample sizes from the data set.



**Figure 10.** The distribution of UK sites sampled for *F. auricularia* (Appendix F); islands are marked in blue, mainland sites in red and symbol diameter is proportional with the number of individuals scored at one or more loci (mean  $12.7 \pm 11.9$  individuals), to reflect sample size.



**Figure 11.** Genotyping success shown by the frequency distribution of population sample size, in terms of the number of single locus genotypes obtained (see Appendix F). Coloured portions of bars indicate populations where at least one individual was scored for all 7 loci (complete multi-locus genotypes; MLG / green) and where the sample size was considered moderate (yellow) using the arbitrary minimum threshold value of 100 for the product of the number of single-locus genotypes multiplied by the number of loci scored. This colouring corresponds to the population colour coding in Figure 12.

### *Private alleles*

Twenty-three (24%) of the 94 alleles identified from the seven loci were found only in single populations. Most of these private alleles were found in the two northern regions around the Firth of Forth and the Farne Islands, where islands populations exhibit particularly high morph ratios (Table 5).

Analysis of variance (ANOVA) on the number of private alleles, with site type (island or mainland) as a random factor, showed that, as expected, more private alleles were found in populations with larger sample sizes ( $F_{1,53} = 12.72$   $P = 0.001$ ). However, by including sample size in the model, and thereby controlling for this effect, there were significantly more private alleles found in island populations compared to mainland populations ( $F_{1,53} = 4.47$   $P = 0.039$ ). A similar analysis was performed using the relative allelic richness (number of alleles per population per locus scored) to see if this

phenomenon extended to the diversity of alleles as a whole, but it did not ( $F_{1,53} = 0.03$ ,  $P = 0.864$ ). The relative measure of allelic richness was used instead of the conventional allelic richness (count of all alleles per population) to control for the heterogeneity in scoring success between populations.

**Table 5. Private alleles found in the 58 sampled sites. Sites from Farnes and Firth of Forth are highlighted in (islands in light orange, mainland in dark orange).**

Locus	Allele	Population	Freq in pop
EW1	230	<b>CRM</b>	4.0%
EW12	194	<b>CGL</b>	7.1%
EW12	203	<b>IKH</b>	3.1%
EW12	204	<b>IKH</b>	3.1%
EW12	216	<b>IMK</b>	50.0%
EW15	304	<b>EWO</b>	16.7%
EW15	276	<b>ICM</b>	3.0%
EW15	302	<b>IGV</b>	4.6%
EW15	318	<b>KRF</b>	16.7%
EW25	210	<b>BAS</b>	16.7%
EW25	212	<b>CGL</b>	4.2%
EW40	109	<b>LAM</b>	2.4%
FA2	153	<b>IKH</b>	2.6%
EW12	189	<b>DAL</b>	2.5%
EW15	306	<b>CML</b>	2.3%
EW35	140	<b>CML</b>	2.1%
EW35	144	<b>CML</b>	2.1%
EW12	212	RVR	10.0%
EW15	300	IWG	50.0%
EW15	314	IWG	25.0%
EW25	198	IWG	50.0%
EW35	148	HIL	12.5%
EW25	209	GIR	1.5%

*Genetic distance between populations*

Neighbour-joining trees constructed with the five different distance measures and rooted with Krakow (KRR) defined as the outgroup, were very similar in topology (e.g. Figure 12). Most of the 56 populations constitute a polytomy with relatively little distance resolved between them, with the exception of a small number of populations that aggregate as a separate clade.  $F_{ST}$  and its SMM derivative  $R_{ST}$  both produced trees that included a second distinct clade within the general polytomy of populations. Using distance measures that assume the SMM or removing the two loci that had non-standard alleles did not alter the tree topology.

The consistent clade outside the general polytomy consists of Forth and Farne mainland and island sites but also excludes many from this region. The clade diverging from within the polytomy suggested by  $F_{ST}$  and  $R_{ST}$  is not consistent with any geographical region or hypothesised relicts. Due to the incompleteness of the data, bootstrapping of trees built from all 56 populations is not possible. Overall, the clearest affinities shown among all the available data for the 7 loci relate to sample size. The 22 populations from which complete multi-locus genotypes were obtained, indicating that DNA from these individuals were of high quality, were placed very closely together in the end of the polytomy (see populations marked in green in Figure 12).

Isolating these 22 populations (Figure 13) and in particular the northern island populations where morph ratios are highest (Figure 14) produced tree topologies that show no geographically consistent clades. Very few populations from the key geographical areas can be included in these analyses and the bootstrap support was generally very low.

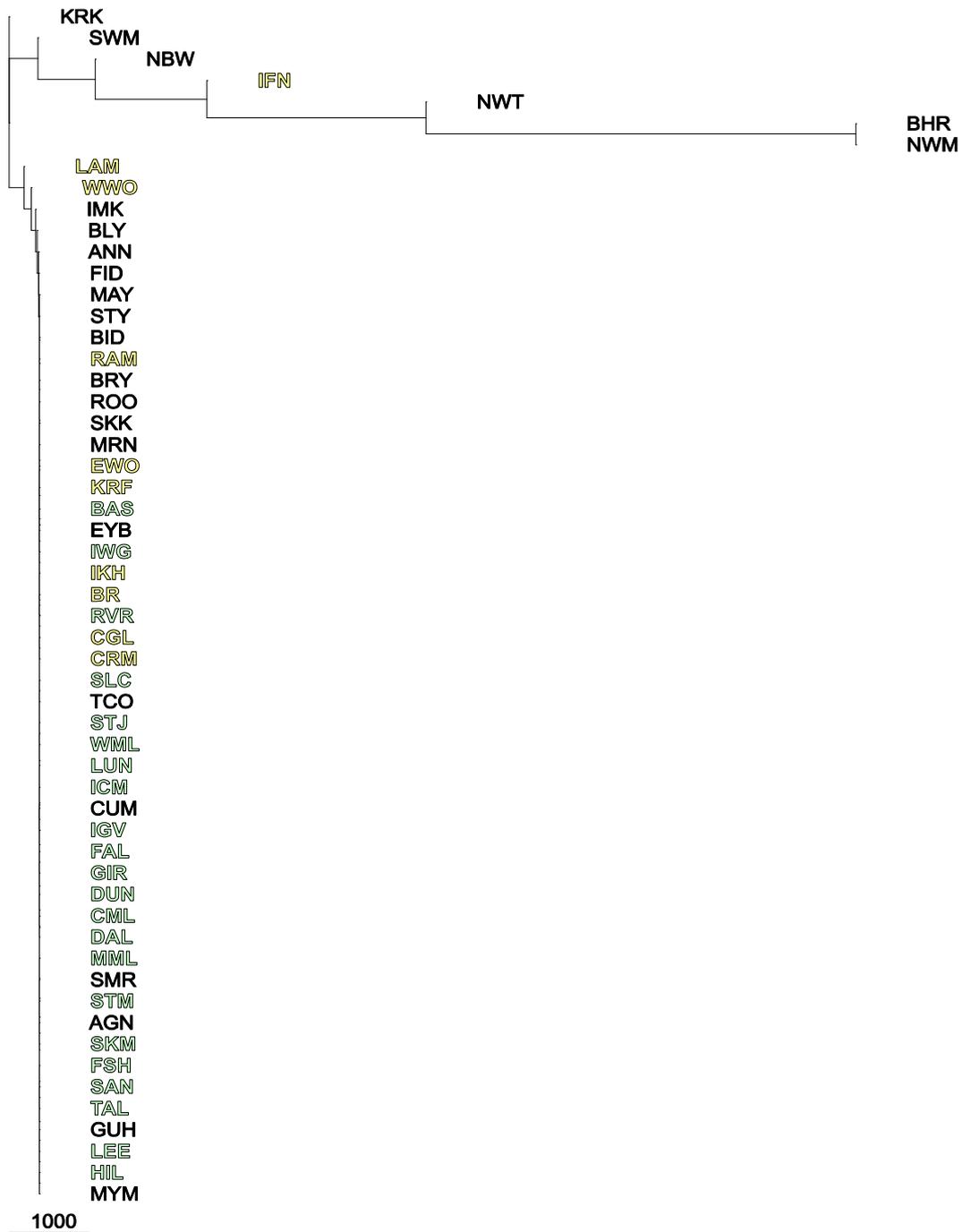


Figure 12. Neighbour-joining tree of chord distance,  $D_c$  (Cavalli-Sforza & Edwards 1967) between populations of *F. auricularia* using 7 microsatellite loci. The tree is rooted using data from a Polish population, Krakow. Populations are labelled using three-letter codes according to appendix A and are coloured according to sample size (Figure 11).

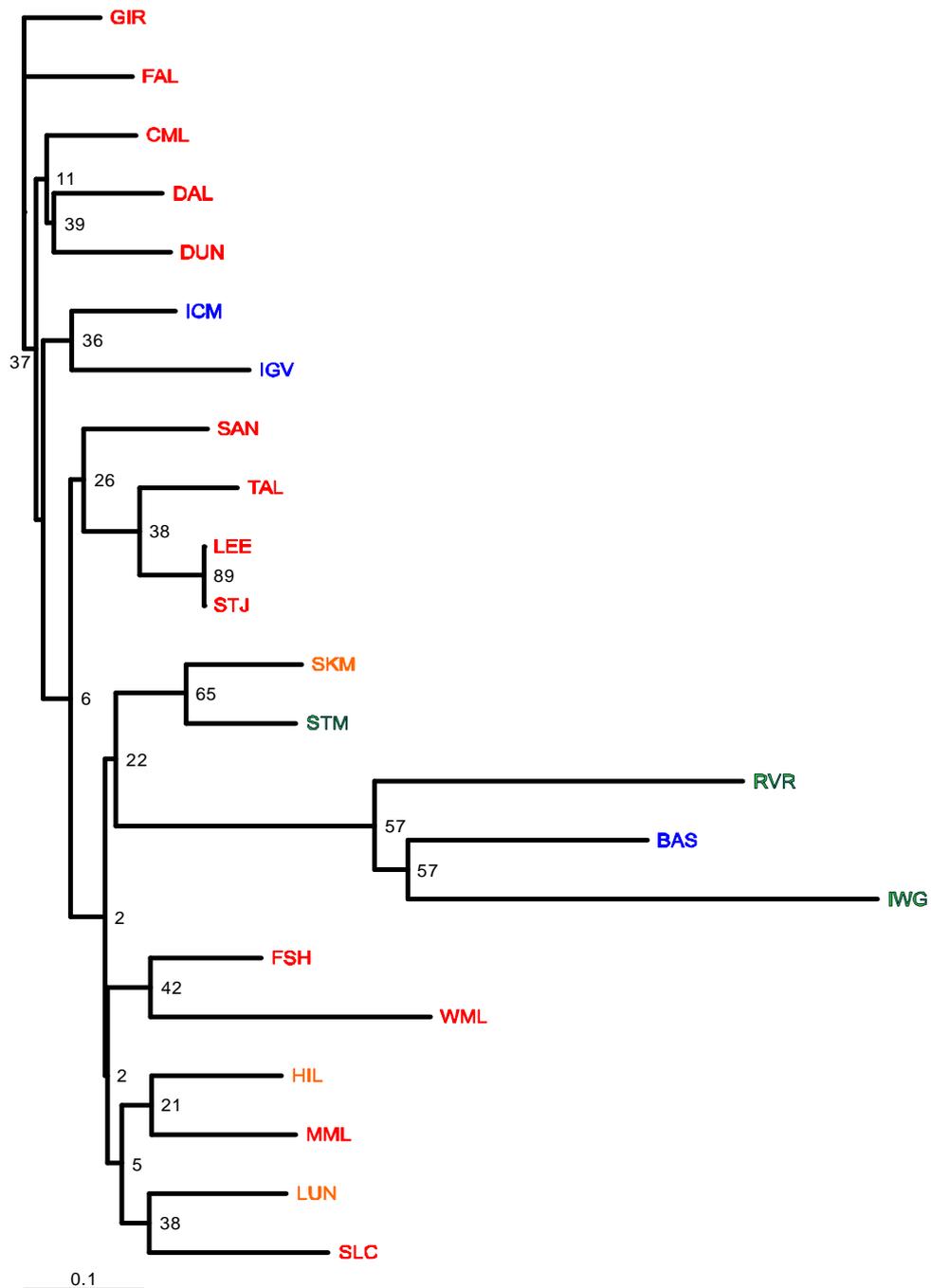
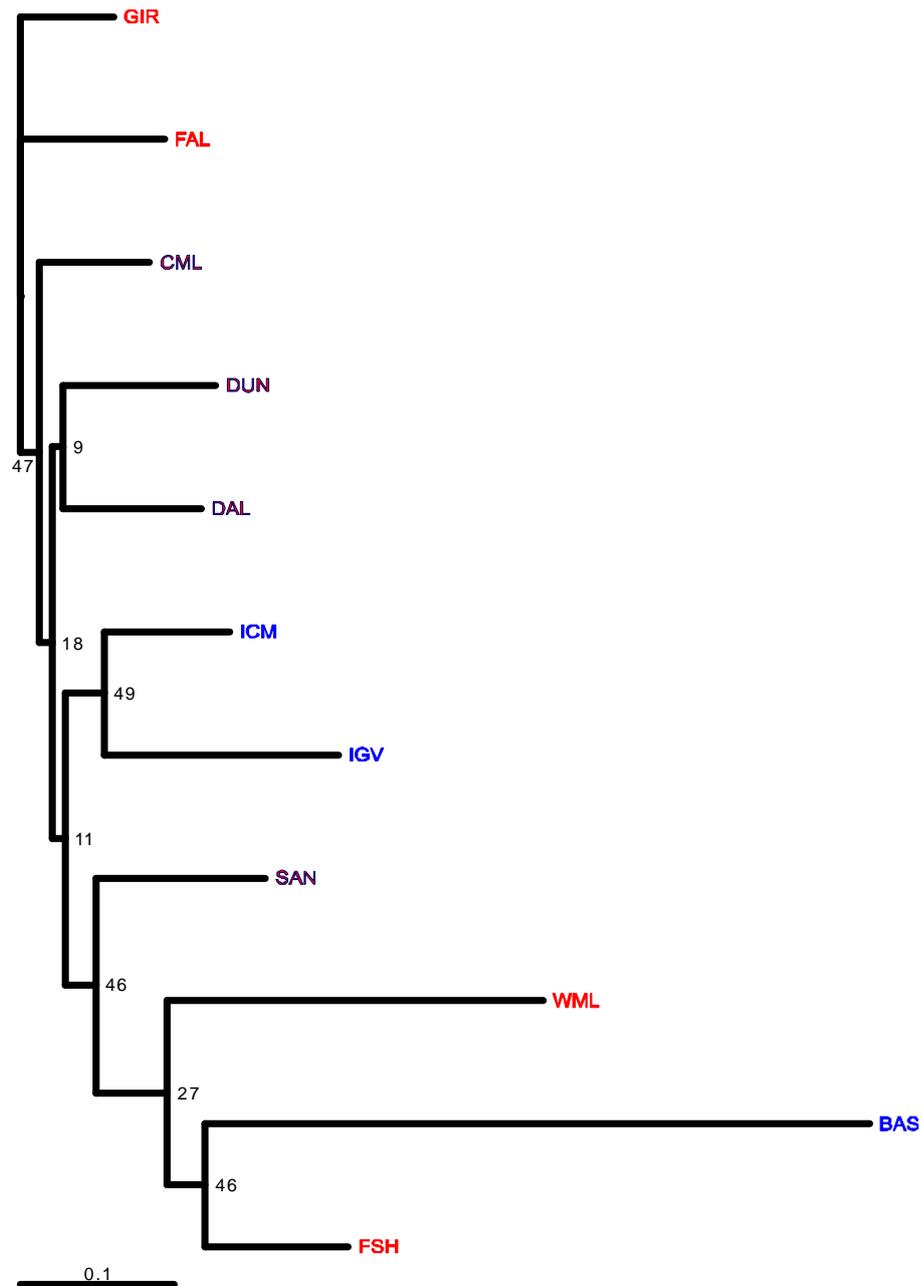
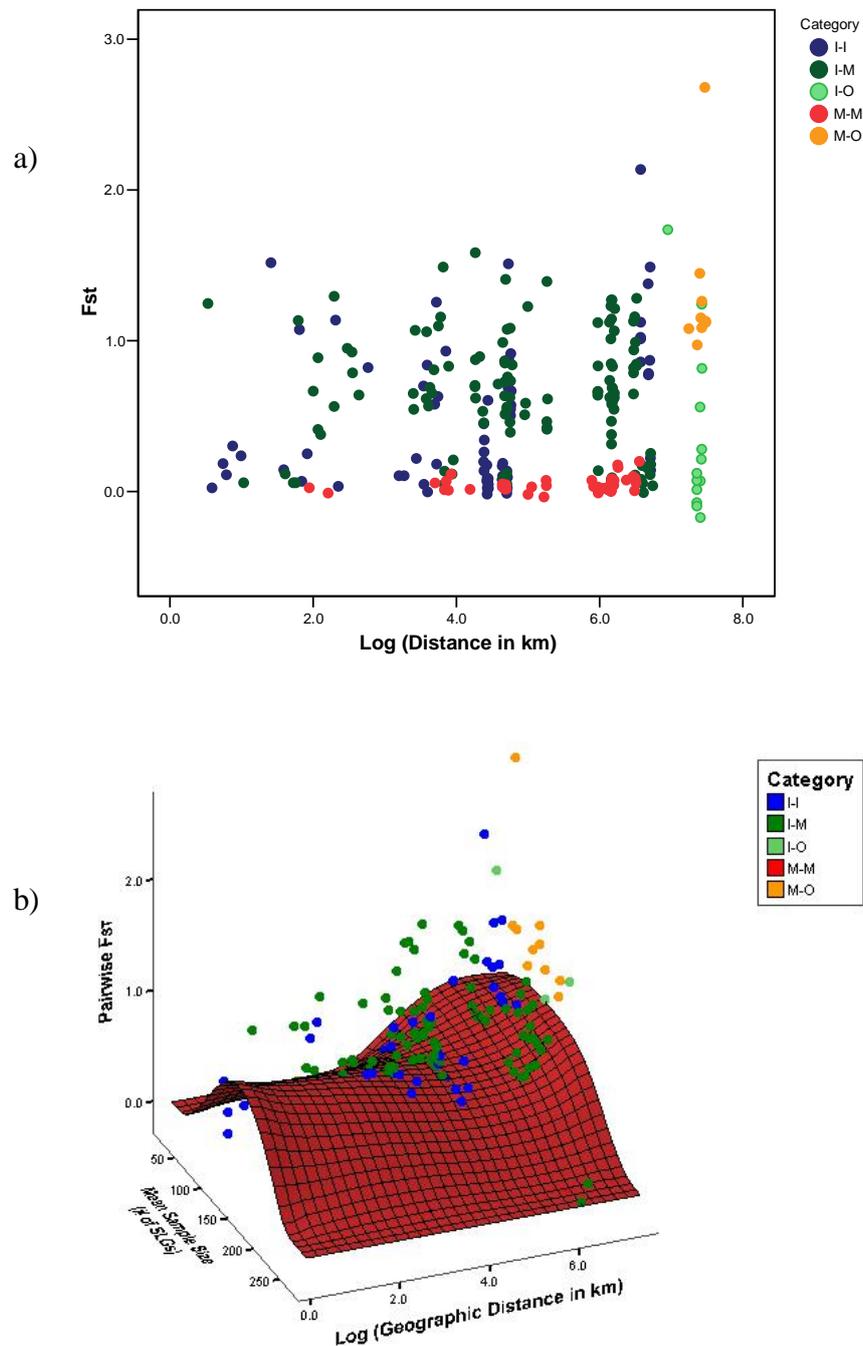


Figure 13. Neighbour-joining tree of chord distance,  $D_c$  (Cavalli-Sforza & Edwards 1967) for 153 individuals from 22 populations of *F. auricularia* where complete multi-locus genotypes were obtained. The tree is rooted by the Girton population from southern England and internal edge labels indicate the percentage bootstrap support from of the 1716 possible bootstraps. Node labels are coloured according to region: Forth islands, blue; Scilly Isles, green; Mainland UK, red; Other UK islands, orange.



**Figure 14.** Neighbour-joining tree of chord distance,  $D_c$  (Cavalli-Sforza & Edwards 1967) for 133 individuals from 11 populations of *F. auricularia* from the Forth islands (blue) and the UK mainland (red; red with blue outline for mainland sites near the Forth) where complete multi-locus genotypes were obtained. The tree is rooted by the Girton population from southern England and internal edge labels indicate the percentage bootstrap support from the 1716 possible bootstraps.

*Isolation by distance*

**Figure 15.** Isolation by distance; a) Scatter plot of genetic distance (pairwise  $F_{ST}$ ) against log transformed geographic distance and b) the same plot displayed with mean sample size, in terms of the number of single locus genotypes per population, as a third axis. Data points are coloured according to the type of sites compared; blue between islands, green/light green between islands and the UK mainland / continental European outgroups, red between mainland sites and orange between mainland sites and outgroups.

A wide variance was found in  $F_{ST}$  values between population pairs, most likely a result of the low sample sizes that pervade the data. Even so very few populations that are geographically close together yielded high  $F_{ST}$  values compared to more distant populations (Figure 15a). However this trend had little statistical support (Mantel test:  $P = 0.090$   $r^2 = 0.007$ ). Considering the data in light of sample size (Figure 15b) showed that indeed low sample size has reduced pairwise  $F_{ST}$  values and whilst the increase in genetic isolation with distance remained, it still failed to gain statistical support.

The genetic distance between mainland UK populations was relatively low compared to the distance between other site types (Figure 15a). Interestingly, the genetic distance between islands and mainland sites is similar to the distance between islands. Furthermore, there are no obvious clusters in the between-island comparisons that would suggest a distinct clade.

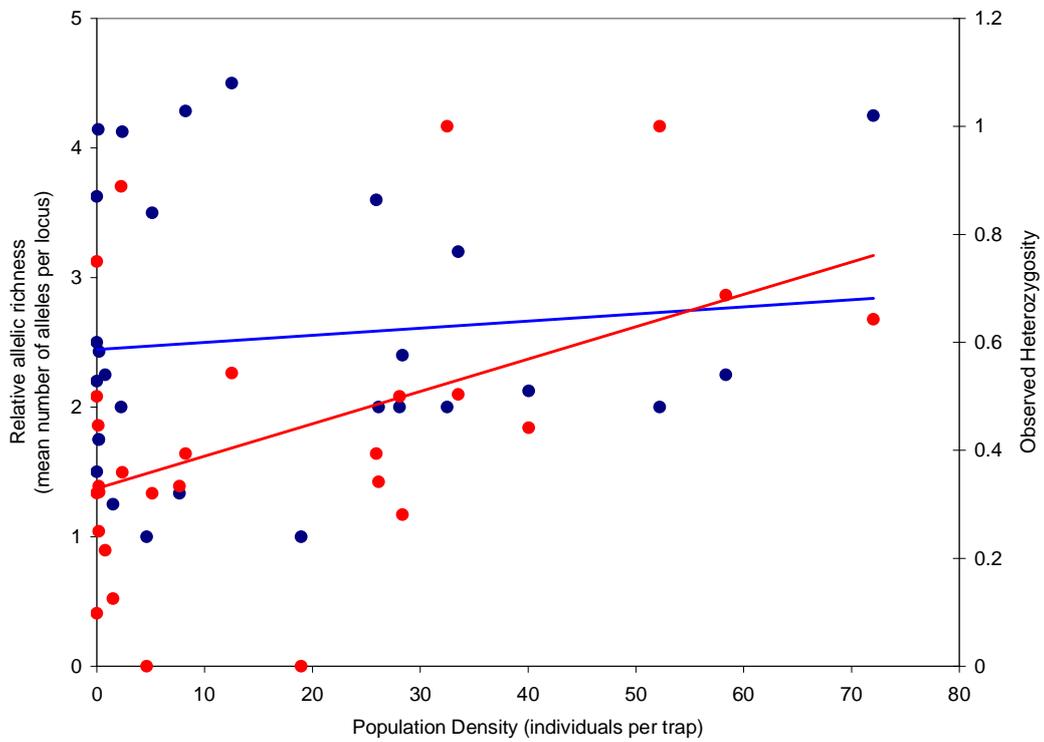
#### *AMOVA*

The populations were assigned to the following 6 groups; the Forth islands, the Farne Islands, the Scilly Isles, all UK mainland sites, other UK islands and the continental European sites (outgroups). The majority of genetic variation in the data is found within the populations (AMOVA  $V_C = 96.43\%$   $F_{ST} = 0.036$   $P < 0.001$ ) and very little variation was found between populations or the assigned groups. To reduce the influence of groups of islands where the dimorphism and population density were not found to be particularly high and of the poorly sampled outgroups, the same analysis was repeated on simply the Forth, Farnes and UK mainland data. This focussed reduction of the data did not greatly alter the outcome of the analysis (AMOVA  $V_C = 94.47\%$   $F_{ST} = 0.055$   $P < 0.001$ ). The low values of  $F_{ST}$  also reflect the low degree of population differentiation in the data.

#### *Genetic Diversity and Population Density*

The observed heterozygosity in UK populations was analysed as the dependent variable in a general linear model, with site type (island or mainland) as a random factor and the following covariates; sample size (Log-transformed count of single locus genotypes), relative allelic richness (number of alleles per locus) and population density (mean number of individuals per trap, see chapter 2). Observed genetic diversity is expected to increase to an asymptote with sampling effort, thus sample size was also included in

the model to control for this effect. The model was reduced to include a single significant interaction between sample size and site type. The heterozygosity observed in the seven accepted loci amongst the 56 populations of *F. auricularia* was higher in island populations than in mainland populations (site type  $F_{1,50} = 8.21$   $P = 0.006$ , sample size  $F_{1,50} = 23.10$   $P < 0.001$ , allelic richness  $F_{1,50} = 22.58$   $P < 0.001$ , population density  $F_{1,50} = 7.48$   $P = 0.009$ , site type \* sample size  $F_{1,50} = 6.40$   $P = 0.015$ ). Additionally, when only islands were considered, for which population density data had been previously recorded (Chapter 2), there was an increase in heterozygosity with density (Figure 16). As an alternative measure of genetic diversity, relative allelic richness correlates with heterozygosity but it does not show the same increase with population density. A multiple regression for island populations, of observed heterozygosity against sample size (number of single locus genotypes), relative allelic richness, and population density, revealed that the relationship between heterozygosity and density was not the consequence of correlations between heterozygosity and allelic richness and sample size and heterozygosity, but rather remained significant in the presence of these other variables (whole model  $F_{3,31} = 6.95$   $r^2 = 0.40$   $P = 0.001$ , Log number of single locus genotypes  $P = 0.073$ , relative allelic richness  $P = 0.008$  and population density  $P = 0.026$ ).



**Figure 16.** Observed heterozygosity (shown in red,  $r^2 = 0.22$ ) increased with *F. auricularia* population density, but the relative allelic richness did not (shown in blue,  $r^2 = 0.01$ ).

## Discussion

### Ecumenicism of the data

In a random mating population, genetic markers that are independently inherited and selectively neutral should exhibit allele frequencies concordant with Hardy-Weinberg equilibrium. Markers in genome regions that are under strong selection, for example, are likely to exhibit allele frequencies biased towards the alleles with the higher fitness. Analysis using such markers is likely to report artefactually strong affinities between populations with similar selective regimes, confounding the underlying phylogenetic signal that is of interest. Therefore, ensuring that populations exhibit allele frequencies concordant with H-W equilibrium is one way of ensuring the markers are independent of selection.

However, it is not unusual for small, isolated island populations to deviate from H-W equilibrium, as having gone through bottlenecking and so show signs of increased inbreeding such as a reduction in heterozygosity and allelic richness. Testing for H-W equilibrium is based on a comparison of the observed heterozygosity with that predicted at equilibrium, given the allele frequencies observed. Therefore, deviation from H-W equilibrium in inbred populations does not necessarily indicate that the genetic markers are inappropriate for phylogenetic analysis. Instead, this deviation from H-W equilibrium is an informative signal reflecting the history of these populations. In this study, the accordance of the mainland populations with H-W equilibrium suggests that the markers are generally independent and likely to harbour a true phylogenetic signal.

The deviation from H-W equilibrium found in individuals collected from Longniddry Bents (LND) is clearly not the result of pooling samples collected in different years. Such deviations in individual populations can be caused by geographically localised null alleles. Null alleles are a common difficulty encountered in the interpretation of microsatellite data, reducing the observed heterozygosity. Another possible explanation for this deviation is sampling bias: If the genotyped individuals mostly originate from a single brood they will be very closely related and will register allele frequencies that deviate from equilibrium in the same way that inbred populations can do. This form of sampling bias is more likely from sites where population density is low and at a time of year when the individuals of a brood have yet to disperse from the protection of the

mother. A sample of the order of 30 individuals was considered satisfactory and broods frequently exceed 20 individuals. Longniddry bents has a relatively low population density, as is the trend amongst mainland sites, and collecting was almost exclusively from hogweed at this site where aggregations of tens of earwigs are frequently found within a single stem. In light of this it seems advisable that collections made from such sites should be made from larger numbers of hogweed stems and with a limited number of individuals taken per stem.

Populations with small sample sizes produced the artefactually high genetic distances shown as a clade in Figure 12. The two indices presented for filtering out populations of small sample size were insufficient to resolve the affinities between populations. Conservatively removing all individuals except those that were scored completely at all loci allowed bootstrap support to be tested; however, too few populations remained to comprehensively approach the questions of interest. The second index of sample size was somewhat arbitrarily defined although it did successfully filter out the very smallest samples, whether they were of few individuals or of limited locus coverage. However, the tree topologies from this larger filtered data set did not help resolve the relationships further and bootstrapping was not possible. This highlights the fundamental weakness of these data.

The non-standard alleles reported in this study fell within the size distribution of the other stepwise alleles of those loci and were therefore unlikely to be PCR artefacts. Microsatellites are characterised by stepwise mutation, which occurs at a higher rate than other mutations in DNA sequence. The rapid rate of mutation makes these markers both useful for detecting recent and small-scale genetic signals but also leads to the loci being most easily modelled in a stepwise manner. The occurrence of non-stepwise alleles can result from mutations in the flanking region of the microsatellite, regions which are not subject to the stepwise mutation process, or similarly rare point mutations within the tandem repeat sequence itself. The analysis of these data was made more difficult because of these non-stepwise alleles being present, however nothing was gained in applying SMM based distance measures when the two loci that bore these non-standard alleles were removed. This is supported by Takezaki and Nei's finding that measures  $D_A$  and  $D_C$ , which assume the infinite allele model (IAM) of evolution,

produce the most reliable estimates of tree topology from simulated microsatellite data (1996).

### Genetic diversity in island populations

The heterogeneity in the distribution of private alleles amongst populations can be indicative of geographic or reproductive isolation. Novel alleles in populations with high gene flow with other populations spread and cease to be private; novel alleles in populations with low gene flow to other populations remain private in these populations for much longer and so are more likely to be found. Alternatively a higher mutation rate in a particular population may give rise to a higher incidence of private alleles, such that at any one time the number of novel alleles yet to spread to neighbouring populations is higher than in other populations with a lower mutation rate. These two explanations are not mutually exclusive and may act in combination. Given the geographical isolation of the island populations in this study, the relative abundance of private alleles from these islands is suggestive of low gene flow in these populations. Furthermore, the consistency with which these high numbers of private alleles were detected on the islands suggests that these are substantial populations.

Another factor for consideration is the greater population density on the islands, which has two implications for the discovery of novel alleles: The probability of a mutation arising in any individual being equal, where population density is greater there will be a proportional increase in the number of mutations that occur in a given geographical area. Secondly, sampling within a similar area of habitat at higher population density is likely to incur a lower probability of collecting two individuals that are immediate familial relatives and therefore a wider range of the alleles present are likely to be sampled. This latter hypothesis of a sample bias in low-density populations is undermined by the finding that allelic richness was not related to overall population density or the island-mainland dichotomy.

It is interesting that the dimorphic island populations exhibit greater numbers of these private alleles. It is tempting to speculate that strong disruptive selection on forceps length in island populations that is associated with the evolution of the ESS threshold of the male dimorphism, might have given rise to greater sequence diversification than in mainland populations. Arguments for the condition-dependence of secondary sexual traits posit that sexual trait expression is dependent on any loci involved in the

conversion of the environment into soma or energy (Tomkins *et al.* 2004b). Hence, it seems possible that mutations even at so-called 'neutral loci' may have some small cost in terms of body size or condition. The disruptive selection on males in species with alternative male reproductive tactics does mean that individuals who bear mutations decreasing their ability to gain large size or competitive ability are retained within the population. Hence, disruptive selection might be a mechanism by which increased numbers of mutations are maintained in a population.

The relationship of higher levels of heterozygosity with higher population density is, on first appraisal, as expected. In high-density populations such as those on the islands where the male dimorphism is so marked, the encounter rate between individuals is expected to be higher than at low density; thus, there will be a greater number of potential mates within a given area. This presents more opportunities to mate and thus it is more likely that an individual will mate with an individual that possesses different alleles to their own. In contrast, at low population density the likelihood that an individual encounters a potential mate that is closely related to them is much higher. Therefore, if comparing two populations with the same allele frequencies but with different population densities; individuals are more likely to produce heterozygous offspring in the high-density population.

This is of course only a transient effect; ultimately, both populations would be expected to tend to H-W equilibrium. If both populations are at equilibrium, they will both exhibit the same levels of heterozygosity and remain so. However, following a disturbance, such as a population bottleneck, that skews allele frequencies away from equilibrium, the higher density population would return to equilibrium sooner after the disturbance abates.

A second aspect of population density is that the effective population size is greater. As the likelihood of a new mutation arising is essentially equal among individuals, the number of new mutations in the population will increase with effective population size. Consequently, the total number of mutations arising in individuals within the area that an individual can search for mates will be greater. Therefore, in the same way that a larger number of private alleles are expected to be detected by sampling a high-density population, an individual in that population is more likely to mate with an individual bearing a novel mutation. The fact that this trend is apparent in these data further

supports the suggestion that these are substantial populations even though they are geographically isolated.

### Genetic affinities between populations

In addition to the general effect of sample-size on tree topology, the continental European population used to root trees (Figure 12) had a very small sample size. It is probable that the topology of the tree presented could change dramatically given a more representative sample from mainland Europe. Irrespective of this, there are two well-sampled sites, Falmouth (FAL) and Girton (GIR), that can be used to draw a comparison between the Forth and Farne islands and the immediately adjacent mainland populations (Figure 13 and Figure 14).

The failure of the genetic data to correlate with geographic distance (Figure 15) or associate with the distinct geographic regions could be interpreted as a lack of sufficient power in the data. However, panmyxis in insect populations has been recently reported in other species (Beveridge & Simmons 2006) and is not necessarily an indication of low power. In the West Australian solitary bee *Amegilla dawsoni* females are monandrous and mated soon after emergence at their natal site, a life history that would be thought to promote population structuring (Beveridge & Simmons 2006). Nevertheless, the bee is a strong flier and liable to have travelled long distances in search of flowers arising from patchy rainfall. It is likely that this dispersal accounts for the gene flow that maintains panmyxis. The European earwig rarely flies, and so it is not immediately obvious how similar levels of gene flow might occur. Despite their apparently poor ability to disperse through flight, earwigs are very easily transported by human activity. Such anthropogenic transport arises from the crevice-seeking habit of the earwig. This 'thigmotactic' behaviour has resulted in the recent arrival of European earwigs in Western Australia having crossed the Nullarbor desert from the East in the folds of tent material; evidenced by outbreaks of earwigs at campgrounds. Anecdotal evidence from the wardens of the Farne islands also suggests that where the densities are high, earwigs are commonly found in clothing and direct evidence of migration has been reported from Brownsman Island to the Ship Inn in Seahouses (Tomkins pers. comm.). Hence, it seems that in pre-industrial Britain, earwigs are likely to have been regularly transported, perhaps large distances, along trade routes amongst goods and

+their packing materials. Shorter distances within rural areas are likely to have been realised through the harvesting and the redistribution of crops.

To what extent island populations are likely to have received introductions of earwigs from the mainland and vice versa through human activity is an open question. Certainly small-holdings were present on some of the Farne and Forth islands and doubtlessly the exchange of some earwigs took place. The collection of eider down and harvesting of seabirds and their eggs was commonplace until the beginning of the 20<sup>th</sup> century; this may have facilitated some transport of some earwigs. Nevertheless the greater frequency of private alleles on island populations does suggest that the mainland population has experienced more mixing and that novel alleles are ‘marooned’ on the islands where they evolved: a pattern consistent with relatively rare transport of earwigs off the islands by whatever means

Returning to the issue of power; trying to find the required level of sampling to satisfactorily test a hypothesis, would not be informative using this data set as it stands. Power analysis simulates analyses based on the mean and variance of the observed data, and the heterogeneous scoring success of the microsatellites has produced unduly high variance in the observed data in this study. Consequently, power analysis of the existing data would predict the need for much larger sample sizes than would be needed if the more successful protocol adopted latterly was followed. Acceptable sample sizes were obtained from mainland sites around the Firth of Forth and from Falmouth and Girton in the south of the UK. Improving the sample sizes from islands in the Firth of Forth would provide an initial data set on which power analysis could provide a useful indication of further work and how best to appropriate effort. Given that the data set shows no sign of structure, it does mean that larger sample sizes are going to be needed in order to be able to satisfactorily accept the null hypothesis of ‘no structure’.

### Origins of the European earwig in the UK

The limitations of the dataset presented are greatly due to the vagaries of preserving earwig DNA. As a result, there are both suggestions of higher genetic diversity and genetic isolation in the male-dimorphic islands of the Forth and Farnes relative the UK mainland and a general lack of genetic differentiation between these islands and the mainland. Additionally, in all loci the most common allele is the same or of similar size across the sampled range of mainland and island sites. It seems unlikely, given the

present data, that the Forth and Farnes are populated by a discrete clade to the population of the monomorphic mainland populations. It is likely that, if such a division existed for such a long period of time (circa 10 000 years) and is associated with such a striking difference in reproductive strategy, the clades would have been discernable in the data collected and yet there is no definitive evidence of this.

This question would be better answered with more complete data, a goal made feasible by the clarification made in this study that DNA extracts from this species should be prepared from fresh, not frozen material. The lack of genetic variation between the gross regions of the sampled area is likely to be improved by this and further by increasing the number of microsatellite loci.

An alternative approach to the questions raised in this chapter would have been to establish a rudimentary phylogeography based on sequence data. As fewer individuals would be required to construct a phylogeny from sequence data (as few as 3 per population), it is likely that sequence data would have been more revealing with the number of serviceable DNA extracts obtained. Furthermore, by using the existing markers, direct comparison could be drawn with previous work resolving the two sister species of *F. auricularia* (Wirth *et al.* 1998) and by comparison with nuclear markers could reveal any sex-specific dispersal. However, given that the 627 base pairs of sequence studied by Wirth *et al.* contained only 44 phylogenetically informative sites between *F. auricularia* and *F. lesnei*, it is likely that the existing mtDNA markers contain insufficient variability to completely resolve the relationships between UK populations of *F. auricularia* alone. Furthermore, the UK populations, being in an area towards the periphery of the range and farthest from the centre of European glacial refugia, are likely to exhibit even lower genetic diversity than found in the more southern sites sampled in Wirth *et al.*'s study (Hewitt 2000).

Therefore, the microsatellites used in this study are likely to be of great value in resolving the fine-scale population genetics of the Forth and Farne Islands with respect to the mainland. Consolidating this view of *F. auricularia* in the UK with the large-scale phylogenetics of the whole European taxon is likely to be achieved most easily by applying the existing mitochondrial sequence-based markers.

The data presented in this chapter do not show any distinct clades of *F. auricularia* in the UK or any correlation between genetic affinity and the dichotomy between mainland and island populations with respect to the male dimorphism. Instead, these data suggest that, in parallel to the high population densities detailed in chapter 2, the island populations are substantial populations, bearing higher heterozygosities than in mainland populations and numerous private alleles. Furthermore, the lower incidence of private alleles on the UK mainland suggests that the island populations exhibit comparatively low gene flow, as expected from their remote geography. Estimates of genetic distance between populations are consistent with a single panmictic population resulting from one or more homogeneous post-glacial invasions of the UK by *F. auricularia*; the islands having diverged independently. This gives support, though not conclusively, to the local adaptation hypothesis of threshold evolution of the dimorphism. Consistent sampling was demonstrated latterly in this study confirming the potential these genetic markers have to further resolve the affinities of these earwig populations. Such quantitative data are essential for the phylogenetic control of any studies of traits associated with population variation in sperm competition risk.

## **Chapter 5: Ejaculate characteristics of the European earwig**

## Introduction

The documented examples of evolutionary change brought about by sperm competition demonstrate the dramatic power of this mode of sexual selection on the organs involved (Simmons 2001) see also Chapter 1). Particularly common among such examples are those traits that allow males to compete by outnumbering the sperm of other males with their own. To outnumber competitors' sperm a male must deliver larger quantities in a given mating compared to that of his rival; in keeping with this notion, greater ejaculate size has been observed where the risk of sperm competition is greater (Gage 1991). The increased number of sperm inseminated must place demands on sperm production, otherwise the number of successful matings that the male will be capable of will be limited. In several intraspecific studies, the testes of dimorphic males that mated in the disfavoured role have been found to be relatively larger (Gage & Baker 1991; Gage et al. 1995; Simmons 2001; Simmons et al. 1999b; Tomkins & Simmons 2002).

The mode of sperm transfer is of key interest to understanding the mechanism of sperm competition. Sperm removal in damselflies is an outstanding example of this; breaking copulating pairs in a time series Siva-Jothy and Tsubaki (1989); reviewed by Simmons (2001) Figure 3.4) revealed sperm numbers dropping as the sperm of rival males was removed and then increasing again as the male inseminates his own sperm. A growing number of studies show how males can tailor their ejaculate characteristics to the socio-environmental conditions (Cook & Gage 1995; Gage & Baker 1991); reviewed by Simmons (2001) Table 7.2), such as the mating status of the female or the number of competing males. Where males transfer a pre-formed spermatophore to the female there might be fewer opportunities for tailoring the ejaculate in this way, unless changes in accessory gland proteins are easily manipulated at the time of mating. In species with free sperm (i.e. sperm are not held in a spermatophore) sperm transfer is likely to be more variable in terms of the amount transferred, particularly if this relates to a behavioural trait such as the duration of copula. This may give males greater ability to alter the number or quality of the sperm that they inseminate depending on the quality of their mate or the competition that they face.

*F. auricularia* has been presented in the preceding chapters as a model for the study of sperm competition as several aspects of the species biology lend themselves to this end. Firstly, the alternative reproductive tactics of the males are likely to confer an inherent

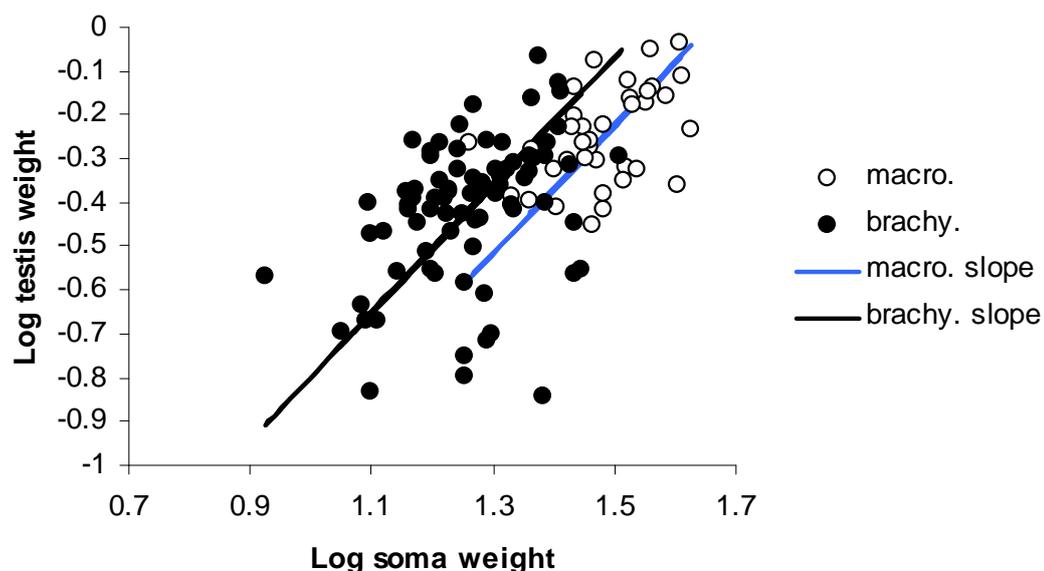
difference in the risk of sperm competition. Males that guard females may successfully control the paternity of all of her offspring; males that cannot guard females must sneak matings and will have to compete with the sperm of the guarding male and any other sneaking males. Secondly, the associated male dimorphism in *F. auricularia* facilitates the identification of the alternative tactics in a population in order to draw these comparisons. The third aspect, and that which makes *F. auricularia* particularly unusual amongst species used to study sperm competition, is the population variation in the proportions of the male morph, and hence the proportions of the guarding and sneaking tactics (Chapter 2). An increase in the relative abundance of sneak-mating males will increase the probability that a guarding male will not completely control all the copulations of the female he guards (Parker 1990b). Therefore, this population variation in the ESS threshold of the male dimorphism represents a natural gradient across which adaptive changes in traits associated with sperm competition can be quantified.

Like many insects, *F. auricularia* females store sperm in a spermatheca after mating. In *F. auricularia* this organ is ordinarily a relatively simple 'banana-shaped' sclerotised vessel with a single opening (Kamimura 2004). When spermathecae are sclerotised their capacity is fixed, and sperm competition tends to occur either through the compaction of earlier male's sperm to the distal end of the spermatheca or through males inseminating enough sperm to remove the sperm of rivals through displacement (Simmons 2001). The simplicity and fixed capacity found in female *F. auricularia* is in contrast to other species of earwig that exhibit complex, multiple spermathecal arrangements that are believed to be the result of strong sexual selection through sperm competition in the males and sperm selection in the females. For example the dermapteran *Diplatys macrocephalus* has a multi-chambered spermatheca with arrays of connecting ducts (Simmons 2001). It is likely that this complexity is a consequence of an arms race between females and males in the control of paternity (Simmons 2001). The spermatheca of *F. auricularia* is known to be subtly variable, increasing in the number and compression of bends such that it ranges from a straight tube, through a 'banana shape' to a tight 'S' shape and may even be bifurcated (Tomkins, pers. comm.). The cause of this variation and its impact on the success of stored sperm from different males are as yet unknown. This simple sclerotised structure can be located with relative ease inside the female and dissected to obtain sperm counts.

Recent analyses have found no significant difference in testis size between macrolabic and brachylabic males of *F. auricularia* from Brownsman Island in the Farnes group (Tomkins & Simmons 2002). However although the analysis of covariance (ANCOVA) recommended by Tomkins and Simmons (2002) improved on the previous use of gonadosomatic index (GSI) to quantify relative testis size in species with alternative tactics, it unfortunately introduced another potential bias. Analysis of covariance is based on the assumption that the independent variable is a series of treatments increasing in effect and determined by the experimenter (Zar 1984). Hence ANCOVA is, strictly speaking, an unacceptable model for allometric studies where the covariate is body size. Conventional least-squares regression underestimates the slope when there are measurement errors in both X and Y (Ricker 1973). When comparing two groups of data, as when comparing allometry of male morphs of a species, this underestimation of the slopes artificially reduces the difference between the intercepts and thereby obscures divergence where it may otherwise be apparent. In situations where lines are to be fitted to measurements of allometry, as in the testes data here, standardised (or reduced) major axis (SMA) estimation is currently viewed as the most appropriate method for doing so (Warton et al. 2006). In particular it allows the intercepts and slopes of the two samples to be compared. Using an analysis of covariance model based on the SMA estimation (Falster *et al.* 2003) significant differences have been found between the morphs from Brownsman (Tomkins in pers. comm.).

At present, it is still somewhat controversial which methodology is appropriate; whatever the statistical approach, it is apparent that there is much scatter and very little in the way of dramatic differences to separate the male morphs in terms of relative testes mass (Figure 17). In fact, the Brownsman Island population is one in which the switch-point is at relatively high status and hence the ratio of sneaks to guards is biased heavily towards sneaks (circa 3:1). The level of sperm competition is predicted to be high in both macrolabic and brachylabic males from this population and hence for them to both invest in adaptations to sperm competition. No difference in testis mass was found between the male morphs of *Onthophagus taurus*, which has a similar morph ratio (Simmons et al. 1999b). Interestingly though, SMA analysis has since suggested that the morphs do differ in testis mass (Tomkins, pers. comm.).

In this chapter I present a study that compares the ejaculate size of the two male morphs from a dimorphic island population of *F. auricularia*. I use two approaches; firstly, the weight lost by males and gained by females during copula is analysed; this allows me to determine whether there is a substantial ejaculate in terms of its proportion of body mass. In the bruchid beetle *Callosobruchus maculatus*, ejaculates of the first mating weigh up to 10% of the male's body mass (Eady 1994) and variation in ejaculate size between and within males is easily quantifiable. Secondly, I count the number of sperm transferred by males of different morphs in relation to copula duration. By employing these two methods for estimating ejaculate expenditure I aim to assess whether male morphs in this population of *F. auricularia* differ both in the number of sperm transferred but also in terms of other ejaculatory components such as accessory gland proteins, which are potentially assessable by looking at changes in weight.



**Figure 17.** Allometry between testes and body size in *F. auricularia* from Brownsman Island (Tomkins, unpubl. data); demonstrated by Log-transformed soma and testis weights with SMA slopes calculated using (S)MATR (Falster *et al.* 2003). Brachylab males have significantly larger testes for their body size (ANOVA:  $F_{1,114} = 23.49$   $P < 0.001$ ).

## Methods

### Collection and Rearing

A random sample of *F. auricularia* adults and nymphs were collected from Brownsman Island in the Farnes, off the coast of Northumberland on 6th July 2004. The nymphs were reared in en masse and were checked daily for eclosed adults, which were removed, sorted by sex based on their forceps morphology (Chapter 1, Figure 2) and confined to single sex holding containers. These single sex rearings were assumed to be virgin as it is thought that only adult *F. auricularia* that have matured for several days are able to copulate (J. Tomkins, pers. comm.). 148 virgin males and 142 virgin females were successfully reared, all having eclosed by 11th August. Of the males, 112 were classed as brachylabic and 84 as macrolabic from visual assessment of forceps morphology (as in Chapter 2). All captive specimens were kept at 16°C in a temperature-controlled room with a L:D cycle of 12:12hrs, equivalent to *in natura* conditions. Individuals were given  $0.5 \pm 0.2$  g pollen 2 days before the first trial, to increase the likelihood of mating. During the mating trials they were deprived of all food to reduce the influence of defecation; high moisture levels were maintained in the holding containers to avoid desiccation.

### Mate trials

Mating trials were performed where a male was presented to a female in a 30mm diameter Petri dish under red lighting at laboratory temperature (mean  $19.5 \pm 1.3^\circ\text{C}$ ). Trials were staggered over 6 non-consecutive days during a 5-week period from 7th October to 11th November 2004. Individuals were weighed before and after trials using an A&D GR202 balance with WINCT v2.0 interface software. Each pair was left to court and initiate copulation and the time at which copulation started was recorded. Pairs were assigned *a priori* to one of six treatments of permitted copula duration; 5, 10, 15, 20, 30 and 60 minutes. The average copula duration of males in this population has been recorded as  $23.2 \pm 29.8$  min (mean and standard deviation;  $n = 53$ , Tomkins unpubl. data); the times chosen amply encompassed the mean for this previous experiment. When a pair had copulated for the assigned period, copulation was interrupted and they were separated into individual micro-centrifuge tubes to prevent any further sperm transfer. When a pair ended copulation before the end of their

assigned period, they were reassigned to an additional, *natural copulation* treatment group.

Trials were allowed to proceed for up to 3 hours (mean  $1.9 \pm 0.8$  hr) during which time pairs were observed continuously and the start and end of copulation was recorded. If no copulation occurred during the trial, the weight change of the male and female was recorded as a control; unmated individuals were reused in subsequent trials, but never with the same mating partner. In addition to the duration of copulation and weight change, the number of sperm present in the female spermathecae was counted, the period of deprivation of food prior to the start of each trial was recorded and the male pronotum width (body size) and forceps length was measured with a *Leica* MZ-5 microscope and SCION IMAGE (*NIH*) software.

## Sperm Counts

Individual spermathecae were dissected out of 95 mated females and 13 control females and the contents suspended in 10 $\mu$ L distilled water. Similar studies involving sperm counts in insects have used Barth's saline (Gurdon 1991) to aid the dispersal of the sperm, which facilitates counting (Gage, pers. comm.; (Gage & Cook 1994). This approach was found to be inappropriate for *F. auricularia* as the number of sperm collected in the spermatheca was several orders of magnitude less than in these other species. Furthermore, the sperm being generally at low density did not need to be dispersed for counting and this simplified the process without the need for careful drying and washing of salt crystals from the slides. The contents of each spermatheca was suspended in 10 $\mu$ L distilled water and mounted on a slide. Where the density of sperm was relatively high, counting was facilitated by adding 40 $\mu$ L distilled water to the original slide and 4 equal aliquots of 10 $\mu$ L were removed and mounted on a separate slide, thus diluting the sperm. In all cases the entire contents of the spermatheca was counted.

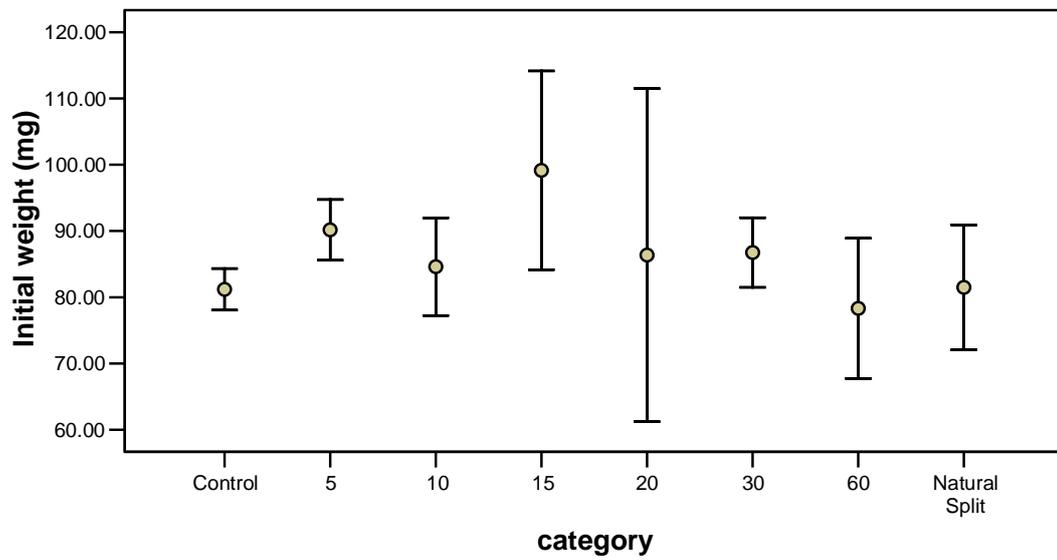
## Results

### Sampling

A total of 210 pairs were observed of which 113 did not mate (controls). Seventeen of the 97 recorded copulations ended naturally (Table 6). There was no detectable weight difference in the females or the males of either morph assigned to the 8 copulation treatment categories (Table 6).

**Table 6. Sample size and mean weight of individuals at the beginning of each trial, per sex and treatment group. Males of both morphs in each of the copulation duration treatment groups did not differ in initial weight, although a significant difference was found in female weight (One-way analysis of variance: Brachylabic  $F_{7,110} = 1.313$   $P = 0.251$ , Macrolabic  $F_{7,84} = 1.566$   $P = 0.157$ , Female  $F_{7,202} = 2.049$   $P = 0.051$ )**

Copulation duration category (min)	Pairs n	Female weight mean / SD (mg)	Brachylabic male weight n	Brachylabic male weight mean / SD (mg)	Macrolabic male weight n	Macrolabic male weight mean / SD (mg)
5	23	90.16 ± 10.60	7	71.85 ± 6.05	16	91.20 ± 8.92
10	16	84.61 ± 13.84	7	71.34 ± 12.41	9	89.27 ± 14.00
15	5	99.15 ± 12.08	3	73.75 ± 11.78	2	96.62 ± 2.81
20	4	86.37 ± 15.79	3	69.58 ± 21.05	1	88.35 ± -
30	20	86.75 ± 11.23	8	63.90 ± 8.65	12	93.66 ± 10.49
60	12	78.31 ± 16.66	9	66.19 ± 11.15	3	97.75 ± 6.40
Natural	17	81.49 ± 18.34	8	67.85 ± 11.77	9	86.01 ± 14.06
Control	113	81.20 ± 16.83	73	62.87 ± 12.38	40	84.48 ± 12.44
Total	210		118		92	

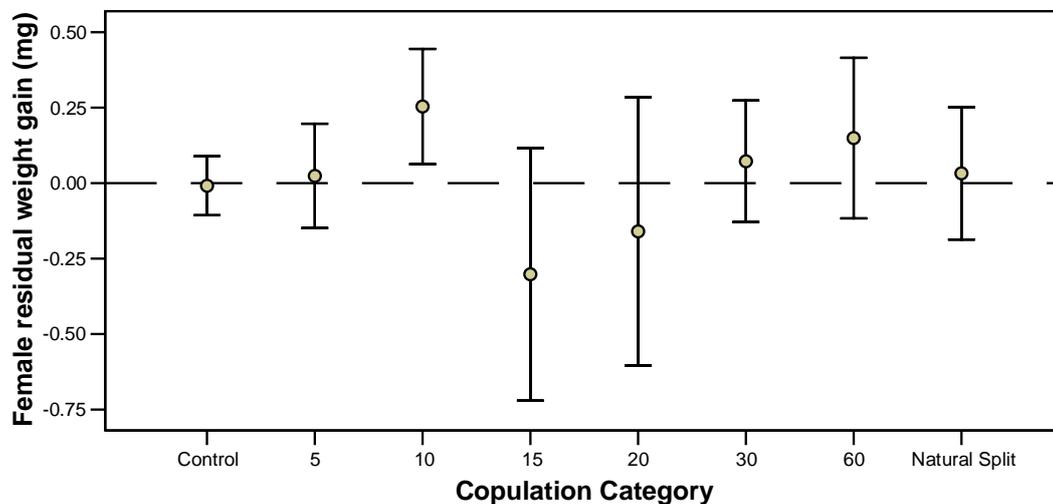


**Figure 18.** Mean initial weight of females ( $n = 210$ ) in each copulation duration category (number of minutes, unmated control or naturally ending bout). Error bars indicate the 95% confidence intervals of the means.

## Weight change

Males and females, even those that did not mate (controls), lost weight during the trials. This non-copulatory weight loss is predicted by a regression including the following terms; initial weight ( $W_1$ ), the period of fasting (the number of hours since individuals last had access to a food supply,  $T_F$ ) and the number of minutes between weighing ( $T_W$ ), with the following relationships: Control female weight change (mg) =  $0.012 W_1 - 0.333 \text{Log}(T_F) + 0.002 T_W - 0.121$  ( $r^2 = 0.26$  whole model:  $F_{3,109} = 12.68$   $P < 0.0005$ ); Control male weight change (mg) =  $0.007 W_1 - 0.113 \text{Log}(T_F) + 0.001 T_W - 0.111$  ( $r^2 = 0.22$  whole model:  $F_{3,109} = 10.27$   $P < 0.0005$ ). Therefore, an individual of mean weight (Table 6) and deprived of access to food for 100 hrs (the average during this study) will lose 0.16 mg (0.20%  $W_1$ ) and 0.19 mg (0.26%  $W_1$ ) per minute, for females and males respectively.

The majority of females that did mate during the trials also lost weight; any gain through sperm transfer is by far outweighed by the loss through baseline defecation, respiration, dehydration etc.



**Figure 19.** The mean residual increase in weight of females in each mating treatment category after the size-specific effects of fasting and duration of the period between weighing is partialled out. The error bars represent the 95% confidence interval of the mean.

Female weight change (mg) was analysed as the dependent variable in a general linear model, with the morph of the pair-male as a fixed factor, the replicate (1-6) as a random factor and the following covariates; initial female weight, Log-transformed female fasting period (hrs), the time between weighing (min), and the mating duration (min). The mating duration was in whole minutes and naturally split pairs were included with the time at which they split. The model was reduced in the absence of any significant interactions between the main effects. The main effects that were previously shown to predict weight loss in females that failed to mate (controls) were likewise significant in this analysis. Having controlled for this non-copulatory weight loss, male morph was unrelated to the weight change in females (morph  $F_{1,86} = 1.64$   $P = 0.204$ , trial  $F_{5,86} = 12.13$   $P < 0.001$ , initial weight  $F_{1,86} = 23.42$   $P < 0.001$ , Log(fasting period)  $F_{1,86} = 0.889$   $P = 0.349$ , weighing interval  $F_{1,86} = 27.41$   $P < 0.001$ , mating duration  $F_{1,86} = 2.72$   $P = 0.103$ ).

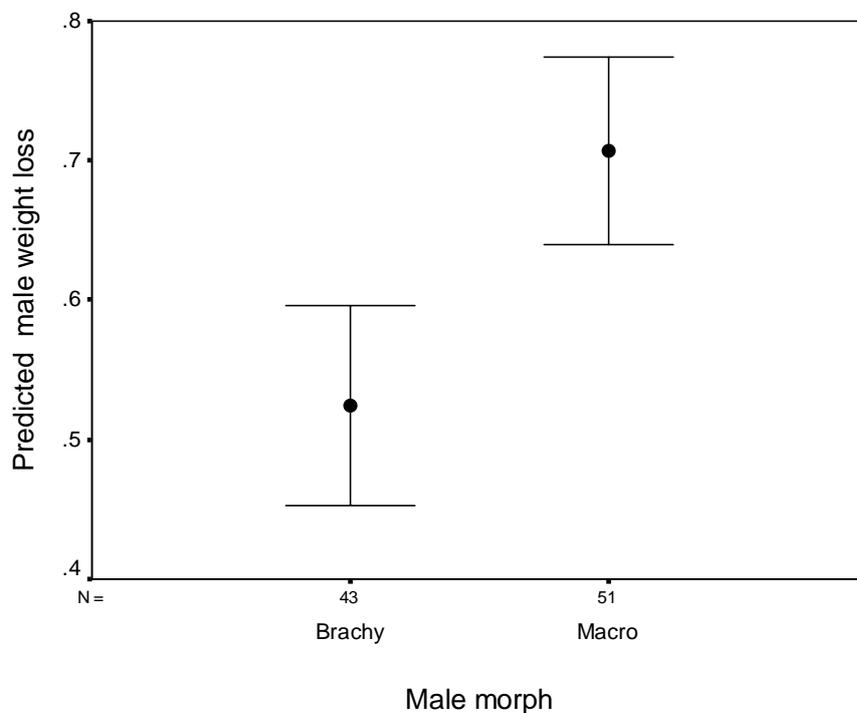
**Table 7. Variables contributing to the loss of weight during mating of the macrolabic and brachylabic males of *F. auricularia*. Dependent variable = raw male weight loss (mg) (i.e. initial weight minus final weight)**

Source	df	MS	F	Sig.
Intercept	1,83.4	0.714 <sup>a</sup>	7.432	0.008
Morph	1,82	0.496 <sup>b</sup>	5.199	0.025
Trial	5,82	0.681 <sup>b</sup>	7.14	0.000
Initial weight	1,82	0.448 <sup>b</sup>	4.698	0.033
Log fast duration	1,82	0.366 <sup>b</sup>	3.837	0.054
Pronotum width	1,82	0.102 <sup>b</sup>	1.071	0.304
Mean forceps length	1,82	0.440 <sup>b</sup>	4.617	0.035
Mating duration	1,82	0.212 <sup>b</sup>	2.229	0.139
Error		0.095		

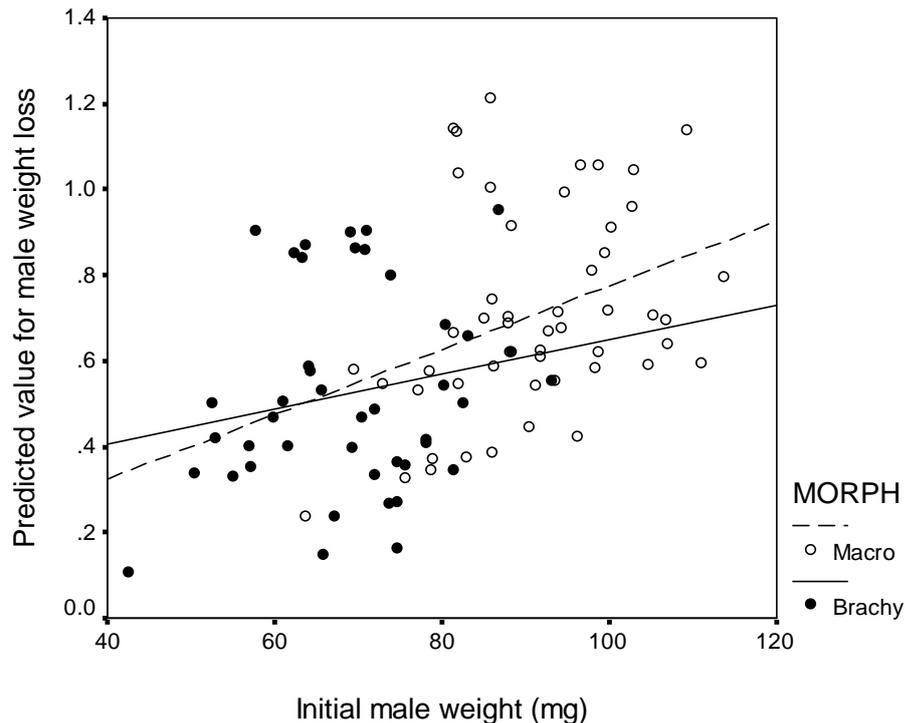
<sup>a</sup> 0.001 MS(Trial) + .999 MS(Error)

<sup>b</sup> MS(Error)

A similar analysis of mated males revealed a significant effect of male morph on the amount of weight lost by males during the trial (Table 7). Macrolabial males lost more weight than brachylabial males (Figure 20). The morph effect exists even in the presence of the pronotum width and initial mass. The inclusion of these variables account for the allometry of weight loss, which appears to suggest that larger males lost more weight (Table 7). Neither male nor females showed any detectable variation in weight change with the duration of copulation



**Figure 20.** Predicted values of male weight loss calculated from the GLM (Table 7), showing the greater weight loss experienced by macrolabial male *F. auricularia* during mating trials. Weight loss = initial weight minus final weight, hence more positive values indicate more weight lost.



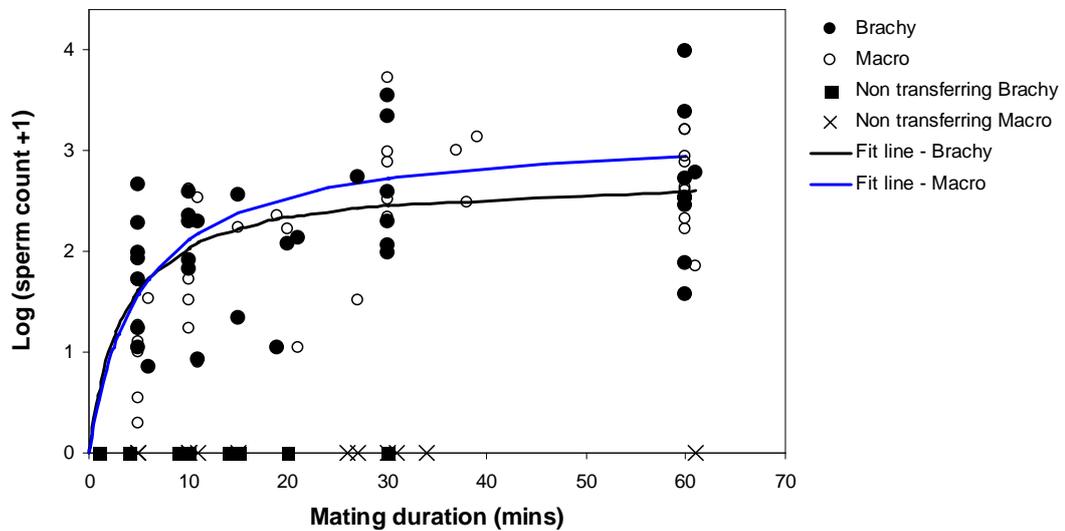
**Figure 21. Allometry of weight loss during mating trials: Larger bodied males lost more mass during mating trials, this effect is independent of the morph effect (Table 7).**

### Sperm Counts

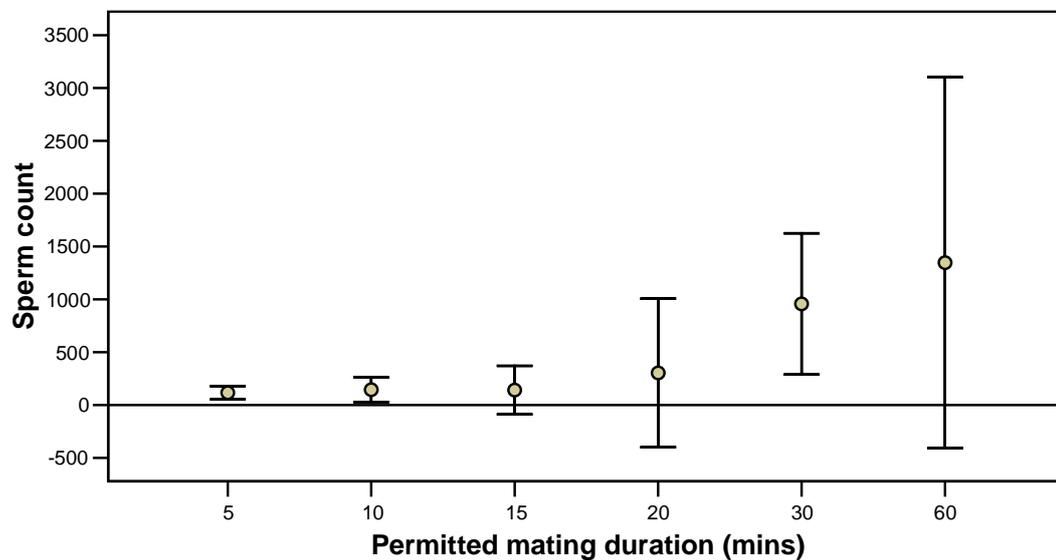
No sperm were found in the spermathecae of 20 of the 95 females that mated and from which sperm count data were obtained; this is a non-sperm representation rate of 20.6%. All 13 control females that were dissected were confirmed as having no sperm in their spermathecae (Table 8).

There were a number of male and female traits, in addition to male morph, that were hypothesised to have contributed to male sperm transfer rate. Males might inseminate larger ejaculates into larger females, and males may transfer more sperm if they themselves are in good condition. Hence in the first analysis, the number of sperm delivered was entered as the dependent variable in a GLM and male morph, mating duration, male morphological traits and female mass entered as covariates. There was no effect of male or female size, or morph (Table 9; Figure 22). Figure 22 gives the impression that sperm numbers reached an asymptote, however this is a visual artefact of the Log-transformation; a difference between one and ten has a much greater difference than between 100 and 200, for instance. Hence, although it appears that

sperm numbers level off, the actual count data suggest that numbers are steadily increasing (Figure 23).



**Figure 22.** Increase in sperm number with copula duration for macrolabial and brachylabial males. Pairs that split naturally are included alongside pairs that were split by the experimenter. The data for males that transferred sperm are fitted with non-linear regression lines assuming rectangular hyperbolae, reflecting the expected intersections with the origin and the apparent asymptotes (Brachy:  $r^2 = 0.23$ , Macro:  $r^2 = 0.53$ ).



**Figure 23.** Mean sperm number for males of both morphs, with error bars denoting the 95% confidence intervals. Sperm numbers continue to increase steadily rather than showing a plateau.

**Table 8. Sperm counts obtained from the spermathecae of mated and unmated (control) *F. auricularia* females from Brownsman Island.**

Copulation duration category (min)	Both Morphs		Brachylabic		Macrolabic	
	n	mean / SD (counts)	n	mean / SD (counts)	n	mean / SD (counts)
5	23	116.4 ± 145.7	7	131.4 ± 161.6	16	109.8 ± 143.4
10	15	144.7 ± 210.2	7	137.0 ± 139.2	8	151.4 ± 267.6
15	5	141.6 ± 184.9	3	127.3 ± 202.6	2	163.0 ± 230.5
20	4	304.0 ± 441.5	3	85.3 ± 74.3	1	960 ± -
30	19	956.8 ± 1387.8	7	938.3 ± 1395.4	12	967.6 ± 1445.4
60	12	1346.8 ± 2760.6	9	1609.1 ± 3168.1	3	560.0 ± 726.6
Control	13	0 ± 0	4	0 ± 0	9	0 ± 0
Natural Split	17	117.3 ± 265.1	8	93.5 ± 190.7	9	138.4 ± 328.1
Total	108		48		60	

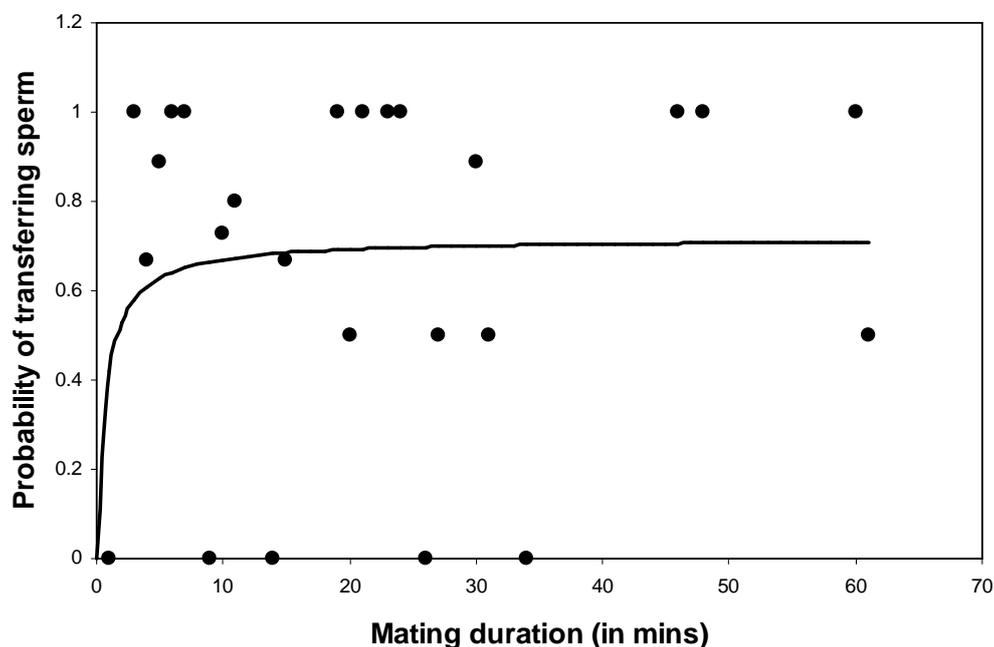
**Table 9. Variables contributing to the number of sperm counted from the spermatheca after mating of the macrolabic and brachylabic males of *F. auricularia*. Dependent variable = Log (sperm count +1).**

Source	df	MS	F	Sig.
Intercept	1,80.6	1.872 <sup>a</sup>	1.70	0.195
Morph	1,80	0.054 <sup>b</sup>	0.05	0.825
Trial	5,80	1.899 <sup>b</sup>	1.73	0.137
Initial female weight	1,80	0.010 <sup>b</sup>	0.01	0.925
Initial male weight	1,80	2.451 <sup>b</sup>	2.23	0.139
Pronotum width	1,80	1.009 <sup>b</sup>	0.92	0.340
Mean forceps length	1,80	1.534 <sup>b</sup>	1.40	0.240
Mating duration	1,80	14.987 <sup>b</sup>	13.67	0.000
Error		1.0967		

<sup>a</sup> 0.002 MS(trial) + .998 MS(Error)

<sup>b</sup> MS(Error)

In order to try to elucidate the high rate of non-sperm representation, males that mated were coded as a 0 if they did not transfer any sperm or as a 1 if they transferred one or more sperm. This dichotomous variable describes the males' ability to transfer sperm to the female and was regressed on mating duration with a logistic regression. The logistic regression showed that there was little change in the *c.* 80% probability that a male would transfer sperm, irrespective of the copula duration or whether the copulation ended naturally or was experimentally interrupted (Wald: mating duration  $\chi^2_1$  0.82,  $P = 0.366$ , end type  $\chi^2_1$  0.30,  $P = 0.582$ , Figure 24).



**Figure 24.** A non-linear regression of the probability of sperm transfer against the duration of mating, calculated using rectangular hyperbola model ( $r^2 = 0.04$ ). The probability that a male will transfer sperm does not change with mating duration after the first 10 minutes, remaining around 70%.

The logistic regression suggests that there is no behavioural indicator of a failure to transfer sperm in terms of copula duration across all matings. In males that mated but which ended without interruption from the experimenter, there was no difference in mating duration between those males that failed to transfer sperm and those that transferred one or more sperm ( $t_{18} = 0.237$ ,  $P = 0.815$ ). This suggests that there is no stimulus to male or female that indicates that a failure to transfer sperm has taken place, and further supports the logistic regression model.

## Discussion

In this chapter I have revealed that male *Forficula auricularia* transfer free sperm to the female rather than transferring a preformed spermatophore. The rate of sperm transfer seems low and does not differ between the male morphs; however macrolabic males do lose more weight during mating than brachylabic males. I have documented 20% non-sperm representation in this species, an important finding for future assays of male reproductive success

### Weight loss

The body-mass lost by males during these mating trials scales allometrically with the size of the individual; individuals lost more weight in proportion with increased underlying body-size, reflected in their pronotum width, and body-mass. Paradoxically, larger-bodied animals typically have a smaller surface area to volume ratio and tend to lose moisture at a slower rate relative to body-mass. An increased rate of water loss might result from a higher metabolic rate in the larger individuals, but there is no evidence to test this hypothesis or an obvious biological reason why this should be.

Defecation is likely to be another major source of weight loss in the animals in this trial and the effect of fasting period on weight loss supports this view. Larger individuals are likely to have larger guts, but there is no reason to expect that gut capacity should increase disproportionately with body-size. If anything, larger individuals are likely to divert relatively more resources into developing larger forceps and so are likely to have fewer resources to increase the size of their larger alimentary tract.

Access to food was only limited after trials had begun and the realisation that defecation was a major source of weight change. From our data it seems that only with defecation and respiratory water loss minimised is the variance in the overall weight change likely to be reduced enough to reveal the more subtle changes in weight due to insemination.

Males of greater body-mass for a given pronotum width are either of better condition or have a greater quantity of matter in their gut. However, no interaction was found between initial weight and pronotum width to suggest that condition (or a heavier gut content) effects ejaculate size or sperm content.

*Difference between morphs*

Macrolabial males are, on average, larger than brachylabial males and thus prone to losing more weight during a trial. However, there was also a significant effect of morph per se on male weight loss, such that for a given body-size, macrolabial males lost more weight than brachylabial males. This effect supports the hypothesis that macrolabial males are delivering a larger ejaculate, but that apparently there is a discontinuity in the scaling with body-size. However, there was no detectable morph effect on the weight increase in females, or in sperm counts, and this rather cautions against an enthusiastic interpretation of these data. Brachylabial males appear to be mating for the same period of time so they were not limited in the amount of time they have access to the female.

One possible explanation for the increased weight loss of mating males in the major morph is that macrolabial males are more active during the trials and so lose more water through increased respiratory rate; it is likely that macrolabials are more active given that, as previously suggested, the courtship time is longer than for brachylabial males.

Forceps length was found to influence only the weight change of males that copulated and not of the females that they copulated with (Table 7). This suggests that forceps length does not influence ejaculate size. It is possible that the analysis has simply captured some of the variance associated with body-size: The dimorphism creates a wider spread of the forceps length data relative to the body-size data and the difference between large-bodied macrolabial males and small-bodied brachylabial males is detected as an effect of forceps length.

*Copula duration*

No effect of copula duration on weight change was found (Figure 19). This may be in part due to the high variance in body-mass within some of the mating duration categories where sample size was smaller (Table 6 & Figure 18). There was also no correlation between weight change and sperm count, which did correlate strongly with mating duration (Table 9). The inconsistency in the effects that influenced male and female weight change and the dissociation with sperm count suggest that this measure is unfortunately not an effective metric of sperm transfer in this species.

## Mating without transferring sperm

Previously unpublished work found that males from Brownsman had a failure rate of sperm transfer to females of 78% (21/27) and WWO of 80% (8/10) (Tomkins, unpubl. data). At the time it thought this was because the females were from a different population, from the Wirral, and that there was some kind of incompatibility during copula that was causing the failure (J Tomkins pers. comm.). These early results, although doubtful at the time support the finding that non-sperm representation is a common occurrence in *F. auricularia* even though the rates in the previous experiments were much higher.

García-González (2004) has recently analysed the frequency of failed matings across a number of insect taxa. His meta-analysis has shown that the failure to transfer sperm in insects that have otherwise mated normally is of a similar magnitude to the study we report here. In his meta-analysis the range of values for the failure to transfer sperm in single matings was from 0 to 60 % with a mean of about 20%. The failure of males to transfer sperm during otherwise normal matings has ramifications for the estimation of paternity. In studies of  $P_2$  the incidence of failed sperm transfer will lead to distributions of  $P_2$  that are trimodal, assuming sperm mixing takes place when both males' sperm is represented. The trimodality arises because, in a proportion of cases, either the first or the second male fails to inseminate the female and sperm precedence is either 100% for the first male or 100% for the last male (Corley *et al.* 2006). With a rate of non-sperm representation of 20%, on average females will be left with no sperm after two matings on  $(0.2*0.2 = 0.04)$  4% of occasions. Hence in such studies, extreme values of  $P_2$  will be encountered quite regularly as a result of this infertility in this species: The second male will not face any competition from the first on  $(0.8*0.2 = 0.16)$  16% of occasions and, as the reciprocal will be equally frequent, thus 32% of  $P_2$  values would be either zero or one.

The probability that male did not transfer sperm during copulation did not change with copula duration. This is very interesting as non-sperm representation is evidently not due to time constraints. It seems likely to be due to male infertility, either mechanical, in terms of genital dysfunction or in terms of sperm manufacture.

It would be interesting to consider male non-sperm representation as a male adaptation rather than as a reproductive failure. This seems immediately counter-intuitive since

males need to transfer sperm in order to have any offspring. However, if males are sperm limited in some way, prudent ejaculation strategies might be adaptive (Wedell *et al.* 2002). In many species virgin females are considered to be of greater value to mating males than females that have mated many times, but this may not be true for the European earwig. Observations over a number of years have found that singly mated females rarely, if ever, produce viable eggs in the lab. It now seems clear that this is because females rarely receive enough sperm from a single mating. Hence, males mating with a virgin female are almost certain to fail to sire her clutch of eggs. Not only is the number of sperm inseminated typically low, but the females also mate polyandrously for months prior to egg-laying. Nevertheless such an adaptationist argument does raise the question of why a male would mate at all with a female of such low value that sperm was withheld. One answer might be that mating is a certain way of mate-guarding (assuming that at some time the male does transfer or has previously transferred some sperm). This is more plausible in earwigs, which form pairs in burrows for some weeks before egg-laying.

Conversely, non-sperm representation may result from female suppression of the delivery of the ejaculate by the male. Such suppression of ejaculate release could allow a female to choose the males that she accepts sperm from without returning an honest signal of her choice. This may allow the female to increase the extra-pair paternity in her offspring without advertising the fact to her guarding male. This would be particularly effective when sneaking males are only able to access the female infrequently compared to the access of the guarding male. Hence, females may be able to realise a benefit from increasing the genetic diversity of their offspring whilst retaining the benefits of being guarded by a single male. If the transfer of sperm is under female control, it does raise the possibility that females are exercising some assessment of the male during copula (Eberhard 1996). Further experiments are required to determine whether non-sperm representation in *F. auricularia* is a male-female interaction, under female control or a repeatable failure of the male.

### Dynamics of sperm transfer

Male *F. auricularia* deliver free sperm at a steady rate rather than aggregated in a spermataphore. This, and the fact that the number of sperm transferred per ejaculate is unusually low, suggests that either few sperm are required to fertilise an entire brood, or

individuals must mate for a very long time, or that they copulate incessantly. Contrastingly, observations of another earwig species suggest that large numbers of sperm are required to maximise brood fertilisation (Kamimura 2003). Furthermore, a spermatheca dissected from a single adult female collected from Bideford (Devon, UK) was found to be packed with sperm (pers. observation). Although no actual count was made, there was at least one order of magnitude more sperm than observed in the single copulation trials presented here. This observation is supported by others (Tomkins pers. comm.) and suggests that the single-copulation sperm counts are small compared to *in natura* whole-season sperm counts and that females are this unlikely to be limited to so few sperm. This lends support to the hypotheses of longer and more frequent copulations: The longest recorded copulation for *F. auricularia* is 7 hours (Tomkins, unpubl. data).

The steady rate and the small total number of sperm transferred also confer a very slow rate of transfer. Furthermore, this rate does not differ between the morphs. The slow transfer rate may be due to the mechanical limitations of extreme genital morphology driven by sexual selection: Extremely long virgae / aedeagi (the male phallus) have been documented in dermapterans (Kamimura 2000; Kamimura 2004). The rate of sperm transfer is likely to be physically limited where this organ is longer and more narrow. Conversely, male weight loss is apparently dependent on morph, being greater in macrolabic males. This suggests that the morphs differ in the quantity of other ejaculate substances that they transfer.

### Timing of mate trials

In the wild *F. auricularia* mates from August until the males die off or the females cease to be receptive with the onset of egg brooding, usually in November. The mating trials in this study were carried out towards the end of this mating period and a seventh trial, carried out on 25th November 2004 was aborted as none of 26 females copulated during the trial even though males were observed to initiate courting behaviour. It seems probable that the incidence of pairs that did not copulate was higher in this study than it might have been had it been carried out earlier in the year.

Further support for this is given by the observations of the aforementioned Bideford female, which was collected on 28th August 2004 and stored in isolation from any males. The spermatheca was dissected 10 days after collection, at which time many of

the sperm observed were still motile. The number and the state of the sperm in this female suggest that the mating season started some time before the mating trials were carried out.

### Future work

No morph-specific differences were found in the number of sperm or rate of transfer in this study. The difference between the morphs in weight change with copulation advocates parallel studies of accessory organ size. Sperm length may also be an important component of sperm competition success, with theoretical models suggesting that increasing SC will lead to longer sperm (Simmons et al. 1999b). Furthermore, we might expect there to be a correlation across populations in the complexity of the female tract and the relative size of the male's testes in response to sperm competition.

## **Chapter 6: Concluding Remarks**

In the work presented in this thesis, several findings of considerable importance have been added to this field of study. The dramatic difference previously observed between populations from the Farne islands and the adjacent mainland was comprehensively surveyed from island and mainland sites around most of the UK. The dimorphism was found to be present on many of the coastal islands sampled and quantification of the morph ratio confirmed the general monomorphy of mainland UK populations and the extreme variability of the islands. Furthermore, population density on these islands was found to correlate with this population variation in ESS threshold; a finding that supports the hypothesis that this population variation is the result of adaptation to local conditions.

Versatile molecular tools were developed, reported in chapter 3 and these were subsequently used to investigate the phylogeographical origins of these intriguing and potentially useful dimorphic populations. Understanding the genetic affinities between these populations is essential for correcting for phylogenetic bias if the population variation in morph is to be harnessed to perform within-species comparative studies of sperm competition risk associated with the dimorphism.

This leads to a central question in this thesis: Do island populations of *F. auricularia* differ with respect to the male dimorphism because of phylogenetic divergence or is it due to local adaptation to population specific environmental conditions? The switch-point defining the dimorphism has been shown to be heritable (Tomkins 1999). Levels of within population genetic variation in switch-point have been found in other arthropod species (Unrug et al. 2004) and the observation of males with brachylabic forceps length and body size expected to be macrolabic, suggests that such variability remains within these island populations of *F. auricularia*.

The levels of genetic diversity observed here are not inconsiderable, yet little differentiation between populations was found across hundreds of kilometres. Such panmixia is not unheard of amongst species of insect that are similarly expected to show signs of isolation between populations; A genetic study of Dawson's burrowing bee, *Amegilla dawsoni*, showed precisely this (Beveridge & Simmons 2006). *A. dawsoni* might be expected to show high levels of inbreeding due to the intensity of local mating as female emerge from brood chambers. Similarly *F. auricularia* is not known for

long-range dispersal, although this has not been studied directly. Although winged, *F. auricularia* has only rarely been documented to fly (Fulton 1924) so it seems likely that the barrier presented by the sea between the islands would have greatly restricted gene flow between the islands. The genetic data gathered showed evidence that the islands support substantial populations with limited gene flow, probably because of their high population density and remote geography. No evidence was found of multiple postglacial invasions to support the hypothesis that the dichotomy between islands and mainland sites with respect to the dimorphism was the result of isolated relicts of distinct clades or subspecies. These findings support the hypothesis that the difference between populations in ESS threshold is the result of adaptation to local conditions. However, a more comprehensive genetic survey is required to confirm this.

The choice of the type of marker used was driven by the intention to maximise potential returns from research funding and reduce effort in developing and optimising alternative markers. As such, microsatellites are suitably versatile markers and have been successfully used in phylogeography studies across similar geographic ranges (Koskinen et al. 2002; Zeisset & Beebee 2001).

The approach was too ambitious in trying to use a single type of marker for a number of different applications; large- and small-scale phylogeography and population genetics, in addition to within-population paternity analysis. Whilst this approach is theoretically possible, it would have been more practical and time effective to have diverted effort at the early stages of the project to sequence based phylogeography. This complementary approach could have provided as much or greater resolution of the population affinities within the U.K. whilst requiring fewer individuals per population to do so. This in turn would have reduced the need to gather such extensive microsatellite data and may have permitted time to comprehensively sample or resample the key dimorphic island populations of the Forth and Farnes. This dual marker approach is common in current literature (Rokas et al. 2003; Trizio et al. 2005) and the benefits from not placing all reliance on a marker type and its associated laboratory technique seem to outweigh the extra initial investment.

Finally, in chapter 5 a study was presented that extended existing work in search of adaptations in *F. auricularia* to sperm competition. Two methods for assaying ejaculate size were employed; change in body weight during copulation and post-copulatory sperm counts. In this study I have demonstrated that *F. auricularia* transfer free sperm; the first demonstration of the mode of sperm transfer in a dermapteran. This differs fundamentally from other insects that produce large discrete ejaculates such as the spermatophores of Orthoptera (Boldyrev 1915) and Lepidoptera (Gage & Baker 1991). From the perspective of the male, concentrating large numbers of sperm into ejaculates or spermatophores may increase the chance of ensuring the paternity of the offspring of individual females. However, transferring sperm incrementally is likely to reduce wastage of sperm when copulation initiates but the transfer of sperm is not successful. Furthermore, fine control of sperm transfer is likely to allow males to distribute sperm amongst an optimally large number of females, maximising reproductive success.

The rate of non-sperm representation observed in the study in chapter 5 was around a fifth of all copulations. Statistically, there appears to be no link between the way in which copulations ended and the probability that the male transferred sperm; copulating pairs that were experimentally separated did not differ in their propensity to transfer sperm than pairs that were permitted to end copulation naturally. It might be expected that if copulations are going to terminate without sperm transfer due to some incompatibility between the pair, termination is likely to occur early on rather than after much time has been wasted. Such early failures with no sperm transfer are unlikely to mate long enough to be experimentally separated. Therefore, it might be expected that a relatively greater proportion of naturally ending copulations would exhibit non-sperm representation, although this was not found.

The occurrence of non-sperm representation in the females' spermathecae after copulation may be an indicator of male potency. There was no difference detected in the mating duration achieved by these males and those that did transfer sperm so it seem unlikely that, if this is due to impotency, females are unable to detect it. Alternatively, this lack of sperm transferred to the female maybe the result of cryptic female choice, signs of which have been observed in *F. auricularia* (Tomkins & Simmons 1998). Females may only permit sperm to be transferred by desirable males yet do not give any indication of this to the male. Evading males in order for females to avoid harassment

by males has been suggested in other species (Shine et al. 2005). In *F. auricularia*, permitting males to copulate but not transfer sperm may be a similar tactic; perhaps it is better to spend time copulating and not to waste energy or risk injury as a result of resisting courtship.

In light of this, it would be interesting to know what quantities of non-sperm components of ejaculate are transferred in such copulations. Males may be transferring large ejaculates whilst not transferring sperm. Given the single opening and relatively simple, sclerotised morphology of the female spermatheca in *F. auricularia*, sperm of the first male to copulate may not have a great chance of successful fertilisation; fertilisations will be allocated on a 'last in, first out' basis (Simmons 2001). Females are likely to acquire more sperm with time during the breeding season, during late spring and summer. Therefore, males should retain most of their sperm for the most opportune times in this season when investing sperm in ejaculates offers the greatest return in reproductive success. As a means of avoiding of this sperm competition, males may transfer larger, low-sperm ejaculates to virgin females early in the season and retain smaller sperm-concentrated ejaculates for females with relatively full spermathecae. Similarly, when females' spermathecae are relatively full later in the season, males will benefit from squeezing the maximum number of sperm into a minimal volume. For this form of control over ejaculates to happen males must be able to assess the copulatory history of females.

Copulation weight-loss is confounded by much greater weight changes, presumably due to respiratory moisture loss and defecation. Thus, this obvious measurement is not a practical metric for overall ejaculate size, as clearly shown in this study. Alternative approaches could involve labelling components of the seminal fluid of males and quantitatively detecting these components in the female after copulation. One such study using radioisotopes to label the ejaculate of male *Scatophaga* dung flies and successfully traced the ejaculates in the female tract after copulation (Simmons et al. 1999a).

Despite ejaculate mass not being discernible from non-copulatory weight loss, macrolabic males were found to lose more weight than brachylabic males during trials. In contrast, no difference was found between the morphs in the number of sperm transferred to the female spermathecae. The weight loss of the males was not detected

as a weight gain in the females, suggesting that the greater weight loss of the macrolabidic males may be in part related to increased activity. This hypothesis is supported by previous findings that, prior to copulating, macrolabidic males court females for longer than brachylabidic males (Tomkins & Simmons 1998). Alternatively, macrolabidic males may be producing ejaculates of greater mass but similar sperm content to the brachylabidic males. Support for this hypothesis could be found through an examination of the male accessory glands. These organs produce non-sperm components of the ejaculate and so may show a corresponding difference in size between the morphs.

This study established that the European earwig transfers free sperm at a steady rate rather than aggregated as in the spermatophore of some other insects. Furthermore, the number of sperm per ejaculate is relatively low, supporting the hypothesis that copula duration in *F. auricularia* is long and occurs frequently in order for females to accrue sufficient sperm to successfully fertilize their eggs. These findings are important to further investigation of sperm competition in this species.

### **Further work**

The versatility of the microsatellite markers presented here has yet to be fully demonstrated. The genetic variation in these focal populations show promise that they could be used to analyse paternity in individual broods; an approach used previously to compare the male reproductive success with multiply-mated females (Simmons 2001).

Preparatory work for two such studies was carried out during the course of this thesis. Both studies were attempts to measure the reproductive success of males of the different morphs, firstly in clutches of eggs laid *in natura* and secondly from controlled two-male mating experiments with virgin specimens as used in chapter 5.

In the first study, guarding males were collected with their paired female and her clutch of eggs, from Farne island populations. A record was made of pairings between individuals and the proximity to other pairs or unpaired individuals (which were also collected). Males in these collections were immediately frozen for subsequent microsatellite genotyping whilst females were kept alive to hatch their eggs. Brood hatching success rate was high and the females and offspring were then frozen along with the males for subsequent paternity analysis.

In the second study, laboratory mate-trials were carried out with virgin specimens collected at the same time as those used in chapter 5. Two males were presented to each female; an initial male for a period of a week, to emulate a guarding male, and a second male which were permitted to copulate with the female once, the duration of which was recorded. P<sub>1</sub> and P<sub>2</sub> males (first and second mating males) were of randomly allocated morph, such that the effect of morph on reproductive success could be tested. No brood was successfully hatched in this second study; mating trials were carried out after the end of the trials carried out in chapter 5, which is likely to have been too late in the season.

In both cases, the method for solving the low genotyping success rate was found too late in the project to complete these studies. This high failure rate of genotyping from the frozen adult earwig specimens may have been due to the activity of microorganisms that are naturally associated with the earwigs in life, but that rapidly degrade the tissues after cellular death. As generalist omnivores, earwigs regularly feed on decaying animal matter and detritus; the gut fauna of an earwig is, therefore, likely to be rich in microbes capable of degrading animal tissues. Indeed the implications of the feeding habits of earwigs are evidenced by the various parasites and parasitoids that attack them by being ingested by the earwig; e.g. tachinid flies, nematodes and protozoan gregarines (Brindley 1918; Kuhlman 1994).

Whilst the most practical solution for working with earwig DNA is clearly to prepare extractions from live tissue, if this hypothesis of microbial degradation is true, there is hope that extractions made from the collected eggs or early instar nymphs may be more successful than from adults that have accrued heavier parasite loads. The potential for juvenile earwigs to be frozen and successfully genotyped has yet to be tested and will not entirely resurrect these two studies given that the adults are likely to be problematic. However, this approach may be of practical use in any repeats of these reproductive success studies.

Additional genetic data are required to supplement those presented here to improve the resolution of the affinities between UK populations. This task is now considerably more tractable as a result of the genetic markers and methods of DNA curation developed during this work. Furthermore, in light of this work it seems that that the most effective approach to move forward would be by applying both the microsatellite

markers detailed here and to extend the use of a previously published mtDNA marker. The two types of genetic marker would permit the problem to be approached from opposite directions; the microsatellites resolving the local affinities between the focal island and adjacent mainland populations in the Forth and Farnes regions, and the mitochondrial markers integrating this fine-scale structure with the broader post-glacial European phylogeography. The improved resolution in the estimates of genetic distance between the focal populations that such extension of this work can provide will be essential for quantitatively partitioning the phylogenetic and local environmental factors that explain inter-population variation in traits associated with sperm competition.

The work presented in this thesis substantially increases the standing of *F. auricularia* as a particularly special model for the study of sexual selection through sperm competition. The relatively commonplace nature, resilience to laboratory rearing in large numbers and annual reproductive cycle of this insect, in conjunction with the singular variability in its male dimorphism, lend *F. auricularia* to natural experiments exploiting the population variation ESS threshold and the associated risk of sperm competition.

## Appendices

- A. Specimens collected
- B. Population density estimates through trapping
- C. Published work
- D. DNA extraction
- E. Processing raw microsatellite data from a *Beckman Coulter* DNA sequencer
- F. Summary of genetic data set

## Appendix A. Specimens collected

Site	Site Code	Site type	Region	Country	Macrolabic	Brachylabic	Female	Morph Ratio	Sex Ratio	OSGB X (m)	OSGB Y (m)	Latitude (°N)	Longitude (°E)	Year
St. Agnes	AGN	Island	Scillies	England	35	84	144	0.29	0.45	88432	8205	5.988	57.020	2002
Annet	ANN	Island	Scillies	England	63	48	114	0.57	0.48	86426	8401	5.697	57.215	2002
Bass Rock	BAS	Island	Forth	Scotland	56	213	292	0.21	0.48	360245	687380	-2.639	56.078	
Big Harcar	BHR	Island	Farnes	England	33	188	116	0.15	0.65	423965	638485	-1.619	55.639	
Bideford	BID	Mainland	Bristol Channel	England	1	166	202	0.01	0.45	243174	127692	-4.237	51.027	2002
Ballycastle	BLY	Mainland	Ireland (north)	Ireland	2	6	5	0.25	0.62	124534	601835	-6.333	55.234	
Brownsman	BR	Island	Farnes	England	206	1396	991	0.13	0.62	423765	637840	-1.622	55.634	
Bryher	BRY	Island	Scillies	England	19	38	81	0.33	0.40	87653	14673	4.798	51.021	2002
Craigleith	CGL	Island	Forth	Scotland	142	679	858	0.17	0.49	355303	686947	-2.718	56.073	2002
Cramond Mainland	CML	Mainland	Forth	Scotland	0	100		0.00		318810	677045	-3.301	55.980	2002 & 2005
Cramond Island	CRM	Island	Forth	Scotland	0	29	41	0.00	0.41	319620	678550	-3.289	55.993	
Greater Cumbrae	CUM	Island	Clyde	Scotland	3	105	228	0.03	0.32	216783	657353	-4.921	55.775	2002
Dalgety Bay	DAL	Mainland	Forth	Scotland	0	10	10	0.00	0.27	316442	683691	-3.341	56.039	2002 & 2005
Dunbar	DUN	Mainland	Forth	Scotland						365165	678725	-2.559	56.000	2005
East Wideopen	EWO	Island	Farnes	England	77	401	293	0.16	0.62	422570	635935	-1.642	55.616	
Eyebroughy	EYB	Island	Forth	Scotland	19	153	96	0.11	0.64	349491	686306	-2.811	56.067	2002
Falmouth	FAL											-5.056	50.158	2005
Fidra	FID	Island	Forth	Scotland	23	331	432	0.06	0.45	351262	686908	-2.783	56.073	2002
Fisherton	FSH	Mainland	Clyde	Scotland	1	18	25	0.05	0.40	229094	617856	-4.701	55.425	2002
Girton	GIR											0.094	52.229	2005
Gugh	GUH	Island	Scillies	England	17	69	95	0.20	0.46	88976	8276	6.091	57.078	2002
Hilbre	HIL	Island	Wirral	England	8	155	194	0.05	0.39	318477	387981	-3.226	53.382	2002
Inchcolm	ICM	Island	Forth	Scotland	0	244	342	0.00	0.35	319079	682612	-3.299	56.030	2002
Inner Farne	IFN	Island	Farnes	England	84	592	670	0.12	0.50	421800	635935	-1.654	55.617	
Inch Garvie	IGV	Island	Forth	Scotland	10	203	256	0.05	0.45	313599	679532	-3.386	56.001	2002
Inchkeith	IKH	Island	Forth	Scotland	4	40	101	0.09	0.30	329211	682847	-3.136	56.033	2002
Inchmickery	IMK	Island	Forth	Scotland	17	107	173	0.14	0.42	320663	680557	-3.273	56.011	2002
Illiswilgig	IWG	Island	Scillies	England								4.527	50.966	2005
Knoxes Reef	KRF	Island	Farnes	England	110	577	334	0.16	0.67	422185	636450	-1.648	55.621	
Krakow	KRK	Outgroup	Poland	Poland	6	13	13	0.32	0.59			19.988	50.066	
Lamb	LAM	Island	Forth	Scotland	10	223	187	0.04	0.55	353484	686595	-2.747	56.070	2002
Lindisfarne	LDF	Island	Farnes	England	0	0	0			413000	642000			
Lee	LEE	Mainland	Bristol Channel	England	1	119	111	0.01	0.52	246941	145735	-4.191	51.190	2002
Longstone End	LGE	Island	Farnes	England	96	169	125	0.36	0.67	424690	638910	-1.608	55.643	
Longniddry Bents	LND	Mainland	Forth	Scotland	2	23	47	0.08	0.35	344132	677390	-2.896	55.986	2002 & 2005
Lundy	LUN	Island	Bristol Channel	England	21	117	152	0.15	0.47	213786	144409	-4.664	51.168	2002
May	MAY	Island	Forth	Scotland	38	236	257	0.14	0.52	365500	699500	-2.556	56.187	
Middlemill	MML	Mainland	Pembrokeshire	Wales	0	6	5	0.00	0.55	181559	224598	-5.174	51.877	2002

Site	Site Code	Site type	Region	Country	Macrolabic	Brachylabic	Female	Morph Ratio	Sex Ratio	OSGB X (m)	OSGB Y (m)	Latitude (°N)	Longitude (°E)	Year
Merens	MRN	Outgroup	France	France	6	5	11	0.55	0.50			1.836	42.654	
Merry Maidens	MYM	Mainland	Scillies	England	13	127	120	0.09	0.54	143306	24637	-5.745	52.056	2002
North Berwick	NBW	Mainland	Forth	Scotland	0	0	0			355465	685180	-2.715	56.057	
North Wamses	NWM	Island	Farnes	England	100	710	443	0.12	0.65	423405	638465	-1.628	55.639	
Newton Links	NWT	Mainland	Farnes	England	0	0	0			416394	635031	-1.740	55.609	
Ramsey	RAM	Island	Pembrokeshire	Wales	0	27	42	0.00	0.39	170655	224067	-5.332	51.868	2002
Rootiagh	ROO	Mainland	Ireland (south)	Ireland	2	1	4	0.67	0.20	-49625	320921	-8.643	52.599	2003
Rosevear	RVR	Island	Scillies	England	0	2	6	0.00	0.25	83910	5975	4.884	55.077	2005
St. Andrews	SAN	Mainland	Fife	Scotland	0	0	0			350700	716600	-2.798	56.339	2005
Skokholm	SKK	Island	Pembrokeshire	Wales	0	8	10	0.00	0.44	173910	205050	-5.272	51.698	2002
Skomer	SKM	Island	Pembrokeshire	Wales	3	54	17	0.05	0.75	172752	209385	-5.292	51.737	2002
Silecroft	SLC	Mainland	NW England	England	0	13	21	0.00	0.38	313128	482279	-3.333	54.229	2002
St. Martin's	SMR	Island	Scillies	England	6	15	29	0.29	0.42	92700	15900	5.530	51.087	
St. Justinian	STJ	Mainland	Pembrokeshire	Wales	0	4	12	0.00	0.25	172430	225170	-5.307	51.878	2002
St. Mary's	STM	Island	Scillies	England	56	106	171	0.35	0.49	91109	11086	5.237	50.672	2002
Staple	STP	Island	Farnes	England	125	755	480	0.14	0.65	423770	637545			
Staynall	STY	Mainland	NW England	England	0	40	44	0.00	0.44	335577	444752	-2.980	53.895	2002
South Wamses	SWM	Island	Farnes	England	103	796	443	0.11	0.67	423510	638265	-1.626	55.637	
Talacre	TAL	Mainland	Wirral	Wales	1	37	27	0.03	0.58	312475	384810	-3.315	53.353	2002
Tresco	TCO	Island	Scillies	England	28	96	115	0.23	0.51	88972	15400	4.995	51.075	2002
Waren Mill	WML	Mainland	Farnes	England	0	82	51	0.00	0.61	414500	634385	-1.770	55.603	
West Wideopen	WWO	Island	Farnes	England	284	335	558	0.46	0.53	422285	635970	-1.646	55.617	
Bamburgh		Mainland	Farnes	England	0	0	0			418060	634930			
Seahouses		Mainland	Farnes	England	0	0	0			421182	632388			

## Appendix B. Population density estimates through trapping

Site	Site Code	Site type	Density (mean earwigs per trap)	No. of traps returned	Trap days	Date traps set
Bass Rock	BAS	Island	40.1	14		
Big Harcar	BHR	Island	28.1	12		
Brownsman	BR	Island	72.0	36		
Craigleith	CGL	Island	12.5	23	24	09-Sep-02
East Wideopen	EWO	Island	33.5	23		
Eyebroughy	EYB	Island	2.3	12	24	09-Sep-02
Fidra	FID	Island	4.6	19	24	09-Sep-02
Gugh	GUH	Island	0.0	4	15	29-Aug-02
Inchcolm	ICM	Island	5.1	23	17	12-Aug-02
Inner Farne	IFN	Island	25.9	54		
Inch Garvie	IGV	Island	2.4	16	24	09-Sep-02
Inchkeith	IKH	Island	0.1	22	24	09-Sep-02
Inchmickery	IMK	Island	7.7	16	24	09-Sep-02
Knoxes Reef	KRF	Island	28.4	36		
Lamb	LAM	Island	8.3	12	24	09-Sep-02
Lindisfarne	LDF	Island	25.6	10		
Longstone End	LGE	Island	32.5	12		
Lundy	LUN	Island	0.2	32	10	24-Aug-02
May	MAY	Island	19.0	28		
North Berwick	NBW	Mainland	0.0	6		
North Wamses	NWM	Island	52.2	24		
Ramsey	RAM	Island	0.2	19	20	20-Aug-02
Skokholm	SKK	Island	1.5	12	10	04-Sep-02
Skomer	SKM	Island	0.2	16		21-Aug-02
St. Mary's	STM	Island	0.8	9		
Staple	STP	Island	56.7	24		
South Wamses	SWM	Island	58.3	23		
Tresco	TCO	Island	0.0	16	14	27-Aug-02
Waren Mill	WML	Mainland	0.0	12		
West Wideopen	WWO	Island	26.2	45		
Bamburgh		Mainland	0.0	12		
Seahouses		Mainland	0.0	12		

## Appendix C. Published Work

Population density drives the local evolution of a threshold dimorphism

The following is a paper co-authored by J. L. Tomkins and G. S. Brown, published in the journal *Nature* (2004, **431**, p1099-1103) that includes data presented in chapter 2.

## Appendix D. DNA extraction

### *Notes on dissection of F. auricularia for DNA extraction*

Cuticle and eye pigments can inhibit PCR (N. LeBas pers. comm.) and extractions of *F. auricularia* that were visibly high in pigments tended not to produce a scorable PCR product. Specimens were dissected by first partially severing the head from the thorax, such that the gut could be drawn out of the body cavity with the head using fine tipped forceps. The ventral integuments of the abdominal carapace were prised away with the tip of scalpel and the forceps of the earwig were then easily torn away with muscle blocks and the lower portion of the gut attached. The forceps muscle and the thorax and legs were severed and used for DNA extraction. This method minimised the amount of contamination from the gut or exoskeleton.

This approach was found to be inappropriate for specimens that have been desiccated, as when stored in organic solvent for an extended period of time. In these the soft muscle tissues are hardened and adhere the abdominal cuticle to the forceps. In this scenario, the abdominal cuticle containing the muscle tissue can be ‘snapped’ off from the forceps. After immersion in the digestion buffer (TNE buffer) for a minute the muscle softens and can be scraped from the abdominal cuticle and returned to the buffer with out the cuticle. Such specimens produced extracts of limited DNA quality.

All dissecting instruments were rinsed in water before being flamed with ethanol to sterilise them. Two sets of instruments were used to allow time to cool before contacting tissue. Specimens and the dissected tissue are kept on ice throughout the dissection process and then extracted immediately thereafter or frozen for later DNA extraction.

*DNA Extraction*

This protocol is a generic ‘salting out’ method of DNA extraction and was developed from several published sources (Aljanabi & Martinez 1997; Sambrook *et al.* 1989; Sunnucks & Hales 1996). This version of the protocol was communicated to the author by G. N. Stone and R. J. Atkinson (Atkinson 2000).

***Materials:***

Use DNA pipettes (not DNA clean PCR pipettes)

1.5 ml eppendorfs (5 per extraction)

Tube rack – 10 x (1 centrifuge load; 12, 18...)

100% Ethanol stored at -20°C

70% Ethanol stored at -20°C

Vortex mixer

Countdown Timer

Bench centrifuge

5M NaCl

***Buffer Recipes***

TNE = (“Squishing buffer”) 10mM TrisHCl  
pH8.2, 1mM EDTA, 25mM NaCl

1ml 1M TrisHCl pH8.2 + 0.5ml 0.2M  
EDTA + 0.5ml 5M NaCl, then make up to  
100ml in  $\delta\text{H}_2\text{O}$ .

SDS = Sodium Dodecyl Sulphate

Proteinase K

milliQ water

	per extraction	45x mix
TNE	400 $\mu\text{l}$	18ml
SDS	25 $\mu\text{l}$	1.125ml
proteinase K	10 $\mu\text{l}$	450 $\mu\text{l}$

***Proteinase-K digest***

For each extraction prepare five 1.5ml eppendorfs (centrifuge tubes). Label the fourth and fifth tubes with complete information (specimen ID), abbreviate info on the other 3.

To each tube add 100µl TNE and ~1 mm<sup>3</sup> of specimen and grind/squish.

Add 400µl TNE, 25µl 10%SDS, 20µl proteinase-K (10mg/ml) and vortex briefly.

Leave to digest: 3hrs @ 55°C or overnight @ 37°C (shaking).

***Removal of non-DNA material***

Spin a 13000-rpm for 5 minutes. Transfer supernatant to 2<sup>nd</sup> eppendorf and discard the pellet of cellular debris.

Add 170µl 5M NaCl, shake hard for 20 seconds by hand, stand on ice for 5 minutes then spin at 13000 rpm for 5 minutes

Transfer clear supernatant to the 3<sup>rd</sup> eppendorf, leaving behind the precipitated salt (and protein).

Stand on ice for 5 minutes, centrifuge at 13000 rpm for 5 minutes and transfer supernatant to 4<sup>th</sup> eppendorf (removing more of the remaining salt and protein)

***Ethanol Wash***

Add 100% Ethanol (from -20°C freezer) to the 4<sup>th</sup> eppendorf at twice the volume of the contents. Mix gently and spin immediately at 13000rpm for 5 minutes (This precipitates only DNA and prevents precipitation of any remaining salt or protein etc)

Discard as much of supernatant as possible with 1ml pipette (blue tip).

Add 200µl of 70% Ethanol (from freezer) to wash DNA pellet. Spin at 13000-rpm for 5 minutes.

Discard as much of supernatant as possible without disturbing the pellet and air dry (aided by a heating block at 40°C)

Re-suspend DNA in 100µl milliQ distilled water (and a short burst of vortexing); remove 30µl of this to the 5<sup>th</sup> eppendorf to serve as a working aliquot. Freeze DNA samples at -20°C for storage.

#### *Storage of F. auricularia*

Genotyping reported in this thesis was mostly carried on DNA extracts made from specimens that had been stored at -20°C (from live) for a period of 4 to 30 months. Where individuals were found not to amplify at any of four loci in the first multiplex group, no attempt was made to genotype them for the remaining four. Latterly, DNA was extracted from fresh specimens from the following populations: SAN, GIR, FAL, DAL, CML, LND, DUN, IWG, RVR. Of these extractions, nearly 100% individuals scored and most scored for all 8 loci.

DNA extracted from fresh tissue was stored at -20°C. It is evident that storing earwigs at -20°C is insufficient to prevent degradation of the DNA within a matter of months or to neutralise destructive microorganisms that are present inside or on the surface of specimens. Therefore, earwig DNA extracts should be prepared from live specimens where possible. Preservation in 100% ethanol or at much lower temperature (-70°C) may abate the rapid degradation seen at -20°C, however these approaches require more expensive equipment and suitably durable containers.

## Appendix E. Processing raw microsatellite data from a DNA sequencer

The following protocol was developed by G. Brown with improvement made based on comments from B. Hutchinson. This protocol permits the operator to extract and manipulate genotype data without relying on the often-unreliable automatic allele-calling facility of the CEQ software supplied with the *Beckman* CEQ8000 capillary sequencer.

### *Preparing and extracting data from CEQ*

Correct calling of the size standard, if not achieved with the default analysis method in CEQ, may be possible by reducing the slope threshold incrementally from 10, to 5 and then in single unit steps down to 1. If this does not correct the calling of the ladder, the system dye spectra can be enabled (instead of the standard calculated dye spectra setting [ADVANCED tab of the analysis method dialogue box]). With 'Sys Dye' selected, the slope threshold should be returned to the default value (10) and a similar iterative reduction performed until the size standard peaks are correctly called. As a rule of thumb, if the operator can discern the pattern of the size standard peaks in a result, by examining the raw data of the chromatogram, the software can usually be modified to correctly call it.

Once the size standard has been correctly called, the fragment filter facility can be used to reduce the number of irrelevant peaks that are called. The filtered peaks are then cropped by manually excluding the remaining irrelevant peaks. The resulting fragment list representing only the allele fragments can then be exported to *MS EXCEL*.

### *Producing a genotype summary*

Within excel, the locus identity can be assigned to the data by setting up a reference worksheet with the locus name, the associated dye colour and size range of each locus. The relevant locus name can then be assigned to each result by using the LOOKUP function.

Adjacent to the data, two columns are set up for each locus. Both columns for each locus are set to the value of the fragment size if the result is assigned to that locus. If the result is of a fragment not of that locus, the first column (minimum query value)

should contain an X and the second column should be blank. This can be achieved using the IF function.

These data are then imported into *MS ACCESS* where a query selects the minimum value from the first column (where blanks are Xs) for each locus per each result name and similarly the maximum value in the second column (where blanks are empty cells). The reasoning behind the two columns is that *ACCESS* will treat a blank cell as zero and consider that smaller than any actual value present elsewhere in the column. All text values are considered greater than infinity in value and therefore will always be ignored when selecting the minimum value. Conversely, if an X is entered in the blanks, this will always be greater than a numerical value and hence be selected instead, when the maximum function is used. Therefore, for the maximum the second column with empty cells for blanks is required.

The resulting query lists every result name (in a simple 'plate setup' this could be the individual name; preferably a consistent code of 3 letters and 3 numbers (or as suits the study). Against each name will be a minimum and maximum fragment size for each locus; similar to a two-column genotype summary.

The final stage is to round the second decimal place fragment sizes appropriately to integers. The rounding procedure can be automated by exporting the fragment query back into *MS EXCEL*, but the exact formulae will depend on the nature of the locus and from which peak it has been scored. A useful approach is to add a standard amount to each fragment size of a locus so that all values can be correctly rounded up or down to the nearest odd or even integer.

## Appendix F. Summary of genetic dataset

The sample sizes of microsatellite genotype data obtained from 735 individuals from the 58 populations sampled. Overall, 1351 unique DNA extracts were run for one or more loci to produce this dataset.

Site	EW1	EW12	EW15	EW2	EW25	EW35	EW40	FA2	* Scored	** All loci	*** Loci used
AGN	3			6		2	6		6		
ANN							2		2		
BAS	5	5	3	9	3	5	12	4	12	2	2
BHR				1			2		2		
BID				4		3	5	1	5		
BLY				1			1	1	1		
BR	6	5	5	14	8	9	8	11	20		
BRY				3		3	7		7		
CGL	11	7	4	14	12	14	7	16	18		
CML	23	24	22	25	24	24	24	20	25	20	20
CRM	25			19			30	2	30		
CUM				1			2	1	2		
DAL	19	20	19	26	18	20	25	21	26	16	16
DUN	19	21	19	22	23	23	23	23	23	13	14
EWO	4		3	18		16	19		22		
EYB	1			1			6		6		
FAL	22	34	19	25	32	35	34	36	36	17	17
FID							1		1		
FSH	6	2	4	18	8	9	21	2	21	1	1
GIR	35	34	33	35	33	36	30	34	36	26	26
GUH	1			4		1	7		7		
HIL	3	4	2	5	5	4	5	6	6	1	1
ICM	37	21	33	39	34	36	43	39	52	10	11
IFN			1	24		17	24	20	27		
IGV	24	24	22	12	27	26	25	26	30	7	16
IKH	12	16		15	16	20	32	19	36		
IMK		1		1			1		2		
IWG	1	3	2	3	3	3	7	5	7	1	1
KRF	3		3	12		10	11		14		

Site	EW1	EW12	EW15	EW2	EW25	EW35	EW40	FA2	* Scored	** All loci	*** Loci used
KRK				3	1	1	4	1	4		
LAM		5	2	13	7	10	21	14	27		
LEE	4	1	1	4	2	1	7	6	8	1	1
LGE				4					4		
LND	17	17	19	26	16	21	30	23	31	13	13
LUN	2	2	4	3	2	3	3	3	4	2	2
MAY				1			3		3		
MML	2	5	4	8	8	8	10	9	10	1	1
MRN	1	1		1	1	1	1	1	1		
MYM	2	1	1	3		1	4		4		
NBW				1			4		4		
NWM	1								1		
NWT							1		1		
RAM		5	5	7	5	5	7	7	7		
ROO		1	1	1		1	1	1	1		
RVR	5	5	3	5	5	6	7	7	7	3	3
SAN	17	15	10	22	12	19	24	23	24	9	9
SKK					1	1	2	1	2		
SKM	3	4	4	6	6	5	6	6	6	3	3
SLC	2	3	2	5	3	3	5	6	6	1	1
SMR	3	2		4		3	4		6		
STJ	6	8	7	12	3	9	14	11	14	1	1
STM	10	6	6	13	7	9	15	16	18	3	3
STY		2	1	1	2	2	2	2	2		
SWM				4		1	1	1	4		
TAL	3	4	4	5	4	4	7	7	7	3	3
TCO	2		1	4		2	6		7		
WML	9	12	12	21	16	21	22	18	24	1	1
WVO				7		13	6	3	16		
totals:									735	155	166

The three rightmost columns give the number of individuals from which:

\* at least one single-locus genotype was scored;

\*\* complete multi-locus genotypes were obtained for all 8 loci optimised in *F. auricularia* (EW1, EW2, EW12, EW15, EW25, EW35, EW40 & FA2)

\*\*\*complete MLGs were obtained from just the 7 loci used in analyses in chapter 4 (EW2 excluded).

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