Open Research Online
The Open University's repository of research publications
and other research outputs

Mate choice and reproductive success in the speckled bushcricket, Leptophyes punctatissima

Thesis

oro.open.ac.uk

Mate Choice and Reproductive Success in the Speckled

Bushcricket, *Leptophyes punctatissima*

Ian Andrew Kilduff (BSc)

A dissertation submitted to the Open University

Department of Biological Sciences

In application for the degree of Doctor of Philosophy

30 September, 2000

For my parents

Abstract

Leptophyes punctatissima is unusual in that both sexes call. The male calls, the female replies and the male performs phonotaxis to the stationary female. Consequently mate choice could occur at either of two stages: first, during the interchange of calls and second, on the basis of proximate criteria once the male has approached. There is no evidence that females choose their mates on the basis of calling behaviour or call characteristics, though males that call more may achieve more matings. There is no evidence that body asymmetry has any effect on mating success for either sex. Males on a protein-supplemented diet do not produce larger spermatophores than males whose diet is not supplemented, but they do mate more often, possibly as a result of female choice but more likely because diet affects the rate at which males can produce spermatophores. Unsupplemented females mate more often than supplemented females, possibly as a result of male male choice or because they are seeking matings so that they can supplement their diet with spermatophores. Males give larger spermatophores to unsupplemented females. Larger males produce larger spermatophores. They also mate more often than smaller males, possibly as a consequence of female choice, success in male-male contests, or because larger males have larger energy reserves and can produce spermatophores more quickly. Larger females mate more often than smaller females but only when their diet was supplemented. Females lay more eggs the more times they mate. Females lay heavier eggs after their first mating than they do in later batches, and unsupplemented females lay more eggs after their first mating than supplemented females do, but otherwise female size, diet or level of asymmetry has no effect on the size or weight of eggs, or the number of eggs laid. The total weight of spermatophores females receive does not affect any measure of female reproductive success: neither fecundity, egg size or egg weight is affected by the weight of spermatophores females consume, irrespective of the diet the females were maintained on. Diet, size or number of matings does not affect female longevity.

Acknowledgements

I would like to thank my supervisors Dr Marion Hall and Dr David Robinson for giving me the opportunity to study for a PhD and for their guidance, encouragement, support and patience, all of which were abundant throughout the course of this research.

I should also like to thank the many people who have assisted me in many ways during this study. Thanks to Mike Gillman and Karen Vines for statistical advice, and to Steve Walters, Dawn Sadler, Lindsey England and Jackie Lancashire for help in the maintenance and care of the insects. Thanks to Leigh Simmons, Richard Palmer and Karim Vahed who gave me advice on several aspects of the project. Thanks to the Departmental secretaries, Dawn Partner, Yvonne Royals, and Heather Holden, and the Departmental clerk, Barbi Pedder, for their assistance in many areas; to Tina Wardhaugh for help in the preparation of slides, to Mike Dodd for the pictures of the crickets. I should like to thank Railtrack for allowing me permission to collect the crickets from their land, and in particular Mike Manley, the Outside Parties Engineer (Kent) for organizing the necessary access permits. To all the friends I have made during my time at the Open University. Particularly Jerry Lea (thanks for letting me rent a room in your flat for 3 and a half years), to Marky Mark Bresler for many good times and lots of football, to John Wilkinson, Diana Maxwell, Derek Martin, Dipesh Chauderhey and Nick White for their convivial company.

A special thanks must go to Jane Loughlin, not only for much sound advice and lots of encouragement during the write up of this thesis, but also for having the fortitude to live under the same roof as me.

I would like to thank the Open University Research Committee for funding this research.

Finally I would like to thank my family for their support, both financial and moral, without which the hard times would have been even harder.

Contents

List of Figures

1 General Introduction

This thesis presents the results of a study of the mating behaviour and reproductive success of the speckled bushcricket *Leptophyes punctatissima* (Orthoptera: Tettigoniidae), a species common in the UK and across Europe. Selective mating has been reported for many species from numerous taxa and is widespread in the Orthoptera (Gwynne, 1982; Wedell & Sandberg, 1995; Brown *et al.*, 1996).

This thesis has three main aims

1. To establish if *L. punctatissima* exhibits mate choice.

2. If non-random pairing is a feature of the mating behaviour of *L. punctatissima*, to investigate the criteria by which potential mates are chosen.

3. To determine the effect of such mate choice on individual reproductive success.

1.1 Natural versus sexual selection

Competition for mates between animals of the same sex is now widely accepted as the major force responsible for the evolution of elaborate male traits. In an effort to explain the evolution of such traits, Darwin (1859), drew a distinction between characters which increase an animal's chances of survival (natural selection), and those which enhance an individual's chances of securing matings (sexual selection). The term natural selection is now used in a wider sense, to cover everything except selection as a result of competition for mates.

Darwin identified two components of sexual selection. He reasoned that male ornaments evolved through a process of female choice (intersexual selection), and that male weaponry

evolved via direct competition between males for access to females (intrasexual selection). Darwin elaborated his theory in the *Descent of Man and Selection in Relation to Sex* (Darwin, 1874). This aspect of Darwin's work proved to be the most controversial of all his evolutionary thinking.

In particular Darwin failed to provide an explanation of how secondary male characteristics could evolve through a process of female choice. It was over 50 years later that, in a highly influential paper entitled 'The Evolution Of Sexual Preference', Fisher (1915), provided theoretical support for Darwin's ideas. Fisher proposed that the evolution of preference has its roots in natural rather than sexual selection. He envisaged a situation in which an uncommon male trait bestows a survival advantage on its bearer. Females that choose to mate with such males will produce fitter offspring and so there will be selection for their preference. Assuming the trait has a genetic basis, male offspring should inherit it and so be chosen more often. These males will accrue a double advantage: first, a higher fitness relative to other males, and second, enhanced mating success. Once female preference for the trait begins to spread through the population, the trait will become more exaggerated until it outstrips its natural selection optimum, in a runaway process. Eventually a point is reached where the natural selection disadvantage outweighs the sexual selection advantage, and runaway ceases.

Fisher's theory contains many assumptions, namely that populations are of an infinite size, that males are unrestrained in the number of females they can mate with, that there are no viability costs to female choice, and that genetic recombination and mutation are regarded as sufficient to explain the co-variance of the male trait and the female preference for it. Although Fisher did not apply a mathematical model to his theory, several authors have since done so, (O'Donald, 1962; O'Donald, 1967; Lande, 1980; Lande, 1981), and have

shown that given certain rules of female choice, elaboration of male traits would indeed occur.

There are other hypotheses that currently exist to explain the evolution of elaborate male traits. The sensory exploitation hypothesis proposes that males evolved conspicuous characteristics as a method of exploiting a pre-existing female bias i.e. the preference for the characteristic pre-dates its origin (Ryan, 1991). The handicap principle (Zavavi, 1975) and the immuncompetence theory (Hamilton & Zuk, 1982) propose that secondary sexual characteristics provide honest information regarding the genetic quality of the signaller. These will be discussed further in Section 1.3.2.

The first empirical evidence for the operation of sexual selection came from Bateman's (1948) seminal work with *Drosophila*. Using genetically marked individuals, he demonstrated that the number of offspring fathered by a male increases relative to the number of females that are inseminated. Females however show no difference in fertility irrespective of the number of times they mate. Bateman argued that this asymmetry is a consequence of the energy investment in sex cells, with females producing limited, large, food rich gametes, and males producing many highly motile microgametes. This disparity in the investment in gametes in anisogamous mating systems led Bateman to draw two important conclusions. First, not all males should achieve their full reproductive potential: success for some males will be at the expense of other males. Second, females should experience little difficulty in securing sufficient sperm to fertilise all of their ova.

Williams (1966) and Trivers (1972) extended Bateman's arguments with the theory of parental investment. Parental investment is defined as the relative investment made by each sex in an individual offspring, which increases its chances of survival, at the expense of the parent's ability to invest in further offspring. Trivers and Williams argued that the

investment in offspring dictates the direction of competition for mates, such that members of the higher investing sex are a limiting resource for which individuals of the lower investing sex compete. Because females generally invest more in reproduction, they tend to be choosy of males. However, in situations where male investment exceeds that of females, male availability may constrain female reproduction and sex roles may be reversed (Smith, 1979.

1.2 How mate choice operates

1.2.1 Species Recognition

When mating, all animals show some degree of discrimination, if only to avoid non-productive matings with members of other species. The selective advantage of mating with a conspecific was for many years postulated to have played an important part in the evolution of elaborate characters and preferences (Andersson, 1994; Andersson & Iwasa, 1996). Wallace (1889) explained the enormous variety of coloration of insects and bird species in these terms. Dobzhansky (1937), Huxley (1942) and Mayr (1942) considered the species isolation function of secondary sex traits as sufficient to account for their persistence. Although the recognition of conspecifics is obviously an important element of choice, it is now recognised that this process alone cannot explain the elaboration of traits well in excess of that which would be required simply for species recognition.

1.2.2 Demonstrating mate choice

Several lines of evidence are required to demonstrate that animals have evolved mechanisms whereby they choose to mate selectively with certain conspecifics. Firstly, it has to be shown that individuals exhibit non-random mating patterns. Second, selective mating must be shown to arise through choice, rather than intrasexual competition. And

finally, individuals who mate with preferred partners must be shown to have a higher relative fitness (Mayr, 1942; Halliday, 1983).

Selective mating has been demonstrated in a variety of animals from many different taxa. Ankey (1977) showed that female snow geese (*Anser caerulescens*) mate with males who have culmen lengths which are larger than their own, with a higher frequency than would be expected by chance alone. Culmen length shows a positive relationship with body size in many bird species, hence, females may be choosing to mate with males that are larger than themselves. Female wolf spiders (*Hygrolacosa rubrofasciata*) discriminate between males on the basis of their signalling rate, and prefer to mate with males who drum their legs with a higher frequency during courtship displays (Rivero *et al.*, 2000). Moller (1992) demonstrated that differences in the degree of fluctuating asymmetry (random deviations from complete symmetry in bilateral traits) were related to male mating success in the male barn swallow (*Hirundo rustica*). Males with increased fluctuating asymmetry (FA) were chosen less often and had higher parasite loads than those males which were more symmetrical. FA is known to be a reliable indicator of developmental homeostasis (Palmer & Strobeck, 1986; Parsons, 1992), so lower levels of FA may be a reliable indicator of individual quality. A negative correlation between FA and mating success has also been shown in the yellow dung fly, *Scatophaga stercoraria* (Ligget *et al.*, 1993), the damselfly *Coenagrion puella* (Harvey, 1993) and the Japanese scorpion fly, *Panorpa japonica* (Thornhill, 1992).

In many cases of mate choice it is probable that individuals are not assessed on the basis of a single character. Sorenson & Derrickson (1994) showed that females of the northern pintail (*Anas acuta*) select males on the basis of a suite of characters, both morphological and behavioural. Female pied flycatchers (*Ficedula hypoleuca*), were shown to have

independent preferences for unmated males, males which were brightly coloured and males who defended nestboxes with small entrance holes (Dale & Slagsvold, 1996).

1.2.3 Active choice versus passive attraction

Many instances of apparent mate choice may simply result from a response to stimuli that make certain individuals easier to locate than others. Parker (1983) terms this process 'passive attraction'. The important distinction between this behaviour and 'active choice' is that, although certain phenotypic traits may result in some individuals gaining a disproportionate number of matings, there need be no particular preference shown by the other sex. Animals simply move towards the most conspicuous conspecific signal.

Arak (1988) argued that female choice in the natterjack toad (*Bufo calamita*) may be a consequence of passive attraction. Females may orient towards louder, more rapid calls, because they are easier to detect in the noisy environment of anuran choruses. Such behaviour may act to reduce the cost of mating, since there is evidence that predation risk may select for rapid mating in anurans (Wells, 1977).

Female tungara tree frogs (*Physalaemus pustulosus*) appear to discriminate between potential mates on the basis of their call. Males of this species produce a complex call consisting of a 'whine' and a 'chuck'. It is thought that the whine component of the song is important in species recognition. Furthermore in a series of playback experiments females were shown to favour the lower frequency calls of larger males (Ryan, 1985). Preference for lower frequency calls has also been reported for many species of cricket (Gwynne, 1982; Bailey, 1985; Brown *et al* 1996). Whether the apparent preference for lower frequency calls is active choice or passive attraction is, however, open to debate. Many playback experiments fail to mimic natural systems. Gerhardt (1982) for example, showed

that female discrimination in the green tree frog was poorer in a complex acoustical environment, because of the effect of multi-male vocalisations.

1.3 The benefits of choice

For sexual selection to operate as a result of mate choice, individuals must benefit by choosing certain mates. These benefits may be direct (non-genetic) or indirect (genetic).

1.3.1 Direct benefits

Choice of certain males may reflect the quality of territory they hold. Campanella & Wolf (1974) showed that mating success in a dragonfly (*Plathemis lydia*) was directly related to the quality of oviposition sites they defended.

In the hangingfly (*Hylobittacus apicalis*), males provision females with dead arthropod prey on which they feed during copulation (Thornhill, 1976b). Females prefer larger males that provide larger gifts. The benefit to females of nuptial feeding is twofold: first, there is a reduction in the time spent foraging and the associated risk of predation (the predation rate of males is twice that of females); second, the larger the gift the greater the female's fecundity.

Females may choose to mate with certain males because they are better able to protect them from predators, or harassment from other males. Female dung flies (*Scatophaga stercoraria*) prefer to mate with larger males because such males are less likely to be interrupted by competitors while copulating (Borgia, 1979). Female choice for males in good condition could reduce the risk of infection inherent in mating with individuals carrying contagious diseases or high parasite loads.

Behavioural complementarity is important in species in which the pair bond is maintained for successive breeding seasons. Kittiwakes (*Rissa tridactyla*) realise an increased reproductive success because of the accumulated breeding experience gained from remaining together. Newly formed pairs that have low breeding success tend to split up and seek new mates (Coulson, 1966).

The benefits of choice may accrue to the female's offspring rather than to the female herself. Nisbet (1977) demonstrated that males of the common tern (*Sterna hirundo*) were less likely to be rejected by potential mates if they fed them efficiently during courtship. He reasoned that the ability of the male to provide food during courtship may be related to his parental ability (i.e. his ability to provide food for the chicks).

Males may also gain by mating selectively with certain females, particularly when fecundity is correlated with body size. Male choice for larger, more fecund females has been reported for a large range and diversity of taxa (Olsson, 1993; Verrel, 1995; Bateman, 1998). Gwynne (1983) demonstrated active male discrimination between females in the mormon cricket (*Anarbus simplex*). In a series of choice experiments, he showed that two thirds of 45 attempted mounts by females resulted in the termination of copulation before the attachment of the spermatophore. Energy investment in the spermatophore is high in this species, such that males incur non-trivial costs in their production (Dewsbury, 1982; Gwynne, 1983). By choosing only the most fecund females, males realise the best return on their investment.

1.3.2 Indirect Benefits

The individuals that make up a breeding population will not all be equally as fit. And when a male's only contribution to the production of offspring is genetic it may pay females to choose those males of the highest quality. Females will benefit from choosing a particular

trait if it is an indication of the genetic quality of the individual possessing it. The 'good genes' process of mate selection was first outlined by Fisher (1915) and redeveloped many years later by Williams (1966) and most famously by Zahavi (1975). Zahavi's premise was that secondary sex traits evolve in response to female choice, as a mechanism to test male quality. Zahavi reasoned that the more elaborate the ornament, the greater the handicap in terms of survival, and the mere fact that such males survived long enough to reproduce was an indication of their phenotypic and genetic quality. The good genes theory of mate selection now exists as various versions of Zahavi's original handicap principle. Hamilton & Zuk (1982) suggested that the condition of male secondary sexual characters may allow females to discriminate against males that carry heavy parasite loads. Such a handicap is presumed to have evolved to indicate a particular, rather than overall condition, such that variation in the condition of ornaments between males could reveal genetic resistance to parasitic infection.

The main theoretical problem with runaway selection and good genes models of mate choice is the intense directional selection imposed by female mate preference. Under these conditions, according to conventional quantitative genetics theory, preferred male characters should reach equilibrium within a few generations, and so nullify any benefits to choice. However genetic variation may be more common in natural populations than was previously assumed. For example, the evolutionary arms race between host immune systems and parasitic infections may promote the continuous generation of new alleles. Rare host genotypes will be at a selective advantage because they will be more resistant than the average genotype in the population (Haldane, 1949). Such frequency-dependent selection may result in single gene substitutions, which affect host-parasite interactions. Genes affecting fitness can remain in a state of high heritability if they co-evolve as a result of biotic interactions between immune systems and parasite infection.

Reproductive success for a breeding pair may depend not only on individual quality, but also on the degree of genetic complementarity that exists between them. The three-spined stickleback (*Gasterosteus aculeatus*) exists in two forms, one is found exclusively in freshwater, while the other form lives mostly in a marine environment, but returns to freshwater to breed (Hay & McPhail, 1975). In a series of choice experiments, females were shown to choose males of their own ecotype 62% of the time. Hay & McPhail argue that offspring from purebred individuals will be more adapted to their environment than those from matings between different ecotypes.

The degree of relatedness between mates is another important component of genetic complementarity. Mating with close relatives increases the chances that any harmful recessive alleles will become homozygous in the offspring and depress reproductive success. Female chimpanzees (*Pan troglodytes*), for example, stop associating with their male siblings when they reach sexual maturity (Pusey, 1980). After oestrus, females move to other groups, seemingly because they are attracted to unfamiliar males, and so avoid mating with close kin. In field crickets (*Gryllus bimaculatus*), femalesspend longer at the burrows of calling males that are unrelated, increasing the likelihood that they will avoid inbreeding (Simmons, 1991).

1.4 Mechanisms of mate choice

If animals do choose between mates, there must be some mechanism by which individuals discriminate between alternative conspecifics. The first theoretical attempt to answer this question came with the work of Janetos (1980). Janetos proposed that females encounter males in a sequential and random manner, and are able to assess the best quality male from a given subset examined, in a retrospective manner (a best of *n* tactic). Real (1990) found that if search costs as well as benefits are incorporated into a model of choice, a best of *n*

tactic yields a lower overall payoff than a threshold sampling strategy, in which females compare males to a critical internal standard and mate with the first male to exceed that standard. Although these models are purely theoretical, there is evidence that Real's threshold mechanism of mate choice may operate in at least some species. Gwynne (1982) showed that in the cricket *Conocephalus nigropleurum*, females reject all males that are below a certain weight but willingly mate with any male whose weight is in excess of a certain threshold.

Some mechanisms of mate choice do not require individuals to compare alternatives directly in order to mate preferentially. This is the case where mate choice is passive rather than active, for example. In other instances, the apparently positive mate choice decisions of animals may actually be a response to the behaviour of other conspecifics. In the fallow deer it has been shown that the rate of female entry into a male's territory increases when he mounts an oestrus female, and when the number of females on the territory increases (Clutton-Brock *et al.*, 1989). Downhower & Lank (1994) showed that mate choice in the mottled sculpin (*Cottus bardi*) was related to a female's previous experience. Females that had been courted on a previous occasion by a smaller male were more likely to spawn, when courted by a subsequent male, than those females that had previously encountered larger males.

1.5 Sex role reversal

Sex role reversal refers to situations in which the usual pattern of courtship, i.e. choosy female and competitive male is switched. In most of the cases of this phenomenon so far reported the underlying cause appears to be an increase in parental investment by males. Under these conditions, the operational sex ratio (the ratio of fertilisable females to sexually active males) (Emlen & Oring, 1977) is female biased, such that male availability

places constraints on female reproductive success. The phalarope (*Phalaropus spp.*) shows complete sex role reversal. Egg incubation in this species is carried out exclusively by males. Females compete vigorously for access to nesting males, and male-male competition is absent (Reynolds & Cote, 1995). In the giant water bug, *Adebus herberti*, males brood eggs which are attached to their backs by females (Smith, 1979). Role reversal in this species occurs only at certain times of the season, when the majority of males are brooding eggs. Role reversal is not complete, as males display to females. In the pipefish *Synganthus typhle* and *Nerophis ophidion*, males brood eggs on their ventral body surfaces. Males of these species do not appear to invest more in the production of offspring than females, and role reversal seems to be due to females being able to produce eggs at twice the rate at which males can brood them (Berglund *et al.*, 1989). Hence, the greater potential reproductive rate of females (Clutton-Brock & Parker, 1992), rather than increased male parental investment, may better explain role reversal in these animals. Food availability has been shown to affect the direction of mate choice in some species of tettigoniids. Under conditions of food stress, males become the choosy sex and females compete with each other for access to receptive males (Gwynne, 1985, 1993; Gwynne & Simmons, 1990; Simmons & Bailey, 1990).

1.6 Cryptic female choice

Inherent in much of the theoretical and empirical literature on mate choice is the assumption that copulation inevitably leads to fertilisation. Recent studies suggest this may not be the case, and that cryptic female choice may be an almost ubiquitous feature of many anisogamous mating systems in which females mate with more than one male (Eberhard, 1996). Cryptic female choice refers to any behavioural, physiological or morphological characteristic that operates after copulation has begun and which results in a selective bias in paternity between conspecific males. The choice is cryptic in the sense

that it would be invisible to anyone using classic Darwinian criteria, which focus on the success of animals in achieving copulations (Eberhard, 1996).

In the katydid *Requena verticalis*, for example, female post-copulatory behaviour may be instrumental in determining which males are successful in gaining fertilisations (Gwynne, 1984b). Males of this species introduce sperm into the female via a complex spermatophore, a bilobed structure consisting of a sperm-containing ampulla, and a sperm-free mass, the spermatophylax. When the male releases the female after copulation, she arches her back, and detaches the spermatophylax from the ampulla and consumes it. During consumption of the spermatophylax, the male's sperm migrate from the ampulla to the female's spermathecae. After eating the spermatophylax the female detaches the ampulla and consumes that also. Females could discriminate between males by removing the ampulla from their genital opening before they have completely consumed the spermatophylax, a situation which has been observed in the cricket *Gryllodes supplicans* (Sakaluk, 1984). Also, any male who produces a smaller than average spermatophore runs the risk of his spermatophylax and ampulla being eaten before all his sperm have migrated to the spermathecae. By imposing these rules female *R. verticalis* favour males which produce larger spermatophores, as these males sire more progeny.

A more subtle form of cryptic choice can be seen in the odonates. Male odonates have the capacity to remove rival sperm from females using special appendages on their genitalia (Waage, 1979). Such an adaptation would appear to offer little opportunity for post-copulatory female choice. However, the structure of some female organs can influence male fertilisation success. An example is the damselfly *Mnais pruinosa*, in which the spermathecal duct is so narrow that it prevents access to male genitalia (Siva-Jothy & Tsubaki, 1989a). A similar situation has been observed in *Agria*, and access to the distal region of the spermathecae is probably impossible (Waage, 1979). Siva-Jothy (1987)

proposes that the arrangement of muscles which line the walls of the bursa and spermatheca may allow females to modify their size and shape, so controlling the amount of sperm an individual male can remove. It is possible therefore, that females may allow some males greater access to their sperm stores, whilst preventing others gaining entry.

1.7 Fluctuating asymmetry as a measure of developmental instability

1.7.1 FA and other asymmetries

Developmental stability refers to the ability of an organism to produce an 'ideal' phenotype under a particular set of environmental and genetic conditions (Zakharov, 1992). Organic structures which exhibit bilateral symmetry are controlled by the same genome, and so if growth is not disrupted during development each side should develop at the same rate and the organism should produce an ideal form. However, an organism rarely, if ever, develops under perfect conditions and may be subject to stress which can shift the trajectory of development producing minor imperfections on either side of the body. Such stresses are broadly grouped under the category of developmental noise and can be both environmental and genetic.

Environmental factors that have been reported to affect developmental stability include temperature and nutrition. Increased temperature, for example, amplifies variation in the fins of the Siberian sturgeon, *Acipeser baeri* (Ruban, 1992). Nutritional stress results in greater developmental instability in *Drosophila* (Parsons, 1964), rats (Sciulli *et al.*, 1979) and mice (Erway *et al.*, 1970).

Reduced heterozygosity increases developmental instability. A decline in the number of unique alleles at a locus can result in a reduced range of enzymatic products with which to resist developmental perturbations as an organism grows. Increased inbreeding produces

more homozygotes and these individuals are less able to buffer their development against random accidents and show increased developmental instability (Phelan & Austad, 1994).

Fluctuating asymmetry (FA) (Ludwig, 1932; Van Valen, 1962) refers to random deviations from perfect symmetry in bilateral traits and can be used to measure how well individuals within a population are able to buffer their development against 'noise'. The level of FA an individual displays could be viewed as the outcome of the conflict between the processes that contribute to developmental stability and those which contribute to developmental noise.

The developmental accidents that lead to a population of organisms displaying FA are random in nature, so the break in symmetry is also randomly distributed, with no one side being consistently larger than the other. Consequently the FA values for a particular population should show a normal distribution of R-L differences around a mean value of zero (Van Valen, 1962). This property of FA distinguishes it from other forms of asymmetry in which a bias to one side is the norm. Directional asymmetry (DA) (Van Valen, 1962), refers to a form of bilateral variation in which asymmetry is consistently in one direction. Distributions of asymmetry values for populations exhibiting DA will be highly skewed in one direction and will not be centred on zero. Antisymmetry (Van Valen, 1962), describes a population in which there is a bias to one side of the body but the distribution of asymmetry is random with respect to which side the bias occurs. The symmetry values for a population displaying antisymmetry will show a platykurtic (broad peaked and short tailed), or bimodal distribution. Traits that exhibit significant levels of DA or antisymmetry have largely been ignored in studies of developmental stability, because they contain a significant but unknown genetic component (Palmer & Strobeck, 1986; Palmer, 1990). Graham *et al.* (1998) argues, however, that under certain conditions

both DA and antisymmetry may accurately predict the level of developmental stability of a population.

1.7.2   FA and mate choice

FA in terms of mate choice is often measured in secondary sexual characteristics. Exaggerated male traits such as ornamental plumage show greater phenotypic variation when compared with homologous traits in females of the same species or males of closely related species which do not develop elaborate ornaments (Moller & Hoglund, 1991). That females may use symmetry to discriminate between potential mates was first demonstrated in the barn swallow (*Hirundo rustica*). By manipulating outer tail length and symmetry Moller (1990, 1992) showed that females preferentially mated with males with longer, more symmetrical tails. These males paired more quickly and produced more offspring per-season than their less symmetric counterparts. There was no evidence that the experimental manipulations affected performance, less symmetric males gathered more food with which to feed nestlings, hence it appears that females were using ornament symmetry as a qualitative measure with which to assess potential mates. Swaddle and Cuthill (1994) showed that female zebra finches mated preferentially with males who were wearing symmetrical leg bands over those whose leg bands were asymmetric. In the bower building cichlid *Cyathophayrnx furcifer*, females mated with males who had long and symmetric pelvic fins more often than would be expected by chance alone (Karino, 1997).

In species that do not develop conspicuous secondary sexual characters, FA may still accurately predict mating success. In the Japanese scorpion fly (*Panorpa japonica*) males with lower forewing FA showed a higher mating success than less symmetrical males (Thornhill, 1992). In this species males provision females with nuptial gifts in the form of dead arthropod prey and copulate with the female as she consumes the gift. In fights for

food items, winners had significantly lower forewing FA than losers and secured more matings (Thornhill, 1992). In the damselfly *Coenagrion puella*, males with lower wing FA enjoyed a higher lifetime mating success than less symmetric individuals, probably because they were more successful in catching females who crossed their territory (Harvey, 1993). These examples illustrate that FA in relation to mate choice can operate in several ways. The intense directional selection responsible for the maintenance of elaborate secondary sexual characters render them more susceptible to perturbations during development than ordinary morphological traits and so more likely to veer from the intended developmental trajectory (Alatalo *et al.*, 1988). Organisms that can maintain expensive structures in the face of stress may directly attract mates because they honestly signal their genetic quality. FA in naturally selected traits may influence mating success indirectly because increased asymmetry affects some aspect of performance.

1.7.3   FA of males versus females

As outlined earlier, homozygous individuals may show greater phenotypic variation than heterozygotes because they are less able to buffer their development against random accidents (Soule, 1979). In many species, males are the hemizygous sex, having only one X chromosome, and so, if any traits that are X linked affect development, males may be more prone to developmental instability and show higher levels of FA than females. Male honeybees (*Apis mellifera*) were shown to exhibit higher levels of wing FA than females (Bruckner, 1976), but studies of FA in the lizard *Uta stansburiana* revealed no sex differences in the level of asymmetry (Fox, 1975). Overall the evidence for sex differences in FA is not compelling. Leamy (1984) found a greater degree of directional asymmetry in the femur of female inbred and hybrid mice than in males, but observed no difference in overall FA between the sexes. In some Amerindian tribes, females were shown to express higher levels of FA than males, possibly because females are subject to greater environmental stress (Harris & Nweeia, 1980). In a review of 10 studies investigating the

association between sex and FA, Palmer & Strobeck (1986) report three which fit the hypothesis, five in which the hypothesis is not supported and two in which the results are equivocal.

1.8 Mating in the Orthoptera

Orthopteran insects use sound to communicate with potential mates. In most cases the male produces a courtship song and the female uses the signal to locate the male. There are variations to this general rule and in some species females have also evolved the capacity to produce sound. In the bladder grasshopper, for example, the system is similar to that in *L. punctatissima*: both males and females call and pair formation is achieved through male phonotaxis (Van Stadden & Romer, 1997).

The orthopteran insects lack an intromittant organ and sperm transfer is via a spermatophore, a package containing sperm which is transferred from the male to the female during copulation (Boldyrev, 1915). The number and size of spermatophores passed to females during copulation varies between different species. In some crickets males pass more than one spermatophore to females during mating. Two or more are transferred in *Gryllus pennsylvanicus* (Alexander, 1961), and up to eight in some species within the genus *Orocharis* (Funk, 1989).

Orthopteran insects show a wide range of mating systems. Some, such as the large weta (*Hemideina femorata*), defend burrows, which contain several females (Field & Sandlandt, 1983). Males of the Australian bush cricket *Kawanaphilia nartee* do not defend females directly, but instead compete for access to prime food sites, through which females are more likely to pass (Simmons & Bailey, 1990). Males of the short tailed cricket were

shown to gain a mating advantage if they called from an elevated position at a certain height from the ground (Walker, 1983).

The criteria for mate choice are also very varied. Large body size increased male mating success in the grasshopper *Sphenarium purparescens* (Del Castillo *et al.*, 1999): larger males were more successful in contests over females and had a greater mating success than smaller males. In the grasshopper (*Melanoplus sanguinipes*), females mate preferentially with males who feed more intensively, and select a more varied diet mix that permits greater food intake (Belovsky *et al.*, 1996]. Belovsky *et al.* argue that such males provide larger spermatophores and so show increased parental investment. Simmons (1988b) showed that large males of the field cricket *Gryllus bimaculatus* have a mating advantage which results from a differential female response to the courtship displays of large and small males. The advantage to females of choosing larger males may be twofold: first, an increase in fecundity since mating with larger males has been shown to increase fecundity in some species; and second an increase in inclusive fitness, since body size in *G. bimaculatus* has heritable genetic variation (Simmons, 1988b).

The characteristics of male song may be an indicator of some aspect of phenotypic quality. Brown *et al.* (1996) showed that female black-horned tree crickets (*Oecanthus nigricornis*), have a preference for the lower frequency calls of larger males, though only during simultaneous playback experiments. They argue that female choice is based on the relative quality of calls that can be sampled simultaneously. In the house cricket (*Acheta domesticus*), females prefer the louder, clearer, chirp rate of dominant males (Crankshaw, 1979). Simmons & Ritchie (1996) argued that the mating success of male *Gryllus campestris* is related to the degree of directional asymmetry in the stridulatory harp. More symmetrical males produce purer tones, and are chosen more often by females. Mating success may be correlated with age in some orthopteran species. There is a positive

relationship between the songs of aged males and female choice in *G. bimaculatus* (Simmons & Zuk, 1992). Older males were shown to have a finer structure to their songs, and they harbour fewer gregarine parasites than younger males. Hedrick (1986) postulated that call bout duration may indicate intrinsic genetic quality, or reflect the parasite load of the calling male (Hamilton & Zuk, 1982), or some other environmental factor such as the nutritional state of a prospective mate.

1.8.1 Nuptial feeding in the Orthoptera

The provisioning of females with nuptial gifts during and after courtship characterises the mating behaviour of many species of Orthoptera (Vahed, 1998). In some the donation may consist of male body parts, a situation which occurs in the genus *Cyphoderris,* in which females consume the fleshy underwings of males during copulation (Morris, 1979). In the genus *Neonemobius*, females feed on glandular secretions, located on the male's tibial spurs, during mating (Mays, 1971). Male tree crickets of the genus *Oecanthus* provide females with protein-rich exudates from specialised metanotal glands (Walker, 1978; Bell, 1980). Bell (1980) showed that female *Oecanthus nigricornis* that fed on glandular secretions for longer periods had greater fecundity.

In the majority of orthopteran species the gift is in the form of a spermatophore which is eaten by the female after mating. In the acridid grasshopper (*Melanoplus sanguinipes)* males pass an average of seven spermatophores to the female during mating (Friedel & Gillot, 1977). Although females eject the spermatophores after copulation, they contain very little soluble protein. Pickford and Gillot (1971) showed that the proteins sequestered from the spermatophores stimulated vitellogenesis and increased female fecundity. By using labelled amino acids, Rice, cited in Gwynne (1983) as pers. comm., showed that the nutrients contained within the spermatophores of an undescribed species of Trigonidiine were utilised by females in the production of eggs. In a series of laboratory trials, males of

M. sanguinipes were shown to choose virgin females over previously mated females as partners, which probably reflects the large investment in the production of spermatophores, with males seeking to ensure that the progeny sired are attributable to them (Pickford & Gillott, 1971). The increase in fecundity resulting from the incorporation of spermatophore proteins into developing oocytes in *M. sanguinipes* and the undescribed species of Trigonidiine would appear to indicate a parental investment function of the spermatophore, as opposed to a simple sperm protection device.

1.9   Mating in the Tettigoniidae

There is a great diversity of mating systems and the criteria important in mate choice within the Tettigoniidae. Feaver (1977), for example, showed that male *Orchelimum nigripes* competed vigorously for perches where females were more likely to be located. Furthermore, females in natural populations were shown to take several hours to move between singing males before actually mating with one, a situation not dissimilar to the lekking behaviour of many vertebrates. Zaprochiline katydids produce ultrasonic signals, the fundamental frequency of which is governed by male size. In common with other sound-producing species, larger males produce lower frequency calls. Gwynne & Bailey (1988) reported apparent female preference for the higher frequency calls of smaller males. They postulated that this anomaly may have arisen because song frequency only provides an accurate measure of body size, and hence spermatophore size, early in the season when food supplies are limited. Alternatively, since higher frequency calls suffer greater environmental attenuation than those of a lower frequency, females may simply have perceived males producing them as being closer. Gwynne (1982) demonstrated a preference for larger males by females of the katydid *Conocephalus nigropleurum*. In laboratory trials females consistently chose the calls of larger males, which may be related to the quantity of nuptial gift available. Simmons & Bailey (1990) showed that competition

between females for the nutrients contained in the male's spermatophore is responsible for the facultative reversal of courtship roles in the bushcricket *Kawanaphilia nartee*. Sex role reversal is apparent early in the season when pollen, the cricket's only food source, is scarce. With an increase in the quantity and quality of pollen supplies later in the season courtship patterns switched to a more conventional pattern. In a field study, Gwynne (1981) also demonstrated that female mormon crickets (*Anarbus simplex*), attracted to the songs of signalling males, competed strongly for access to them. Males showed no aggression towards each other, and were shown to reject smaller, less fecund females. The mormon cricket produces a spermatophore that constitutes 27% of a male's body weight, which may explain male discriminatory behaviour.

1.9.1 Duetting and phonotaxis

As with most other orthopteran insects pair formation in most tettigoniid species is achieved when a female performs phonotaxis towards the signal of a stridulating but stationary male (Hartley, 1993). However, in two sub-families, Phaneropterinae and Ephipiggerinae, females have developed sound producing apparatus independently of males and are known to stridulate in response to a male call (Spooner, 1968). This duetting method of communication has led to a modification in phonotaxis for many species within the Phanerinopterinae, with males orienting to the stridulatory response of receptive females (Heller & Von Helverson, 1986; Robinson *et al.*, 1986; Robinson, 1990). This method of courtship involves a bidirectional flow of information with both male and female in acoustic contact as the male performs phonotaxis towards the female's signal (Hartley *et al.*, 1974; Heller & Von Helverson, 1986; Zhantiev & Korunovskaya, 1986).

Successful pair formation relies on females responding to the signal of a stridulating male within a narrow neuronal time window at the end of each call (Heller & Von Helverson, 1986; Robinson *et al.*, 1986; Zimmerman *et al.*, 1989). This time window is short and

exact and shows remarkable inter-individual consistency, and as such it seems likely that it acts as a species identification mechanism (Robinson *et al.*, 1986). The female's latency period relative to a male's call can be extremely brief, e.g. in *Andreiniimon nuptialis* it is only 15ms (Heller & Von Helverson, 1986).

1.9.2 Nuptial feeding in the Tettigoniidae

As with other orthopterans, transfer of sperm in the Tettigoniidae is achieved when a male transfers a spermatophore to a female during copulation. The spermatophores of the tettigoniids are complex bilobed structures consisting of a sperm containing ampulla which is inserted into the female during mating and a sperm-free mass, the spermatophylax, which remains external, and which the female eats upon the termination of copulation (Boldyrev, 1915). Investment in the spermatophylax shows a large interspecific variation, ranging from 2%-40% of male body mass (Gwynne, 1983)

Nuptial feeding in the Tettigoniidae is through the spermatophylax, which the female eats after copulation. There are two hypotheses that exist to explain the evolution and maintenance of costly male gifts, although neither is mutually exclusive. The sperm protection hypothesis proposes that the large spermatophore is selected to function solely to allow sperm to migrate from the ampulla before the female removes and eats it (Sakaluk, 1984). The paternal investment hypothesis on the other hand advocates that selection maintains the spermatophylax to benefit the nurturant male's offspring (Gwynne, 1988).

The observation that radiolabelled proteins from the spermatophore of the donating male have been found in the developing oocytes and soma of the bushcricket species *Requena verticalis* (Bowen *et al.*, 1984), *Decticus verucivorus* (Wedell, 1993a) and *Kawanaphilia nartee* (Simmons & Gwynne, 1993), would appear to support the paternal investment

hypothesis. However, they may be an incidental effect of a primarily sperm protection function of the spermatophore (Vahed, 1998). Consumption of the spermatophylax has been shown to affect reproductive success in some but not other species of bushcricket. In *Requena verticalis* (Gwynne, 1984a) and *Kawanaphilia nartee* (Simmons, 1990; Simmons & Bailey, 1990), spermatophylax consumption increases both fecundity and egg weight The effect is evident in females maintained on both normal and low quality diets; however, see Gwynne *et al.* (1984). In *K. nartee* the effect is only significant when females are nutrient stressed. In the tettigoniid species *Poecilimon veluchianus* (Reinhold & Heller, 1993), *Leptophyes laticauda* (Vahed & Gilbert, 1996b) and the wartbiter, *Decticus verrucivorus* (Wedell & Arak, 1989), there is no observable effect of spermatophylax consumption on female fecundity. In the last two species, the effect is consistent irrespective of the nutritional condition of the female.

If the spermatophylax acts as more than a sperm protection device, then males of species in which consumption of the spermatophylax appreciably enhances female reproductive output are expected to produce larger spermatophores (as a proportion of a male's overall body mass) with a higher protein content. There is evidence for this difference in size/quality of the spermatophore in some species of bushcricket, though not in others. In *R. verticalis*, a species in which spermatophore consumption increases female reproductive success, males produce a spermatophore representing 12.5-19% of overall body mass (Gwynne, 1986, 1990) with a protein content of 13.5% wet mass (Bowen *et al.*, 1984). In *D. verrucivorus*, a species in which the spermatophylax does not contribute to female reproductive success, the spermatophore is both smaller (9% male body mass) (Wedell & Arak, 1989) and has a lower protein content (4.3% wet mass) (Wedell, 1994). However the relationship between large spermatophylaces and enhanced female fecundity is not ubiquitous. The spermatophylax of *L. laticauda* constitutes 26% of male body mass (Vahed & Gilbert, 1996b) and that of *P. veluchianus* 23% of male body mass (Heller &

von Helversen, 1991); in neither of these cases does consumption of the spermatophylax have any effect on female fecundity.

In a comparative study of 22 bushcricket species, Wedell (1993b) concluded that a sperm protection function of the spermatophylax best explained its evolution. Although there was a higher total amount of protein contained within larger spermatophylaces there was a negative correlation across species between spermatophylax size and the concentration of protein. Wedell hypothesised that this may have been a result of males seeking to enhance gift size cheaply, i.e. by increasing water content at the expense of more costly proteins; see Gwynne (1995), however, who criticised the study because it failed to control for common phylogenetic history. Will & Sakaluk (1994), proposed that the spermatophylax of the cricket *Gryllodes sigillatus* represented a sham in terms of a food gift. They found no observable benefit to females from consumption of the nuptial meal, with neither female fecundity nor the mass of the eggs laid by females being affected by spermatophylax consumption.

Whether the donating male stands to benefit from the consumption of his spermatophore depends to a large extent on how many of the subsequent eggs are fertilized by his sperm. In species where females mate multiply, males risk being cuckolded by other males whose sperm may fertilize eggs to which he has contributed nutrients. In the wartbiter, females may mate with several males following an initial mating (Wedell, 1993a). In this species stored sperm from several males mix within the spermathecae, and fertilization success is a function of the numerical representation of the sperm from each of the males. Consequently any male's spermatophore donation is likely to contribute to ova fertilized by another male (Wedell, 1993a). In other species similar patterns have been observed. In *Steropleurus stali* there is last male sperm precedence (Vahed, 1995), so males that mate

later are likely to benefit from the investment of males that mated previously with the female.

If the spermatophylax functions as mating effort then the gift should be just large enough to allow all the sperm to migrate from the ampulla before the female removes and eats it. Gwynne (1986) concluded that, in *R. verticalis*, sperm and substances from the ejaculate which influence a female's remating interval were transferred from the ampulla to the spermathecae when the female had consumed approximately 50% of the spermatophylax. Consequently a mating effort function did not appear to explain the large spermatophylax in this species. Vahed (1995) compared the sperm remaining in the ampulla (as a percentage of mean sperm number) against ampulla attachment time (as a percentage of mean spermatophylax consumption time) between *R. verticalis* and *L. punctatissima*, which produces a small spermatophore about 5.6 % of male body mass, and found no difference. He concluded that complete sperm transfer actually correlates well with mean spermatophore consumption time, and the effect is consistent even in species that produce relatively large spermatophores (Simmons & Gwynne, 1991; Heller & Reinhold, 1994; Vahed, 1995).

If larger males produce larger ejaculates then it might be expected that there will be a concomitant increase in the size of the spermatophylax to ensure complete sperm transfer. Corroborative evidence for this hypothesis comes from a comparative study of 46 species of European tettigoniids (Vahed & Gilbert, 1996a). This study demonstrated that there is a positive correlation between the mass of the spermatophylax and both the size of the ampulla and sperm number across taxa after controlling for the effects of body size and common ancestry. Prolonged mating duration in the genus *Meconema* provides indirect evidence that the spermatophylax acts as a sperm protection device. The spermatophylax is no longer apparent in species from this genus, hence, increased copulation duration may

function in an analagous way to the to large spermatophylax of other species i.e. males have increased the time they spend in copula to ensure all their sperm are transferred (Vahed, 1996).

1.10 Mate choice and reproductive success in *Leptophyes punctatissima*

There is little known about *L. punctatissima* in the wild, apart from a brief description of the general biology of the species by Duncan (1960). The communication system has been comprehensively analysed (Hartley & Robinson, 1976; Robinson, 1980; Robinson *et al.*, 1986; Zimmerman *et al.*, 1989). Pair formation is achieved through male phonotaxis to the response call of a receptive female (Hartley & Robinson, 1976). The flow of information between the sexes is very brief, with the male call rarely being longer than 15-25ms and the female response only 1ms. Successful pair formation relies on the female responding to the call of the male within a time window of 30-50ms after the start of the male call (Hartley & Robinson, 1976). This represents one of the fastest acoustico-motor responses yet described for any insect species (Zimmerman *et al.*, 1989).

This system could, in theory, afford the opportunity for both sexes to choose a mate without coming into contact with them. Females could assess a male's quality via his call and males could choose whether or not to approach a responding female. It has been argued, however, that the short time scales involved in the *L. punctatissima* communication system would not allow detailed neuronal processing of the characteristics of the call, and so choice of a male based on the properties of his call may not be possible (Robinson *et al.*, 1986). Because of the short latency period for the female response required for successful phonotaxis, the female reply to a male call is a reflex response once her tegmina are raised. However, the female can still choose to raise her tegmina or not in response to a male call. It may also be possible to process call characteristics in a post-hoc

manner and females may choose to close their tegmina and so halt phonotaxis if the call of the male is perceived not to be of a high quality. Thus it is possible that calls could be used as a criterion for mate choice in both sexes.

If the call is not used as a criterion of mate choice then individuals could still assess mates once they meet, using sight, smell or touch. In other species important criteria of choice seem to be both male and female size, size and/or quality of the spermatophore, which in turn is related to the size and nutritional condition of the male, and asymmetry. Reproductive success often depends heavily on the ability to get matings in males, and on fecundity in females. In both males and females, size, diet and asymmetry may be important factors in determining reproductive success.

In order to achieve the aims of this study, I therefore looked at variation in size, asymmetry and call characteristics and how this variation relates to differences in diet. I also investigated the interrelationships between mating success, fecundity, survival and diet and the variation in physical and call characteristics I observed.

2 Methods

2.1 Introduction

This chapter sets out all the methods used in this project. It describes the collection and laboratory maintenance of the insects, the techniques used for measuring and marking them, the culture and measurement of eggs, the design of all experiments, the methods of behavioural observation, and the recording and analysis of the characteristics of male song. It also describes the methods used in statistical analysis where these apply to more than one chapter.

2.2 Field collection of insects

All insects used in this study were collected from the wild in 1998 and 1999 during June, usually when they were in their second or third instar, occasionally in their first or fourth instar (*L. punctatissima* has six instars). The main collecting site was the Warren nature reserve in Folkestone, Kent, UK. The Warren is a long, thin strip of land, which runs along the coastline for 2-3km on the east side of Folkestone. It supports a large population of *L. punctatissima* at a relatively high density. The vegetation of the Warren consists of a strip of deciduous woodland inland, with a variety of herbs and shrubs, mostly buddleja (*Buddleja davidii*) and bramble (*Rubus fruticosus*), on the seaward side. All insects were collected from the coastal strip of scrub. Here, insects in their first four instars were found in the greatest numbers on low vegetation growing underneath mature shrubs, mostly on wild sage (*Salvia nemorosa*) with smaller numbers on bramble, buddleja and stinging nettle (*Urtica dioica*), all of which are common along the coastal scrub.

In 1999 some insects were also collected from similar habitats at two sites in Milton Keynes: the Ouzel Valley park and the grounds of the Open University. These sites support

much smaller and lower density populations of *L. punctatissima* than the Warren. In the Ouzel valley park, insects were usually found on stinging nettle and bramble. Around the grounds of the Open University they were found in the greatest numbers on snowberry (*Symphoricarpos albus*). At all three collecting sites, it was most common to find insects, especially the younger instars, on young, 'fresh' leaves.

The animals were collected using a pooter (Fig.2.1), an entomological collecting aid that consists of a hollow tube corked at both ends. Each cork is drilled and has a thin plastic tube inserted through it, one of which is attached to a rubber tube. Sucking through the rubber tube allows the insects to be drawn through the tubing at the other end and trapped in the main portion of the apparatus.
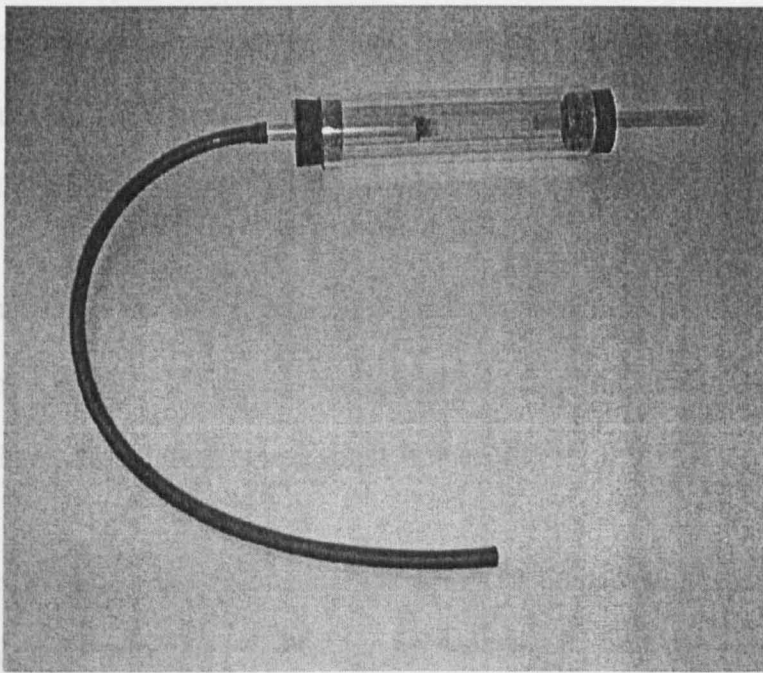
Figure 2.1 A pooter used to collect insects

After capture the insects were transferred to ventilated holding tins containing bramble and sage leaves as a food source, and returned to the laboratory.

2.3 Maintenance of wild-caught insects in the laboratory

In the laboratory, insects were transferred from the collecting containers to seed propagators (50cm long x 31cm wide x 23cm high) and to round breeding cages (20cm diameter x 40cm high; manufactured by Watkins & Doncaster; hereafter referred to as large WD cages) or steel breeding cages (54cm high x 32cm wide x 20cm deep; manufactured by Griffin & George; hereafter referred to as steel GG cages). Animals were segregated into groups on the basis of size. This increased the likelihood that individuals were at the same stage of development and would reach sexual maturity at a similar time. It also reduced the risk of cannibalism of smaller individuals by larger ones, since *L. punctatissima* can be cannibalistic in captivity (Duncan, 1960). Animals were kept at a low density (maximum 1 insect per 800cm^3), with 15 individuals in the large WD cages, 20 in the steel GG cages and 25 in the seed propagators, to further reduce the risk of cannibalism. Keeping animals at low densities also reduced the risk that they would be injured during moulting. This can occur because a non-moulting individual who encounters one who is moulting will often nibble at the moulter.

When females needed to be housed separately for experimental purposes, each female was placed in a 12cm diameter x 23cm high round breeding cage (manufactured by Watkins and Doncaster; hereafter referred to as a small WD cage).

In 1998, animals were kept in a constant temperature (25°C) and controlled light / dark cycle of 12 hours on, 8 hours off (20.00 to 04.00). In 1999, a controlled laboratory environment was unavailable and animals were housed in natural light conditions in an unheated laboratory, thus temperature varied with time of day and from day to day.

The insects were fed on a mix of bramble, buddleja, stinging nettle and garden sage (*Salvia officinalis*, purchased from a local garden centre). Conical flasks filled with water were

used as containers for the vegetation bunches. The stems of each bunch of vegetation were wrapped in cotton wool before being placed into the flask, so that the neck of the flask was completely blocked. This prevented the insects from falling into the flask and drowning. The cotton wool was kept moist to provide a water source for the insects. The vegetation in the breeding cages was changed every 4-5 days to ensure the animals always had access to fresh vegetation.

2.4 Manipulation of diet

At the penultimate instar stage the animals were segregated by sex to ensure they remained virgin. From this point on, half the individuals of each sex had an ad-libitum protein supplement added to their diet. The supplement was administered in two forms, buddleja and buttercup (*Ranunculus* spp.) flower heads and granules of bee collected pollen. Insects were observed to feed readily on both kinds of supplement. Consequently, from their penultimate instar stage, half of the insects were maintained on a supplemented diet (S) and half were maintained on an unsupplemented diet (US).

2.5 Measurement and marking of adults

Within 7 days of its final moult, each adult was weighed and various body measurements taken (Fig.2.2; Table 2.1). It was then marked with a number so that it was individually recognizable.

A: overall body length
B: pronotum length
C: tegmen length
D: tibia length
E: femur length
F: ovipositor depth
G: ovipositor length

male

female
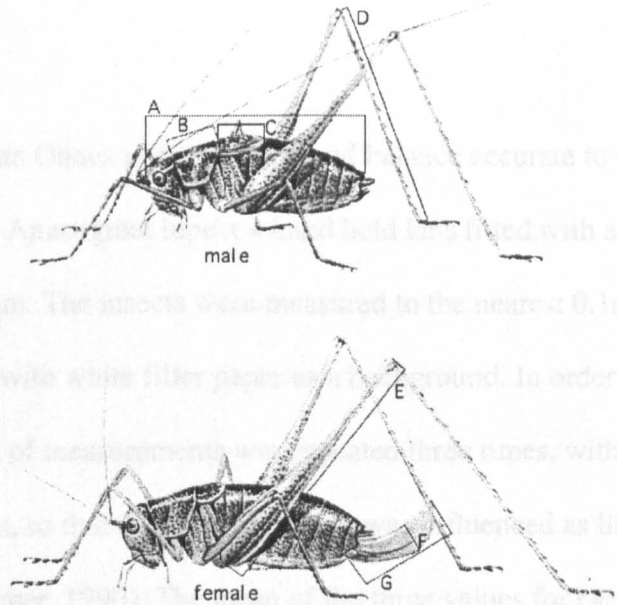
Figure 2.2 The body measurements taken for each insect

Table 2.1 Definition of body measurements shown in Fig. 2.2

Overall body length	Measured from front of head, between antennae, to end of last abdominal segment. Taken immediately after anaesthetised, while insect fully relaxed. Measured from above.
Pronotum length	From most rostral to most caudal point of pronotum, measured from above.
Tegmen length	Separate measurements for left and right tegmen, measured from above from most caudal point of pronotum to end of tegmen.
Tibia length	Separate measurements for left and right tibia. Measured from relevant side, from edge of 'knee' at proximal end to junction between tibia and tarsus at the distal end.
Femur length	Separate measurements for left and right femur. Measured from relevant side, from most proximal point of femur (excluding trochanter and coxa) to junction with tibia.
Ovipositor depth	Measured from left side. Measured at right angles to tangent of curve of ovipositor at widest point.
Ovipositor length	Measured from left side. Measured from tip of ovipositor to 'V' formed at point where ventral edge of ovipositor meets ventral abdomen.

Before being weighed and measured each animal was placed in a pooter which was attached to a cylinder of CO_2 by a length of rubber tubing. The animals were then anaesthetised by passing a stream of CO_2 gas through the pooter. Complete anaesthesia took approximately 20 seconds and was considered complete when the insects had ceased

49

moving. The process was repeated if the animals recovered before the measurements were completed.

The insects were weighed once on an Ohaus portable advanced balance accurate to 0.001g. Measurements were made using an Anastigmat lupe x 4 hand held lens fitted with an eyepiece graticule accurate to 0.1mm. The insects were measured to the nearest 0.1mm, under a clear perspex petri dish lid with white filter paper as a background. In order to reduce measurement error, each set of measurements was repeated three times, without reference to previous measurements, so that each measurement was influenced as little as possible by previous measures (Palmer, 1990). The mean of the three values for each measure was then calculated. After being weighed and measured, each animal was marked with an individual number on the dorsal surface of the abdomen using yellow Humbrol enamel paint.

For comparison with the laboratory population, I also analysed a similar set of weight and body measurement data collected in the field from a population of *L. punctatissima* near Nordkirchen in northern Germany in 1998 by M. Hall, D. Robinson and J. Rheinlaender. The data set consists of the same range of body measurements and weight as I collected from my study animals. Each body measurement was defined in the same way and was measured in the same way as in my study, using duplicate equipment.

2.6 Measuring asymmetry

Each adult measured in 1998 and 1999 was assessed for its degree of asymmetry by comparing right versus left sizes for three characters: tibia length, femur length and tegmina length. Asymmetry was calculated as right length minus left length for each character. A combined (mean) asymmetry score was also calculated for each individual as

total asymmetry (i.e. all absolute R-L values for the relevant characters added together) divided by the number of characters.

2.6.1 Testing for measurement error and directional asymmetry

To be considered as exhibiting true fluctuating asymmetry (FA) the signed difference (R-L) of characters should show a normal distribution around a mean of zero (Van Valen, 1962). Measurement error can seriously bias estimates of FA because it displays the same properties i.e. it has a normal distribution with a mean of zero, consequently it is necessary to assess the impact of measurement error on the actual between-sides variance. Palmer & Strobeck (1986) recommend a two-way mixed model ANOVA as a first step in any study of FA. The model consists of 'sides' as the fixed factor and 'individuals' as the random factor with three repeated measures of each character. The mean square (MS) value of the 'sides' term estimates if there is a consistent size bias to one side of the body (i.e. directional asymmetry). Non-directional asymmetry (i.e. FA and antisymmetry) present in the sample relative to measurement error is estimated by testing the MS of the interaction term, 'sides' x 'individual', against the MS of the error term. It is important to note that the ANOVA cannot distinguish between FA and antisymmetry and some other test must be performed to establish whether the sample contains individuals that exhibit FA or antisymmetry. The test also yields an index of FA (FA10) for each sample which is the only one which tests for the presence of FA relative to the amount of measurement error present.

2.6.2 Detecting outliers

Extreme outlying values of asymmetry in a sample may arise from factors other than developmental instability (Palmer, 1990; Tomkins & Simmons, 1995), and as such may be legitimately removed from the analysis. I used the method of Grubbs (1969), cited in Sokal & Rohlf (1995), to identify extreme values of asymmetry. For sample sizes greater than 30

the test is simply a matter of subtracting the sample mean from the value of the suspected outlying value and dividing by the sample standard deviation. I tested whether the directional asymmetry that was evident in some of the samples after performing the ANOVA was due to extreme outlying values. Five outliers were removed from the supplemented male population, five from the unsupplemented male population, three from the supplemented female population and five from the unsupplemented female population.

2.6.3 Testing samples for a normal distribution

I calculated kurtosis and skewness values for each sample. Antisymmetry is typified by negative values of kurtosis which indicate a broad (platykurtic) or in extreme cases bimodal distribution. Leptokurtosis is characterized by positive kurtosis values, and has a narrow centrally peaked distribution with long tails either side. Negative and positive skewness values indicate long tails to either the right (positive skewness) or to the left (negative skewness). I used a one-sample Kolmogorov-Smirnov test to investigate if any of the samples deviated significantly from normality. I also re-tested the four samples from which I removed outliers for DA using a one-sample t-test to check if the distributions were centred on zero.

2.6.4 Tests for size dependence and FA

The extent to which size and asymmetry are correlated can influence any inferences that are made concerning the level of FA in a sample (Palmer & Strobeck, 1986), consequently it is important to quantify the relationship between these two variables. To investigate if there was a relationship between the size of the characters under investigation and the level of FA expressed, I carried out regression analyses using the mean size of the trait (i.e. $(R+L)/2$) as the independent variable, and absolute asymmetry ($|R-L|$) as the dependent variable.

2.6.5 Comparing FA among samples

To investigate if the degree of FA differed between the different groups I used Levenes test (Palmer, 1990), Levenes test allows the comparison of three or more samples to be carried out consecutively.

2.7 Recording calls

The male call in *L. punctatissima* consists of a chain of 5-8 pulses (syllables), each about 1ms in duration, while the female call usually consists of one, occasionally two, 1ms syllables (Fig. 2.3).
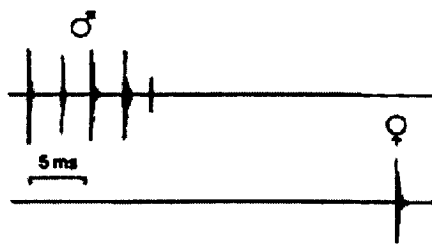
Figure 2.3 Oscillogram of the male call and female reply

The calls are ultrasonic with a carrier frequency of 40kHz and as such cannot be recorded directly onto audio-tape. To tape calls they first have to be passed through a bat detector set to the appropriate frequency. The bat detector modulates the frequency of the signal, which can then be recorded. To investigate the relationships between call characteristics, other physical characteristics, mate choice and reproductive success, I recorded the calls of *L. punctatissima* during the summers of 1998 and 1999.

All calls were recorded between early August and early September each year in an anechoic chamber measuring 1.2m long x 1.2m wide x 1.3m high. The microphone of a bat detector (Ultra Sound Advice) was suspended from the ceiling of the anechoic chamber 20cm above a 25ml flask containing a sprig of bramble. The bat detector was a modified

version of the standard model with a frequency range between 10-107kHz. The bat

detector also had a 3m extension flex connected to the microphone, which made it possible

to suspend the microphone from the ceiling of the anechoic chamber at a set distance from

each insect. The bat detector was connected to a tape recorder. To record a male, he was

placed on the sprig of bramble, then the bat detector was set to 40kHz and switched on.

When the male began calling the tape recorder was switched on and recording began.

In 1998, the tape recorder was a Sony WM D6C professional portable cassette recorder

recording onto Sony high-fidelity chrome tape, and all males were recorded for a period of

2 min. A total of 23 males were recorded, of which 12 were supplemented and 11 were

unsupplemented

When the recordings were completed the tape recorder was connected to an Ultravox audio

filter and the male calls were transferred to a computer using the Ultravox ultrasonic sound

analysis software (version 2.0). This analysis package allowed two parameters of the calls

to be investigated: call length and inter-call interval (see Table 2.2).

Table 2.2 Definition of the call parameters analysed for the males in the study

Parameter	Definition
Call length	The length of time between the beginning and end of a single call.
Inter-call interval	The length of time between the end of one call and the beginning of the next.
Syllable length	The length of time between the beginning and end of a single syllable
Syllable separation	The time between the end of one syllable and the beginning of the next within a single call
Frequency	The carrier frequency of the call

In 1999, the tape recorder was a TEAC A-2300SX reel-to-reel, recording onto Quantegy

456 studio master audio-tape. The reel-to-reel tape recorder allowed recordings to be

played back at a slow speed so that the structure of each call could be studied in greater

detail. Calls recorded at normal speed (183.75 cms^{-1}) on the reel-to-reel tape recorder were

played through a Gould DSO 400 oscilloscope at half speed (91.88 cms^{-1}). This allowed call length, number of syllables per call and inter-syllable interval to be calculated (see Table 2.2). Six separate calls were recorded for each male and a total of 36 males were recorded, of which 18 were supplemented and 18 were unsupplemented.

In 1999, one call from each male was also recorded onto a portable ultrasound processor (Ultra Sound Advice) via an S-25 bat detector. This allowed the frequency of the male call to be determined (see Table 2.2).

In both 1998 and 1999, it proved impossible to record from all the experimental males, since some males failed to call under the conditions required for recording, despite repeated attempts.

Female *L. punctatissima* only call in response to a male's call or to something approximating a male call. To stimulate a female to call, an artificial male call was synthesized on a Global Specialties 4001 pulse generator. To record a female's call she was placed on the sprig of bramble in the anechoic chamber and a series of synthesized male calls were played through an ultrasonic speaker located 40cm from the centre of the chamber. Both the male call and the female reply were recorded onto Sony chrome audio tape using the Sony portable cassette recorder. Unfortunately, the number of females that responded to the synthetic call was not sufficient to allow me to make any meaningful comparisons between the call characteristics of different females.

2.8 Collection, culture and measurement of eggs

2.8.1 Collection of eggs

After mating, each female was housed separately in a small WD cage, so that the number of eggs she laid could be counted. In the wild, female *L. punctatissima* are thought to

oviposit in crevices in tree bark (Duncan, 1960; Deura & Hartley, 1982). In the laboratory, each female was therefore supplied with oviposition substrates that mimicked natural substrates in that they provided layers between which eggs could be laid. These oviposition substrates consisted of (a) five circular discs of polythene stapled together in the middle and (b) a strip of corrugated cardboard. The polythene was placed under the flask in the cage of each female so that it protruded by about 5 cm all around; the strips of cardboard were laid flat on the floor of the cages. The oviposition substrate was inspected for the presence of eggs at least every 48 hours.

Eggs were removed from the polythene discs by teasing each layer apart by hand and removing the eggs with a pair of storkbill forceps. The cardboard was inspected by running cold tap water over each strip and separating out the different layers, to expose any eggs which had been deposited. The cotton wool stoppers and the vegetation from each cage were also checked for the presence of eggs. The total number of eggs collected from each female during each inspection was recorded.

2.8.2   Culture of eggs

After collection the eggs were plated, i.e. separated and placed on a sheet of filter paper, which was then put on top of a layer of moistened cotton wool in a petri dish to prevent the eggs from desiccating. The eggs of different females were plated in separate petri dishes and only eggs laid within the same seven-day period were plated together. When plating the eggs, care was taken to handle them directly as little as possible. The eggs are prone to a fungal growth in culture, which can kill them, and contact with the skin tends to encourage fungal infection. Each petri dish was labelled to show female identity and the period in which they were laid.

Eggs were kept at room temperature (22°C) for 15 weeks after which they were transferred to an incubator set to 8-10°C for 12 weeks; thereafter eggs were incubated at 15°C until they hatched. This schedule was based on those used by Deura (1982). During the incubation period the eggs were checked at least once a week. The cotton wool was re-moistened as necessary by injecting water into it using a syringe. Any dishes containing eggs infected with fungus were replated onto fresh filter paper and any badly affected eggs were discarded.

2.8.3   Measurement of eggs

The eggs of *L. punctatissima* are known to take up water, which could affect their size and weight, but this occurs mainly after the pre-embryonic diapause, which begins about 60 days after laying or later (Warne, 1972). All measurements of eggs collected from experimental females were therefore completed in the first 6 weeks of the 15-week period during which they were stored at room temperature. Random samples of 10 eggs from each of the females were weighed and measured. The eggs of *L. punctatissima* are flattened and ovoid in shape with neither end being discernibly wider than the other. Two measurements were taken for each egg: the length, and the width across the widest part of the egg. Each egg was measured three times, without reference to previous measures. Mean length (L) and mean width (W) were calculated from the three measurements taken in each case. A measure of overall egg size, based on the area of an oval with maximum radius 0.5L and minimum radius 0.5W was then calculated using the formula:

$$\frac{\pi \times W \times L}{4}$$

After the completion of measurements each batch of 10 eggs was weighed once on an Ohaus portable balance accurate to 0.001g.

2.8.4 Hatching

During the final stage of egg culture (incubation at 15°C), the eggs were checked daily and any hatchlings were removed to rearing cages. However, hatching success was poor and only 10 individuals emerged successfully from all of the eggs that were incubated. This made it impossible to compare hatching success between females.

2.9 Maintenance of laboratory-bred insects

Eggs collected by M. Hall and D. Robinson in the summer of 1996 were cultured with the aim of establishing a laboratory-bred population of *L. punctatissima*. On eclosion in spring 1997, newly hatched immatures were housed in large WD cages with others that hatched in the same 7-day period. Initially they were fed on the same diet as the wild-caught insects and were maintained in the same way. However, in the first week after eclosion, newly emerged insects suffered mortality rates that ranged from 75% to 100% per cage. Various changes in regime were tried to try to reduce this mortality rate, including increasing the humidity and decreasing the numbers of individuals per cage. These had negligible effects. Observations of feeding behaviour of wild-caught insects suggested that dietary preferences change with age: younger instars (2-3) show a preference for sage while older instars (4 onwards) show a preference for bramble. It was thought therefore that the diet provided to first instars could be unsuitable and this might be the cause of the high mortality rates. Observations of the feeding behaviour of newly eclosed insects showed that they feed in a different manner to older individuals. Older instars (3 onwards) eat entire sections of leaves whereas younger instars graze on the cuticle, perhaps because their mouthparts are not strong enough to break through the leaf. The leaves of bramble and sage on which the insects were initially maintained are quite tough, and it is possible that younger insects may find it difficult to feed off these two plants efficiently.

I therefore experimented with other plant material in an effort to overcome this problem. I used a mix of vegetation types, common in the natural habitat of the insects. Buddleja and stinging nettle, which are both softer in texture than bramble and sage, were added to the diet of newly emerged insects. Buddleja flower heads, the petals of which are very soft, were also provided. For the first 3 weeks after hatching the food was changed every 48 hrs to ensure it remained very fresh. Thereafter the vegetation was replaced every 4-5 days as usual for the wild caught insects. The change in the dietary regime of the early instars reduced the death rate considerably, with rates dropping to around 40% mortality. However, as a consequence of the difficulty involved and the amount of time required to rear hatchlings to adulthood, plans to establish a laboratory culture of *L. punctatissima* were abandoned, and all the insects used in my experiments were collected from the wild.

2.10 Preliminary behavioural observations

2.10.1 Male calling activity

So that mating experiments could be timed to coincide with the insects' more sexually active periods, I monitored sexually mature male adults from the laboratory population of *L. punctatissima* for 10 minutes at hourly intervals, and noted any calling activity. The observations took place from 08.00 BST (British Summer Time = GMT + 1 hour) until 20.00 BST over three consecutive days in July 1998. These preliminary observations indicated that more males called between the hours of 10.00 to 13.00 BST and 15.00 to 18.00 BST (Fig. 2.4) than at any other time during daylight hours.
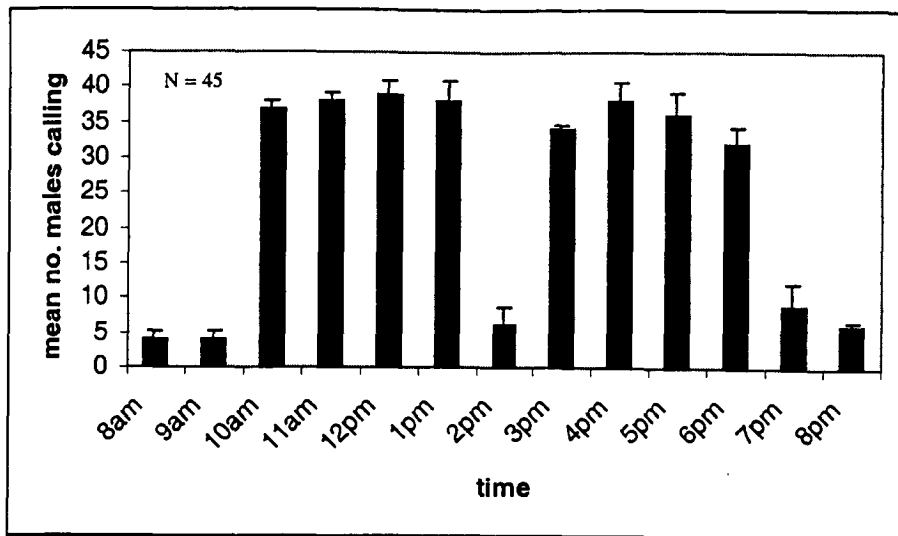
Figure 2.4 *Mean number of males calling with respect to time of day*

Observations of a wild population of *L. punctatissima* in Germany indicate that male

singing activity peaks between the hours of 09.00 to 11.00 local time (German Standard

Time, which is equivalent to BST), 14.00 to 19.00 GST and 00.00 to 04.00 GST (M. Hall,

pers. comm.). The two daytime peaks thus occurred at similar times in the lab and in the

wild, though, as no observations of male calling behaviour were made during the hours of

darkness, it is not clear whether the laboratory population showed a nocturnal peak of

calling activity.

2.10.2 The refractory period between matings

In order to plan mating experiments I needed to know the refractory period of males and

females between matings. The refractory period is the time interval following mating

before an individual is willing to mate again. I took 10 males and placed them in a 40cm

long x 20cm wide x 25cm high glass tank. The males were then presented with 10 females

and observed for 30 min. During this initial observation period three males and three

females mated. The insects that mated were removed from the arena and placed in separate

holding cages. Insects that did not mate were also removed and returned to the general

laboratory population. Two hours after the end of the initial observation period, the males

and females that had mated were re-introduced into two separate 40cm long x 20cm wide x 25cm high glass tanks with males in one tank and females in the other. They were then presented with five new partners of the opposite sex. The insects were observed for a period of 30 min for any signs of mating activity. If no mating activity occurred during this period the insects were removed from the arena and placed back in their holding cages. I continued to repeat this procedure, at intervals of 2.5h, throughout the daylight hours from 07.30 BST to 19.00 BST during the period 29/6/97 to 2/7/97 and recorded any mating behaviour. Although no males or females re-mated within any one observation period, three males were observed calling 4h after mating for the first time and two females were seen to reply to the calls of these males. Two of the males and two of the females re-mated 24h after mating for the first time, and the third male was observed calling but failed to mate. Experiments were therefore designed to allow individuals at least 24 hrs to recover between matings.

2.11 Breeding population experiment

The experiment was designed to simulate real breeding populations in that individuals were given a large choice of mates and the opportunity to mate throughout the whole season. However, as it was not possible to observe for 24 hours a day for several weeks, the real life situation was approximated by taking a group of insects and putting them together so as to give individuals the opportunity to mate, at set intervals. Between 'group' sessions the sexes were separated so that no mating could take place. It was therefore possible to record all the matings that took place within each group. Three groups were set up to represent three different breeding populations, each with different nutritional circumstances. The S group (supplemented group) was the equivalent of a population in an area with high-quality food available, the US group (unsupplemented group) a population

with poorer-quality food, and the half and half group a population where different individuals had access to food of different quality.

After being measured, 10 males and 10 females were randomly assigned to each of the three groups (see Table 2.3). Randomization was achieved by writing the identification numbers of the insects on pieces of paper and drawing them from a hat. Within each group the individuals were all approximately the same age (they emerged as adults within the same 7-day period). All the insects used in the experiment were wild caught and the same animals were used throughout. Any animals that died during the course of the experiments were not replaced.

Table 2.3 The allocation of insects to the three breeding groups

Group	Males	Females
S	10 supplemented	10 supplemented
US	10 unsupplemented	10 unsupplemented
Half and half	5 unsupplemented 5 supplemented	5 unsupplemented 5 supplemented

The experiment was carried out from 17/7/99 to 13/8/99 (see Table 2.4). Groups were put together, so that individuals had the opportunity to mate, twice every 72 hours. The second group session was at least 24 hours after the previous one, to ensure that individuals would be ready to remate if they had mated in the previous session (see Section 2.10.2). Each group session lasted 3 hours and they were timed to coincide with the peak periods of male calling, 10.00 to 14.00 BST or 15.00 to 18.00 BST (see Section 2.10.1). Each group had one morning session and one afternoon session within each 72-hour period, to control for the possible effects of time of day on mating activity. Between group sessions males and females were returned to their appropriate holding cages. Males were housed together with others from the same dietary regime in large WD cages; females were caged individually in small WD cages so that the number of eggs each female laid could be recorded.

Table 2.4 Timing of group sessions for the breeding groups

	First session	Final session	Total no. sessions
S	17/7/99	12/8/99	19
US	16/7/99	12/8/99	19
Half and half	17/7/99	13/8/99	19

A glass tank measuring 120cm x 38cm x 38cm was used for the group sessions. At the beginning of each session the tank was divided centrally with a cardboard sheet and the insects were introduced at either end, males at one end and females at the other end. Three flasks of bramble were placed in the tank, spaced about 25cm apart along its length, to provide males with an elevated position from which to call, and a natural substrate on which pairs could mate. After introduction into the tank the insects were given 2 min to acclimatize to their surroundings. The divider was then removed and the observations were started.

An interval sampling method was used to record male calling activity. Every 3 min, the tank was scanned and the males were checked for movements of the tegmina, i.e. rapid opening and closing of each tegmen. The scan lasted for 1 min and if he made at least one call during this 1-min period, a male was recorded as singing; if no call was made the male was recorded as silent.

All occurrences of behaviours defined in Table 2.5 were recorded. A Smiths bench stop-clock was used for all timings and all data were recorded on behavioural check-sheets. Table 2.6 lists the data that were recorded for each type of behaviour.

Table 2.5 Definitions of behaviours shown by *Leptophyes punctatissima*

Behaviour	Definition
Male calling	A male stridulates by rapidly opening and closing his tegmina.
Female reply	A female responds to a male call, by rapidly opening and closing her tegmina within 30-50 ms of the onset of the male's call.
Male rejection of female	Male rejects a copulation attempt by a female either by walking away from the female after approaching her, kicking her or making kicking movements towards her as she attempts to mount him, or failing to cooperate in achieving genital coupling.
Female rejection of male	Female refuses to mate with a male after he has approached her, either by walking away from him, kicking him as he attempts to mate, or dismounting before genital coupling is achieved.
Antennation	One individual touches another with its antenna.
Backing under	One individual moves backwards towards the head of another individual, and pushes underneath them, effectively making the other individual mount them.
Mounting	One individual climbs onto the back of another, usually approaching from the rear and moving along its back.
Palpating back	During mounting, the mounter palpates the dorsal cuticle and tegmina of the individual being mounted as it moves up its back.
Copulation	There is genital coupling between a mating pair during which time a spermatophore is transferred from the male to the female. Copulation ends when pair's genitals separate and the mounter dismounts.
Spermatophore consumption	A female bends forward and begins to eat the spermatophore by pulling pieces off it while it remains attached to her genital region.
Tremulation	A male moves its body in a violent up-and-down motion while its feet remain in contact with the substrate.
Interference	A third individual attempts to prevent a pair from copulating by physically interfering with them, either by attempting to dislodge the female, kicking the pair, or attempting to mount the female.
Intrasexual aggression	Kicking movement of the legs directed at another individual of the same sex.
Pseudocopulation	The genitals of two individuals of the same sex come into contact after one mounts the other, during which time a spermatophore may be passed between two males. Ends when the pair's genitals separate and the mounter dismounts.

Table 2.6 Behavioural data recorded

Behaviour	Data recorded
Male calling	Identity of male Whether singing or not in each 3-min intervals
Rejection	Identity of male and female Time rejection occurs Nature of rejection behaviour.
Mounting	Identity of the mounter and mountee Time mount occurs
Copulation	Identity of copulating pair Time copulation occurs
Spermatophore consumption	Identity of the individual Time begins to consume spermatophore Duration of spermatophore consumption
Tremulation	Identity of tremulator Time begins tremulation.
Interferes	Identity of interferer Identity of pair interfered with Nature of interference behaviour Whether successful (i.e. prevents another pair from copulating) Whether interferer then successfully copulates with a member of the pair
Pseudocopulation	Identity of pseudocopulating pair Time pseudocopulation begins Whether spermatophore is produced

2.12 Factors affecting fecundity: single versus multiple mating experiment

2.12.1 Introduction

Like many orthopteran insects *L. punctatissima* is known to mate multiply. Multiple

matings by both males and females have been observed by Vahed (1995) in the laboratory

and by Hall (pers. comm.) in a wild population in Germany. This experiment was designed

to investigate the effect on fecundity of the number of times a female mated, how other

factors such as diet, female size, spermatophore size, and male size influence female

fecundity, the effect of multiple mating on male spermatophore production, and the

relationship between spermatophore production and other male characteristics such as size.

2.12.2 Experimental protocol

Females were divided into two sets: in one set (single matings) females were allowed to mate only once; in the other set (multiple matings) females were allowed to mate up to three times. Within both single and multiple mating sets I randomly assigned females and males to each of four mating groups, each representing a different combination of male and female diets (Table 2.7). The males in the experiment mated between 2 and 4 times.

Table 2.7 The allocation of insects to the four groups

	Males	Females
Group 1	supplemented	supplemented
Group 2	unsupplemented	unsupplemented
Group 3	supplemented	unsupplemented
Group 4	unsupplemented	supplemented

Before the experiment was carried out each animal was measured and marked (see Section 2.5) and all insects were initially virgin. Animals were given the opportunity to mate in four mating arenas, one for each mating group, each of which consisted of a glass tank measuring 90cm long x 40cm wide x 50cm high. Twenty-file ml conical flasks containing bramble were placed in each mating arena to provide the insects with a natural substrate on which to mate. At the beginning of each experimental period four males and four females from the same mating group were introduced into each of the tanks, and were monitored for mating activity. The experimental periods were timed to coincide with the insects' more sexually active periods i.e. between the hours of 10.00 to 13.00 BST and 15.00 to 18.00 BST (see Section 2.10.1).

When a mating took place, both the male and the female were immediately removed from the tank. Females were housed separately in small WD cages. The cages were marked to indicate the dietary regime the female belonged to, whether she was singly or multiply mated, the identity of the male she mated with in each case and the dates mating took

66

place. At the end of the observation period all males were returned to the general laboratory population with others from the appropriate dietary regime. Females that were still virgin at the end of an observation period were returned to the general laboratory population with other females from the appropriate dietary regime. Females from the single mating set were left in their individual cages after mating once and allowed to lay eggs until their natural death. Twenty-four hours after their first mating, females from the multiple mating set were re-introduced into the glass tank for their group, along with three other females and four males from the same group. The four males never included one that had previously mated with any of the females in the tank. This ensured that each female mated with a different male on each subsequent mating. After mating, and at the end of the observation period, males and females were treated as outlined above. This procedure was repeated at 24-hour intervals until the females had mated a maximum of three times.

This whole procedure was repeated for all males and females in each set, with the aim of mating as many females as possible in each set, spread evenly between the four mating groups. The number of females actually mated for each group in each set is shown in Table 2.8.

Table 2.8 Number of females mated singly and multiply for each dietary combination group.

Group	No. females mated singly	No. females mated twice	No. females mated 3 times
1	7	0	8
2	8	2	5
3	8	3	5
4	6	2	5

The cage of each female was checked for the presence of eggs at least every 48 hours and the eggs were treated as described in Section 2.8. At the end of the experiment, and after

all the females had finished laying, a random sample of 10 eggs for each female was weighed and measured as described in Section 2.8.3.

2.13 Measurement of spermatophore size

Each male used in the experiment was weighed on an Ohaus portable balance accurate to 0.001g immediately before being placed in the mating arena. Any male that mated was removed from the mating arena immediately after copulation had ceased and re-weighed. Their post-mating weight was then subtracted from their pre-mating weight to give an estimate of the weight of the spermatophore passed to the female. Without removing the spermatophore from the female, which was not possible in this experiment, this was the only way to estimate spermatophore weight. It is however subject to error as a result of weight loss by the male due to e.g. defaecation, respiration, water loss and weight gain due to ingestion of water or food. The size of the error is likely to increase with the length of time between first weighing and mating.

After they had been re-weighed males were kept in large WD cages with other males from the same dietary regime and with the same mating history. Males were given at least 24 hours to recover before being given the opportunity to mate again.

2.14 Statistical analyses

Data were analysed using parametric tests where possible. Where sample sizes were small or the data deviated from a normal distribution, non-parametric tests were employed. Analysis of variance techniques were used to examine the relationships between various attributes of the animals in the study and their reproductive success. Paired comparisons were analysed using paired t-tests, or their non-parametric equivalent. All statistical analysis was carried out on a personal computer using SPSS version 7 software.

When conducting multiple correlations using the same characteristic care should be taken to guard against false positive results. I used the Bonferroni correction which adjusts p values at the table wide level of significance.

All means are quoted ± SE (Standard Error), and error bars on graphs represent ± SE. All tests are two-tailed unless otherwise stated.

3 Ecology, life history and general behaviour

3.1 Introduction

In this chapter I give an overview of the ecology, life history and general behaviour of *L. punctatissima*, based on a combination of previously published information and observations made during my own study.

3.2 The species

Leptophyes punctatissima (Orthoptera: Tettigoniidae) is a small to medium sized bushcricket. Marshall & Haes (1988) give figures of 9-11mm for body length in males and 11-18mm for that in females, although as you will see in Chapter 4, I found individuals outside these size ranges both in the laboratory animals collected in the UK and in the German population. In the laboratory, the range in body length was 11.1-16.6mm for males and 12.7-18.3 mm for females. In the German population it was 10.3-13.5 mm for males and 12.3-16.0 mm for females. The range of body lengths quoted for females exclude the length of the ovipositor, which I found to be in the range 5.2-8.0 mm, similar to the published figures in Marshall & Haes.

Adults are predominately green in colour and males have a dark brown stripe running along their dorsal surface, which is absent in females (Fig. 3.1, 3.2). Both sexes have brown speckles which cover most of their body area. The insects are flightless and their greatly reduced forewings (tegmina) have a leathery appearance. Hindwings are absent. The cerci are short, lack teeth and point inwardly and the curved ovipositor is broad and flattened (Marshall & Haes, 1988).

Figure 3.1 Male *L. punctatissima*
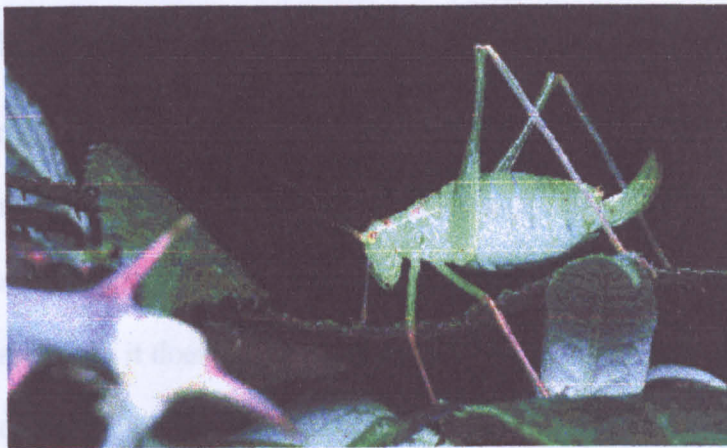
Figure 3.2 Female *L. punctatissima*

In the wild *L. punctatissima* are relatively inactive (though they still may feed) during the

hours of 03.00 to 09.00. They may also show reduced activity during the early part of the

afternoon, especially if temperatures are high. During these periods, insects may move

slightly deeper into the vegetation or hide under a leaf (M. Hall, pers. comm.). This

behaviour may act to regulate temperature, keeping the insects warm during the early part

of the day and cooler during the hot afternoons. It probably also functions to hide the

insects from predators.

71

3.3 Phylogeny of *Leptophyes punctatissima*

L. punctatissima is an ensiferan Orthopteran belonging to the family Tettigoniidae, the group that contains all bushcrickets. It is a member of the sub-family Phaneropterinae, a group in which, in many species, both sexes have evolved the capacity to stridulate (Spooner, 1968). The genus *Leptophyes* belongs to the tribe Barbitistini, a group that contains 18 other genera of phaneropterines, including *Ancistrura, Barbistes, Isophya, Melaplastes, Orthocercodes, Poecilimon* and *Polysarcus*. The genus *Leptophyes* contains 14 species, of which only *L. punctatissima* is present in the UK.

3.4 Distribution

The distribution of *L. punctatissima* is described by Marshall & Haes (1988). The species is common in Europe, occurring from Spain to southern Scandinavia and eastwards to Yugoslavia and western Russia. In England *L. punctatissima* is common throughout the south and east though it does not occur in areas of high moorland in Devon. There are large populations around coastal areas of Wales but it is rare inland. There are small isolated colonies on the Galloway coast of Scotland but otherwise it is absent. The main distribution in Ireland is around Dublin, but Cotton (1980) reported one or two other separated colonies. The Channel islands of Jersey and Guernsey have widespread populations and it is still well established in its single location on the Isle of Man. The occurrence of *L. punctatissima* on a number of British offshore islands suggests a natural distribution rather than a chance introduction to the UK.

3.5 Diet preferences

The reported movements of wild *L. punctatissima* with respect to age (Duncan, 1960), and my observations of insects in the laboratory indicate that there may be a change in food

preferences with age. These changes may be related to the insects' increasing ability, with age, to deal with tougher vegetation (see Chapter 2).

Observations of wild populations in the UK and Germany indicate that juvenile food preferences may also change with location. Insects collected at Folkestone in the UK were found in the greatest numbers on wild sage. At the two other collection sites in the UK, Ouzel valley park and the grounds of the Open University, wild sage was absent but there were other vegetation types that were common to all three areas, namely stinging nettle and bramble. In the Ouzel Valley Park, juveniles were found in the greatest numbers on stinging nettle, whereas at the Open University young instars colonised the flowering shrub snowberry in preference to either stinging nettle or bramble. In the German population juveniles appeared in the largest numbers on stinging nettle, but moved to trees and shrubs as they matured (M. Hall, pers. comm.). It is not clear from these observations whether populations are locally adapted to the available vegetation types or whether insects are simply generalists, eating whichever plant is most suitable in their location.

3.6   Life history

Females begin to oviposit within a few hours following mating in captivity, and show a similar time lag in the wild (J. Rheinlaender, pers. comm.). In the wild eggs are laid in crevices or between the bark of trees (Duncan, 1960). They are ovoid in shape and light brown in colour and are approximately 3mm long by 1.5mm wide (Marshall & Haes, 1988). The species may be univoltine or biennial (Hartley & Warne, 1972). The embryo develops slowly and, in addition, usually enters a period of diapause, which may last from 2 months to more than 6 months, depending on temperature. Eggs, particularly those laid late in the year, may take over 18 months to hatch.

Most nymphs hatch in late May to early June (Duncan, 1960), and first instars are light green in colour and resemble immature capsid bugs or large aphids. The juveniles go through six instar stages and emerge as adults by late July to early August (Marshall & Haes, 1988). However, hatching times may vary depending on temperature and other prevailing environmental conditions, such as rain, and a few juveniles may still be found in late August in the wild (M. Hall, pers. comm.). Each instar lasts approximately 7 days at a constant temperature of 25°C in the laboratory. In the wild the timing is more variable and each instar can take up to 2 weeks. Sex differences are apparent from about the fourth instar. Adults achieve sexual maturity approximately 1 week after their final moult. Males tend to emerge as adults before females so initially there is an excess of males. This is not uncommon in insects (Rutowski, 1982), and is probably connected to male competition for females, (see Chapter 5).

Duncan (1960) reported that *L. punctatissima* juveniles were found in low-lying vegetation, moving to the tops of trees as late instars and adults. He also described females descending from these elevated positions to oviposit in low lying vegetation. However, observations of a wild population in Germany (M. Hall, pers. comm.) show that some juveniles may be found in elevated positions and adults at any height. Since juveniles move around very little, and stay close to where they hatched, this suggests that females may sometimes oviposit in elevated positions, as well as close to the ground. Also adults of both sexes may travel long distances, both between levels within the vegetation and from one area to another, ranging up to at least 50m away from their natal site. The reason for these large-scale movements is unclear since movement by both sexes is not necessary to avoid inbreeding.

The length of time the mating season lasts is also unclear. In the laboratory the animals used in the mating experiments showed a reluctance to mate towards the end of the

experiment, which lasted 4 weeks. This may not necessarily reflect the natural situation. In the laboratory both male and female insects were kept together at a high density and may have had a greater number of mating opportunities than individuals in the wild. Males have been heard calling in late October in Germany (J. Rheinlaender, pers. comm.). Whether this indicates a prolonged breeding season or simply reflects the late hatching of some individuals is not clear.

By the end of October, most adult individuals are dead (Duncan, 1960).

3.7 Calling behaviour

As with other orthopteran insects, *L. punctatissima* advertise their willingness to mate using sound. The sound-producing structures are located on the modified forewings (tegmina). As the forewings open and close, the costal margin (plectrum) of the right tegmen impacts against a row of stridulatory pegs (the file) located on the left tegmen and produces the call. It is possible that the length of the tegmina could have an effect on call characteristics; for instance, longer tegmina may contain a greater number of stridulatory pegs, which may produce longer calls.

The call of *L. punctatissima* is ultrasonic, with a carrier frequency of 40kHz, and only the faint modulation envelope is audible to the human ear (Warne & Hartley, 1975). The male call is 15-25ms long, and consists of a chain of 5-8 pulses, each about 1ms long. This is amongst the shortest recorded for any orthopteran species (Zimmerman *et al.*, 1989). Like some other members of the phaneropterine sub-family, females have also evolved the capacity to stridulate. They have a different stridulatory apparatus from males, however, with a plectrum and file on both tegmina, indicating that females developed the ability to call independently of males (Hartley & Robinson, 1976). Females normally call only in

response to a male call (Hartley & Robinson, 1976). The female reply is extremely brief and usually consists of a single pulse of 1ms duration, though occasionally there is a second pulse (Robinson *et al.*, 1986; Zimmerman *et al.*, 1989; Robinson, 1990). As with other duetting phaneropterines, male phonotaxis towards the female depends on the female replying to the male signal within a neuronal time window after each individual call. The temporal window of *L. punctatissima* is highly specific and successful phonotaxis by the male requires female response times that fall within a time frame of 30-50ms after the onset of the male call (Heller & Von Helverson, 1986; Robinson *et al.*, 1986; Zimmerman *et al.*, 1989).

Males show peaks of calling in late morning, late afternoon and for a few hours after midnight (see Section 2.10.1). If a calling male detects a reply from a conspecific female he increases his calling rate and moves towards her (Hartley & Robinson, 1976). The male will only respond if he detects the female reply within a critical time window of 30-50ms from the start of the male call. If the female fails to respond within this time window, the male stops performing phonotaxis. This behaviour is contrary to that observed in most species of Orthoptera in which females move towards the stridulating but stationary male. Once the male has approached the female, mating usually takes place quickly.

3.8   Copulation in *L. punctatissima*

Once a calling male has approached a responding female, the pair antennate each other. The male usually then turns away from the female and pushes his posterior region under the female's head, arching his back downwards as he does so. The female then mounts the male, palpating the male's dorsal surface as she walks up his back. When the female has mounted the male she nibbles the dorsal tergites immediately underneath the male's tegmina (Vahed, 1995). This often leads to the male lurching forward and can result in the

76

termination of copulation, perhaps because the female bites too hard. The tips of the males

cerci then couple with grooves on both sides of the base of the ovipositor. In orthopteran

insects males lack an intromittant organ and the transfer of sperm is via a spermatophore.

In the Tettigoniidae this spermatophore is a complex bilobed structure consisting of a

sperm containing ampulla which is inserted into the female during copulation and a sperm

free mass the spermatophylax, which remains external (Boldyrev, 1915).

Mean copulation duration in my study was 3.35 min (SE = 0.01, N = 72). Upon

termination of copulation the male and female separate and either the male or the female

may walk away, although they may also remain in close proximity. However, after mating

the male and female do not associate with each other.

After copulation the female arches forward and begins consumption of the spermatophore,

the mean time before the onset of spermatophore consumption in my study was 18.3 min

(SE = 0.9, N = 72). The mean duration of spermatophore consumption was 41.min (SE =

2.38, N = 72). During this period the male's sperm migrate from the ampulla to the

female's sperm storage organ (spermatheca), and it is from here that fertilization of the ova

takes place. Mean spermatophore weight (calculated as the difference between a male's

pre-mating weight and his post-mating weight) of the males in my study was 0.013g (SE =

0.0006, N = 53).

Approximately 2 min after the end of mating the male begins to groom his genital region.

Then, about 1 min after genital grooming has ceased, the male starts to tremulate for

approximately 30s. The significance of this behaviour is unclear. It could simply be some

kind of reflex reaction related to the physical process of mating. It could also relay

information on the mating status of the male or the female, since the substrate-borne

vibrations resulting from this behaviour are likely to be perceived by other individuals in

the immediate vicinity. However, if this is the reason for this behaviour, the benefits are unclear. Males in this species, like others that produce costly nuptial gifts, enter a refractory period immediately after mating, during which time they replenish their accessory glands with materials needed to produce another spermatophore. Consequently they are incapable of further matings until this process is complete. Advertising this condition to other insects would not appear to be advantageous. Nor does there seem to be any advantage in advertising the female's status, since the male does not appear to guard the female and usually does not stay close to her for very long. There are also possible costs associated with this behaviour: apart from the energy required, tremulation is more likely to alert predators to the location of the male, both because of the vibration and the highly visible movements involved, thus increasing the chances that he will be eaten.

3.9 Pseudosexual behaviour

In my study, males were seen to engage in stereotypical mating behaviour patterns normally associated with females relatively often. Pairs of males were observed pseudocopulating, with the mounter using the same behavioural repertoire as females use during mating. In the supplemented breeding group (N = 10), 60% of the males were observed to mount another male; 40% of males in the unsupplemented group (N =10) and 70% of males in the half and half breeding group (N = 10) did so.

Pseudosexual behaviour, also called heterotypical behaviour (Haug, 1990), has been described in many species including red deer (*Cervus elaphus*) (Hall, 1983), the smooth newt (*Triturus vulgaris*) (Halliday, 1974), the lizard *Cnemidophorous uniparens* (Moore *et al.*, 1985), and the bushcricket *Kawanaphilia nartee* (L. Simmons, pers. comm.).

It is possible that pseudocopulatory behaviour is an artefact of captivity and that crowded conditions may induce aberrant behaviours which are not observed in the natural situation. Cole & Townsend (1983) for example argue that pseudocopulation in females of the parthenogenic lizard *C. uniparens* is a response to population density in captivity. Male-male mounting behaviour has however been observed in the wild in the bushcricket *Kawanaphilia nartee* (L. Simmons, pers. comm.). Simmons observed that, after mounting, the mounting male continues to call while the mounted male ceases singing and tries to couple with the mounting male. He points out, however, that although one of the males is silent, if a female were to arrive then both males would still be present and should have an equal chance of securing a mating.

The number of times I observed male-male mounting behaviour during the course of these experiments does suggest that it may have a functional basis. If two males are in close proximity to a receptive female, a mounting male may gain an advantage by fooling the other male into believing he is being mounted by a female and inducing him to produce a spermatophore. It may not be necessary for a full spermatophore to be produced, merely for the process to be started. If a mounted male reaches a point where spermatophore production cannot be reversed, i.e. a point of no return, then the recovery time required for him to generate another spermatophore may benefit the mounting male who can mate with the female unopposed.

The benefits for the mounter in pseudocopulation are thus potentially high, given that another male may be excluded from the competition for mates for at least 24 hours. The costs of the behaviour are probably small, requiring relatively little time and energy. Benefits may be reduced, however at high densities. If there are several males in the vicinity of a receptive female, a male may lose an opportunity to mate by indulging in pseudocopulation, since while he is 'getting rid' of one male, another may be mating with

the female. Observations of a natural population of *L. punctatissima* in Germany, however, indicate that individuals are thinly distributed (M. Hall, pers. comm.), making it likely that mounting another male is advantageous.

3.10 Aggressive behaviour in *L. punctatissima*

Although overtly aggressive physical interactions between males for direct access to females have not been observed in *L. punctatissima*, either by myself or others, males may physically interact with each other if they come into contact. In particular males engage in a kind of boxing behaviour with their forelegs. I observed this behaviour on 16 occasions. It is most common when two males are in the vicinity of an elevated position, such as the top of a branch. Males may therefore be competing for the best calling sites, a situation which has been noted in the short tailed cricket (Walker, 1983).

Males may also interfere with each other during mating. On six occasions, I observed a male attempting to dislodge a female that was copulating with another male. Take-overs, where the mating male is supplanted by another male, have been reported for several insect species (Parker, 1970). In the yellow dung fly *Scatophaga stercoraria*, take-over success is correlated with male size (Borgia, 1979), with larger males able to successfully out-compete smaller males. I did not observe any instances in which a male managed successfully to dislodge a mating female. This behaviour could be aberrant behaviour in response to the high population density experienced in the experiments compared with densities found in the wild. It could however be an alternative mating strategy, with a male who happens upon a mating couple simply trying his luck in securing a mating. Even if he does not manage to separate the couple completely, he might prevent the spermatophore attaching to the female, leaving the male unable to mate and the female still receptive to other mating attempts.

In none of the observation periods did I observe any overtly aggressive interactions between females. Females tended to move around the arena a lot less than males, although they would occasionally move between the different food plants in the tanks. The only interaction that was noticeable between females was a tendency to climb over each other if they met whilst moving from plant to plant.

4 Variation in *Leptophyes punctatissima*

4.1 Introduction

Variation within a species is the raw material upon which evolution can work. For mate

choice to operate there have to be differences between individuals which make up the

breeding population. As discussed in Chapter 1, body size, fluctuating asymmetry, diet,

spermatophore size and quality, and calling characteristics can be important factors in mate

choice and reproductive success in many tettigoniid species. In this chapter, I therefore

look at variation in *L. punctatissima* in body size, FA, spermatophore size and calling

characteristics in relation to each other and to diet. I also compare the data obtained from

my own laboratory population with comparable data collected from a wild population in

Germany.

4.2 Results

4.2.1 Variation in body measurements

The correlations between all body measurements are given for males in Table 4.1 and for

females in Table 4.2. There was good overall concordance between most body

measurements and statistical significance in many cases was at the $p < 0.001$ level

Table 4.1 Correlations between male body measurements

N=79	B/lgth	L/fem	R/fem	L/tib	R/tib	L/teg	R/teg	P/num	Wght
B/lgth		0.305**	0.288**	0.232*	0.169	0.125	0.193	0.214	0.779***
L/fem	0.305**		0.930**	0.842**	0.83**	0.347**	0.402**	0.570**	0.634**
R/fem	0.288**	0.930**		0.802**	0.782**	0.341**	0.391**	0.476**	0.596**
L/tib	0.232*	0.842**	0.802**		0.897**	0.313**	0.362**	0.542**	0.544**
R/tib	0.169	0.803**	0.782**	0.897**		0.392**	0.435**	0.799****	0.479**
L/teg	0.125	0.347**	0.341**	0.313**	0.392**		0.944**	0.418**	0.317**
R/teg	0.193	0.402**	0.391**	0.362**	0.435**	0.944**		0.544**	0.365**
P/num	0.214	0.570**	0.476**	0.542**	0.499**	0.418**	0.544**		0.497**
Wght	0.779**	0.633**	0.596**	0.544**	0.479**	0.317**	0.365**	0.497**	

* p<0.05 ** p<0.01 *** p<0.001.

B/lgth: body length; L/fem: left femur length; R/fem: right femur length; L/tib: left tibia length; R/tib: right tibia length; L/teg: left tegmina length; R/teg: right tegmina length; P/num: pronotum length; Wght: weight.

Table 4.2 Correlations between female body measurements

N=85	B/lgth	L/fem	R/fem	L/tib	R/tib	L/teg	R/teg	P/num	Wght	Ovip L	Ovip D
B/lgth		0.408**	0.392**	0.360**	0.357**	0.215*	0.225*	0.242*	0.920**	0.318**	0.069
L/fem	0.408**		0.918**	0.844**	0.831**	0.189	0.225*	0.623**	0.595**	0.474**	0.327**
R/fem	0.392**	0.918**		0.835**	0.844**	0.143	0.186	0.621**	0.567**	0.443**	0.321**
L/tib	0.360**	0.844**	0.835**		0.926**	0.184	0.211	0.658**	0.543	0.511**	0.445**
R/tib	0.357**	0.831**	0.844**	0.926**		0.205	0.218*	0.666**	0.544**	0.245*	0.454**
L/teg	0.215*	0.189	0.143	0.184	0.205		0.644**	0.051	0.281*	0.517**	0.188
R/teg	0.225*	0.180	0.186	0.211	0.218*	0.664**		0.197	0.312**	0.024	0.242*
P/num	0.242*	0.623**	0.621**	0.658**	0.666**	0.051	0.197		0.408**	0.514**	0.372*
Wght	0.920**	0.595**	0.567**	0.543**	0.544**	0.281*	0.312*	0.408**		0.458**	0.221
Ovip L	0.318**	0.474**	0.443**	0.511**	0.517**	0.188	0.245*	0.514**	0.458**		0.436**
Ovip D	0.069	0.327**	0.321**	0.445**	0.454**	0.319**	0.319**	0.372**	0.221	0.436**	

p<0.05 ** p<0.01.

B/lgth: body length; L/fem: left femur length; R/fem: right femur length; L/tib: left tibia length; R/tib: right tibia length; L/teg: left tegmina length; R/teg: right tegmina length; P/num: pronotum length; Wght: weight; Ovip L: ovipositor length; Ovip D: ovipositor depth.

Pronotum length was used as a measure of overall size in all further analyses. It showed a strong relationship with most other traits in both males and females and is less prone to error than two other possible measures of overall size: body length and body weight. Body length correlates relatively poorly with other body measurements, especially in males, probably because it was difficult to measure accurately. When insects are anaesthetised they tend to curl their abdomen, making precise measurements difficult. Body weight, although it does correlate well with other body measures, is probably not a reliable

indicator of overall size because it fluctuates substantially as the insect gains and loses water, eats and defaecates. Pronotum length is often used as a measure of overall body size in insects (Fox, 1993b).

There was a difference in overall body size between the males collected in the two years, with males from 1998 being significantly larger than males collected in 1999 (Table 4.3, 4.4). There was no difference in size between supplemented and unsupplemented males, nor was there any interaction between diet and year (two-way ANOVA diet*year, diet: $F = 0.620$, df = 1,105, $p > 0.05$; year: $F = 20.579$, df = 1,105, $p < 0.001$; interaction term diet*year: $F = 0.190$, df = 1,105, $p > 0.05$).

Table 4.3 Variation in body measurements for supplemented and unsupplemented males and females from 1998

Body measurement	Supplemented males (N = 15)			Unsupplemented males (N = 15)		
	Mean	SE	Range	Mean	SE	Range
Body length (mm)	12.73	0.21	11.10-13.90	13.48	0.17	12.23-14.77
Weight (g)	0.158	0.004	0.122-0.182	0.183	0.005	0.154-0.214
Right femur (mm)	14.07	0.11	13.47-14.87	14.25	0.08	13.73-14.70
Left femur (mm)	14.13	0.12	13.50-15.06	14.26	0.18	13.63-15.23
Right tibia (mm)	16.60	0.12	15.70-17.37	16.52	0.16	15.00-17.43
Left tibia (mm)	16.42	0.14	15.37-17.33	16.35	0.17	15.00-17.47
Right tegmina (mm)	2.78	0.03	2.47-30.00	2.74	0.04	2.50-3.00
Left tegmina (mm)	2.78	0.03	2.53-2.93	2.72	0.04	2.40-3.00
Pronotum (mm)	2.44	0.02	2.30-2.60	2.43	0.04	2.10-2.60
Body measurement	Supplemented females (N 15)			Unsupplemented females (N = 15)		
	Mean	SE	Range	Mean	SE	Range
Body length (mm)	14.85	0.27	12.67-15.90	14.97	0.21	13.47-16.47
Weight (g)	0.288	0.009	0.226-0.346	0.290	0.009	0.234-0.366
Right femur (mm)	14.88	0.09	14.30-15.43	14.74	0.14	13.90-15.90
Left femur (mm)	14.97	0.09	14.30-15.43	14.84	0.18	14.40-15.80
Right tibia (mm)	17.20	0.16	16.03-18.43	17.31	0.20	15.80-18.57
Left tibia (mm)	16.99	0.17	15.70-18.00	17.30	0.14	16.50-18.23
Right tegmina (mm)	1.60	0.03	1.30-1.80	1.61	0.05	1.40-1.90
Left tegmina (mm)	1.58	0.03	1.30-1.80	1.63	0.05	1.43-2.00
Pronotum (mm)	2.90	0.03	2.73-3.20	2.90	0.02	2.70-3.13
Ovipositor width (mm)	1.88	0.04	1.70-2.07	1.95	0.032	1.70-2.20
Ovipositor length (mm)	7.04	0.12	5.57-7.53	7.08	0.09	6.50-7.56

Table 4.4 Variation in body measures for supplemented and unsupplemented males and females from 1999

Body measurement	Supplemented males (N = 38)			Unsupplemented males (N = 33)		
	Mean	SE	Range	Mean	SE	Range
Body length (mm)	13.64	0.15	11.33-16.57	13.74	0.13	12.20-16.00
Weight (g)	0.174	0.004	0.126-0.232	0.177	0.004	0.126-0.234
Right femur (mm)	13.89	0.12	12.50-15.27	13.88	0.10	13.00-15.20
Left femur (mm)	13.82	0.12	12.37-15.33	13.89	0.12	12.30-15.27
Right tibia (mm)	15.90	0.13	14.23-17.53	15.80	0.16	14.00-18.50
Left tibia (mm)	15.70	0.16	12.67-17.50	15.88	0.15	14.00-18.13
Right tegmina (mm)	2.75	0.04	1.50-3.33	2.71	0.05	1.80-3.20
Left tegmina (mm)	2.80	0.04	1.70-3.33	2.73	0.05	1.80-3.17
Pronotum (mm)	2.28	0.03	1.87-2.47	2.29	0.03	1.90-2.60
Body measurement	Supplemented females (N=36)			Unsupplemented females (N = 38)		
	Mean	SE	Range	Mean	SE	Range
Body length (mm)	15.83	0.21	12.70-17.70	15.82	0.22	13.33-18.33
Weight (g)	0.310	0.005	0.200-0.420	0.308	0.005	0.180-0.450
Right femur (mm)	14.50	0.12	12.43-15.77	14.60	0.13	13.03-15.70
Left femur (mm)	14.55	0.12	12.27-15.60	14.62	0.13	13.30-15.70
Right tibia (mm)	16.62	0.16	14.23-18.83	16.61	0.15	15.00-18.50
Left tibia (mm)	16.56	0.17	14.00-18.60	16.55	0.18	15.00-18.40
Right tegmina (mm)	1.66	0.02	1.40-2.03	1.64	0.02	1.40-1.83
Left tegmina (mm)	1.67	0.02	1.50-1.87	1.67	0.02	1.40-2.10
Pronotum (mm)	2.75	0.03	2.02-3.00	2.70	0.04	2.30-2.90
Ovipositor dpth (mm)	1.98	0.03	1.70-2.73	1.96	0.03	1.70-2.20
Ovipositor lgth (mm)	6.89	0.07	6.00-8.00	6.88	0.08	5.20-8.00

The same pattern was observed in females, with individuals collected in 1998 being significantly larger than those captured the following year (Tables 4.3 and 4.4). There was no effect of diet on female size and there was no interaction between diet and year (two-way ANOVA, diet*year, diet: $F = 0.246$, df = 1,111, $p>0.05$; year: $F = 35.114$, df = 1,111, $p<0.001$; interaction term diet*year: $F = 0.126$ df = 1,111 $p > 0.05$).

Data from 1998 and 1999 were therefore analysed separately for all further analyses; however, data from individuals within years on different diets were combined where appropriate.

Although individuals were larger in 1998, they were more variable in size in 1999. The difference in pronotum length between the smallest and the largest supplemented female for 1998 is 0.47mm whereas for 1999 it is 0.98mm. The figures for unsupplemented females are 0.43mm versus 0.60mm, for supplemented males 0.30mm versus 0.60 and for unsupplemented males 0.50 versus 0.70mm.

Insects reared in the laboratory in 1998 were significantly larger than those from the 1998 German population (Table 4.5; Students t-test, male size: $t = 7.55$, $df = 66$, $p<0.001$; female size: $t = 5.33$, $df = 56$, $p<0.001$).

Table 4.5 Variation in body measurements for UK and German males and females from 1998

Body measurement	UK males (N = 30)			German males (N = 32)		
	Mean	SE	Range	Mean	SE	Range
Body length (mm)	13.11	0.15	11.10-14.77	11.92	0.13	10.30-13.50
Weight (g)	0.170	0.004	0.120-0.210	0.126	0.002	0.100-0.140
Right femur (mm)	14.17	0.07	13.47-14.87	13.42	0.12	11.57-14.53
Left femur (mm)	14.19	0.08	13.50-15.23	13.49	0.12	11.57-14.80
Right tibia (mm)	16.56	0.10	15.00-17.43	15.44	0.13	13.07-17.10
Left tibia (mm)	16.39	0.11	15.00-17.47	15.38	0.14	13.13-16.93
Right tegmina (mm)	2.76	0.03	2.47-3.00	2.99	0.03	2.57-3.30
Left tegmina (mm)	2.75	0.03	2.40-3.00	2.98	0.03	2.67-3.30
Pronotum (mm)	2.44	0.02	2.10-2.60	2.15	0.03	1.50-2.50
Body measurement	UK females (N = 30)			German females (N = 28)		
	Mean	SE	Range	Mean	SE	Range
Body length (mm)	14.89	0.17	12.67-16.47	13.72	0.22	12.30-16.00
Weight (g)	0.289	0.006	0.226-0.366	0.211	0.006	0.150-0.300
Right femur (mm)	14.83	0.09	13.90-15.90	14.60	0.07	14.00-15.37
Left femur (mm)	14.92	0.08	14.30-15.80	14.59	0.07	13.60-15.13
Right tibia (mm)	17.28	0.13	15.80-18.57	16.27	0.10	15.53-17.97
Left tibia (mm)	17.16	0.12	15.70-18.23	16.27	0.10	15.37-17.97
Right tegmina (mm)	1.60	0.03	1.30-1.90	1.65	0.02	1.47-1.90
Left tegmina (mm)	1.60	0.03	1.30-2.00	1.65	0.02	1.43-1.87
Pronotum (mm)	2.91	0.11	2.70-3.20	2.70	0.03	2.40-3.00
Ovipositor wdth (mm)	1.92	0.13	1.70-2.20	2.01	0.05	1.50-2.90
Ovipositor lgth (mm)	7.07	0.40	5.57-7.56	6.89	0.08	6.00-8.00

The German population was, however more variable in size than the UK population. The difference in pronotum length between the smallest and the largest German male is 1.00mm whereas for the UK population it is 0.50mm. The difference for German females is 0.60mm while that for UK females is 0.50.

4.2.2 Variation in fluctuating asymmetry

4.2.2.1 Differences between years

There were no significant difference in asymmetry values for tibia, femur or tegmen between insects measured in 1998 and 1999. For each character I used the absolute

(unsigned) asymmetry value which was then log transformed to remove any scale effects

(R. Palmer, pers. comm.). Differences between years were tested using a three-way

factorial ANOVA using diet, sex and year as the grouping variables (Table 4.6). I therefore

combined data from both years for all further analyses.

Table 4.6 Asymmetry differences between year, diet and sex

	Femur	Tibia	Tegmen
Year	df = 1,216; F = 0.121; NS	df = 1,216; F = 0.561; NS	df = 1,216; F = 0.507; NS
Sex*year	df = 1,216; F = 0.004; NS	df = 1,216; F =1.250; NS	df = 1,216; F = 0.070; NS
Diet*year	df = 1,216; F = 1.387; NS	df = 1,216; F = 0.139; NS	df = 1,216; F = 0.127; NS
Diet*sex*year	df = 1,216; F = 0.080; NS	df = 1,216; F = 2.426; NS	df = 1,216; F = 0.223; NS

4.2.2.2 Asymmetry of the insects used in the study

The mean levels of asymmetry found for each character, the relationship between

asymmetry and size, and the results of tests for skewness, kurtosis, normality and

directional asymmetry are given for all males in Table 4.7 and for all females in Table 4.8.

Before conducting any studies on FA it is important to test for any departures from

normality (see Section 2.6). In this study there was significant directional asymmetry in the

tibia and tegmina of supplemented males, the femur of supplemented females and the

tegmina of unsupplemented females. In the German population the between-sides variation

of the tegmina for both males and females was indistinguishable from measurement error.

Consequently none of these characters were used in any further analyses.

Table 4.7 Asymmetry in males

Group	Character	Mean R-L asymm (SE)	Skewness (SE) kurtosis (SE)	Tests of normality (KS test*)	DA test (from ANOVA FA10)
S males	Femur	0.014 (0.026)	-0.206 (0.327) 0.170 (0.644)	Z=1.003 p= 0.267	F(1,57) = 1.48 p = 0.228
	Tibia	0.117 (0.04)	0.640 (0.327) 3.316 (0.644)	Z=0.186 p= 0.001	F(1,57) = 12.2 p= 0.001
	Tegmen	-0.04 (0.026)	-0.916 (.0327) 0.170 (0.644)	Z=1.697 p= 0.006	F(1,57) = 8.251 p = 0.006
US males	Femur	-0.03 (0.03)	0.185 (0.347) 4.785 (0.681)	Z=1.005 p= 0.265	F(1,50) = 0.058 p= 0.810
	Tibia	-0.009 (0.037)	-0.508 (0.347) -0.311 (0.681)	Z=0.617 p= 0.027	F(1,50) = 0.803 p= 0.375
	Tegmen	-0.006 (0.013)	-0.161 (.0347) -0.198 (0.681)	Z=1.464 p= 0.027	F(1,50) = 0.277 p= 0.601
German males	Femur	-0.07 (0.062)	-1.277 (0.414) 3.835 (0.809)	Z=0.783 p= 0.571	F(1,31) = 1.443 p= 0.239
	Tibia	0.061 (0.055)	0.438 (0.414) 1.179 (0.809)	Z=0.736 p= 0.651	F(1,31) = 1.269 p= 0.269
	Tegmen	0.013 (0.014)	0.120 (0.414) -0.046 (0.809)	Z=0.712 p= 0.691	F(1,31) = 0.813 p= 0.374

*Kolmogorov-Smirnov test.

Table 4.8 Asymmetry in females

Group	Character	Mean R-L asymm (SE)	Skewness (SE) kurtosis (SE)	Tests of normality (KS test*)	DA test (from ANOVA FA10)
S females	Femur	-0.080 (0.027)	-0.596 (0.337) 0.686 (0.662)	Z= 0.936 p= 0.345	F(1,52) = 3.406 p= 0.071
	Tibia	0.059 (0.044)	0.604 (0.337) 1.222 (0.662)	Z= 0.745 p= 0.635	F(1,52) = 0.775 p= 0.135
	Tegmen	-0.008 (0.014)	0.165 (0.337) 0.248 (0.662)	Z= 1.599 p= 0.012	F(1,52) = 0.126 p= 0.724
US females	Femur	-0.020 (0.029)	-0.194 (0.311) 1.329 (0.613)	Z= 1.071 p= 0.202	F(1,61) = 0.656 p= 0.424
	Tibia	0.083 (0.033)	-0.513 (0.311) 0.317 (0.613)	Z= 0.777 p= 0.582	F(1,610) = 1.268 p= 0.264
	Tegmen	-0.02 (0.0089)	-0.206 (0.311) -0.182 (0.613)	Z= 1.462 p= 0.028	F(1,61) = 8.626 p= 0.005
German females	Femur	0.024 (0.051)	0.986 (0.414) 3.119 (0.809)	Z= 0.558 p= 0.914	F(1,31) = 0.222 p= 0.641
	Tibia	0.052 (0.054)	0.220 (0.414) 4.150 (0.809)	Z= 0.880 p= 0.421	F(1,31) = 0.917 p= 0.346
	Tegmen	0.038 (0.01)	0.551 (0.414) 0.126 (0.809)	Z= 1.028 p= 0.242	F(1,31) = 0.000 p= 1.00

* KS: Kolmogorov-Smirnov test.

4.2.2.3 Size versus asymmetry

The level of FA in the traits examined as a percentage of trait size was low, being 1.04% in the femur, 1.24% in the tibia and 3.14% in the tegmina

There was no relationship between the size of the characters used in this study and the level of FA they expressed (Figs 4.1 to 4.12).

Figure 4.1 Regression of absolute (unsigned) asymmetry against mean femur length, i.e. (R + L)/2 for supplemented males. (Regression equation = -0.237 + 0.028*mean femur length; R square = 0.023, p = 0.281).
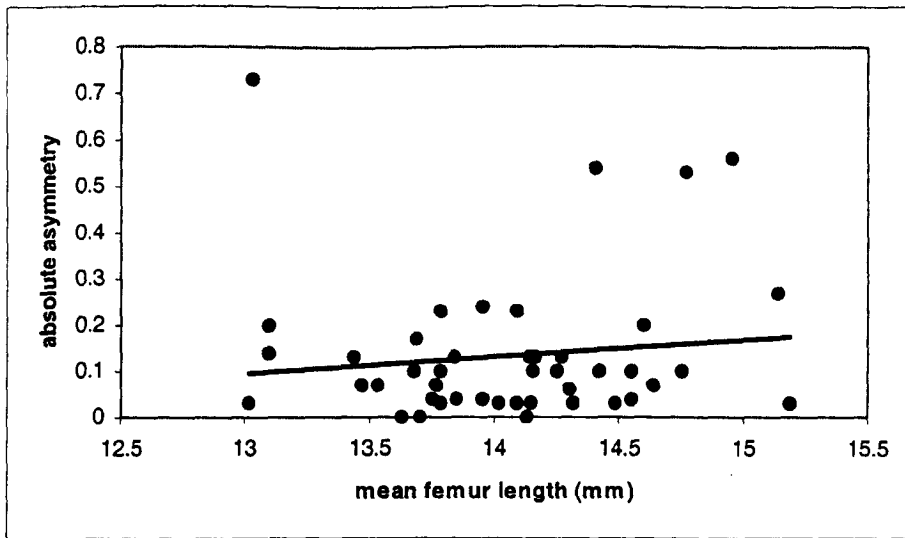
Figure 4.2 Regression of absolute (unsigned) asymmetry against mean femur length, i.e. (R + L)/2 for unsupplemented males. (Regression equation = -0.353 + 0.035*mean femur length; R square = 0.014, p= 0.434).

Figure 4.3 Regression of absolute (unsigned) asymmetry against mean tibia length, i.e.(R + L)/2 for unsupplemented males. (Regression equation = -0.513 + 0.044*mean tibia length; R square = 0.05,7 p= 0.107).
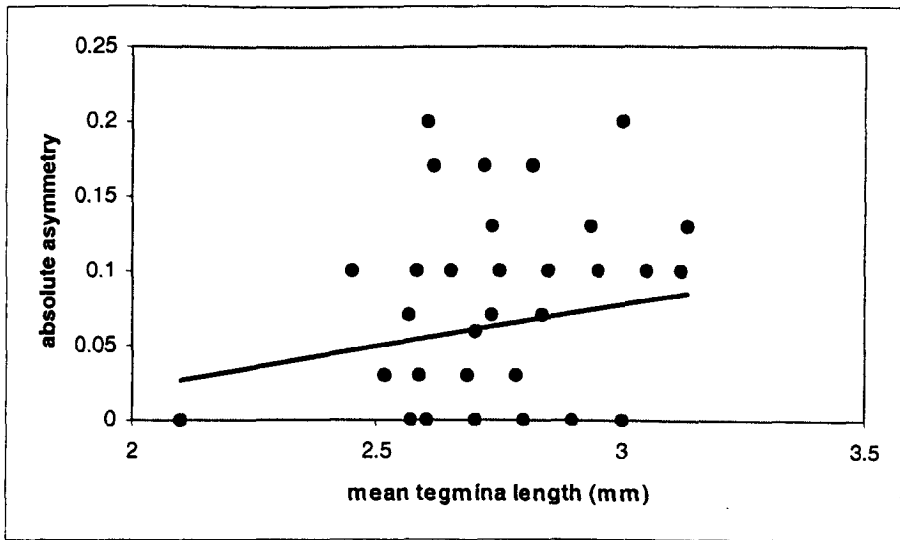
Figure 4.4 Regression of absolute (unsigned) asymmetry against mean tegmen length, i.e.(R + L)/2 for unsupplemented males. (Regression equation = -0.093 + 0.057*mean tegmina length; R square = 0.031, p = 0.233).

Figure 4.5 Regression of absolute (unsigned) asymmetry against mean tibia length, i.e.(R + L)/2 for supplemented females. (Regression equation = 0.800 – 0.034*mean tibia length; R square =0.020, p = 0.325).

93
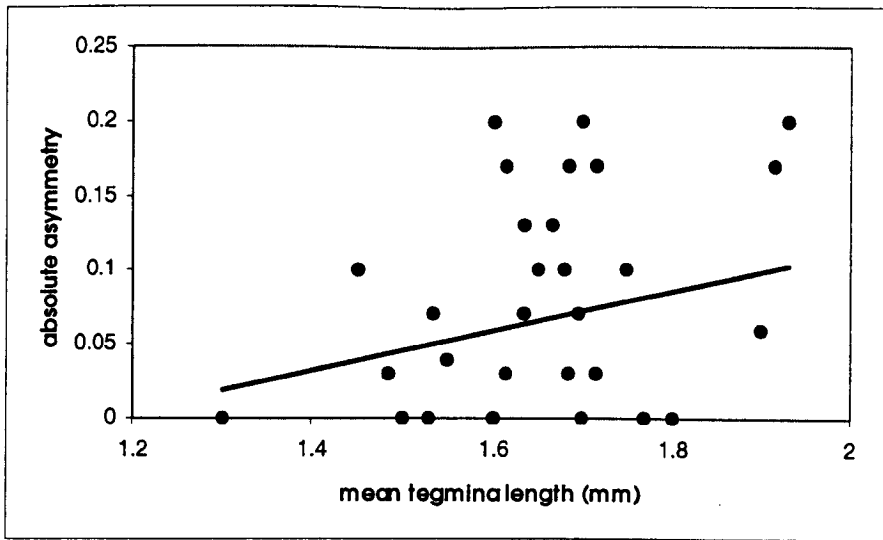
Figure 4.6 Regression of absolute (unsigned) asymmetry against mean tegmen length, i.e.(R + L)/2 for supplemented females. (Regression equation = -0.154 + 0.133*mean tegmina length; R square = 0.04, p = 0.138).
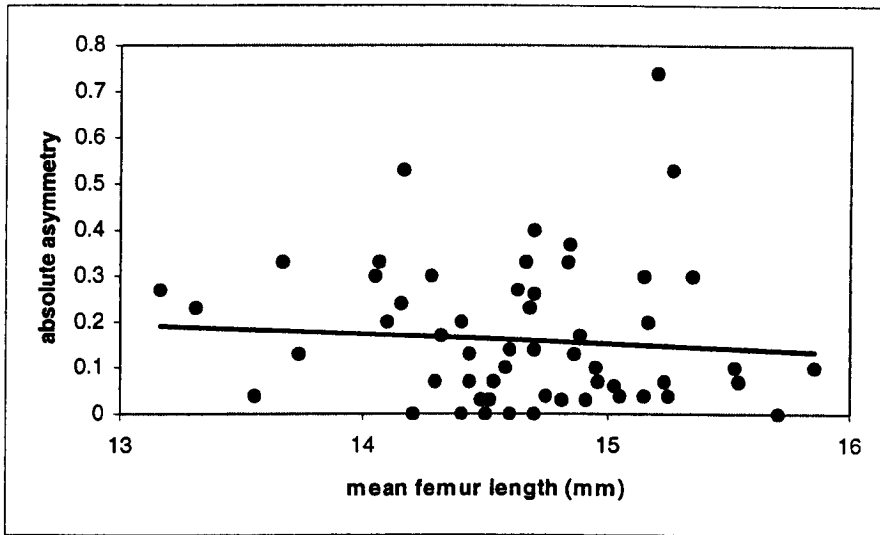
Figure 4.7 Regression of absolute (unsigned) asymmetry against mean femur length, i.e.(R + L)/2 for unsupplemented females. (Regression equation = 0.472 – 0.021*mean femur length; R square = 0.74, p = 0.579).
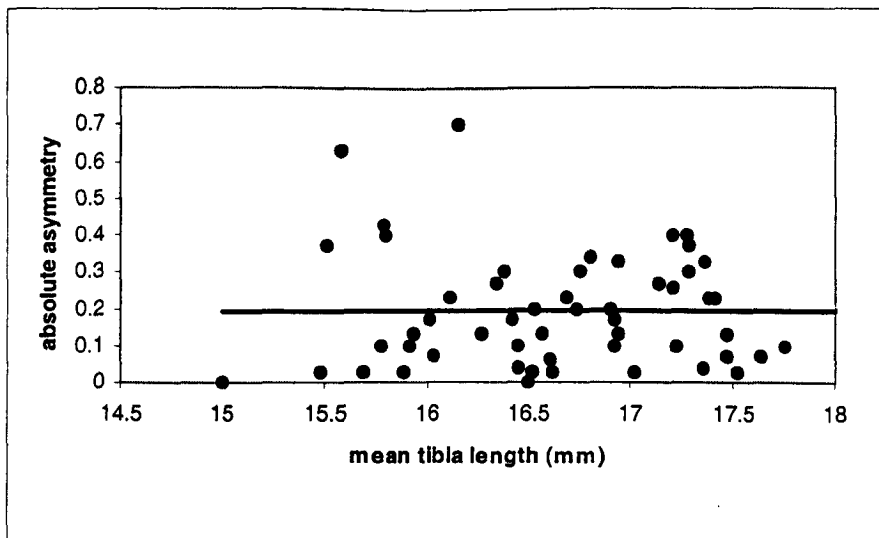
Figure 4.8 Regression of absolute (unsigned) asymmetry against mean tibia length, i.e.(R + L)/2 for unsupplemented females. (Regression equation = 0.180 + 0.00097*mean tibia length; R square = 0.000, p = 0.972).
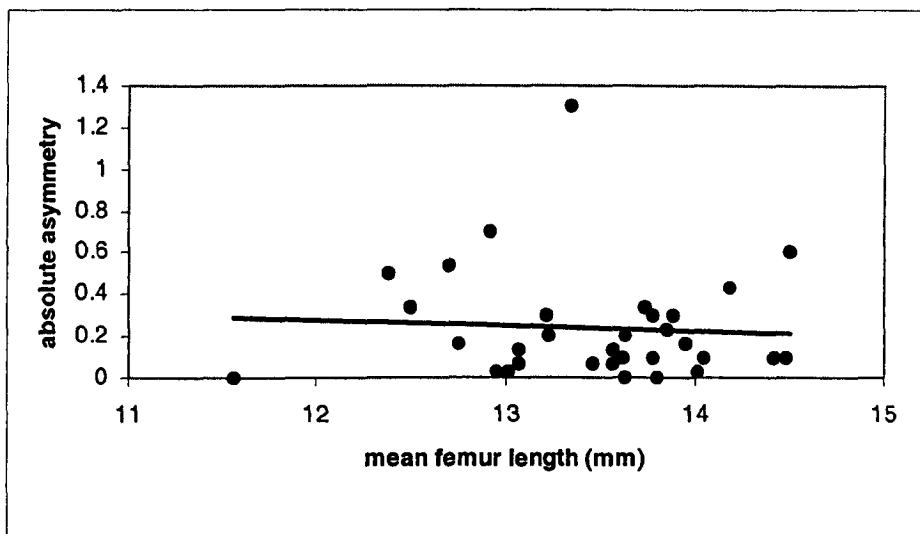
Figure 4.9 Regression of absolute (unsigned) asymmetry against mean femur length, i.e. (R + L)/2 for the German male population. (Regression equation = 0.553 – 0.023*mean femur length; R square = 0.00,3 p = 0.757)
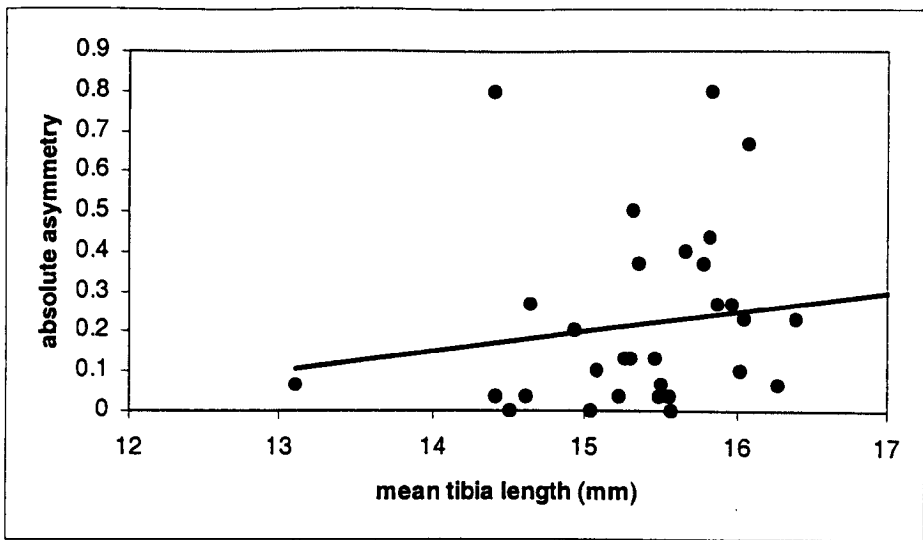
Figure 4.10 Regression of absolute (unsigned) asymmetry against mean tibia length, i.e. (R + L)/2 for males of the German population. (Regression equation = -0.532 + 0.049*mean tibia length; R square = 0.025, p = 0.386).
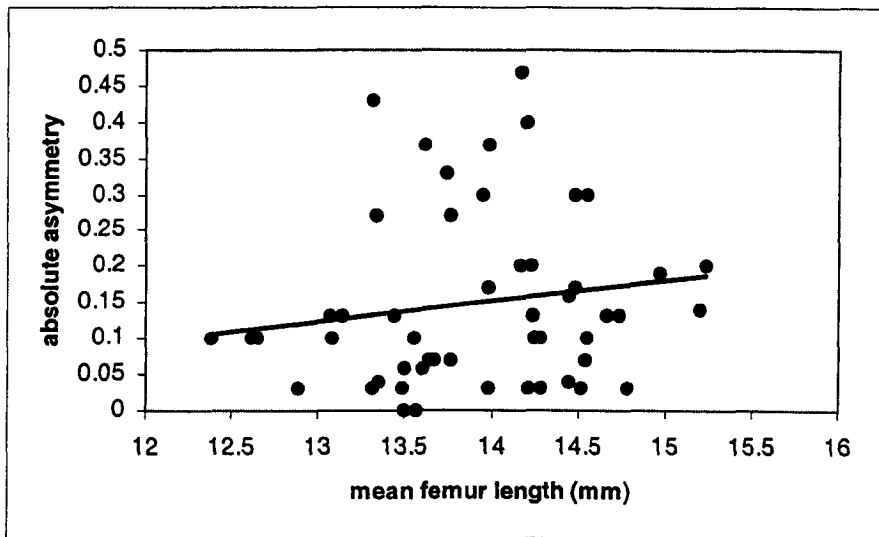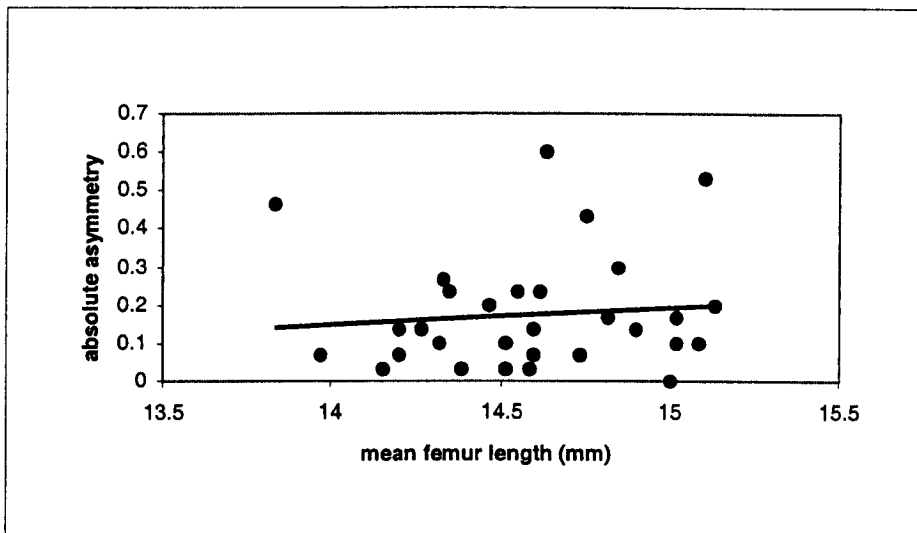

Figure 4.11 Regression of absolute (unsigned) asymmetry against mean femur length, i.e. (R + L)/2 for the German female population. (Regression equation = -0.448 + 0.043*mean femur length; R square = 0.00, p = 0.613)
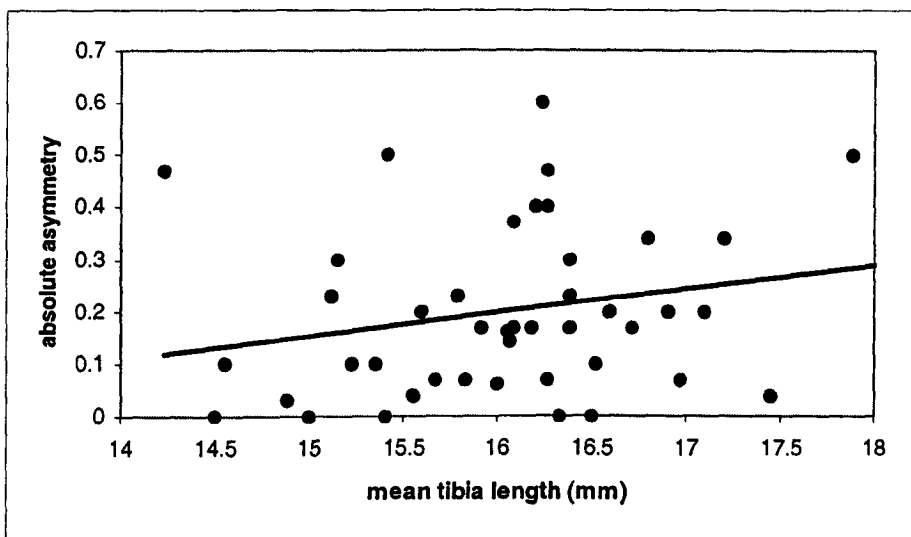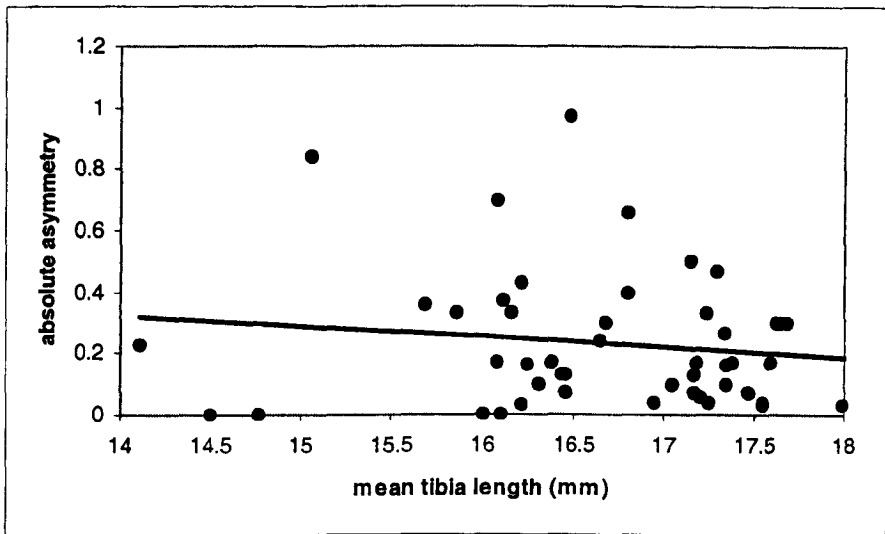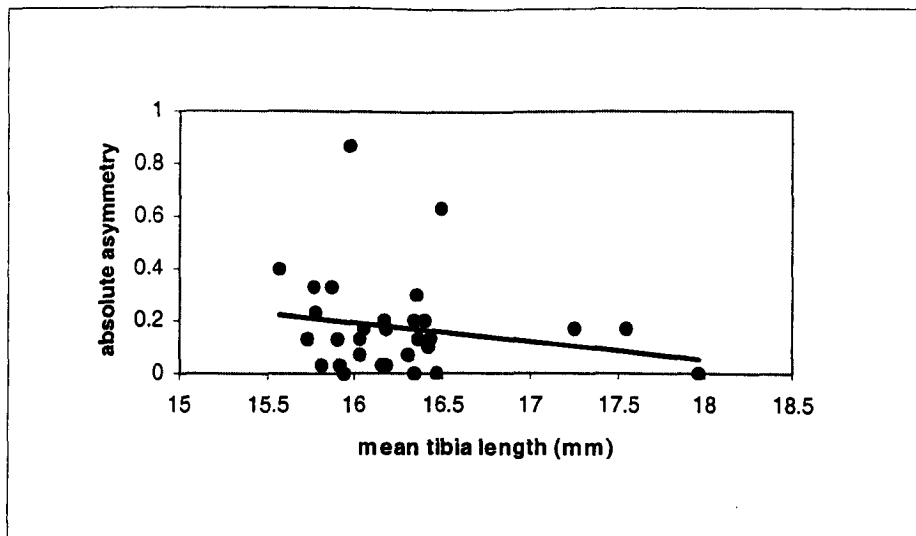
Figure 4.12 Regression of absolute (unsigned) asymmetry against mean tibia length, i.e. (R + L)/2 for the German female population. (Regression equation = 1.296 – 0.069*mean tibia length; R square = 0.035, p = 0.313).

4.2.2.4   FA differences between groups

There was no difference in the level of FA expressed in the femur, tibia or tegmina by any of the groups in this study (i.e. supplemented or unsupplemented, male or female, German or UK; Table 4.9).

Table 4.9 Levenes test of asymmetry differences between groups

Character	Between groups MS*	Within groups MS	df	F value
Femur	0.061	0.029	4,217	2.137; NS
Tibia	0.017	0.035	4,214	0.495; NS
Tegmen	0.000077	0.0045	1,95	0.017; NS

*Mean square.

4.2.3   Variation in calling characteristics

There was no significant difference between supplemented and unsupplemented males in mean call length but there was a significant difference between years, with males in 1999 having a significantly longer call length than males from 1998 (Tables 4.10 and 4.11; two-way ANOVA year*diet, diet: $F = 0.329$, $df = 1,55$, $p > 0.05$; year: $F = 22.558$, $df = 1,55$, $p < 0.001$). There was no interaction between year and diet on male call length ($F = 3.276$, $df = 1,55$, $p > 0.05$).

97

Table 4.10 Calling characteristics for males from 1998

Calling characteristic	Supplemented males (N 12)			Unsupplemented males (N= 11)		
	Mean	SE	Range	Mean	SE	Range
Call length (ms)	9.81	0.87	5.00-15.60	7.78	0.90	2.50-12.50
Inter-call interval (s)	3.55	0.33	2.40-5.71	8.21	0.28	1.68-29.70

Table 4.11 Calling characteristics for males from 1999

Calling characteristic	Supplemented males (N=20)			Unsupplemented males (N=16)		
	Mean	SE	Range	Mean	SE	Range
Call length (ms)	14.62	1.27	7.39-28.86	18.53	2.13	7.60-36.50
Inter-call interval (s)	28.56	2.64	15.78-65.20	4.05	0.31	1.61-6.81
No. syllables	8.00	0.49	4.00-12.00	8.00	0.56	3.00-12.00
Syllable length (ms)	0.74	0.08	0.22-1.56	0.74	0.08	0.24-1.44
Syllable separation (ms)	1.40	0.25	0.46-4.62	1.05	0.16	0.24-2.22
Frequency (kHz)	38.22	0.44	35.00-42.00	38.47	0.77	35.00-43.70

There was no significant difference between supplemented males and unsupplemented males in inter-call interval in 1998 (Mann-Whitney U test, U = 43, p>0.050); but supplemented males in 1999 had a significantly longer inter-call interval than unsupplemented males (U = 0, p<0.05). Supplemented males in 1999 also had a significantly longer inter-call interval than supplemented males in 1998 (U = 0.000, p<0.05) but there was no significant difference between the inter-call interval of unsupplemented males in 1998 and unsupplemented males in 1999 (U = 92, p>0.05).

There were no significant differences between supplemented and unsupplemented males from 1999 in syllable number, (one-way ANOVA, F = 6.368, df = 1,35, p>0.05), syllable length (F = 0.001, df = 1,35, p>0.05), syllable separation (F = 0.546, df = 1,35, p>0.05), or call frequency (F = 0.616, df = 1,35, p>0.05).

Call characteristics did not vary with male size. There was no correlation between male size and call length in 1998 (Fig. 4.13; r = -0.210, N = 23, p>0.05;combined data from

supplemented and unsupplemented males); or in 1999, (Fig. 4.14; r = -0.123, N = 36, p>0.05).
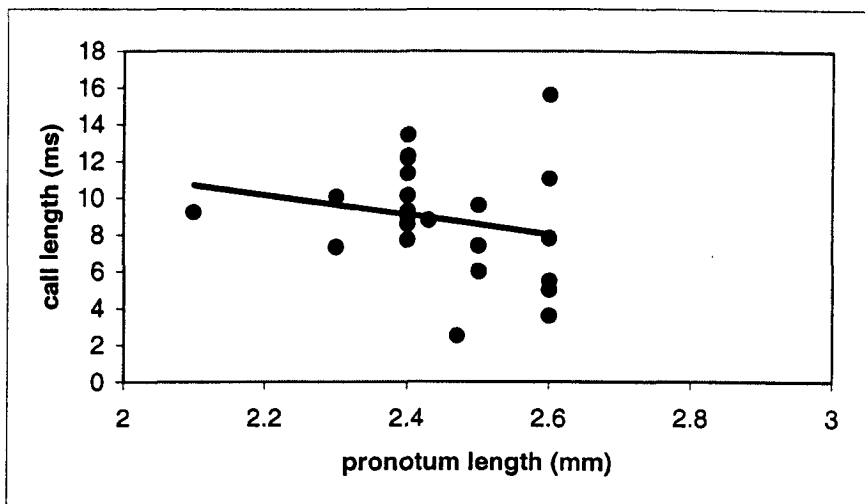
Figure 4.13 The relationship between male size and call length for 1998 males
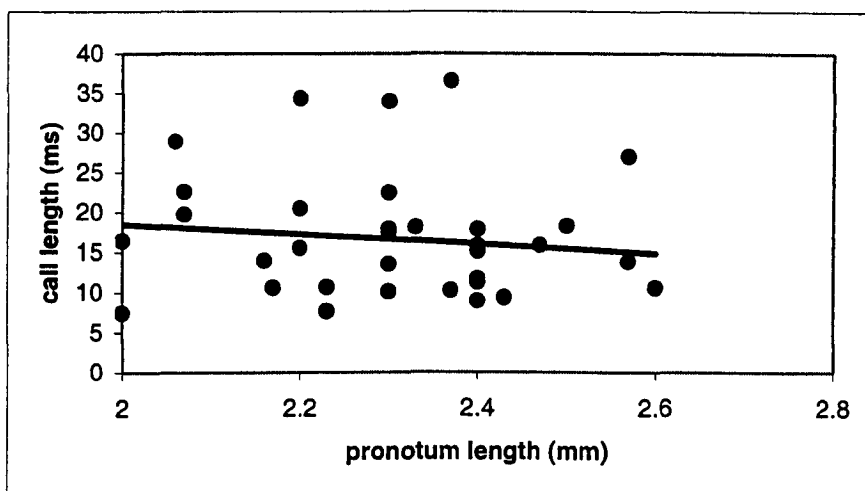
Figure 4.14 The relationship between male size and call length for 1999 males

Inter-call interval was not correlated with male size, either for supplemented or unsupplemented males in 1998 or 1999 (S males 1998 (Fig. 4.15): r = 0.184, N = 12, p>0.05; US males 1998 (Fig. 4.16) r = 0.483, N = 11, p>0.05; S males 1999 (Fig.4.17): r = -0.250, N = 18, p>0.05); US males in 1999 (Fig. 4.18): r = 0.114, N = 18, p>0.05).
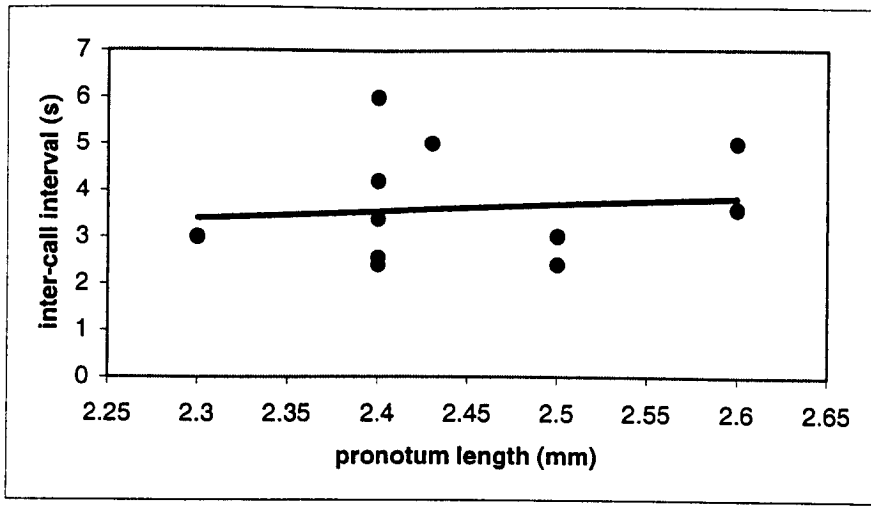
Figure 4.15 The relationship between male size and inter-call interval for 1998 supplemented males
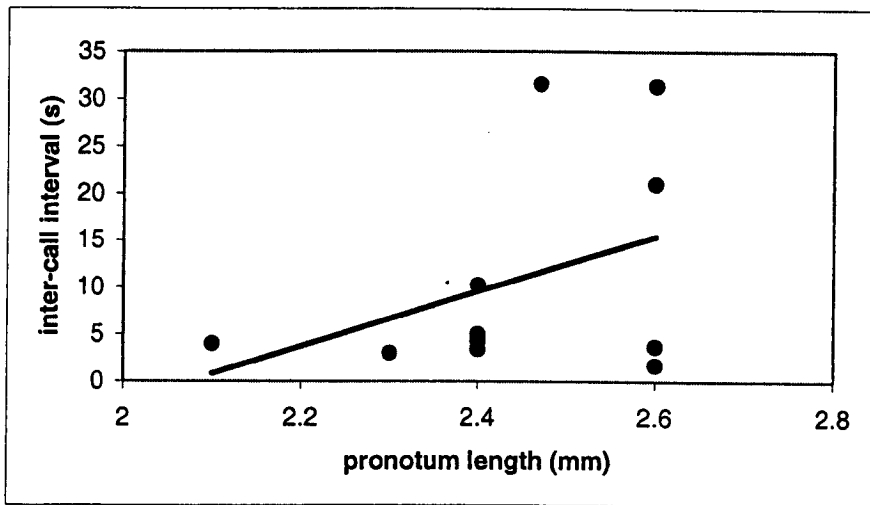
Figure 4.16 The relationship between male size and inter-call interval for 1998 unsupplemented males
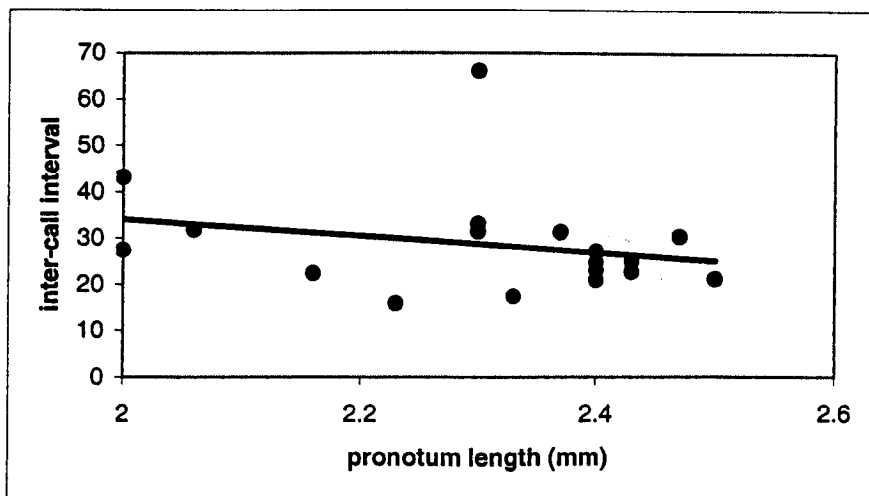
Figure 4.17 The association between male size and inter-call interval for 1999 supplemented males
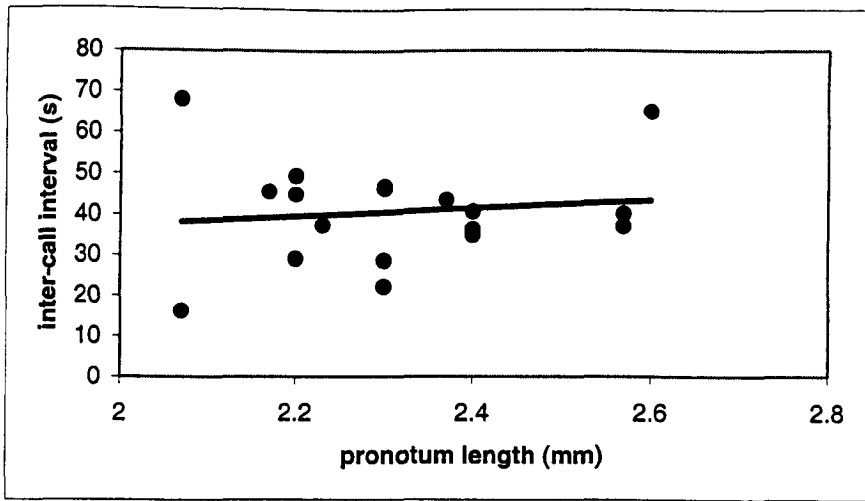
Figure 4.18 The relationship between male size and inter-call interval for 1999 unsupplemented males

There was no correlation between male size and the number of syllables contained within a call (Fig. 4.19; r = 0.039, N = 36, p >0.05, combined data from supplemented and unsupplemented males from1999); syllable length (Fig. 4.20; r =184, N = 36, p >0.05); syllable separation within a call (Fig. 4.21; r = 0.154, N = 36, p>0.05); or frequency (Fig. 4.22; r = -0.105, N = 36, p>0.05).
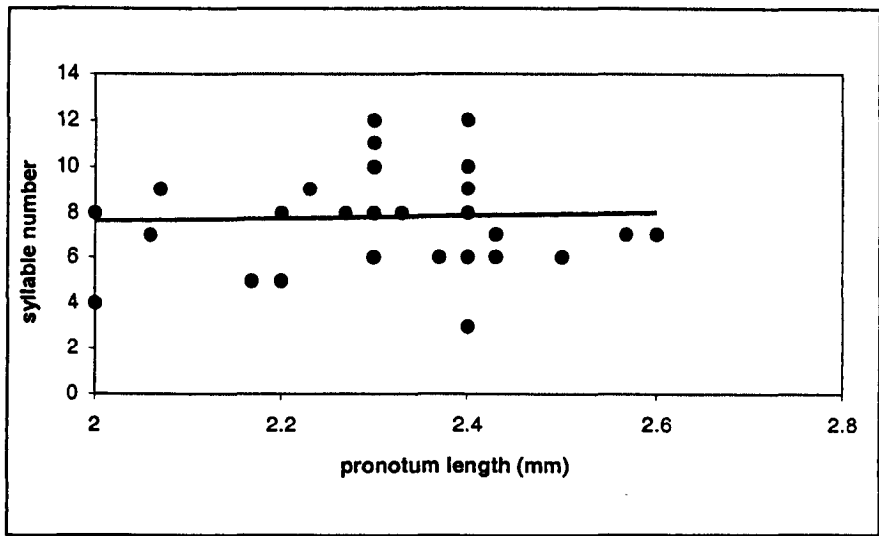
Figure 4.19 The relationship between male size and the number of syllables contained within a call.
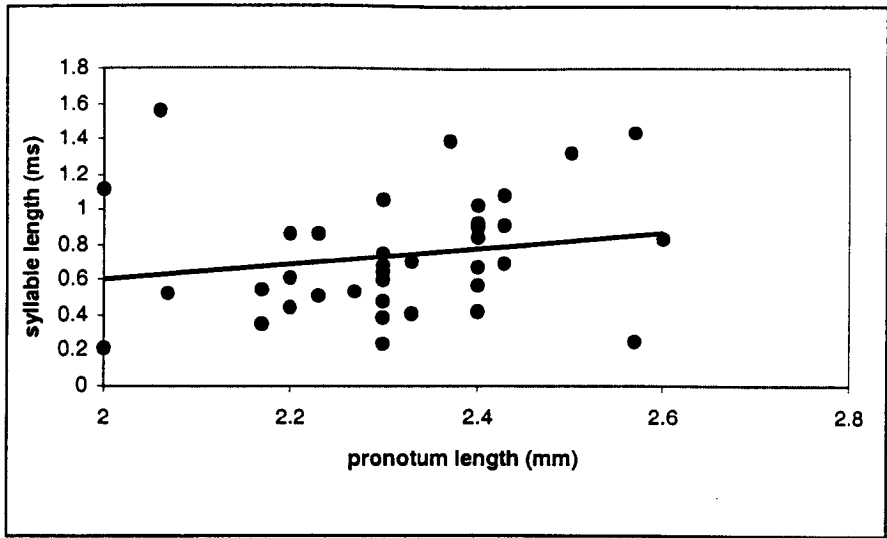
Figure 4.20 The relationship between male size and the length of syllables contained within a call.

Figure 4.21 The relationship between male size and the separation of syllables within a call.

Figure 4.22 The relationship between male size and call frequency

Bout lengths of calling were calculated for all the males in the three breeding population groups (see Section 2.11), where a bout was defined as a series of calls separated from another such bout by an interval of at least 5 min. The first and last bouts for a particular male in any session were excluded if they included the first interval of the session or the last interval of the session respectively. This was to allow for the possibility that a bout that ran from the start of the session may simply have been a continuation of a bout begun earlier, while a bout that ended with the end of the session might have continued had it not been interrupted.

Mean calling bout length was not correlated with male body size in any of the three breeding groups (Spearman rank correlation, S group (Fig. 4.23): $r_s = 0.351$, N = 10, p>0.05; US group (Fig. 4.24): $r_s = -0.611$, N = 10, p>0.05; half and half group (Fig. 4.25): $r_s = 0.248$, N = 10, p>0.05).
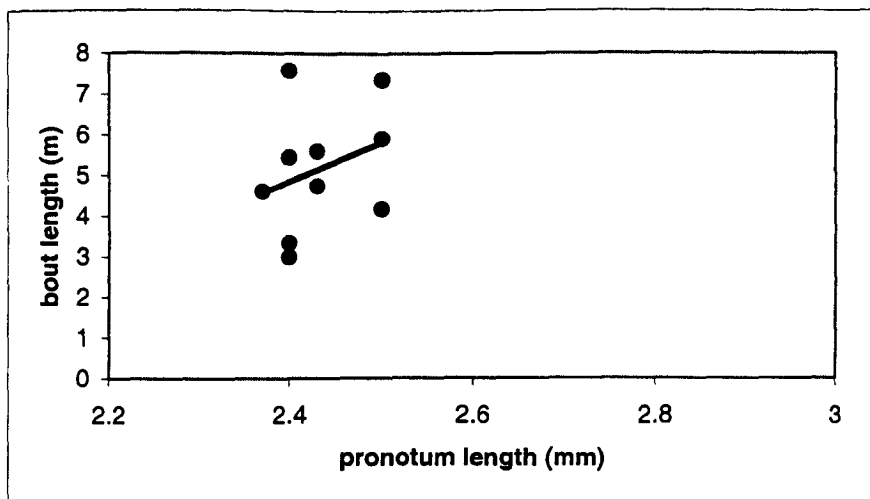
Figure 4.23 The relationship between size and calling bout length for males in the supplemented breeding group
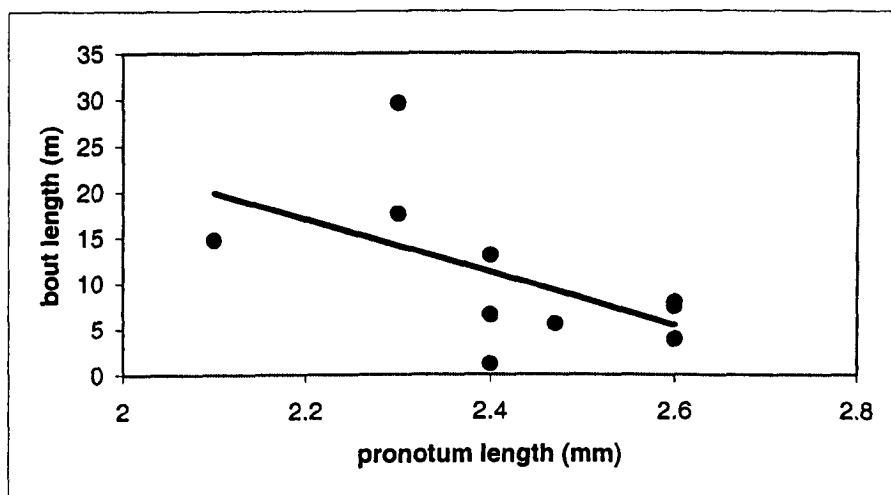
Figure 4.24 The relationship between size and calling bout length for males in the unsupplemented breeding group
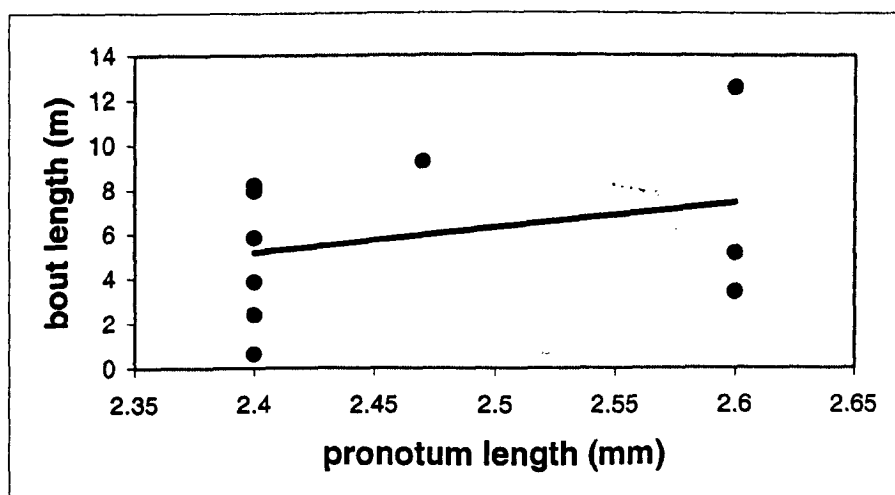
Figure 4.25 The relationship between male size and calling bout length in the half and half breeding group

Percentage time spent calling was calculated for each male in each breeding group session. This was defined as the number of 3-min intervals in which the male was recorded as calling, as a percentage of the 60 3-min intervals in each 3-h session. Percentage time spent calling was not correlated with male body size in any of the breeding groups. (Spearman rank correlation, S group (Fig. 4.26): $r_s = -0.019$, N= 10, p>0.05; US group (Fig. 4.27): $r_s = 0.380$, N= 10, p>0.05); half and half group (Fig. 4.28: $r_s = 465$, N= 10, p>0.05).
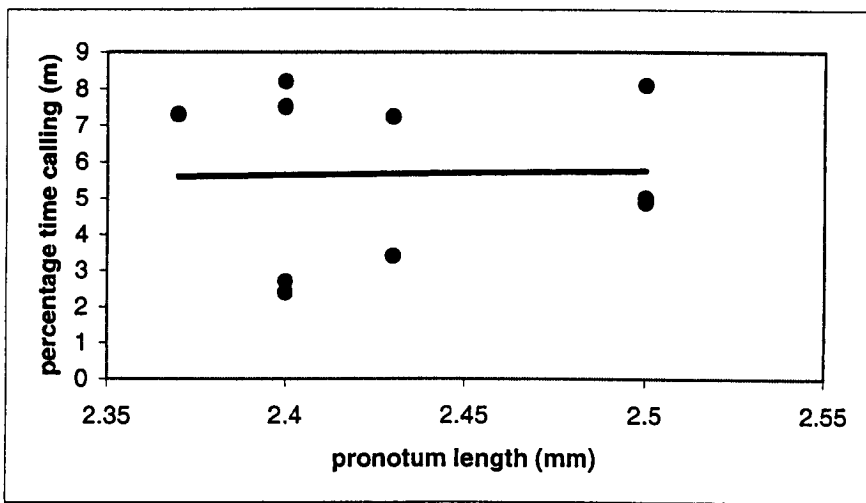
Figure 4.26 The relationship between male size and percentage time calling for the supplemented breeding group
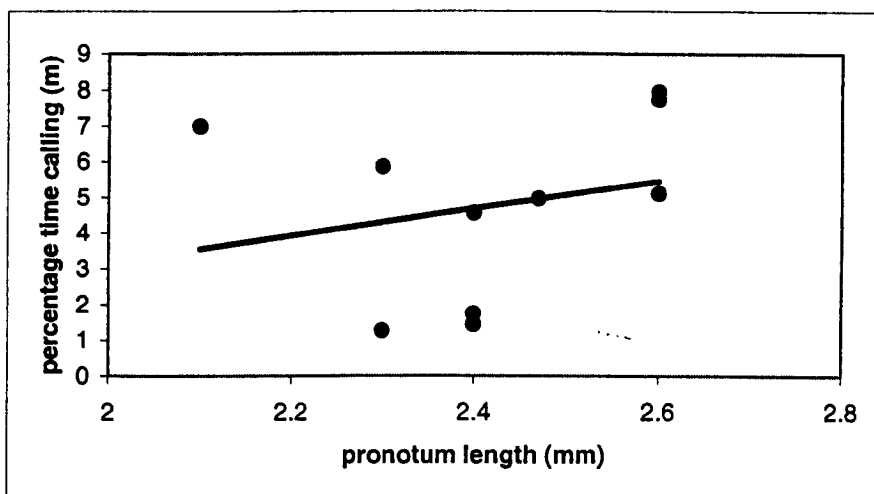
Figure 4.27 The association between male size and the percentage time calling for the unsupplemented breeding group
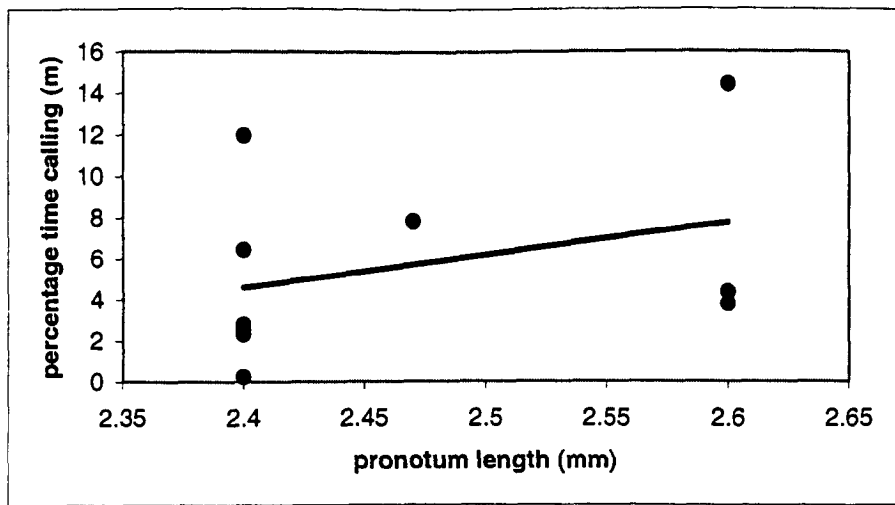
Figure 4.28 The relationship between male size and percentage time calling for the half and half group

Mean calling bout length did not differ significantly between supplemented and unsupplemented males (Mann Whitney U = 111, p>0.05; data from all males from the breeding group experiment). Nor was there any significant difference between supplemented and unsupplemented males in the percentage time they spent calling (U =76, p>0.05; data from all males from the breeding group experiment).

There was no relationship between the degree of asymmetry and any of the call characteristics measured. I used the mean asymmetry of the males in the study to investigate the relationship.

As there were differences in call length between the males in 1998 and 1999 I analysed the data for each year separately, although, as call length did not differ between supplemented and unsupplemented males within years I combined these data sets. There was no correlation between call length and FA in either year (Spearman rank correlation, 1998 (Fig. 4.29): r_s = -0.222, N = 23, p>0.05; 1999 (Fig. 3.30): r_s = 0.086, N = 36, p>0.05).
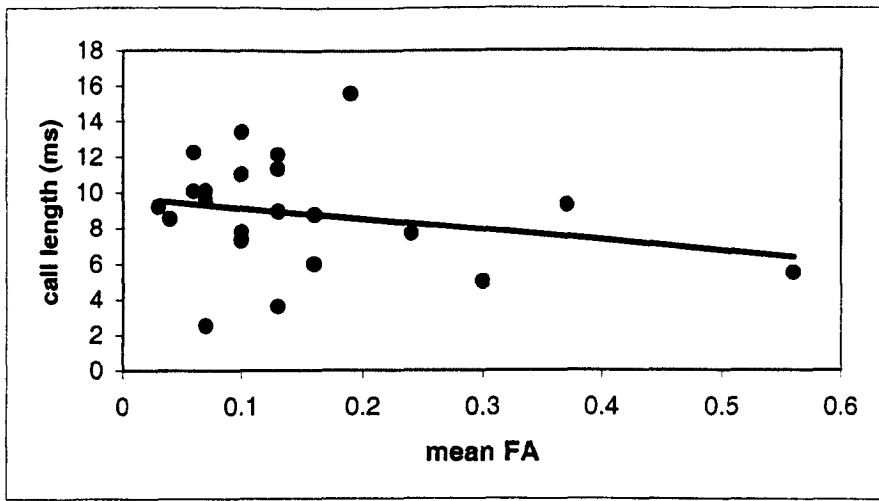
Figure 4.29 The relationship between FA and call length for males from 1998
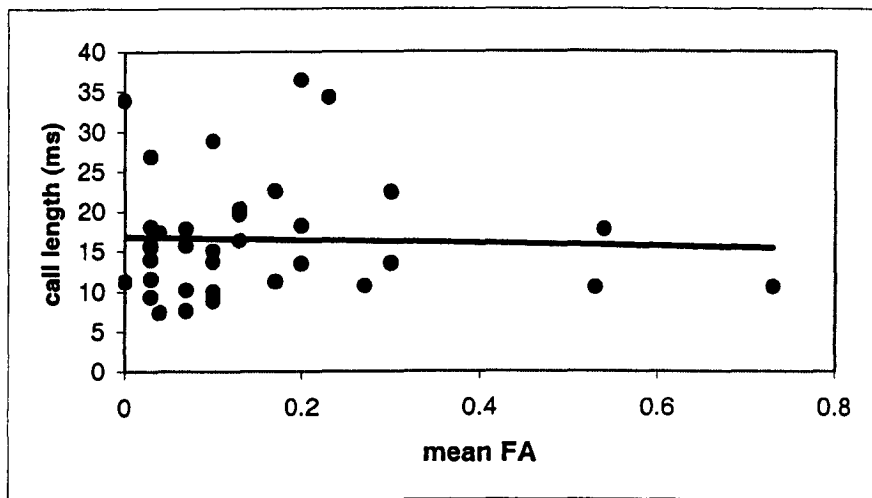
Figure 4.30 The relationship between FA and call length for males from 1999

As inter-call differed significantly both between supplemented and unsupplemented males

and between years I analysed the data for each set of males separately. There was no

correlation between FA and inter-call interval for any of the sets of males (Spearman rank

correlation, S males in 1998 (Fig. 4.31): $r_s = 0.514$, N = 12, p>0.05: US males in 1998

(Fig. 4.32): $r_s = 0.392$, N = 11, p>0.05; S males in 1999 (Fig. 4.33): $r_s = 0.255$, N = 18,

p>0.05; US males in 1999 (Fig. 4.34): $r_s = 0.077$, N = 18, p>0.05).
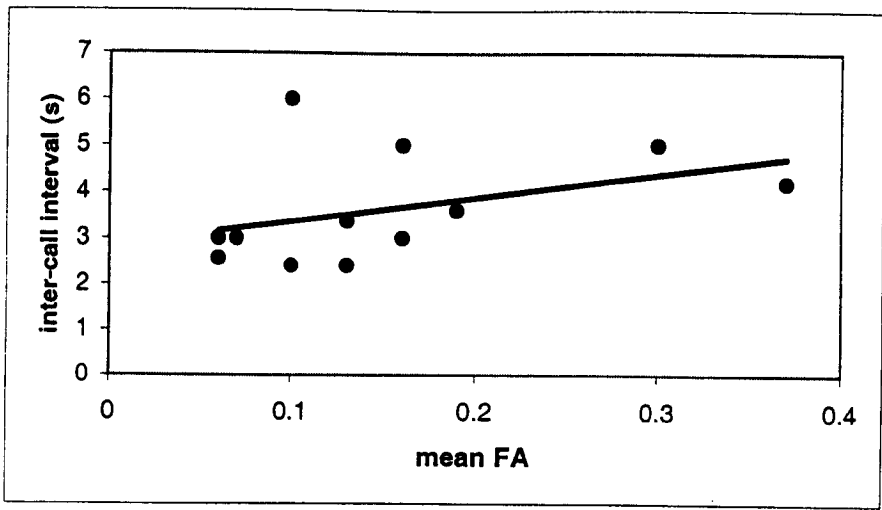
Figure 4.31 The relationship between FA and inter-call interval for supplemented males in 1998
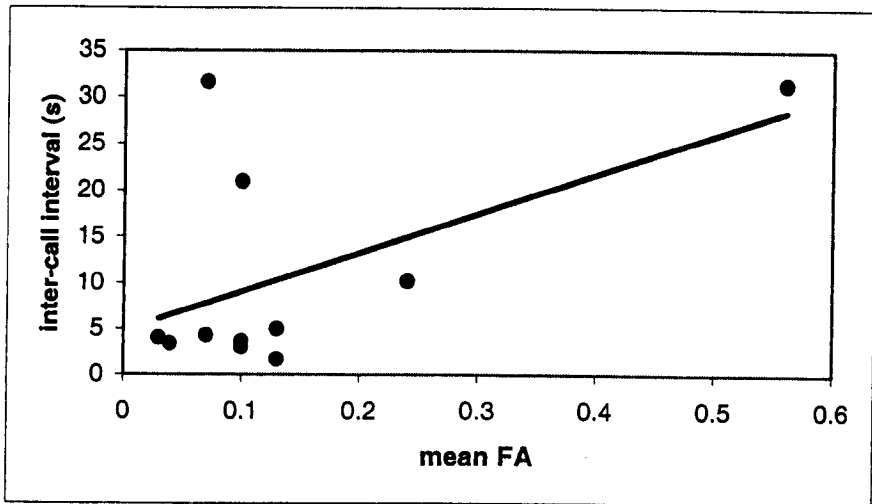
Figure 4.32 The relationship between FA and inter-call interval for unsupplemented males in 1998
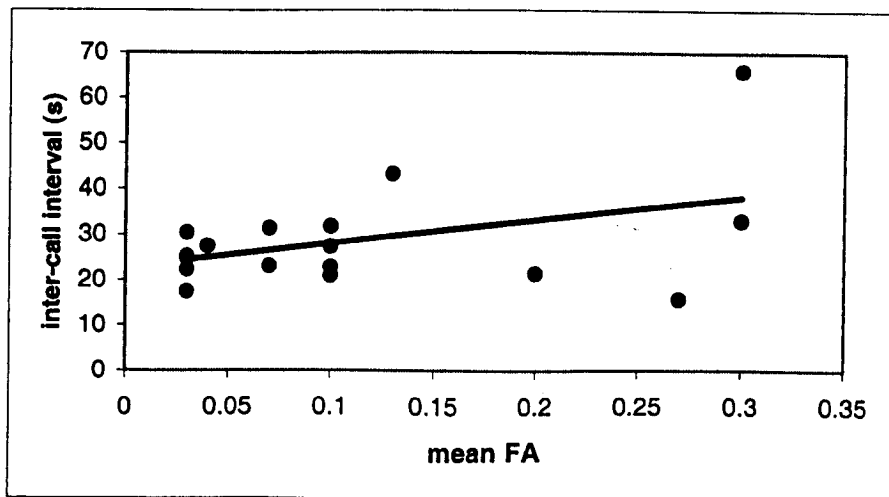
Figure 4.33 The relationship between FA and inter-call interval for supplemented males in 1999
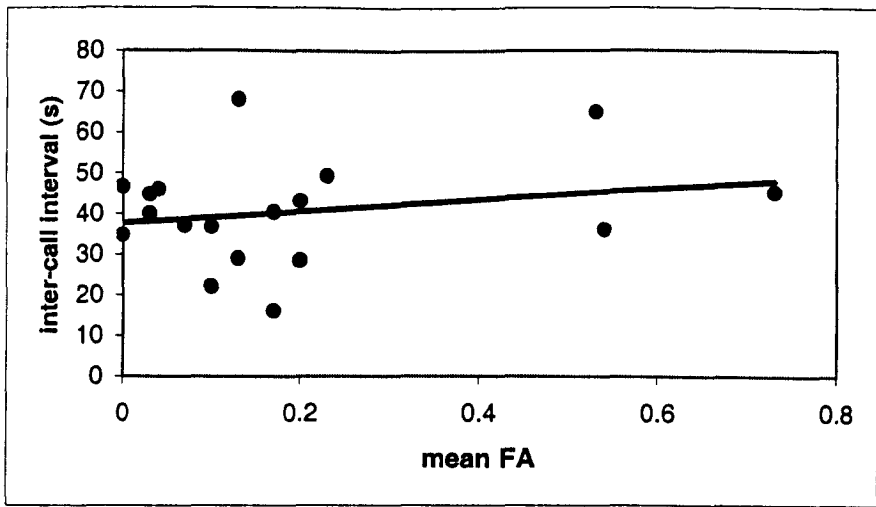
Figure 4.34 The relationship between FA and inter-call interval for unsupplemented males in 1999

There were no significant correlations between mean FA and any of the song characteristics measured in 1999 (Spearman rank correlation, no. syllables (Fig. 3.35): $r_s =$ -0.033, N = 36, p>0.05; syllable length (Fig. 3.36): $r_s = 0.010$, N = 36, P > 0.05; syllable separation (Fig. 3.37): $r_s = -0.256$, N = 36, p>0.05; frequency (Fig. 3.38): $r_s = 0.203$, N = 36, p>0.05).
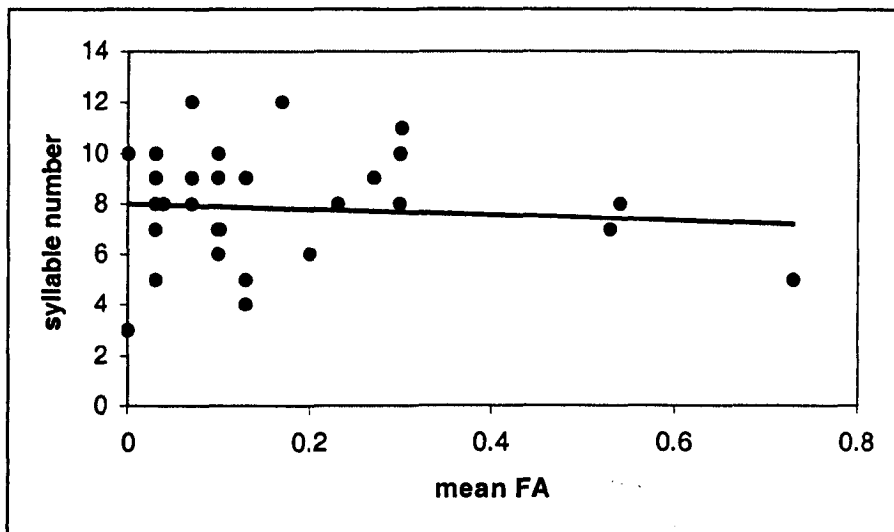
Figure 4.35 The relationship between FA and the number of syllables contained within a call
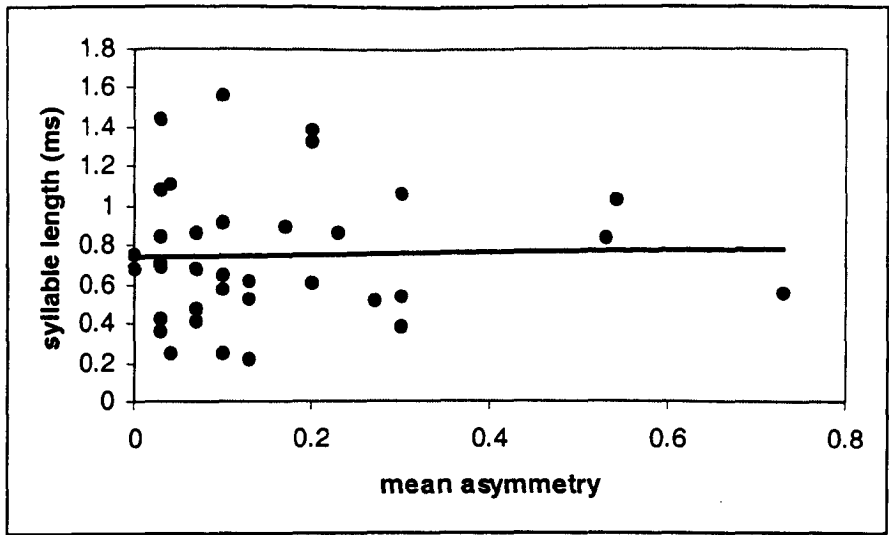
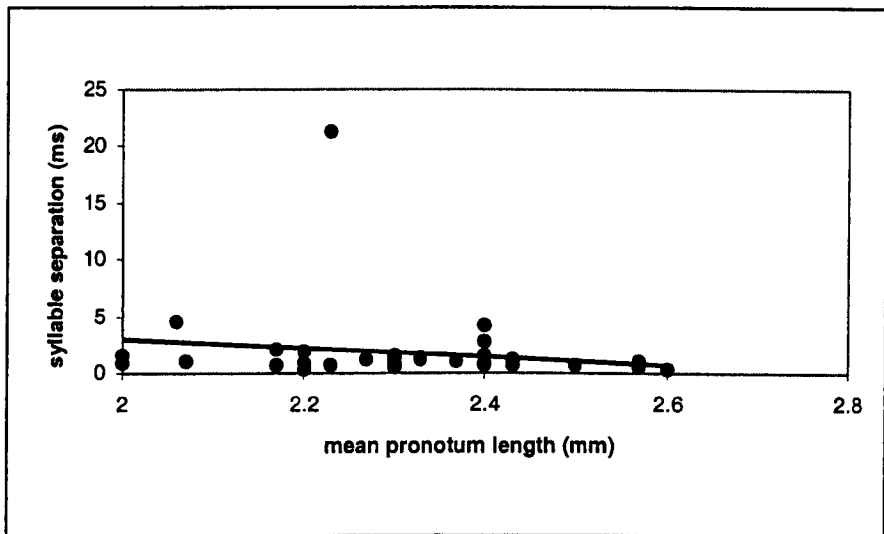Figure 4.36 The relationship between FA and the length of syllables contained within a call

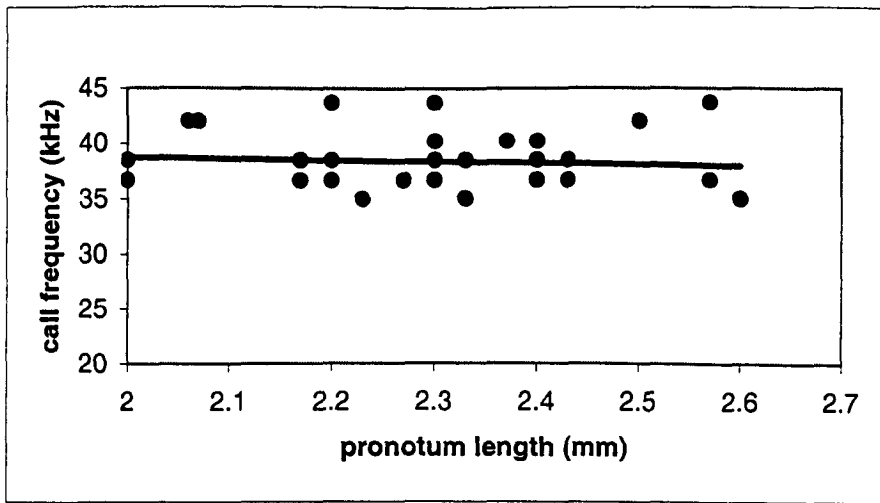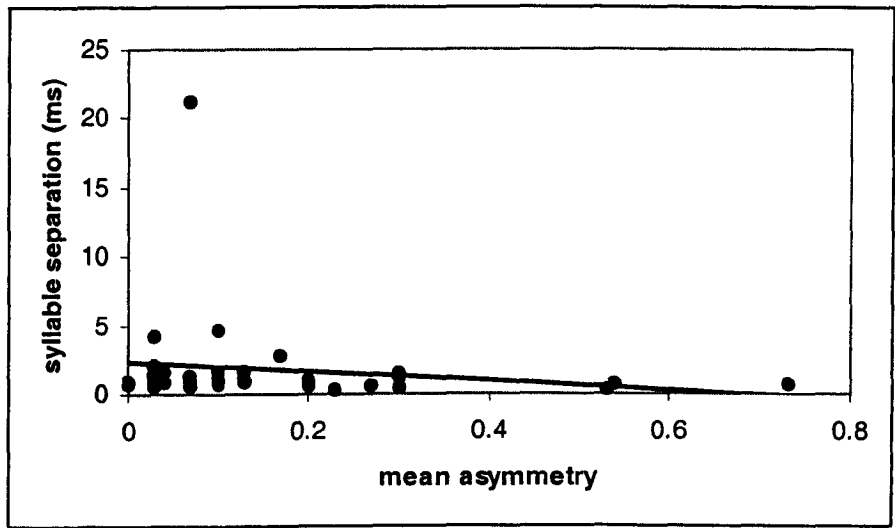Figure 4.37 The relationship between FA and the separation of syllables within a call
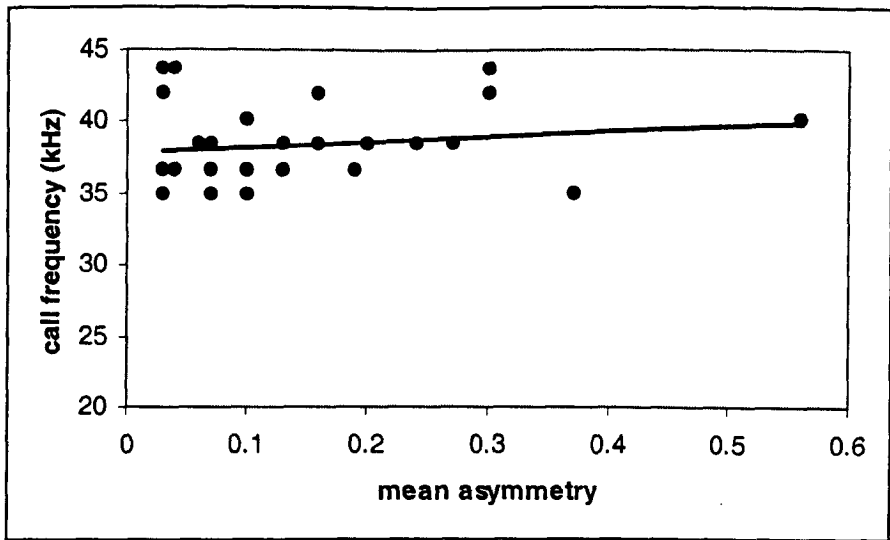
Figure 4.38 The relationship between asymmetry and call frequency

There was no correlation between FA and the mean calling bout length of males

(Spearman rank correlation, data from all males in 1998, $r_s = 0.003$, N = 30, p>0.05; Fig.

4.39); nor was FA related to the percentage amount of time males spent calling ($r_s = 0.006$,

N =30, p>0.05; Fig. 4.40).
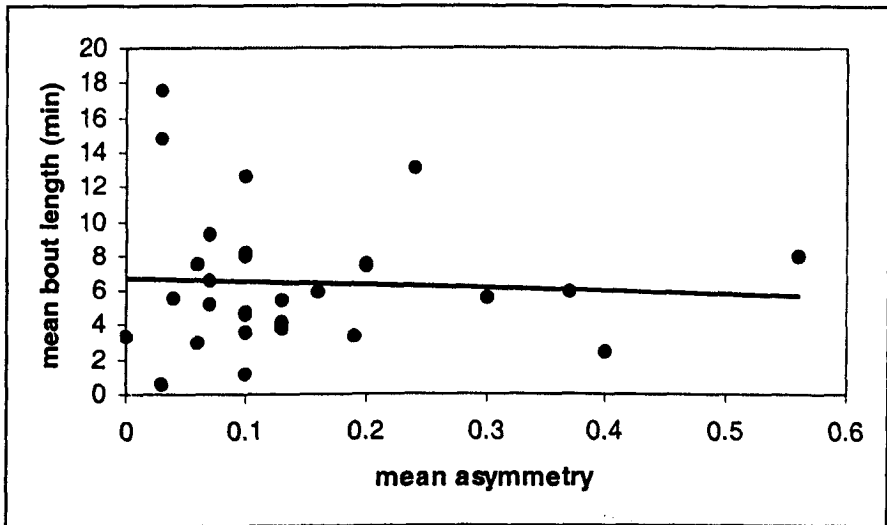
Figure 4.39 The relationship between FA and mean calling bout length
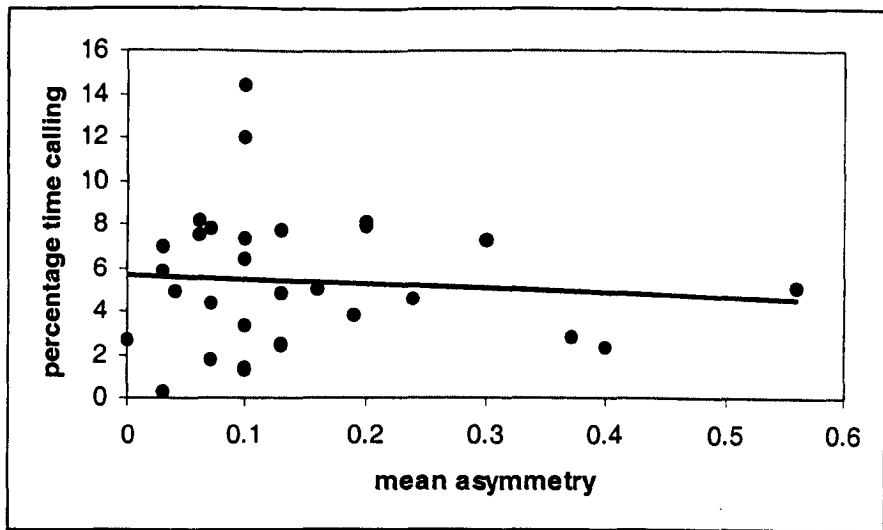
Figure 4.40 The relationship between FA and the percentage time spent calling

4.3 Discussion

4.3.1 Variation in body measurements

Most body measurements were significantly correlated with each other. One exception was the tegmina of females, which showed no correlation with several of the other traits in the study. Characters that are under sexual rather than natural selection are likely to exhibit greater phenotypic variation than other morphological traits because they are less highly canalized during development (Waddington, 1940), consequently they may not display the same linear growth patterns as other characters. Females do respond to calling males in this species, so if there is any selection pressure on the female response, it is possible that sexual selection is operating on the tegmina. It is not, however, possible to reach any firm conclusions on the basis of this present study.

There were no differences in size between insects maintained on a protein enhanced diet and those maintained on a standard diet of leaves. Nutritional stress has been shown to be a major factor leading to decreased body size in several species. In the stalk eyed fly (*Cyrtodiopsis daminni*) larvae raised under conditions of poor food quality showed reduced adult size relative to larvae whose diet was of a higher quality (Bjorksten *et al.*, 1999).

112

However the insects in this study were not nutrient stressed throughout their entire development: the protein supplement was provided only from the penultimate instar stage. It is therefore not unexpected that differences in size do not result from differences in diet affecting only a short part of the overall period of growth and development.

Insects were larger in 1998 than they were in 1999 (based on pronotum length). There are many reasons why individuals may differ in size between years. Habitat quality and therefore food quality may vary between years. Although the insects used in this study were maintained in captivity for the majority of their lives, and were provided with fresh food regularly, differences in food quality between years may have adversely affected individuals at an early stage of development. In the bushcricket *Metrioptera bicolor* extreme weather conditions, which affected the quality of food available, led to a reduced body size in adults (Kindvall, 1995). Another factor which may have affected size differences between years is a difference in temperature, both in the wild when the insects were at an early stage of development and in the laboratory after they were captured. The growth rate of insects is temperature dependent (Schneiderman & Gilbert, 1964). In 1998 the insects were kept in a laboratory in which the temperature was held at a constant 25°C, whereas in 1999 they were kept in an unheated laboratory so the temperature fluctuated considerably, especially between night and day, with the average temperature likely to be considerably below 25°C.

The insects in the 1998 laboratory population were larger than the German population in 1998. This again may be due in part to differences in temperature. Insects collected in the UK were kept at a constant, relatively high temperature after capture, while the German population developed under natural conditions and were likely to have been subjected to a lower average temperature and to temperatures that fluctuated throughout the day and from one day to the next.

Despite being smaller, UK insects in 1999 were more variable in size, compared with those in 1998, as were wild German insects compared with the 1998 laboratory population. This could also be as a result of the differences in rearing conditions between the three populations. It is possible that fluctuating or lower average temperatures lead to a greater range of sizes, perhaps because some individuals are less well able to cope with these conditions than others.

Diet may have been another factor in the observed size differences. In the laboratory, insects were provided with a variety of fresh foodstuffs every 48h and the range of plant material may have provided higher nutritive value than that available to the natural population. This would not explain the differences between the 1998 and 1999 laboratory populations. However, there may also be differences between populations from different areas. In 1998 all the animals were collected from one site, the Warren nature reserve in Folkestone Kent. In 1999 the insects were collected from several different sites and there may have been differences between these areas in habitat quality which was reflected in the size differences and the degree of variability in size between the two years.

4.3.2   Variation in fluctuating asymmetry

I found no differences in the levels of FA shown by the laboratory populations in different years or between laboratory reared and wild populations in 1998, though it might be expected that animals in a totally natural environment would be exposed to greater environmental stress than those in which environmental conditions are held constant. Temperature fluctuations, such as those experienced by the German population and the 1999 laboratory population, have been correlated with increased FA in several species. Bradley (1980) demonstrated that in *Drosophila*, flies raised in environments in which temperatures were experimentally manipulated to fluctuate between 20°C and 29°C showed greater developmental instability than insects raised at a constant 25°C. So even

114

though the average temperature was not different, the fluctuations above and below the average appeared to affect the developmental pathways of the insects. Average temperatures may also affect the degree of FA. Temperatures below the optimum led to greater developmental instability in the Australian sheep blowfly (*Lucilia cuprina*) (Clarke & McKenzie, 1992; McKenzie & Yen, 1995), increased water temperatures increased asymmetry in the fins of rainbow trout (Leary *et al.*, 1992) and Siberian sturgeon (*Acipenser baeri*) (Ruban, 1992), and decreased temperatures increased dental asymmetry in mice (Siegel & Doyle, 1975).

One reason why there may have been no difference in the level of asymmetry expressed by the three populations may be that the laboratory-reared insects completed a substantial part of their development under natural conditions. The pattern of asymmetry may be set early in development and the change to a constant temperature environment for the 1998 laboratory population may have occurred too late to alter it.

Interestingly, apart from the tegmina in which between-sides variation was indistinguishable from measurement error for both males and females, the other morphological traits in the wild population showed the properties of true FA. This was not the case for several of the characters in the laboratory population in which instead there was significant directional asymmetry. In many cricket species, the right tegmen overlaps the left and this has resulted in the evolutionary retrogression of the right tegmina (Masaki *et al.*, 1987). In *L. punctatissima* the opposite pattern is observed and the tegminal arrangement is left over right, so that any directional bias should be to the left with the right tegmina being significantly smaller. I recorded such directional asymmetry in the tegmina of two groups of insects in this study: supplemented males and unsupplemented females.

I also observed directional asymmetry in the femur for supplemented females and the tibia for supplemented males, with a bias to the left and to the right respectively. It seems unlikely that a consistent size difference to one side of the body in an important functional trait would confer any advantage on an individual. On the contrary, if the bias is large enough to affect locomotion significantly individuals may be more susceptible to predation and therefore be less likely to reach adulthood. The predator free environment of the laboratory population would exclude this form of selection hence; highly asymmetric individuals may survive until adulthood, which would not be the case for wild insects. If this were the case then one might expect the laboratory population to exhibit greater FA than the wild population. I found no difference in FA between wild and captive insects. The directional asymmetry present in the laboratory population could be a result of the small sample size being disproportionately influenced by a few highly asymmetric individuals that happened to be asymmetric in the same direction.

Supplemented and unsupplemented males showed no difference in levels of FA. Nutritional stress has been postulated to be a major environmental influence increasing developmental instability (Kirpichnikov, 1981). Swaddle & Witter (1994) showed that a poor nutritional state led to increased FA during a moult in the tail feathers of the European starling. Differences in food quality between habitats increased FA differences between populations of the Montana grizzly bear (*Ursus arctos horribilis*) (Picton *et al.*, 1990). It is possible that poor nutrition may simply not cause greater developmental instability in *L. punctatissima*. Bjorksten *et al.* (1999) reported a lack of correlation between FA and diet in the stalk eyed fly (*Cyrtodiopsis dalmanni*), even when diet is strictly controlled throughout development. Animals reared in food stressed conditions showed reduced adult size when compared to individuals whose diet was of a better quality, but FA did not differ. Arnqvist & Thornhill (1998) also reported similar findings in the water strider. However it is possible that any effects of nutrition on FA were limited, either because the diet of the

116

insects was not controlled prior to collection and supplementation was only given from the fifth instar, or because even the unsupplemented diet was sufficient not to cause nutritional stress.

4.3.3 Variation in calling characteristics

There was a difference in the call length of males between years, which was not related to the diet males, were maintained on or to male size. Interestingly males captured in 1999 had longer calls than the 1998 males even though the 1998 individuals were, on average, larger. This suggests that call length is not correlated with the size of the stridulatory apparatus as one might expect.

Neither call length or the number of syllables contained within each call differed between males in relation to size or diet. One possible reason may lie in the problem facing a responding female in knowing precisely when to reply to a stridulating male. If her response overlaps the male's signal it will not be perceived, rendering the male effectively deaf (Hedwig & Elsner, 1985; Wolf, 1985). In many reciprocally singing species the male song reaches a brief intensity maximum at the end of each call which acts as a trigger for the female response (Nickle, 1976; Heller, 1984). For instance in *Ancistrura nigrovittata* the trigger for the female reply comes approximately 400ms after the main syllable group (Dobler *et al.*, 1994). In species such as *L. punctatissima* that have a very brief call, the entire male song is the stimulus that elicits a female response (Heller & Von Helverson, 1986). In such a system there may be selection pressure which acts to produce an optimum call length, since if a male produces calls that extend beyond this optimum then his signal may temporally overlap a female response, and he will not be able to hear it.

Inter-call interval varied between males and this was related to both the year the males were collected in and the diet they were maintained on. Calling has been shown to be an

energetically expensive process in several species within the Orthoptera, (Bailey *et al.*, 1993), consequently, one might predict that individuals with greater energy reserves may call at an increased rate i.e. they will have a shorter inter-call interval. There was, however, no difference in the inter-call interval between supplemented and unsupplemented males in 1998 and in 1999 supplemented males had longer inter-call intervals than both unsupplemented males from the same year and supplemented males from the previous year. Males of this species are known to increase their rate of calling when they perceive a female reply (Robinson, 1980). The males in 1998 were recorded in a laboratory in which there were females present, whereas the males in 1999 were screened from the effects of female replies. The difference in the inter-call interval between supplemented males from different years may be a consequence of this. The difference between 1999 supplemented and unsupplemented males in inter-call interval is probably real since the data from 1999 are more reliable than those in 1998. It is, however difficult to explain. The shorter inter-call interval of unsupplemented males in this year may indicate that the calling rate of males is not energetically expensive and is therefore not limited by nutritional status, at least not at the levels of nutrition provided in this study.

It might also be expected that nutritional status could affect the amount of uninterrupted calling males are able to perform. Males on a poor diet might concentrate their calling to short but intense bouts, whereas males on a better quality might be able to call for longer uninterrupted periods or call for a greater proportion of their time. There was, however, no evidence that from this study that this might be happening, with supplemented and unsupplemented males showing no differences in bout length or the proportion of time spent calling.

Nor was there any correlation between male size and calling bout length. Body size is often correlated with calling bout length in crickets (Hedrick, 1986) because larger body size

equates with a greater amount of stored energy. The lack of association between size and bout length and the observation that unsupplemented males can actually call at an increased rate is further evidence that calling in *L. punctatissima* is not limited by energy availability.

There were no differences between males in relation to size or diet for any of the other song characteristics investigated. *L. punctatissima* is therefore unusual in that its shows no differences in call frequency in relation to size, unlike many other Orthoptera (Gwynne, 1982; Bailey, 1985).

4.4 Summary

Body size varies in *L. punctatissima* between years, probably as a result of variations in weather conditions. A high protein diet does not influence body size, at least if only available towards the end of the growth period. There was no difference between males in their calling characteristics in relation to size, though unsupplemented males called at a faster rate than supplemented males. The amount of time males spent calling was not influenced by male size or nutritional condition. Levels of fluctuating asymmetry did not vary between years or between wild and laboratory populations and may not be affected by diet.

5 Effects of size on mate choice and reproductive success

5.1 Introduction

Competition between members of the same sex for access to mating partners is a common feature in many species and mating success is often related to body size. The skew in mating success may result directly because larger individuals have a physical advantage in intrasexual contests, whether in fights over prospective mates (Simmons, 1986), or in the defence of territories which increase their chances of encountering sexual partners (Campanella & Wolf, 1974).

Alternatively, individuals may gain a mating advantage because their size renders them more attractive to members of the opposite sex. Size assortative mating is a common phenomenon in many species. Such mating patterns may arise if larger males monopolize larger females (Crespi, 1989), or if there is mutual mate choice between the sexes for larger mating partners (Gwynne, 1981; Rutowski, 1982; Otronen, 1993). Both possibilities effectively result in the same outcome, that is smaller individuals are excluded from mating with larger sexual partners.

There may be benefits to both sexes in mating with larger partners. Fecundity is often correlated with size so that mating with large females may increase male reproductive success (Ridley, 1988; Del Castillo et al, 1999). In many species males furnish females with nuptial gifts (Thornhill, 1976a). Often the size of these gifts is related to body size and correlates with an increase in some measure of the reproductive success of females (Bowen et al., 1984; Gwynne, 1984a).

Greater size is related to greater longevity in many species with a concomitant increase in the reproductive lifetime of larger individuals. There may also be a disparity between different sized individuals in the cost of mating. For instance the reduced longevity of small males of *Drosophila melanogaster* is thought to arise from the investment in ejaculates being relatively greater for smaller individuals (Partridge & Farquhar, 1983).

In tettigoniids the quantity of nuptial gift a male is able to produce is usually a function of his body size, with larger males producing relatively larger spermatophores (Sakaluk & Smith, 1988; Weddel, 1993b). This disparity between large and small males in the size of their nuptial gifts may have implications for individual mating and reproductive success. If males produce spermatophores that are too small they run the risk of the female eating the spermatophylax before all of their sperm have migrated to the spermatheca (Wedell, 1991). This problem is compounded by the fact that smaller spermatophores tend to contain fewer sperm (Vahed & Gilbert, 1996a). In situations where sperm competition is present (i.e. polyandry) a male's sperm will be numerically underrepresented if a female re-mates with a larger male (Wedell, 1991). Simmons (1988b) and Sakaluk & Smith (1988) showed that smaller males in the field crickets *Gryllus bimaculatus* and *Gryllus sigillatus* respectively invested proportionately more of their overall body mass in the production of each spermatophore. Consequently smaller males have a longer post-copulatory refractory period relative to larger males, with a concomitant decrease in lifetime mating success. Furthermore, as body size has a significantly heritable component in these species (Simmons, 1987; Sakaluk & Smith, 1988), smaller males that do manage to fertilise the eggs of females produce sons with reduced body size and hence have a lower inclusive fitness relative to larger males.

It is also possible that larger males could achieve greater fertilization success as a result of cryptic female choice (Eberhard, 1996). For example, females do not usually begin to

consume the spermatophore immediately after copulation ends, and the time lag before they do begin to eat it varies considerably (Chapter 3). The greater the time lag, the greater the number of sperm likely to be transferred, and the greater the male's reproductive success is likely to be. Thus if females were to allow a greater time lag after mating with a large male, this could increase his reproductive success relative to other males.

In this chapter, I look at the effects of body size on male and female mate choice and reproductive success and try to answer a number of questions. Do males prefer to mate with larger females and females larger males and if so does their choice affect their reproductive success? Do larger females lay more or better quality eggs and do larger males produce larger spermatophores? Do smaller males produce relatively larger spermatophores in proportion to their own body size and does investment in spermatophores by an individual male vary depending on the circumstances? Do females show any evidence of cryptic choice? And finally, does the size of the spermatophore she receives affect the female's reproductive success?

5.2 Analysis of data

Data on mating success and mating behaviour were analysed separately for the supplemented breeding group and the unsupplemented breeding group. This was necessary because in effect mating success was not measured absolutely but relative to the other members of the group. For example, the number of matings or number of different partners a particular male achieved was dependent on the behaviour and characteristics of the other males and females in his group, and would probably have been different had that individual been assigned to another breeding group. Similarly, if cryptic female choice operates in this species, mating behaviour may be affected by other members of the group.

Consequently it was not possible to combine data on mating success or mating behaviour from different breeding groups.

Data on mating success and mating behaviour from the half and half breeding group were not included in the analyses because of the confounding factor of diet (see Chapter 6) and because sample sizes were too small to analyse data from this group separately for supplemented and unsupplemented individuals.

5.3 Results

5.3.1 Male size and mating success

Although the correlation between male size and the total number of matings achieved in the breeding group experiment was not significant (Spearman rank correlation, S group (Fig. 5.1): $r_s = 0.294$, $N = 10$, $p > 0.05$; US group (Fig. 5.2): $r_s = 0.552$, $N = 10$, $p > 0.05$), there was a trend for larger males to mate more frequently than smaller males and a comparison between the total number of matings achieved by the five largest males in the group and those achieved by the five smallest revealed a significant difference (S group: $\chi 2 = 5.56$, $p < 0.05$, Table 5.1; US group: $\chi 2 = 13.1$, $p < 0.001$, Table 5.2).

Figure 5.1 The relationship between male size and total number of matings for males in the supplemented breeding group.

Figure 5.2 The relationship between male size and the total number of matings for males from the unsupplemented breeding group

Table 5.1 The distribution of matings with respect to body size in the supplemented breeding group

	5 smallest females	5 largest females	Totals
5 smallest males	11	15	26
5 largest males	11	35	46
Totals	22	50	72

Table 5.2 The distribution of matings with respect to body size in the unsupplemented breeding group

	5 smallest females	5 largest females	Totals
5 smallest males	5	5	10
5 largest males	20	14	34
Totals	25	19	44

A significant effect of size on the number of matings achieved was also revealed by a logistic regression analysis, which was used to analyse the mating pattern of males from the supplemented and unsupplemented breeding groups across the experimental period. This analysis tests the effects of a series of continuous variables (i.e. male size and time) on a binomially distributed data set (i.e. whether males mated or not in a particular observation period). In both the supplemented and unsupplemented breeding groups, larger males mated more frequently than smaller males and there was a significant decrease in mating activity with time (Tables 5.3, 5.4).

Table 5.3 Logistic regression analysis of mating frequency for males in the supplemented breeding group. Male size and time are entered as covariates.

	B	SE	df	EXP (B)	p
Male size	7.79	3.30	1	2418.18	0.018
Time	-0.09	0.03	1	0.91	0.0028

Table 5.4 Logistic regression analysis of mating frequency for males in the unsupplemented breeding group. Male size and time are entered as covariates

	B	SE	df	EXP(B)	p
Male size	3.79	1.35	1	44.33	0.005
Time	-0.087	0.036	1	0.92	0.014

Further analysis revealed that in both the S and US groups the effect of male size on mating frequency was apparent only in the first half of the breeding season. In the second half of the season there was no significant effect of male size on mating frequency and smaller males were equally as likely to mate as their larger counterparts (Tables 5.5, 5.6).

Table 5.5 Logistic regression analysis of mating frequency for males in the supplemented breeding group in the first and second half of the breeding season.

		B	SE	df	EXP(B)	p
1st half season	Size	11.13	4.63	1	68356.19	0.0163
	Time	-0.17	0.08	1	0.841	0.0352
2nd half season	Size	4.31	4.91	1	74.23	0.381
	Time	-0.18	0.099	1	0.833	0.064

Table 5.6 Logistic regression analysis of mating frequency for males in the unsupplemented breeding group in the first and second half of the breeding season.

		B	SE	df	EXP(B)	p
1st half season	Size	4.21	1.81	1	67.11	0.02
	Time	-0.009	0.091	1	0.99	0.92
2nd half season	Size	2.52	2.06	1	12.42	0.22
	Time	-0.38	0.14	1	0.68	0.005

Larger males in the unsupplemented breeding group mated with significantly more different partners than smaller males (Fig. 5.4; Spearman rank correlation, $r_s = 0.637$, N = 10, p<0.05), but there was no correlation between male size and the number of different partners mated with in the supplemented breeding group (Fig. 5.3; $r_s = 0.155$, N = 10, p>0.05).
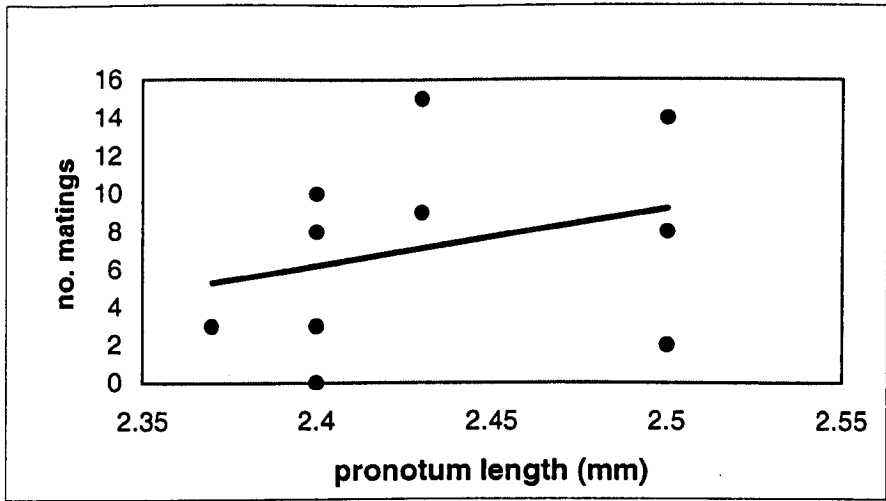

Figure 5.3 The relationship between male size and the number of different partners for males in the supplemented breeding group

Figure 5.4 The relationship between male size and the number of different partners for males from the unsupplemented breeding group.

5.3.2 Female size and mating success

There was no correlation between female body size and the total number of matings in either the supplemented group (Spearman rank correlation, $r_s = 0.338$, N = 10, p>0.05; Fig. 5.5) or the unsupplemented group ($r_s = -0.089$, N = 10, p>0.05; Fig. 5.6). Nor was there any correlation between body weight and the number of matings females achieved (Spearman's rank correlation, S group: $r_s = 0.031$, N = 10, p>0.05; US group: $r_s = 0.163$, N = 10, p > 0.05). However, there was a trend for larger females to get more matings in the supplemented breeding group and a comparison between the five largest females and the five smallest females revealed a significant difference (Table 5.1, $\chi2 = 5.44$ p <0.05). There was no significant difference between the total number of matings obtained by the five largest females and the five smallest females in the unsupplemented breeding group (Table 5.2; $\chi2 = 0.82$ p >0.05).
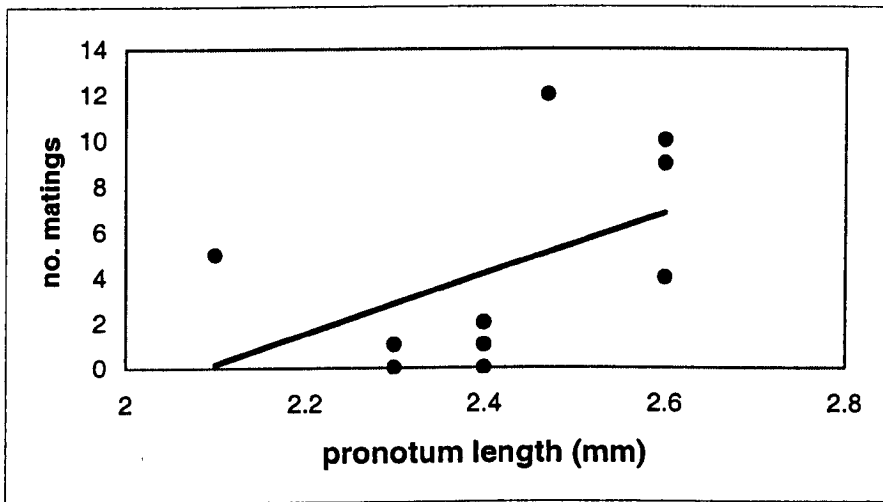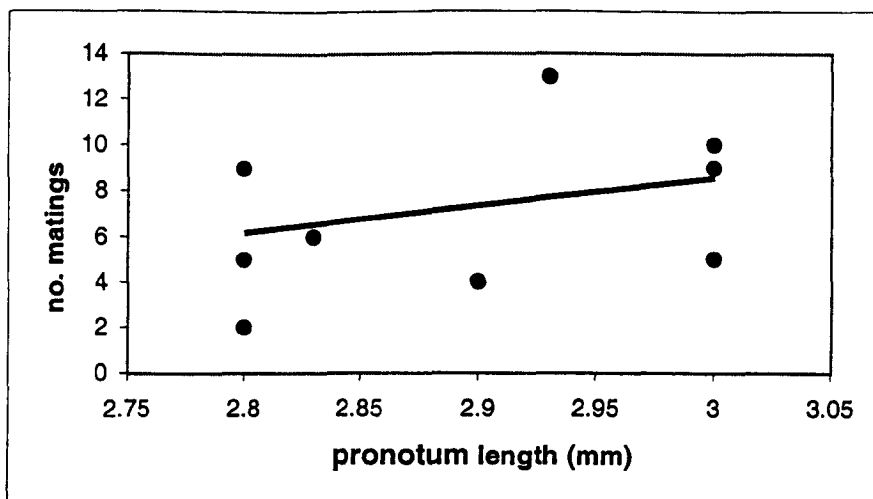
Figure 5.5 The relationship between female size and the total number of matings for females from the supplemented breeding group
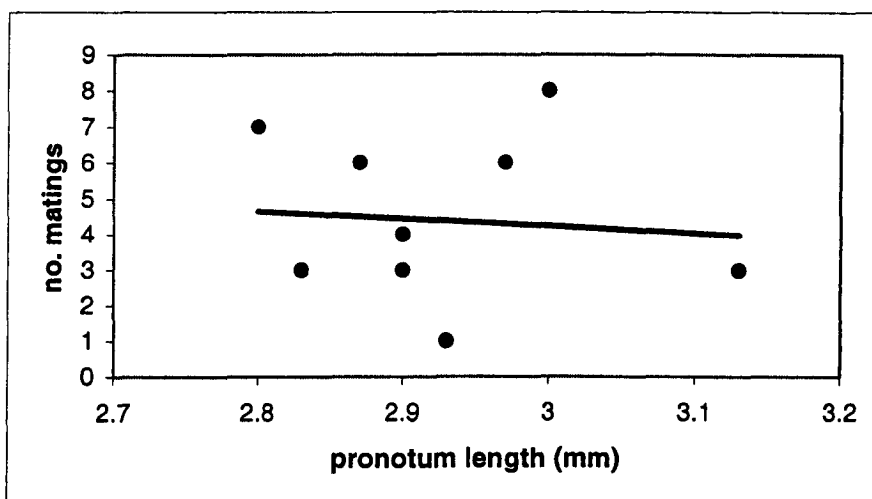
Figure 5.6 The relationship between female size and the total number of matings for females from the unsupplemented breeding group

Larger females did not mate at a higher frequency than smaller females (Tables 5.7, 5.8). A logistic regression analysis was carried out for female matings in the same way as was done for males. This showed no difference in mating frequency between large and small females in either the supplemented or unsupplemented breeding groups. There was a significant effect of time in both the supplemented and unsupplemented breeding groups with the frequency of mating diminishing significantly as the season progressed, especially in the unsupplemented group.

Table 5.7 Logistic regression analysis of mating frequency for females in the supplemented breeding group. Time and female body size are entered as covariates

	B	SE	df	EXP(B)	p
Female size	1.68	1.83	1	5.39	0.36
Time	-0.58	0.03	1	0.94	0.049

Table 5.8 Logistic regression analysis of mating frequency for females in the unsupplemented breeding group. Time and female body size are entered as covariates

	B	SE	df	EXP(B)	p
Female size	-1.11	2.08	1	0.33	0.59
Time	-0.10	0.03	1	0.91	0.004

Larger females did not mate with significantly more different partners than smaller females, in the supplemented group (Spearman rank correlation, $r_s = 0.299$, N = 10, p>0.05; Fig. 5.7) or the unsupplemented group ($r_s = 0.013$, N = 10, p>0.05; Fig. 5.8). Nor was there any correlation between body weight and the number of different partners females mated with (Spearman rank correlation, S group: $r_s = 0.068$, N = 10, p > 0.05; US group: $r_s = -0.075$, N = 10, p > 0.05.
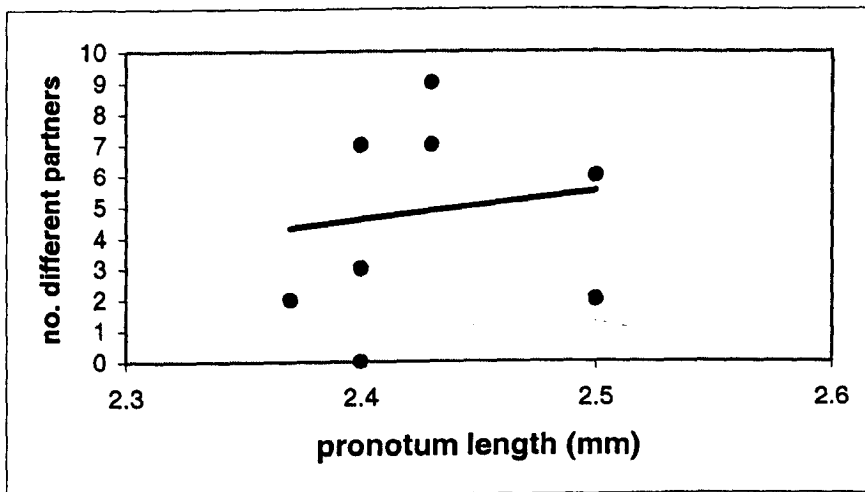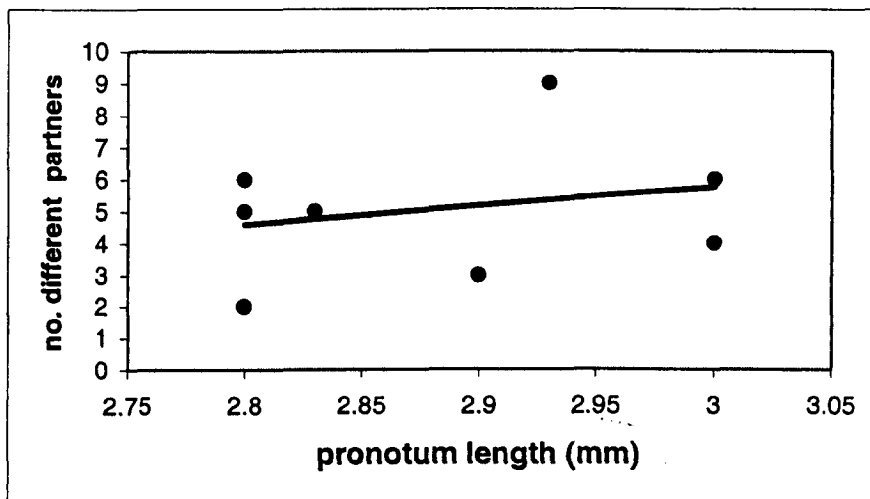

Figure 5.7 The relationship between female size and number of different partners for females from the supplemented breeding group
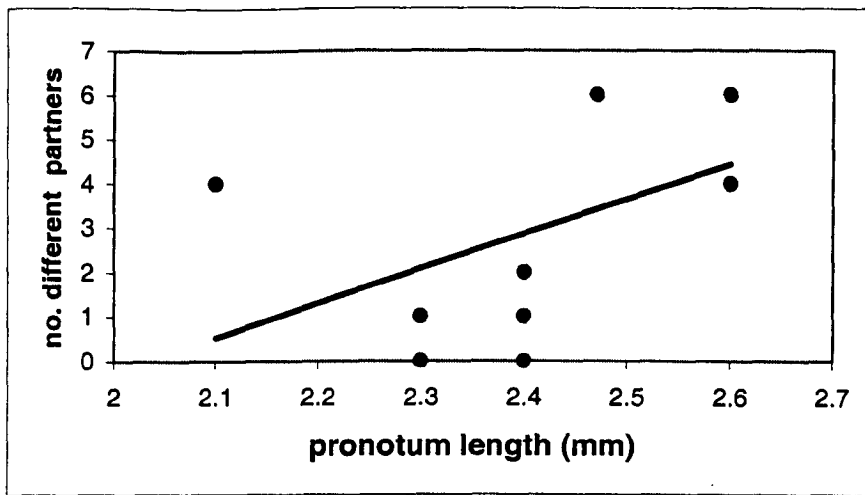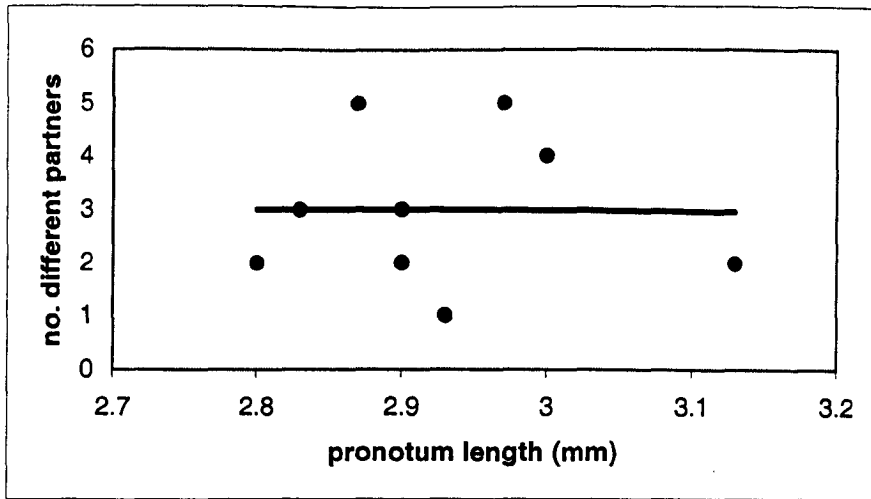
Figure 5.8 The relationship between female size and number of different partners for females from the unsupplemented breeding group

5.3.3 Assortative mating with respect to size

There was no assortative mating with respect to body size in the supplemented breeding group (Spearman rank correlation, $r_s = 0.156$, N = 72, p>0.05; Fig. 5.9) or the unsupplemented breeding group ($r_s = -0.010$, N = 44, p>0.05; Fig. 5.10).

Figure 5.9 The relationship between male size and female size for all matings in the supplemented breeding group
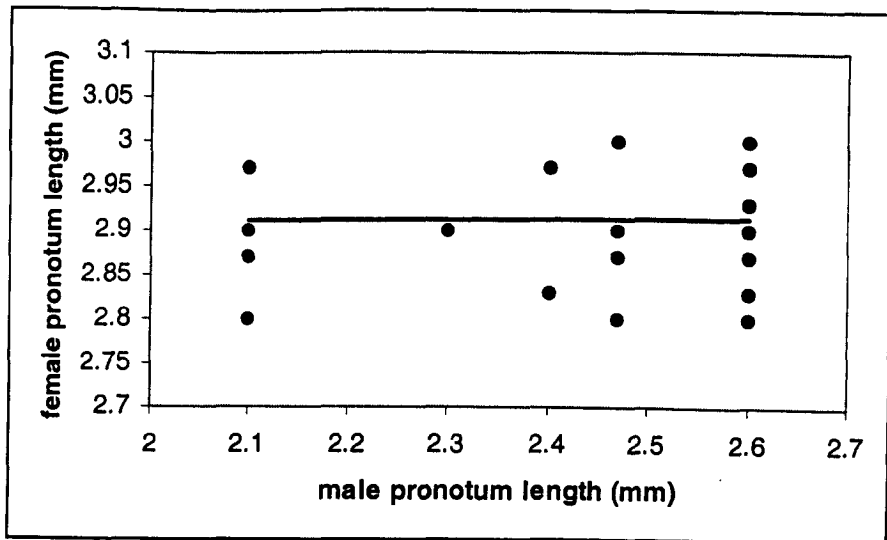
Figure 5.10 The relationship between male size and female size for all matings in the unsupplemented breeding group

Nor was there any evidence that animals mated assortatively on their first mating (Spearman rank correlation, S group (Fig. 5.11): $r_s = 0.252$, N = 10, p>0.05; US group (Fig. 5.12): $r_s = 0.646$, N = 10, p>0.05). Both male and female were virgin in all first matings.
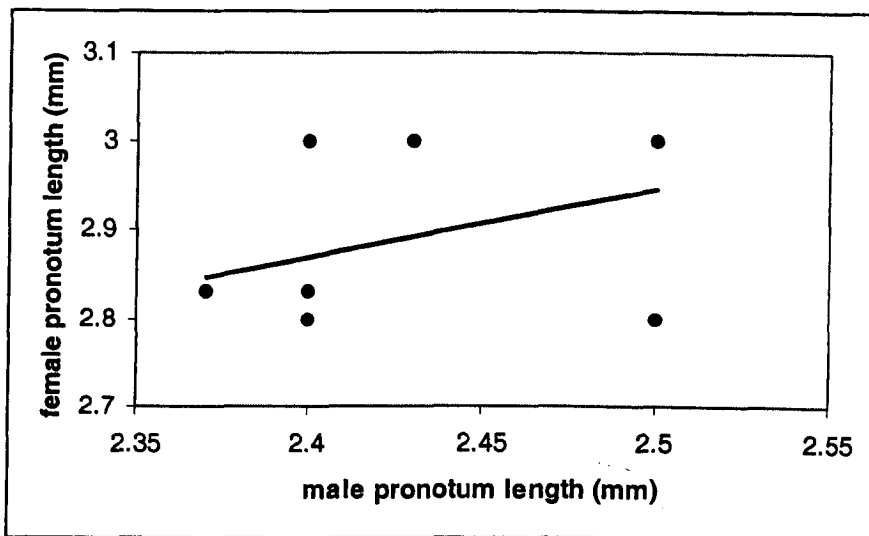
Figure 5.11 The relationship between male size and female size for first matings in the supplemented breeding group
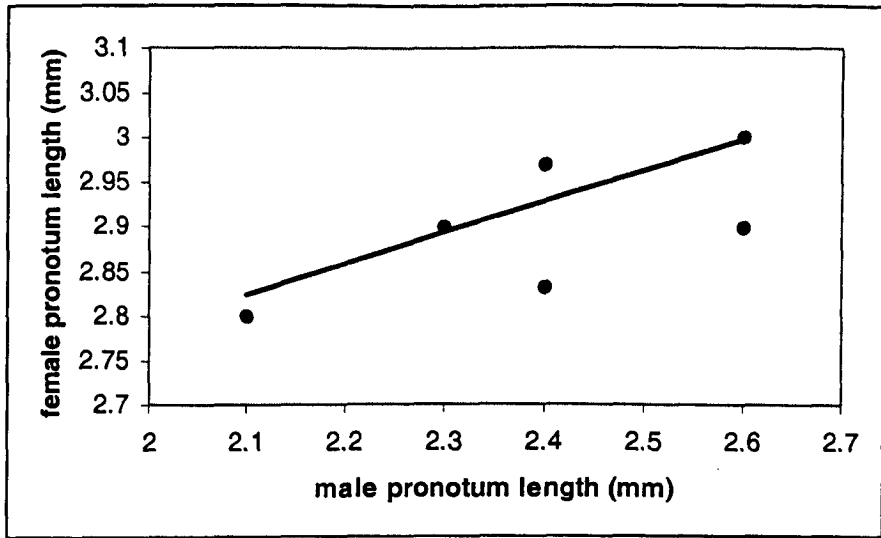
Figure 5.12 The relationship between male size and female size and first matings in the unsupplemented breeding group

5.3.4 Size and mating behaviour

Body size was not correlated with the length of time spent in copula for males either in the supplemented breeding group (Spearman rank correlation, $r_s = 0.108$, N = 72, p >0.05; Fig. 5.13); or the unsupplemented breeding group ($r_s = 0.099$, N = 44, p>0.05; Fig. 5.14). The time lag after copulation before females began to eat the spermatophore was not related to the size of the male they mated with in either the supplemented breeding group, ($r_s = 0.065$, N = 65, p>0.05; Fig. 5.15), or the unsupplemented breeding group, ($r_s = -0.080$, N = 40, p>0.05; Fig. 5.16). The time taken for females to eat the spermatophore was not related to the size of the male they mated with in the supplemented breeding group ($r_s = 0.173$, N = 59, p>0.05; Fig. 5.17), or the unsupplemented breeding group ($r_s = -0.140$, N = 36, p>0.05; Fig. 5.18). Nor was there any correlation between male size and total spermatophore attachment time (S group: $r_s = 0.096$, N = 72, p > 0.05; US group: $r_s = 0.036$, N = 44, p>0.05).

132
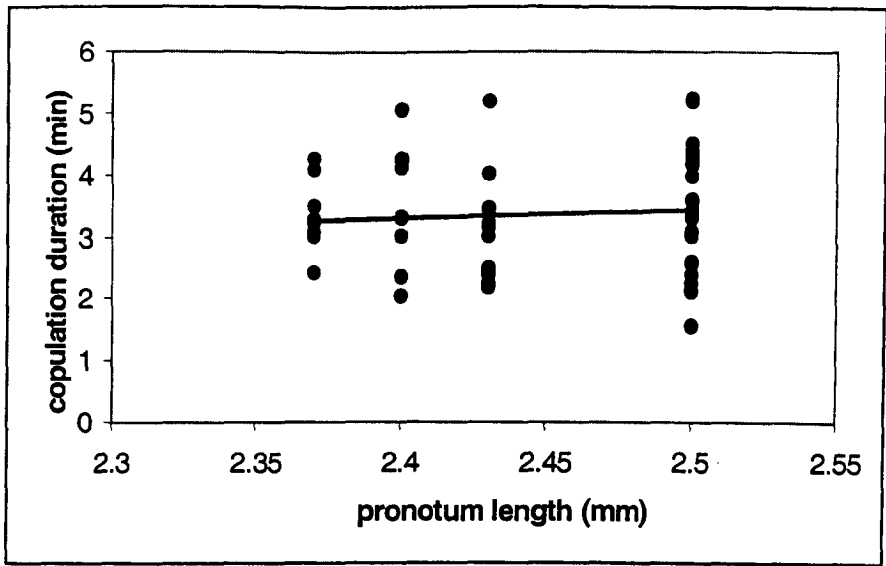
Figure 5.13 The relationship between male size and copulation duration for all matings in the supplemented breeding group
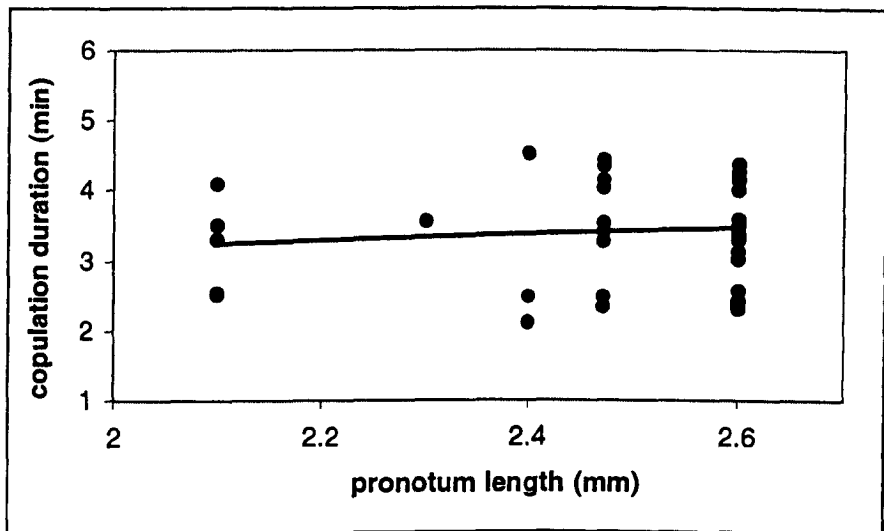
Figure 5.14 The relationship between male size and copulation duration for all matings in the unsupplemented breeding group
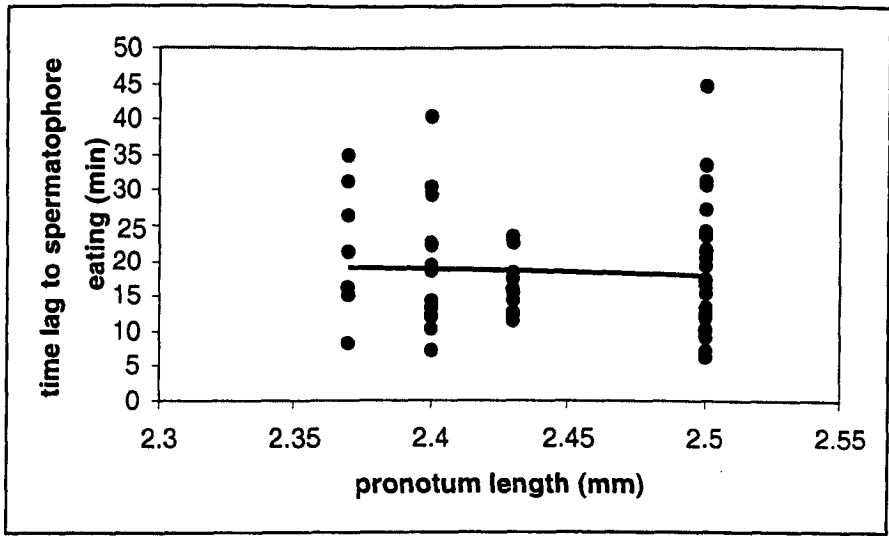
Figure 5.15 The relationship between size of male mated with and the time lag between the end of copulation and the onset of spermatophore consumption in the supplemented breeding group
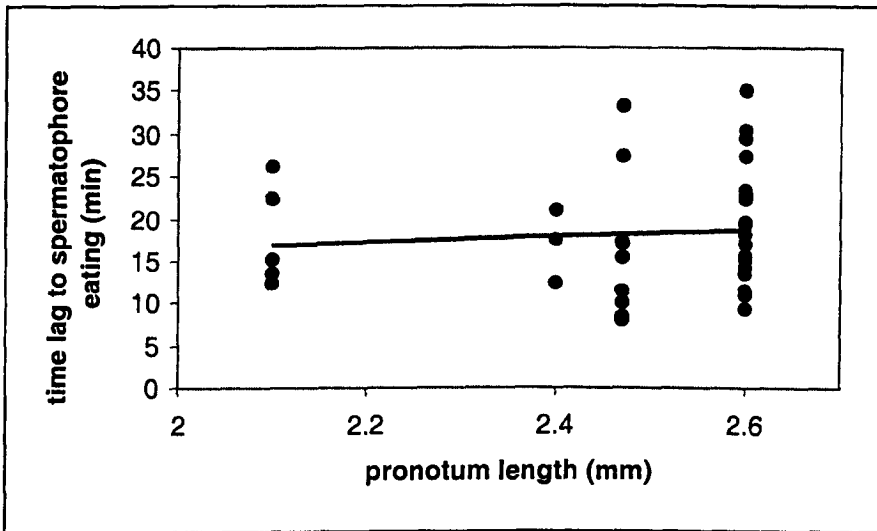
Figure 5.16 The relationship between size of male mated with and the time lag between the end of copulation and the onset of spermatophore consumption in the unsupplemented breeding group
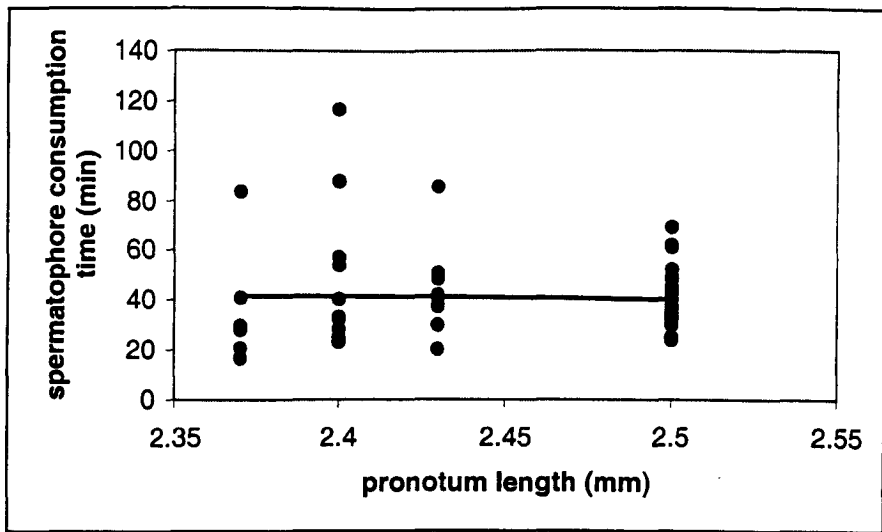
Figure 5.17 The relationship between size of male mated with and the time taken for the spermatophore to be consumed in the supplemented breeding group
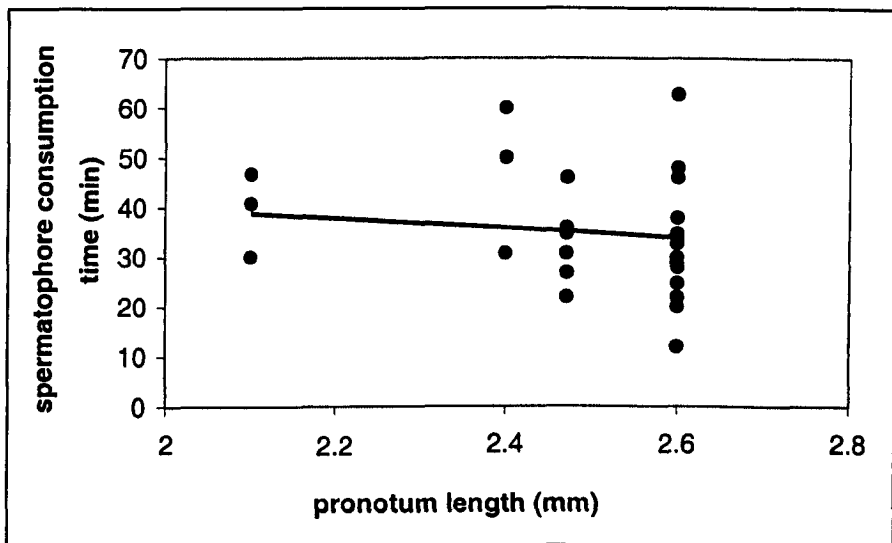
Figure 5.18 The relationship between size of male mated with and the time taken for the spermatophore to be consumed in the unsupplemented breeding group

Copulation duration was not associated with female size in the supplemented breeding group (Spearman rank correlation, $r_s = 0.106$, N = 72, p>0.05; Fig. 5.19) or the unsupplemented breeding group ($r_s = -0.072$, N = 44, p>0.05; Fig. 5.20). The time lag before the onset of spermatophore consumption was not related to the size of the female in the supplemented breeding group ($r_s = -0.120$, N = 65, p>0.05; Fig. 5.21) or the unsupplemented breeding group, ($r_s = -0.080$, N = 40, p>005; Fig. 5.22). The time taken to eat the spermatophore was not related to female size in the supplemented breeding group

135

$(r_s = 0.259, N = 59, p>0.05;$ Fig. 5.23) or the unsupplemented breeding group $(r_s = 0.181, N = 40, p>0.05;$ Fig. 5.24). Total spermatophore attachment time was not related to female size (S group: $r_s = 0.135, N = 65, p > 0.05;$ US group: $r_s = -0.219, N = 40, p > 0.05$).
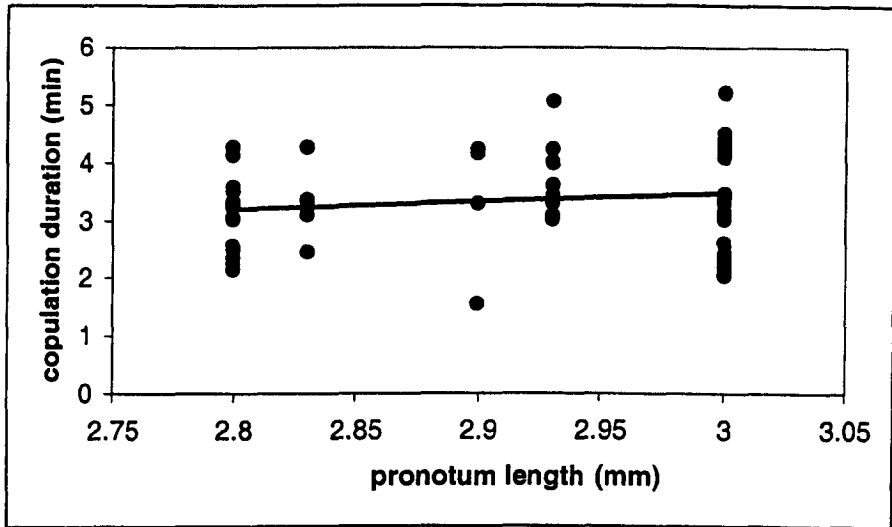
Figure 5.19 The relationship between female size and copulation duration for all matings in the supplemented breeding group
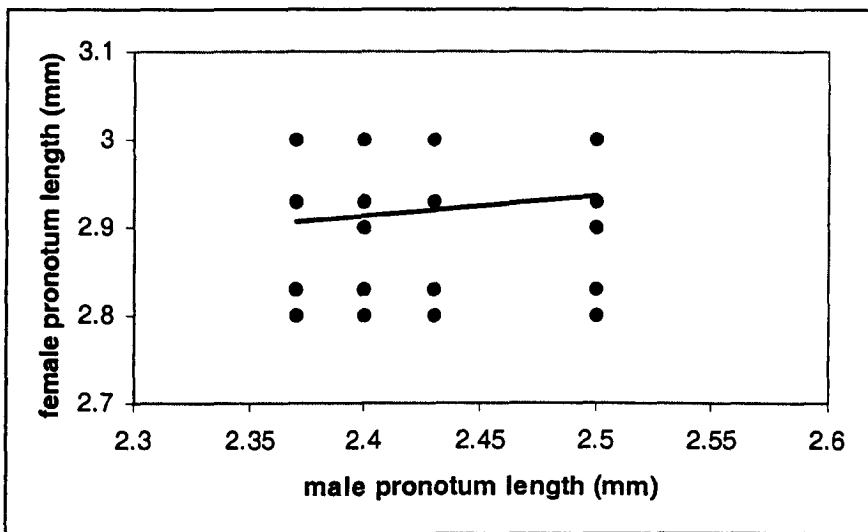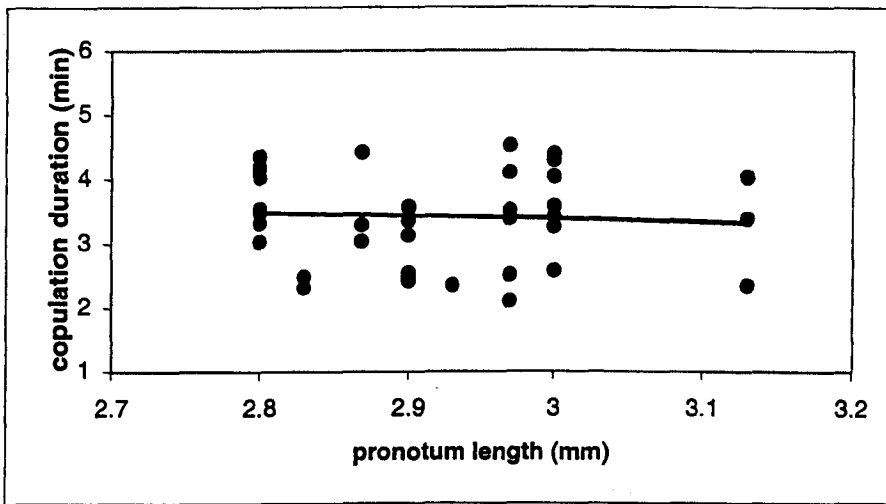

Figure 5.20 The relationship between female size and copulation duration for all matings in the unsupplemented breeding group
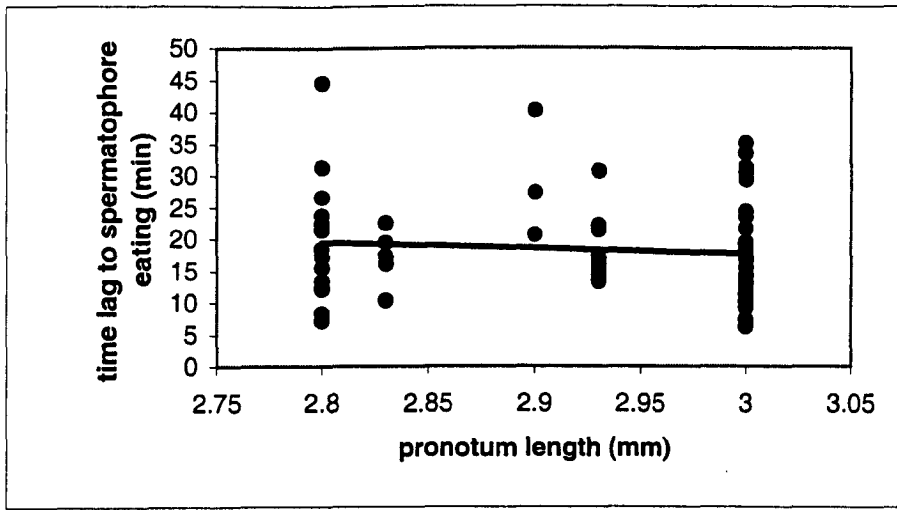
Figure 5.21 The relationship between female size and the time lag between the end of copulation and the onset of spermatophore consumption in the supplemented breeding group
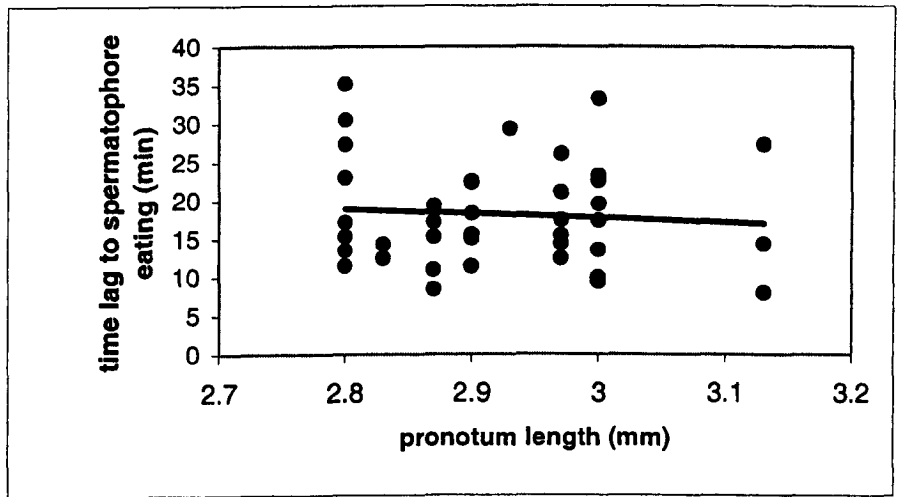
Figure 5.22 The relationship between female size and the time lag between the end of copulation and the onset of spermatophore eating in the unsupplemented breeding group
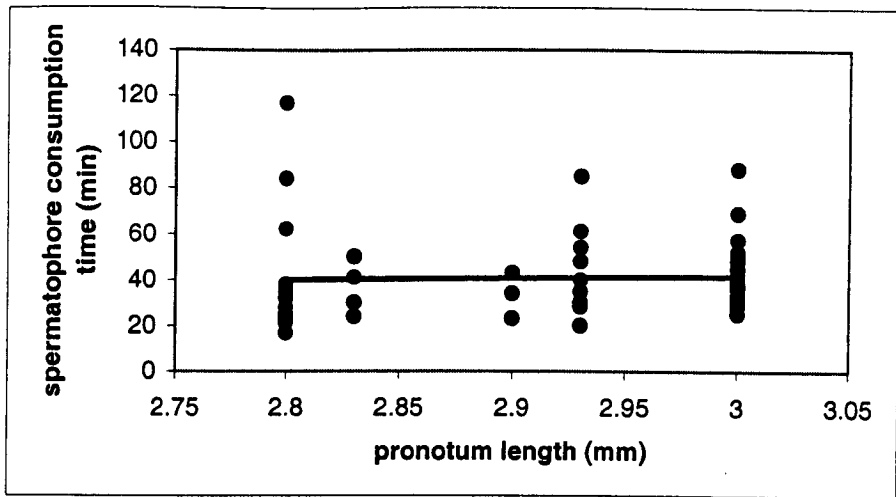
Figure 5.23 The relationship between female size and the time taken for the spermatophore to be consumed in the supplemented breeding group
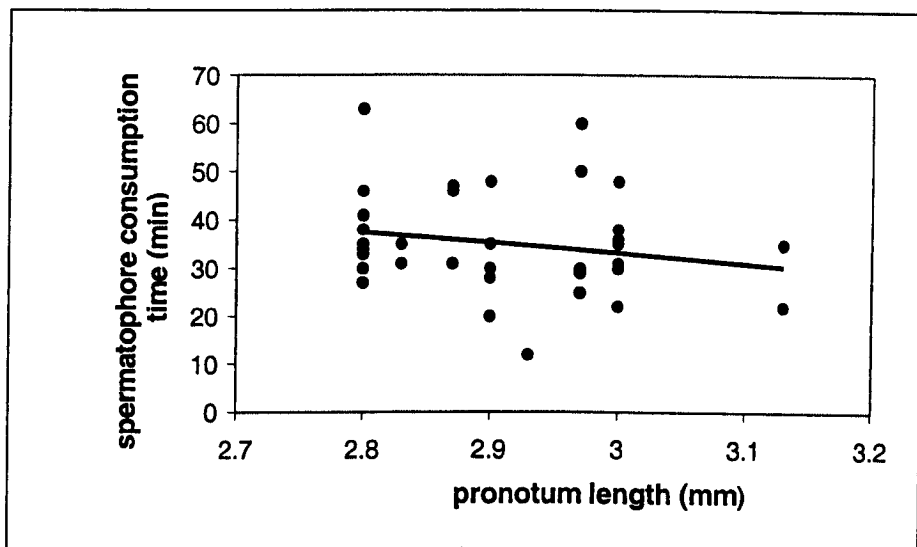
Figure 5.24 The relationship between female size and the time taken for the spermatophore to be consumed in the unsupplemented breeding group

5.3.5   Size and rejection as mates

There was no relationship between male size and the likelihood that they would be rejected as mates by females. The number of times a male was rejected as a proportion of his total mating attempts was not significantly correlated with his size in either the supplemented breeding group (Spearman rank correlation, r_s = -0.284, N = 9 p>0.05; Fig. 5.25) or the unsupplemented breeding group (r_s = -0.307, N = 8, p>0.05; Fig. 5.26).

138
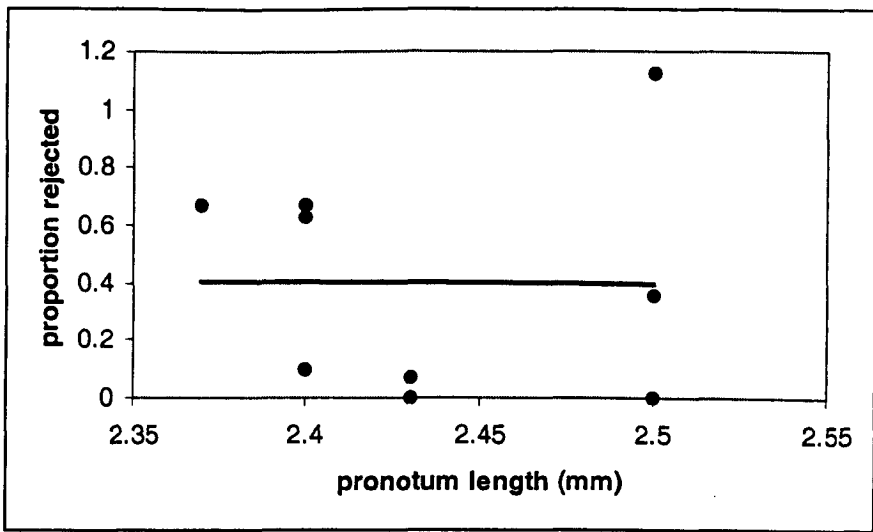
Figure 5.25 The relationship between male size and the proportion of times they were rejected as mates for males in the supplemented breeding group
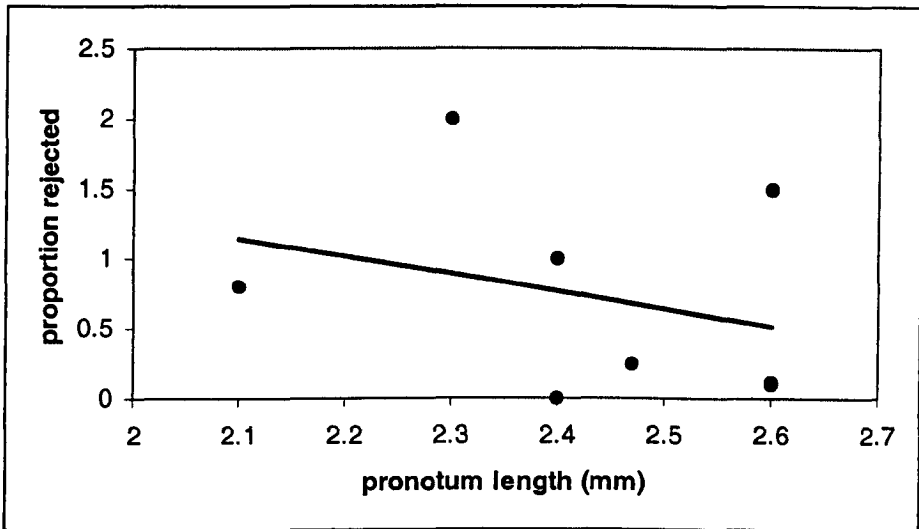
Figure 5.26 The relationship between male size and the proportion of times they were rejected as mates for males in the unsupplemented breeding group

There was no relationship between female size and the proportion of times they were rejected as a mates in the supplemented breeding group (Spearman rank correlation, r_s = -0.226, N = 10, p>0.05;Fig. 5.27) or the unsupplemented breeding group (r_s = -0.145, N = 10, p>0.05; Fig. 5.28).
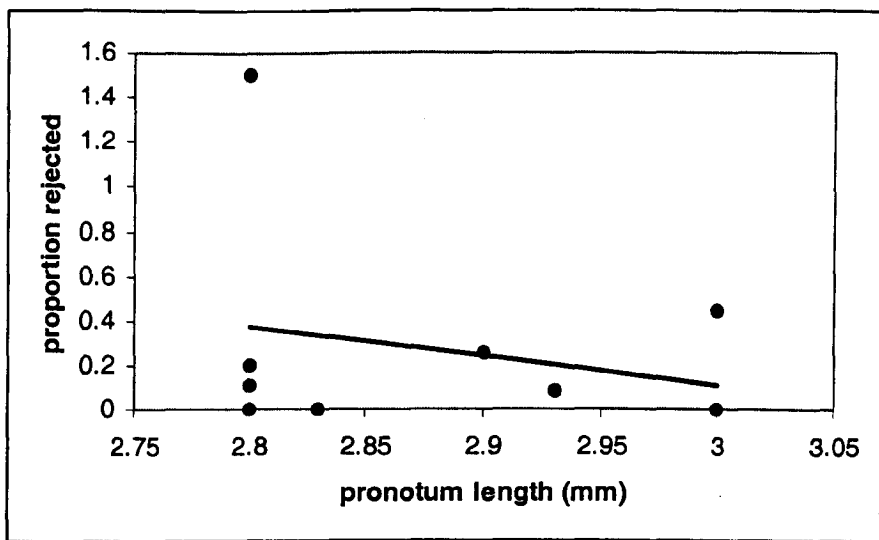
Figure 5.27 The relationship between female size and the proportion of times they were rejected as mates for females in the supplemented breeding group
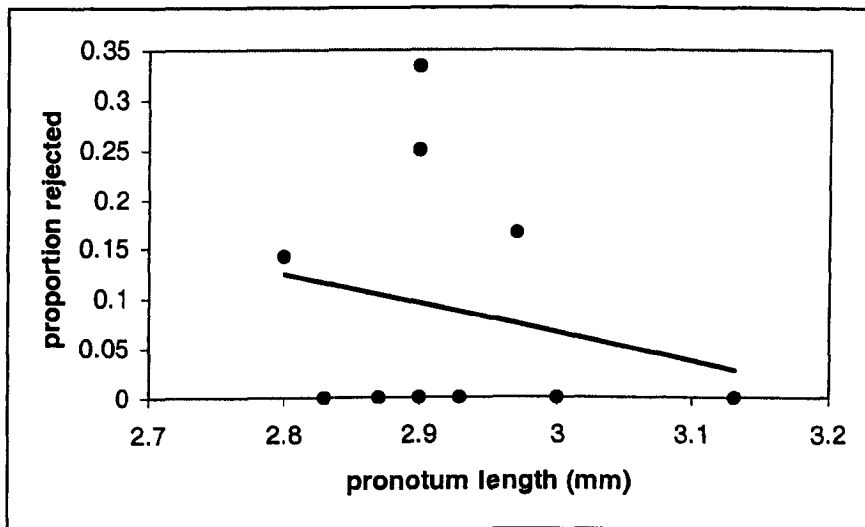
Figure 5.28 The relationship between female size and the proportion of times they were rejected as mates for females in the unsupplemented breeding group

5.3.6 Male size and satellite behaviour

On a total of 14 occasions, males were observed to achieve a mating without previously calling (defined as not calling for at least 30-min before copulation took place. There were 10 occasions in the supplemented breeding group, one in the unsupplemented breeding group and three in half and half breeding group. This 'satellite' behaviour was not related to male size: the total number of satellite matings achieved by the five smallest males in

the supplemented breeding group was not significantly different from that achieved by the
five largest males ($\chi2 = 0.09$, p>0.05)

5.3.7 Male size and pseudocopulatory behaviour

Pseudocopulatory behaviour was observed on a total of 32 occasions: 10 times in the
supplemented breeding group, 9 times in the unsupplemented breeding group and 13 times
in the half and half breeding group. There was no correlation between male size and the
performance of male-male mounting behaviour. The number of times the five smallest
males mounted another male was not significantly different from the number of times the
five largest males mounted another male, either in the supplemented group ($\chi^2 = 0$,
p>0.05), the unsupplemented group ($\chi^2 = 1.0$, p>0.05) or the half and half group ($\chi^2 =
0.38$, p>0.05). Nor was male size associated with being the recipient of a male-male
mount. The number of times the five largest males were mounted by another male was not
significantly different from the number of times the five smallest males were mounted,
either in the supplemented breeding group ($\chi^2 = 1.0$, p>0.05), the unsupplemented breeding
group ($\chi^2 = 1.0$, p>0.05),or the half and half breeding group ($\chi^2 = 0$, p>0.05).

5.3.8 Male size and spermatophore weight

Larger males produce larger spermatophores. There was a positive correlation between
male body size and mean spermatophore weight (r = 0.508, N = 53, p<0.001; Fig. 5.29).
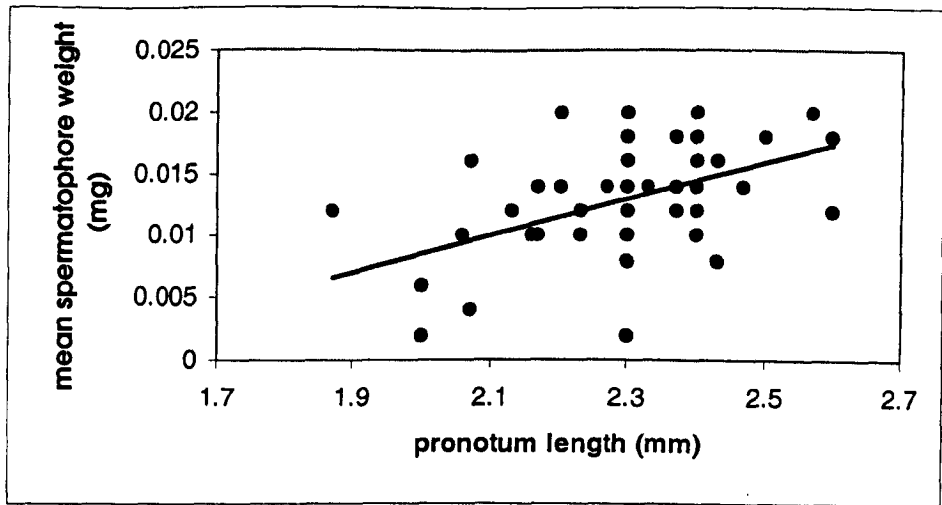
Figure 5.29 The relationship between male size and mean spermatophore weight

Spermatophore weight showed significant variation between matings and co-varied with male size (ANCOVA, mating frequency with male size as a covariate; male size: $F = 6.574$, $df = 1,97$, $p<0.05$; matings: $F = 6.192$, $df = 2, 97$, $p<0.05$). Tukey's HSD multiple pairwise post hoc comparisons indicated that the difference in spermatophore size between matings became apparent only after the second spermatophore was produced. The first and second spermatophores were of approximately equal size, but the third spermatophore was significantly smaller (Fig. 5.30)
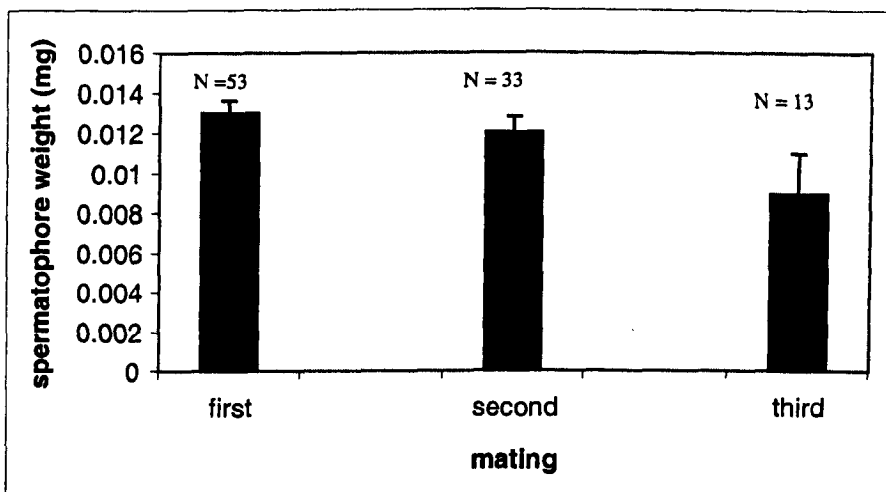
Figure 5.30 The effect of the number of previous matings on spermatophore size

There was no evidence that male size influenced the relative investment in spermatophores: larger males did not produce relatively larger spermatophores with respect to their body size (ANOVA, relative spermatophore weight (calculated as spermatophore weight as a percentage of body weight, arcsine transformed) with male size: F = 0.413, df = 1,97, p>0.05).

There was no association between spermatophore weight and the size of the female the male mated with (Fig. 5.31; r = 0.206, N = 29, p >0.05; data from singly mated females from the single versus multiple mating experiment).
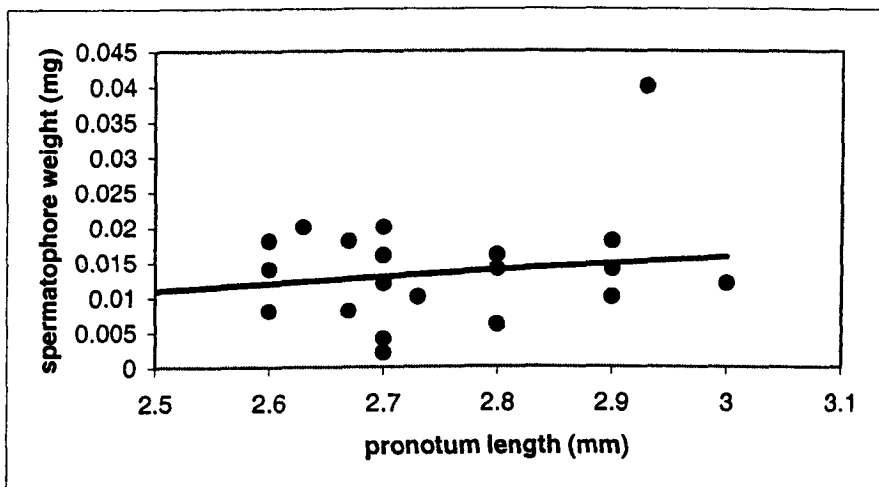
Figure 5.31 The relationship between weight of spermatophore and the size of the female the male mated with for singly mated females

5.3.9   Size, number of matings and female fecundity

Females varied enormously in their fecundity. One female laid no eggs at all and was excluded from further analyses of fecundity, but for those females that laid eggs the mean number laid was 38.5 and the range was 3-106. This variation was not related to the size of the females: there was no correlation between female size and the total number of eggs laid either among all females (r = 0.159, N = 80, p>0.05; analysis includes data for all females from the single versus multiple mating experiment and the breeding groups experiment) or among singly mated females (Fig. 5.33; r = -0.274, N = 29, p>0.05; analysis includes data

for all singly mated females from the single versus multiple mating experiment). There was a significant negative correlation between female body mass and fecundity (Fig. 5.32; $r = -0.361$ N = 74 p > 0.05, analysis includes data for all females from the breeding group experiments and the single versus multiple mating experiments). However, there was no correlation between female body weight and fecundity in singly mated females ($r = -0.319$, N = 22, p>0.05)
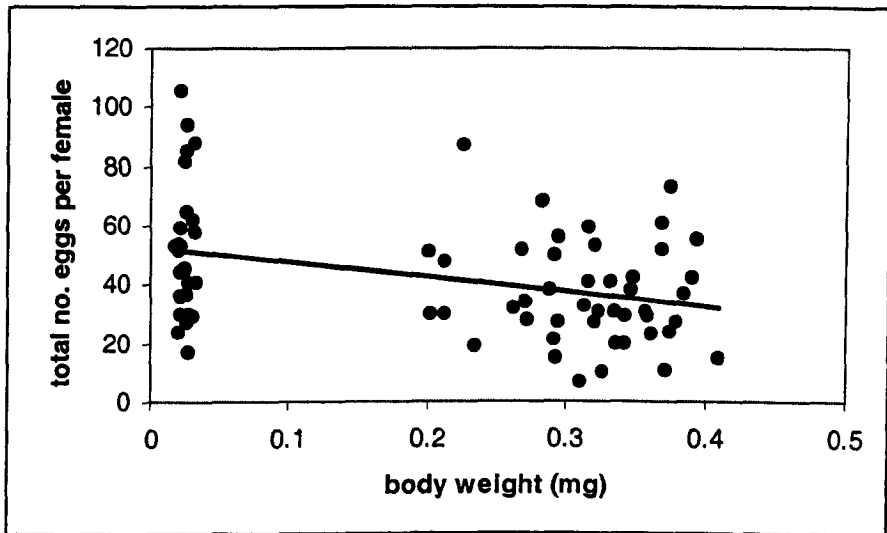
Figure 5.32 Female body weight and fecundity for all females
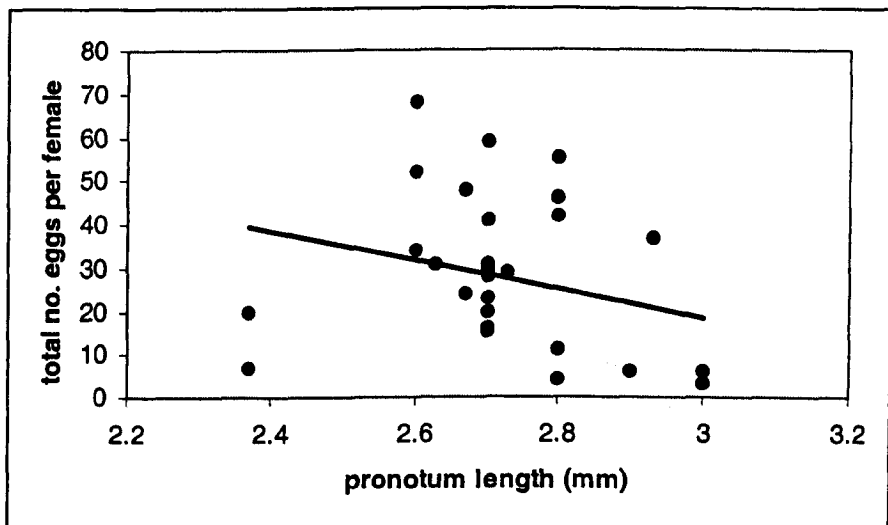
Figure 5.33 The effect of female body size on fecundity for singly mated females

The number of matings did, however, have a significant effect on fecundity, with females laying more eggs the more times they mated (Fig. 5.34; ANCOVA, mating frequency with

female size as a covariate, mating frequency: F = 6.782, df = 3,76, p<0.001; body size: F = .0398, df = 1,76, p>0.05). Tukey's HSD post-hoc multiple pairwise comparisons also revealed that there was a significant difference in fecundity between females that had mated once and those that had mated four or more times.
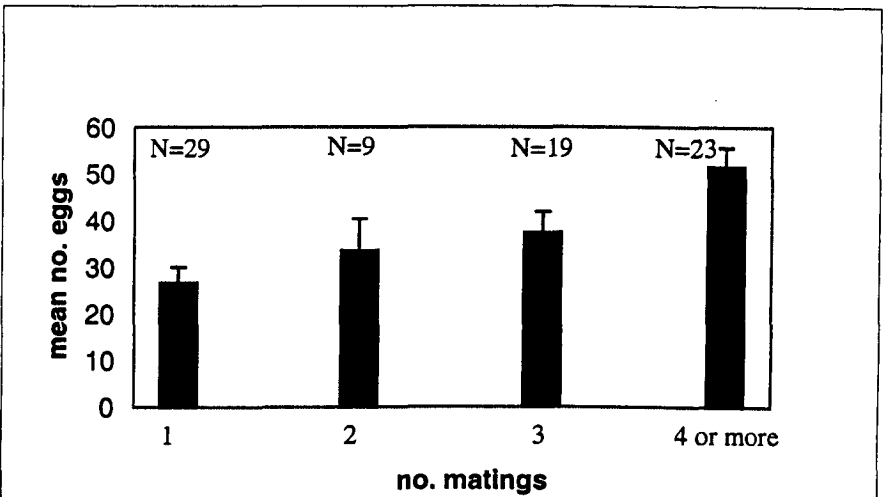
Figure 5.34 The mean number of eggs laid by females in relation to the number of times they mated

This increase in fecundity with number of matings was not related to the amount of spermatophore material a female consumed during her reproductive lifetime. Fecundity was not correlated with the total weight of spermatophores a female received (Fig. 5.35; r = 0.244, N = 52, p>0.05; data from single versus multiple mating experiment only).
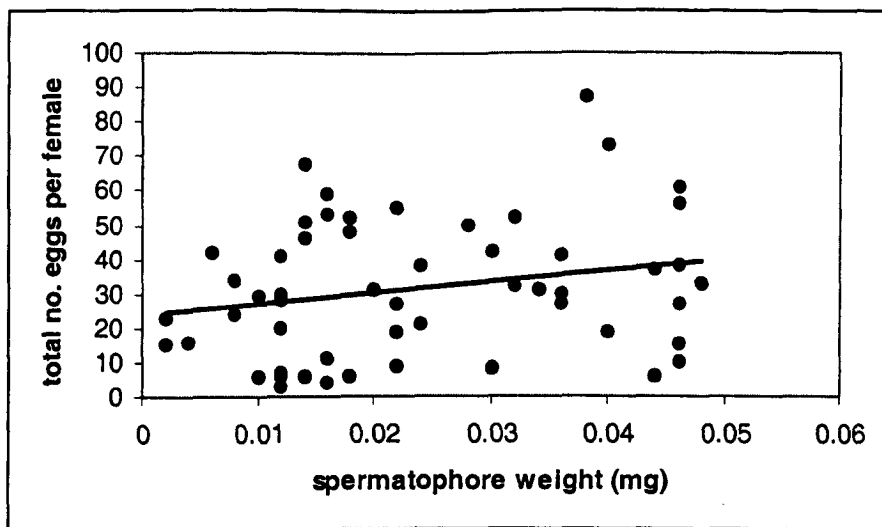
Figure 5.35 The effect of total amount of spermatophore material consumed on female fecundity

5.3.10 Female size, number of matings and egg weight and size

There was no effect of female size on egg weight but egg weight did differ between females mated singly and multiply, with singly mated females laying significantly heavier eggs. (Mean egg weight singly mated females = 0.025mg, SE = 0.001; mean egg weight multiply mated females = 0.021mg, SE = 0.0009) (ANCOVA, number of matings with size as a covariate; number of matings: F = 11.719, df = 1,43, p<0.05; body size: F = 1.977, df = 1,43, p>0.05; data from the single versus multiple mating experiments). This implies that the first batch of eggs a female lays (i.e. after her first mating) is heavier than later batches.

There was no effect of female size or number of matings on egg size. (Mean egg size singly mated females = 4.43mm, SE = 0.05; mean egg size multiply mated females = 4.51mm, SE = 0.04) (ANCOVA, number of matings with size as a covariate, number of matings: F = 0.578, df = 1,46, p>0.05; size: F = 0.411, df = 1,46, p>0.05; data from the single versus multiple mating experiments).

There was no significant effect of the amount of spermatophore material females consumed during their reproductive lifetime on the weight of the eggs they laid. (ANCOVA, number of matings with total spermatophore weight received as a covariate,

number of matings: F = 7.911, df = 1,43, p<0.05; spermatophore weight: F = 1.392, df = 1,43, p>0.05; data from single versus multiple mating experiments) or on the size of eggs they laid (number of matings: F = 1.026, df = 1,43, p>0.05; spermatophore weight: F = 0.103, df = 1,43, p>0.05; data from single versus multiple mating experiment).

5.3.11 Female size, number of matings and longevity

There was no relationship between female size and longevity, where longevity was defined as the number of days from emergence as an adult to death (Fig. 5.36; r = 0.210, N = 49, p>0.05; data from single versus multiple mating experiments).
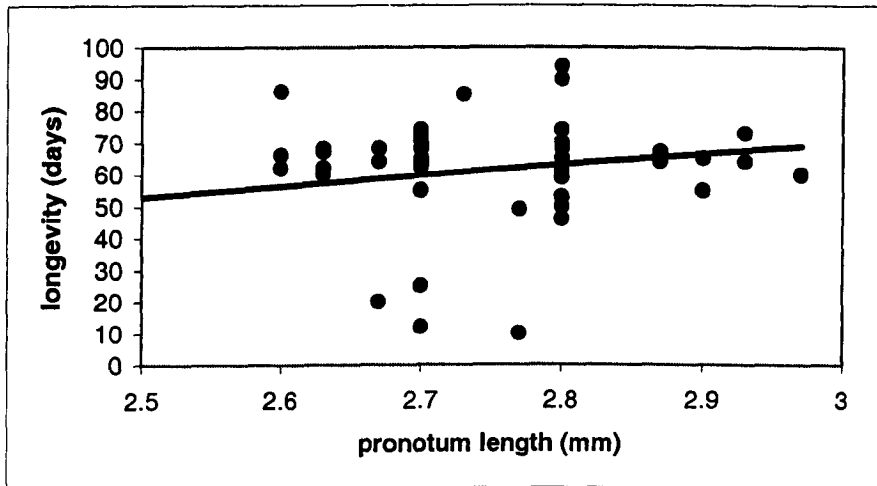
Figure 5.36 The relationship between body size and longevity in females

Nor was there any difference between singly mated females and multiply mated females in the length of time they lived (Fig. 5.37; t-test, t = -1.117, df = 47, p>0.05; data from single versus multiple mating experiments).
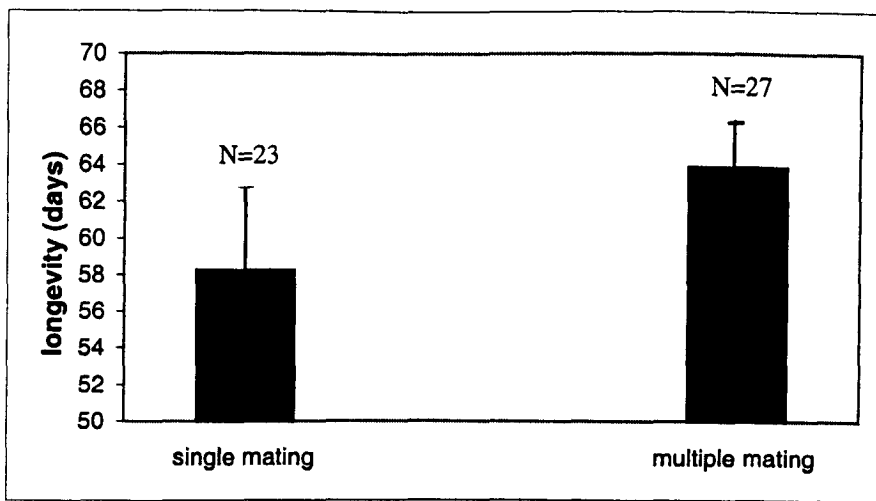
Figure 5.37 The longevity of singly mated females and multiply mated females

5.4 Discussion

Larger males get more matings than smaller males and also mate with more different partners, though this difference is only seen in the first half of the breeding season. The increased mating success of larger males could be a consequence of female choice for larger partners, greater success in male-male competition, a shorter refractory period between matings, or a combination of any or all of these factors.

My experiments did not test directly for female choice and further work is necessary to decide whether or not females do discriminate between mates on the basis of size.

It is possible that the effect is a consequence of male-male competition for access to the best calling sites, as discussed in Chapter 3: if larger males have greater success in these interactions, they may be able to improve their chances of being heard by females (Walker, 1983).

It could also be a consequence of females being willing to mate as often as they can but males being limited in how quickly they can produce spermatophores. Fecundity increases the more times females mate, therefore it might be expected that females will be highly

motivated to mate. There is no evidence that smaller males invest a relatively greater proportion of their body mass into the production of spermatophores as has been observed in other species (Sakaluk & Smith, 1988; Simmons, 1988b) but larger males may have more resources at their disposal than smaller males which may enable them to produce spermatophores more quickly. The fact that there is no apparent difference in the mating success of small and large males in the second half of the breeding season may be a consequence of larger males concentrating their reproductive effort into the early part of the season and 'running out' of resources later on. This is supported by the fact that the third spermatophore produced by a male is smaller than the first two.

One advantage to males of mating more often early in the season is an increase in the probability of mating with a virgin female. Calos & Sakaluk (1998) demonstrated a first male mating advantage in males of the decorated cricket (*Gryllodes sigillatus*). Males that mate with virgin females are more likely to have exclusive access to the batch of eggs laid in the first 24 hours following mating, because their sperm are not in competition with the sperm of other males within the spermathecae. The advantage to males would only be apparent if females fail to re-mate before laying their first batch of eggs. I have observed female *L. punctatissima* re-mating immediately after eating the spermatophore, however, the mating frequency of females may be elevated in captive conditions. Female *G. sigillatus* were shown to mate with a mean frequency of 2-2.5 times per 24 hour period when confined continuously with a male in the laboratory (Calos & Sakaluk, 1998), whereas under natural conditions they mate on average less than once in any 24 hour period (Sakaluk, Eggert & Sneddon, unpublished data). Although females were not continuously confined with males during the course of this study they were kept at densities which probably did not reflect those of natural populations. Observations of a natural population of *L. punctatissima* in Germany by M. Hall and D. Robinson indicate

that adults are relatively thinly distributed, consequently there may be less chance of a female re-mating before her first oviposition bout.

Males may accrue a further advantage from mating more often early in the season. Singly mated females in this study, although less fecund than multiply mated females overall, laid heavier eggs, which suggests that females lay heavier eggs in their early oviposition bouts. Increased egg size is often correlated with traits that affect early offspring fitness, particularly offspring size (Weigensberg *et al.*, 1998), which in turn is correlated with greater survival (Reinhold, 1999).

Males of the wartbiter *Decticus verucivorus* transfer larger spermatophores to virgin females and virgin females lay more eggs in their refractory period than females that have mated previously (Wedell, 1992). Whether male *L. punctatissima* can tailor their spermatophores to reflect the mating status of females was not tested directly in this study. All males mated with a virgin female on their first mating, so whether the first spermatophore is large because males have more resources at their disposal or because the female is virgin is not clear. However, there is no difference in size between the first and second spermatophores a male produces, which suggests the former explanation is more likely to be true. It seems likely therefore, that males produce their largest spermatophores early on, and that the change in size is a result of a depletion of resources.

If males are limited in how quickly they can produce spermatophores, then pseudocopulatory behaviour could be a useful strategy for any male to pursue, and this may be the reason why the behaviour is not related to size. Stimulating the process of spermatophore production in another male effectively removes them as a competitor until they are ready to produce another spermatophore.

Similarly, interrupting a mating couple could be a useful strategy for any male to pursue: it is not necessary to separate the mating pair completely but simply interfere with spermatophore transfer. If the spermatophore fails to attach properly as a result of the interference, then the interferer may be able to mate with the female himself. Even if he does not, the other male will be excluded from competition for mates until he can produce another spermatophore. I only observed interference on six occasions, and no male managed to take over the female and mate with her himself. The data were too few to analyse statistically but it is possible that larger males could have a better chance of interfering successfully. Such a strategy has been shown in the yellow dung fly *Scatophaga stercoraria* (Parker, 1970; Borgia, 1979).

If males try to choose more fecund females, it might be expected that they would prefer heavier females, since body weight is likely to be related to the number of eggs the female is carrying. There was no relationship, however, between a female's body weight and the number of times she mated and, strangely, there was a negative relationship between female body weight and fecundity. These results may have been a consequence of the problems inherent in measuring body weight accurately in such small animals (Section 4.2.1).

In the supplemented breeding group, larger females mated more often than smaller females, whereas in the unsupplemented breeding group, there was no relationship between size and number of matings obtained. There may be several reasons why larger females get more matings than smaller ones. First, larger females may enjoy greater success in intrasexual competition than smaller females. Second, larger females may have a greater motivation to mate, perhaps because they require more spermatophore material to achieve full fecundity than smaller females. Finally, there may be male mate choice for larger females.

As discussed in chapter 3, I never observed any instances of female-female competition in *L. punctatissima,* so it seems unlikely that this would explain the observed difference in mating success.

It also seems unlikely that larger females in general are more highly motivated to mate than smaller females, since there was no difference in the number of matings between large and small females in the unsupplemented breeding group. It will be shown in Chapter 6 that unsupplemented females mate more often than supplemented ones, possibly as a result of increased motivation to mate. One possibililty is that smaller unsupplemented females might suffer more than larger unsupplemented females as a result of their poor diet, so that their motivation to mate increases even more relative to larger females, cancelling out the effects of larger size on motivation as seen in the supplemented breeding group.

If there is male mate choice for larger females, however, it is strange that it was only observed in the supplemented breeding group. It is possible that this may have been a consequence of supplemented males having more resources at their disposal than unsupplemented males. As already discussed, this might enable them to produce spermatophores at a greater rate, thus giving them the 'luxury' of choice - they can afford to be choosy about their mates 'knowing' that they will soon be able to mate again, whereas unsupplemented males are less discriminating because it could be several days before they can mate again. There is some evidence against male mate choice of larger females in that, although both supplemented and unsupplemented males do occasionally reject a female, this is not related to her size.

It is not possible, on the basis of the data I have, to determine whether larger supplemented females mate more because they are more highly motivated, or because they are chosen by males, or a combination of the two.

There was no evidence evidence that individuals mate assortatively. Assortative mating with respect to body size is a feature of the mating system of many species of arthropods (Crespi, 1989). Assortative mating should be apparent if reproductive success increases as a function of size; under these conditions mutual mate choice for larger partners may maximize the reproductive fitness of both sexes. In this study there was no evidence that fecundity increases with female size, nor is fecundity influenced by the amount of spermatophore material females receive during their reproductive lifetime. There is therefore no advantage to males in mating with larger females or females with larger males.

A female's fecundity is mainly dependent on the number of matings she achieves. This relationship is not due to the weight of spermatophore material she consumes, which has no effect on her fecundity, egg size or weight. A similar situation exists in the congeneric bushcricket *Leptophyes laticauda*. In this species nuptial feeding has no effect on either female fecundity or egg weight (Vahed & Gilbert, 1996b; Vahed, 1998). Females denied any spermatophylax material do not appear to be compromised in their reproductive output.

The increase in fecundity that results from multiple mating may be due to the mechanical act of mating: copulation has been shown to stimulate oviposition in several insect species (Wigglesworth, 1972). Or it might result from an increase in oviposition stimulants which accumulate as females mate more often (Ridley, 1988). The observation that singly mated females laid significantly heavier eggs than multiply mated females would appear to discount any cumulatively beneficial effects of spermatophore consumption on female reproductive output.

Repeated mating by females is often necessary to achieve full fecundity in polyandrous insects (Ridley, 1988). It is possible that singly mated females are less fecund than multiply mated females because the former do not have enough sperm to fertilise all their eggs. Vahed (1995), showed that double mating increases fecundity in *L. punctatissima* but not in *L. laticauda*. Although, overall, the lifetime fecundities of the females are not different, male *L. laticauda* transfer around six times more sperm, relative to male body weight, than *L. punctatissima* (Vahed, 1995). In this study the steady increase in fecundity with additional matings may be a consequence of the accumulation of sperm. If there were advantages to multiple mating over and above increasing fecundity then one would expect multiply mated females to lay better quality eggs. For example, an increase in egg size as a consequence of additional matings has been demonstrated in the beetle *Callosobruchus maculatus* (Fox, 1993a). In this study, however, the reverse is true, with females laying heavier eggs early in the breeding season.

There was no evidence that post-copulatory female choice was operating in any of the three breeding groups, at least not in terms of the variables measured in this study. Females did not always begin spermatophore consumption immediately following copulation, but the time lag was not correlated with the size of the male the female had mated with. However, it is possible that there may be some criteria other than male size by which females exercise this form of cryptic choice.

Reproductive success in females also depends on their longevity since multiply mated females continue to lay eggs until they die. However, longevity is not related to size or to the number of matings a female has.

5.4.1 Summary

Larger males mate more often than smaller males although the difference is only evident in the first half of the season. This difference may be a consequence of female choice for larger males, the greater success of larger males in male-male competition or because larger males are less constrained in their ability to produce spermatophores. Larger supplemented females mate more than smaller supplemented females. This may be the result of male choice for larger females or greater motivation to mate in larger females. However, no such difference was observed in the unsupplemented breeding group. There is no evidence for assortative mating in relation to size. Female reproductive success (fecundity, egg size or egg weight) is not associated with female body size. Number of matings is an important fitness component for females since fecundity increases with additional matings. Larger males produce larger spermatophores, but the amount of spermatophore material females receive does not affect their reproductive success. Females lay heavier eggs in their early batches and this is one possible reason why males might invest more in mating (mating more often and producing bigger spermatophores) in the first half of the breeding season. There is no evidence for cryptic female choice nor does size have any affect on female longevity.

6 Effects of diet on mate choice and reproductive success

6.1 Introduction

Sexual selection theory predicts that the relative investment in offspring will largely dictate the direction of competition for access to members of the opposite sex (Trivers, 1972). On a gamete for gamete basis males invest proportionally less in reproduction than females, hence, males are usually the competitive sex and females are discriminative (Trivers, 1972). However sperm are rarely if ever transmitted individually rather they are packaged as ejaculates, complex parcels each of which contains millions of sperm and a variety of accessory material (Dewsbury, 1982). In bushcrickets sperm are transferred via spermatophores (Boldyrev, 1915), large gelatinous structures which are expensive to manufacture, consequently males incur non-trivial costs in their production (Dewsbury, 1982). Emlen & Oring (1977) predicted that ecological factors could influence the intensity of sexual selection. In conditions in which female reproduction is limited by male availability such as increased male parental investment, the operational sex ratio (the ratio of fertilizable females to sexually active males) will be skewed in favour of females (Emlen & Oring, 1977). Under these conditions the normally observed sex roles of choosy females and competitive males may be reversed. Gwynne (1985, 1993) proposed that sex role reversal in the bushcrickets *Anabrus simplex* and *Metaballus litus* was a consequence of resource availability within the habitat. When resources were scarce males were limited in the number of spermatophores they could produce which placed a limit on female reproduction. In habitats where resources were more freely available male and female sexual behaviour reverted to the more conventional pattern. The plasticity in sexual behaviour in response to prevailing ecological conditions has been noted in another species of bushcricket *Kawanaphilia nartee* (Simmons & Bailey, 1990). In this species role

reversal was evident only in the early part of the season when pollen, the insects' exclusive food source, was of a low quality; later in the season, when animals had access to pollen with a higher nutritive value, role reversal was absent (Simmons & Bailey, 1990). The facultative nature of this behaviour indicates that females were foraging for additional spermatophores to supplement their diet. In this species consumption of spermatophores increases the fecundity of females when food resources are limited, hence, male parental investment increases under conditions of nutrient stress with an associated reversal in the normally observed sex roles (Simmons, 1990; Simmons & Bailey, 1990). Under conditions of nutrient limitation females are subject to increased sexual selection over short time scales. This increase in selection pressure on females is brought about not only by a shortage of receptive males but also by an increase in female-female competition and mating frequency as they forage for additional nuptial meals (Gwynne & Simmons, 1990).

This chapter examines the effect of diet on both mate choice and reproductive success. It will seek to answer the answer the following questions. Does diet influence mate choice and reproductive success in males or females? Do females on a better quality diet lay more or better quality eggs and do supplemented males produce larger spermatophores? Does the diet of the male affect the length of time he needs to recover between matings or the reproductive success of the female he mates with? Do males produce larger spermatophores if they mate with a supplemented female? And finally, does diet affect reproductive success by affecting longevity?

6.2 Results

6.2.1 Diet and mating success

The total number of matings observed in the supplemented breeding group was 72, in the half and half group 54 and in the unsupplemented group 44. These differences are significant ($\chi^2 = 7.1$, df $= 2$, p <0.05).

Within the half and half group (Table 6.1), unsupplemented females mated more often than supplemented females ($\chi^2 = 4.74$, p<0.05), whereas unsupplemented males mated less often than supplemented males ($\chi^2 = 4.74$, p<0.05).

Table 6.1 The distribution of matings with respect to diet in the half and half group

	S males	US males	Totals
Supp females	11	8	19
Unsupp females	24	11	35
Totals	35	19	54

There was no significant difference between supplemented males and unsupplemented males in the number of different partners they mated with (Mann-Whitney U test, $U = 3.5$, p >0.05), nor was diet a factor in the number of different partners females mated with ($U = 3.5$, p>0.05).

6.2.2 Assortative mating with respect to diet

There was no assortative mating with respect to diet. Given that supplemented males mated more often than unsupplemented males and unsupplemented females mated more often than supplemented ones, both males and females mated randomly with respect to the diet of their partner (Table 6.1; $\chi^2 = 0.61$, df $=1$, p>0.05).

6.2.3 Diet and mating behaviour

Mean copulation duration was 3.66 min (SE = 0.11, N =37) for supplemented males and 2.67 min (SE = 0.17, N = 18) for unsupplemented males in the half and half breeding group. This difference in copulation duration between supplemented and unsupplemented males was significant, with supplemented males spending longer copulating than unsupplemented males. (two-way ANOVA diet with number of previous matings, diet: F = 4.773, df = 1,37, p<0.05). There was no effect of the number of previous matings on the length of copulation (F = 1.425, df = 11,37, p>0.05), nor was there any interaction between diet and number of previous matings (F = 1.553, df = 5,37, p>0.05).

Copulation duration did not differ significantly between supplemented and unsupplemented females in the half and half breeding group. Mean copulation duration was 2.84 min (SE = 0.13, N = 19) for supplemented females and 3.19 min (SE = 0.12, N= 35) for unsupplemented females, nor did it vary across matings (two-way ANOVA, diet with number of previous matings diet: F = 0.269, df = 1,36, p>0.05; number of previous matings: F = 0.423, df = 11,36, p>0.05; diet*number of previous matings: F = 0.258, df = 5,36, p>0.05).

There was no difference between supplemented and unsupplemented males in the half and half breeding group in the time taken for the females they mated with to eat the spermatophore. Mean spermatophore consumption time was 40 min (SE = 1.89, N = 34) for supplemented males, and 34.69 min (SE = 3.29, N = 16) for unsupplemented males). Nor did the time taken to consume the spermatophore change across matings (two-way ANOVA, diet with number of previous matings; diet: F = 0.002, df = 1,32, p>0.05; matings: F= 0.911, df = 11,32, p>0.05; diet*number of previous matings: F = 1.260, df = 5,32, p>0.05). The time interval between the end of copulation and the onset of spermatophore consumption by the female that a male mated with, was not related to the

diet of the male, nor were there any differences in this time lag across matings. The mean time lag was 21.51min (SE = 1.55, N =34) for supplemented males and 18.13 min (SE = 1.37, N = 16) for unsupplemented males (two-way ANOVA, diet with number of previous matings; diet: F = 2.247, df = 1,35, p>0.05; number of previous matings F = 0.293 df = 11,35 p>0.05; interaction diet*number of previous matings: F = 0.857, df = 5,35 p>0.05). There was no difference between supplemented and unsupplemented males in the total length of time the spermatophore was attached to the female. Mean attachment time was 55.02 min (SE = 2.68, N = 36) for supplemented males and 51.00 min (SE = 3.64, N = 17) for unsupplemented males. Nor were there any differences in attachment time across matings (two-way ANOVA, diet with number of previous matings; diet: F = 0.335, df = 1,35 p, >0.05; number of previous matings; F = 0.955 df = 11,35, p >0.05; interaction term diet*number of previous matings: F = 0.856 df = 5,35 p>0.05).

There was no difference between supplemented and unsupplemented females in the half and half breeding group in the length of time taken to eat the spermatophore. Mean spermatophore consumption time was 35.40 min (SE = 2.33, N = 17) for supplemented females and 40.25 min (SE = 2.26, N = 2.26) for unsupplemented females. Nor did the time taken to eat the spermatophore vary across matings (two-way ANOVA, matings with diet; diet: F = 0.253, df = 1,32, p>0.05; number of previous matings: F = 1.078, df = 11,32, p>0.05; diet*number of previous matings F = 0.218, df = 4,32, p>0.05).

There was no effect of female diet on the time interval from the end of copulation until the female began to consume the spermatophore. The mean time lag was 19.75 min (SE = 1.84, N = 17) for supplemented females and 20.96 min (SE = 1.53, N = 32) for unsupplemented females. Nor did this time lag vary across matings (two-way ANOVA, diet with matings; diet: F = 0.587, df = 1,34, p>0.05; number of previous matings: F = 0.940, df = 11,34, p>0.05; diet*number of previous matings: F = 0.440, df = 5,34, p>0.05).

There was no difference between supplemented and unsupplemented females in the total time the spermatophore was attached following copulation. Mean attachment time for supplemented females was 50.00 min (SE = 3.72, N = 19) for supplemented females and 54.9 min (SE = 3.72, N = 36) for unsupplemented females. Nor did total attachment time vary across matings (two-way ANOVA, diet with matings; diet F = 1.966, df = 1,37, p>0.05; number of matings F = 0.863, df = 11,37, p >0.05; interaction term; diet*matings F = 0.891, df = 5,37, p>0.05).

6.2.4 Diet and rejection as mates

There was no evidence that males or females were rejected as mates on the basis of their diet. There were 16 rejections by females in the half and half group, which constituted 23% of attempted matings by males (Table 6.2), but there was no difference in rejection rates between supplemented and unsupplemented males (χ^2 = 0.3, df = 1, p>0.05). Only five rejections by males of females were recorded in the half and half group but these were evenly split between supplemented females (2 instances) and unsupplemented females (3 instances). There was no significant difference between the supplemented breeding group and the unsupplemented breeding group in the proportion of mating attempts that were rejected, either by males (Mann-Whitney U test: U = 25.5, p >0.05), or by females (U = 39.5, p >0.05).

Table 6.2 Rejections of males as mates by females in the half and half breeding group

	S	US males	Totals
Number of matings	35	19	54
Rejected by female	9	7	16
Proportion of attempted matings rejected	0.21	0.27	0.23

Table 6.3 Rejections of females as mates by males in the half and half breeding group

	S	US males	Totals
Number of matings	19	35	54
Rejected by male	2	3	5
Proportion of attempted matings rejected	0.10	0.079	0.085

6.2.5 Diet and calling behaviour

There was a significant difference in male calling activity between the supplemented and unsupplemented breeding groups, with a greater number of males calling on average in each group session (see Section 2.11) in the unsupplemented breeding group compared with the equivalent group session in the supplemented group (Figure 6.1, Mann-Whitney U test: $U = 92$, $p<0.05$). There was also a significant difference between supplemented and unsupplemented males within the half and half group, with more unsupplemented males calling on average in each group session (Figure 6.2, $U = 54$, $p<0.05$).
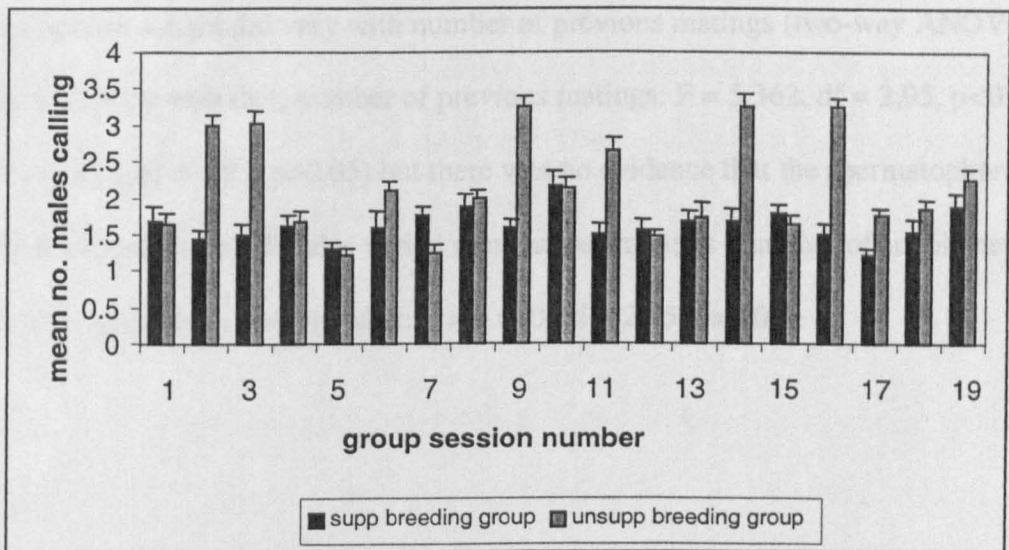
Figure 6.1 The mean number of males calling in each group session in the supplemented and unsupplemented breeding groups

162
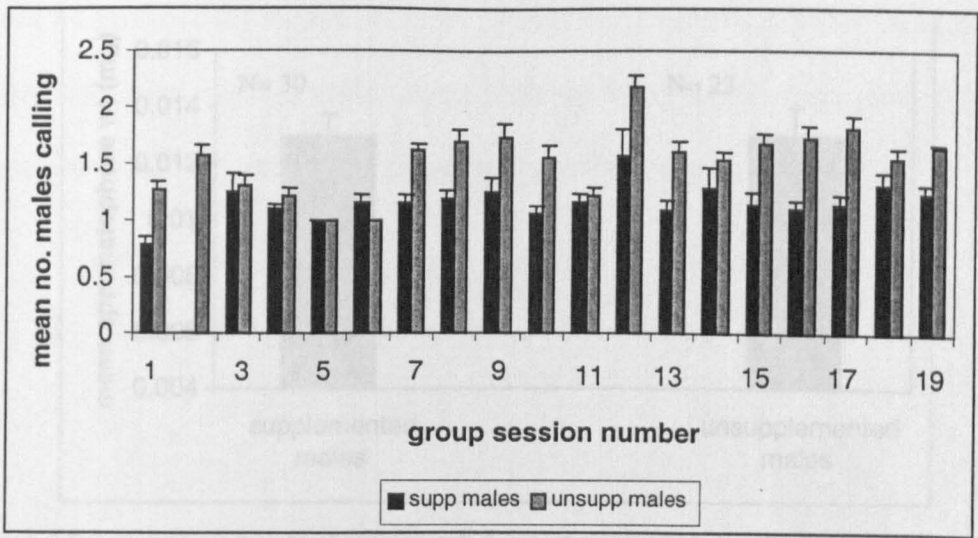
Figure 6.2 The mean number of supplemented and unsupplemented males calling in each group session in the half and half breeding group

6.2.6 Male diet and spermatophore weight

There was no difference in spermatophore weight between supplemented and unsupplemented males from the single versus multiple mating experiment (Figure 6.3). Spermatophore weight did vary with number of previous matings (two-way ANOVA, mating frequency with diet; number of previous matings: $F = 5.362$, df $= 2,95$, $p<0.05$; diet: $F = 0.810$, df $= 1,95$, $p>0.05$) but there was no evidence that the spermatophore weight of unsupplemented males varied more across matings than that of supplemented males (interaction term matings*diet: $F = 0.610$, df $= 2,95$, $p>0.05$).
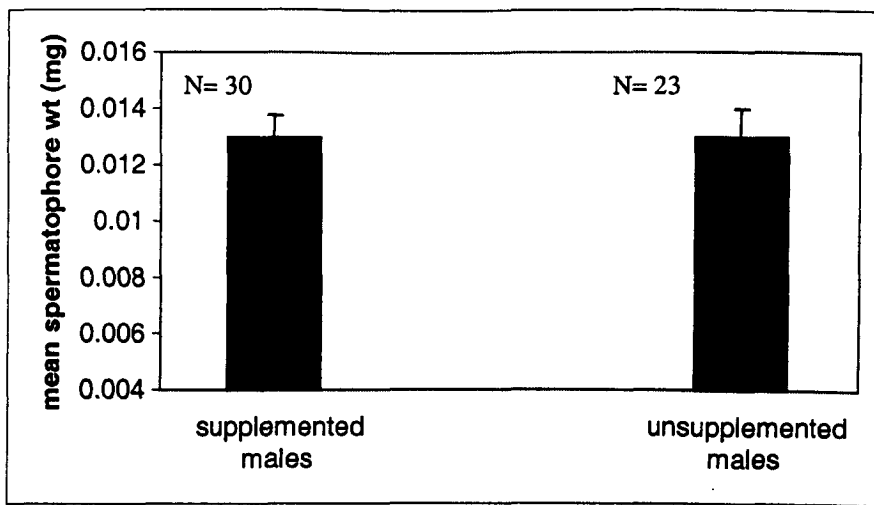
Figure 6.3 Spermatophore weight of supplemented and unsupplemented males from the single versus multiple mating experiment

There was no difference between supplemented and unsupplemented males in relative spermatophore weight, i.e. the weight of the spermatophore as a proportion of body weight (arcsine transformed). Mean relative spermatophore weight was 0.07 (SE = 0.003, N = 56) for supplemented males and 0.06 (SE = 0.004, N = 45) for unsupplemented males. But relative investment in spermatophores declined across matings (two-way ANOVA, number of previous matings with diet; diet: F = 2.958, df = 1,95, p>0.05; number of previous matings: F = 8.104, df = 2,95, p<0.05). There was no evidence that relative spermatophore weight of unsupplemented males varied more across matings than that of supplemented males (interaction term, mating*diet: F = 1.797, df = 2,95, p>0.05).

6.2.7 Diet and female fecundity

Since female fecundity is related to number of matings (Chapter 5), the effect of diet on fecundity was analysed for singly and multiply mated females separately, in each case using data from the breeding group experiment and the single versus multiple mating group experiment combined.

There was a significant difference in fecundity between supplemented and unsupplemented singly mated females, with unsupplemented females laying significantly more eggs than supplemented females (t-test, t = -2.500, df = 27, p<0.05; Fig. 6.4).
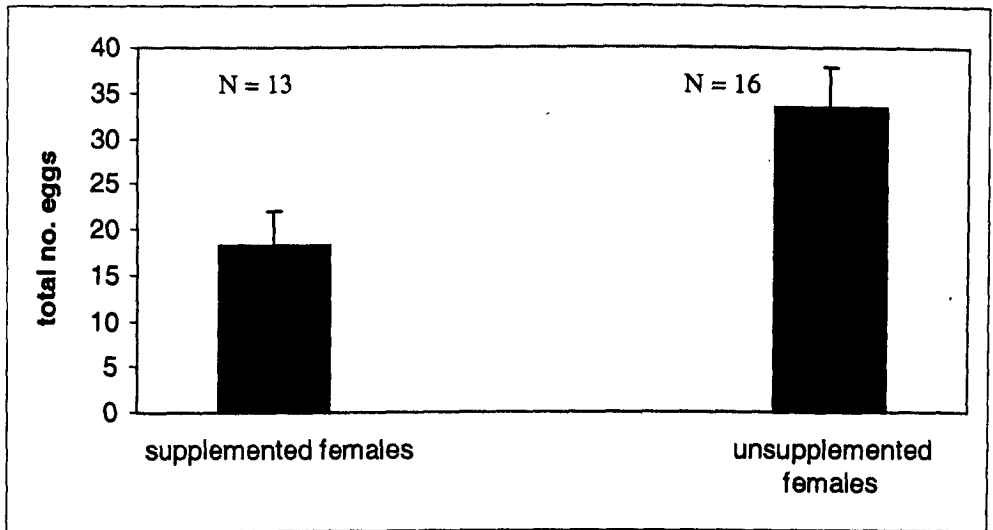
Figure 6.4 The fecundity of supplemented and unsupplemented singly mated females

There was no difference in fecundity between supplemented and unsupplemented multiply mated females (t-test, t = 1.536, df = 56, p>0.05; Fig.6.5).
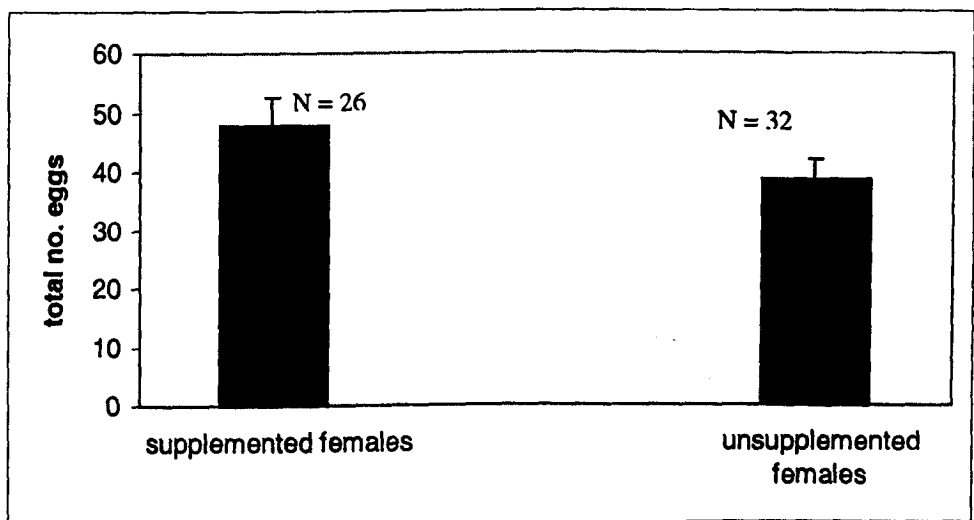
Figure 6.5 The fecundity of supplemented and unsupplemented multiply mated females

6.2.8   Diet and egg weight and size

The effects of female diet on both the size and weight of eggs were analysed separately for singly and multiply mated females, using data from the single versus multiple mating experiment. There was no significant difference between supplemented and unsupplemented singly-mated females, either in the weight of eggs (t-test, t =-0.700, df = 19, p >0.05; Fig. 6.6), or the size of eggs (t = -0.396, df = 19, p>0.05; Fig. 6.7), though there was a trend for unsupplemented females to lay larger, heavier eggs.
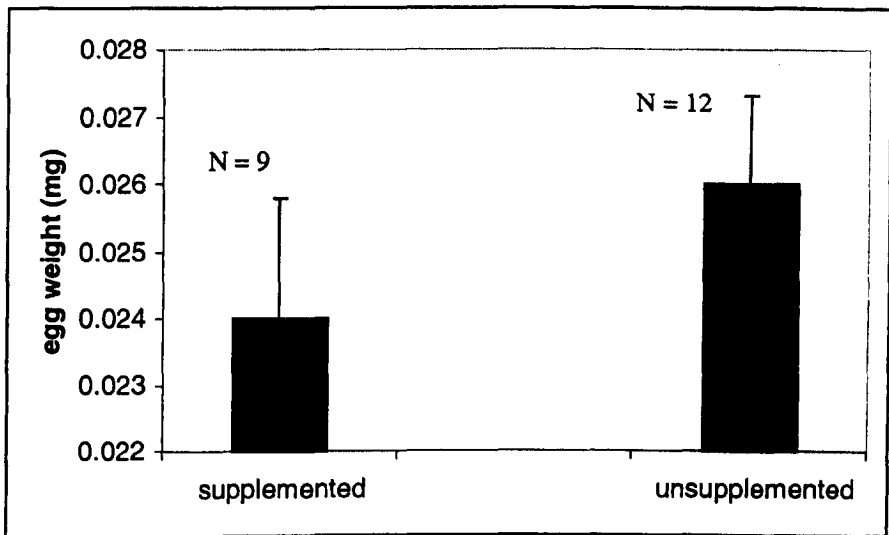
Figure 6.6 Egg weight for supplemented and unsupplemented singly mated females
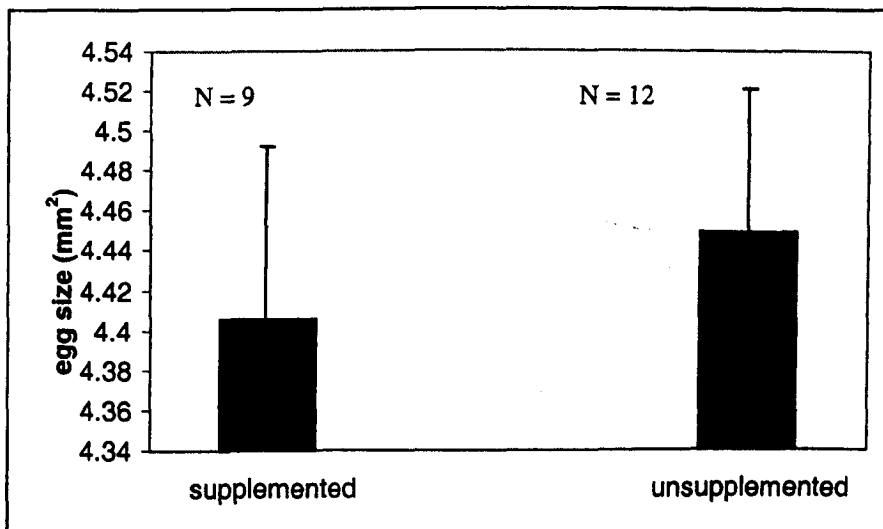
Figure 6.7 Egg size for supplemented and unsupplemented singly mated females

There was no significant difference between supplemented and unsupplemented multiply mated females either in egg weight (t-test, t = -1.310, df = 23, p>0.05; Fig. 6.8) or egg size (t = -1.710, df = 23, p>0.05; Fig. 6.9), though again unsupplemented females tended to lay larger, heavier eggs.
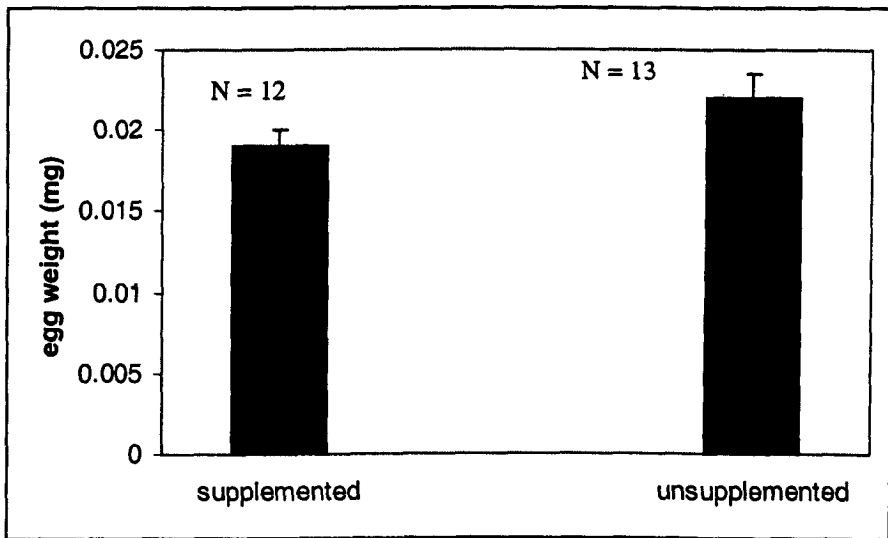
Figure 6.8 Egg weight for supplemented and unsupplemented multiply mated females
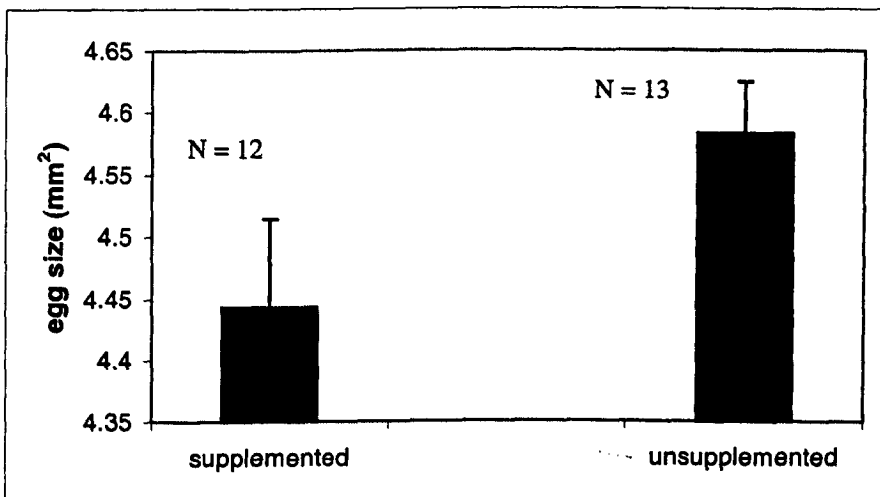
Figure 6.9 Egg size for supplemented and unsupplemented multiply mated females

6.2.9 Spermatophore weight received and fecundity

The relationship between the weight of spermatophore received and the fecundity of the female was investigated for supplemented and unsupplemented females separately, using

data from the single versus multiple mating experiment. There was a significant negative relationship between spermatophore weight and fecundity for supplemented singly mated females (Spearman rank correlation, $r_s = -0.585$, N = 13, p<0.05; Fig. 6.10). Fecundity was not significantly correlated with spermatophore weight in unsupplemented singly mated females ($r_s = 0.414$, N = 16, p>0.05; Fig. 6.11).
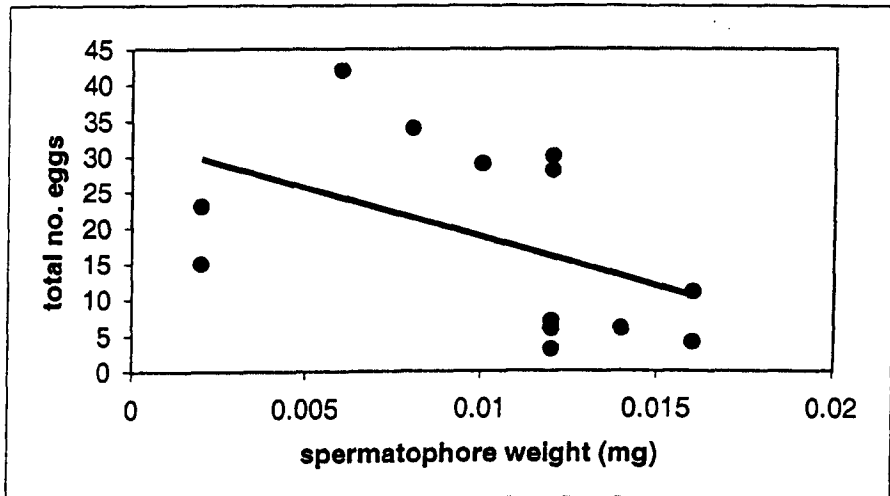
Figure 6.10 The relationship between spermatophore weight received and fecundity for singly mated supplemented females
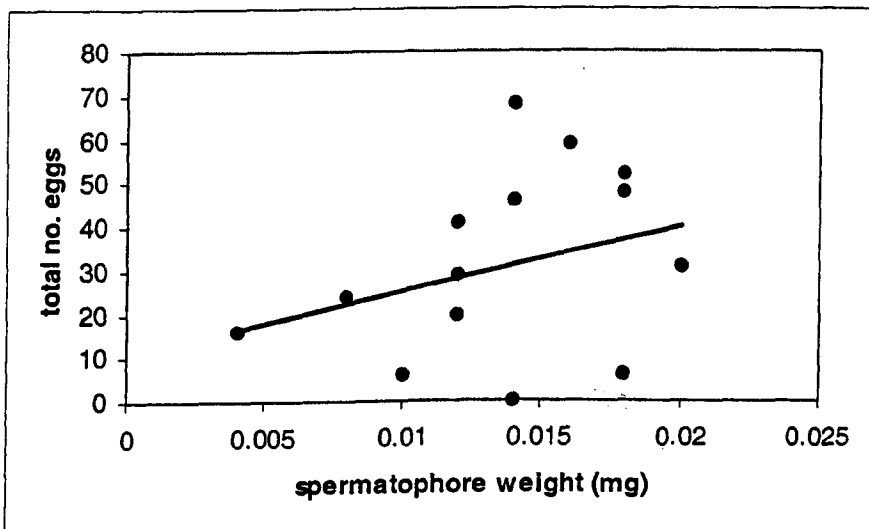
Figure 6.11 The relationship between spermatophore weight received and fecundity for singly mated unsupplemented females

There was no correlation between fecundity and the total weight of spermatophore material received for multiply mated females, either for supplemented ($r_s = -0.028$, N = 14, p>0.05; Fig. 6.12) or unsupplemented females ($r_s = -0.079$, N = 15, p>0.05; Fig. 6.13).
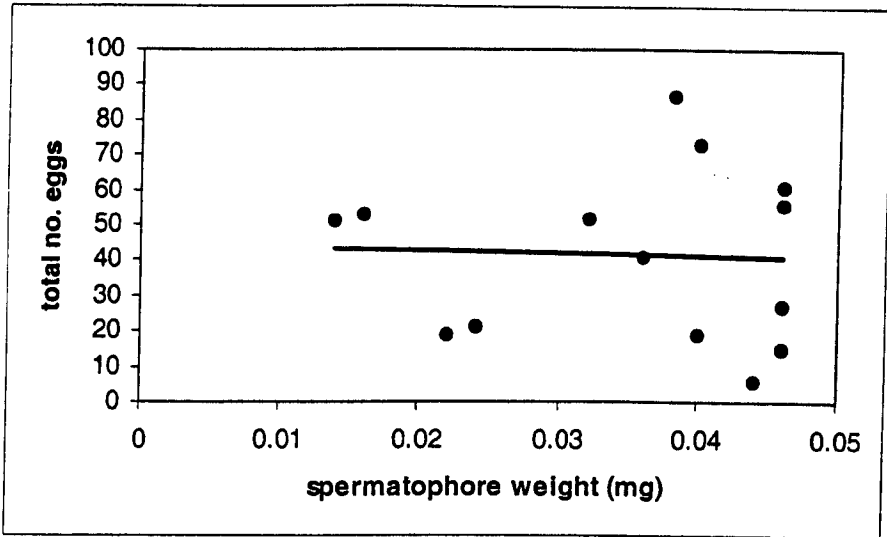
Figure 6.12 The relationship between spermatophore weight received and fecundity for multiply mated supplemented females
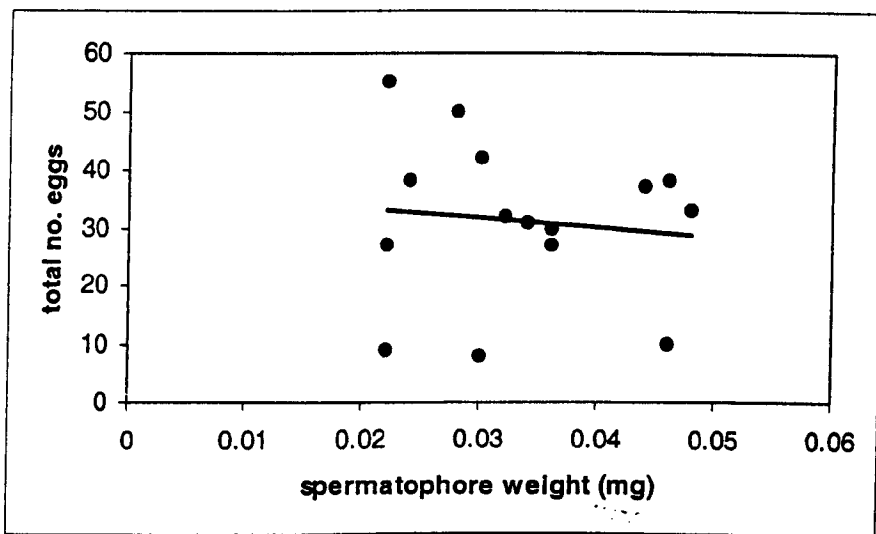
Figure 6.13 The relationship between total spermatophore weight received and fecundity for multiply mated unsupplemented females

6.2.10 Spermatophore weight received and egg weight and size

The analyses carried out in Section 6.2.8 were repeated for egg weight and egg size, again using data from the single versus multiple mating experiment. There was no correlation

between spermatophore weight received and egg weight (Spearman rank correlation, r_s = - 0.391, N = 9, p>0.05; Fig. 6.14); or spermatophore weight and egg size (r_s = –0.170, N = 9, p>0.05; Fig. 6.15) for singly mated supplemented females.
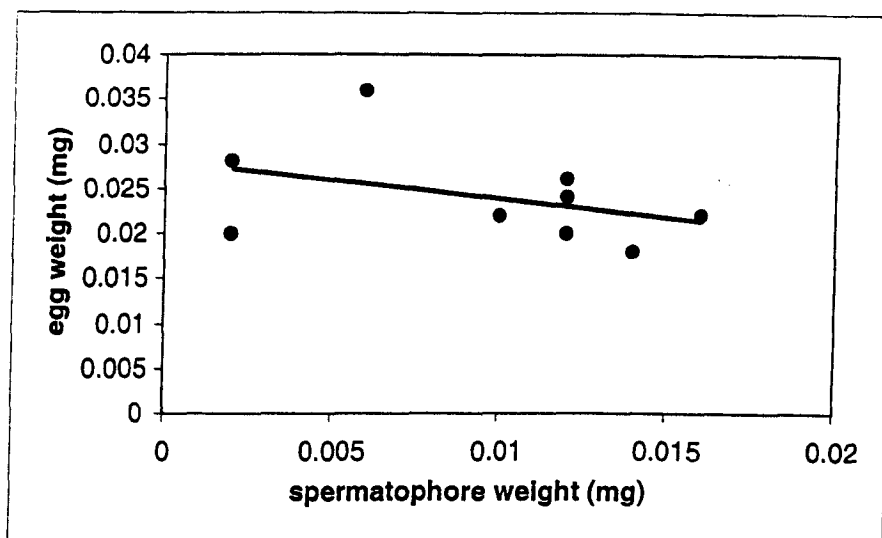
Figure 6.14 The relationship between spermatophore weight received and egg weight for singly mated supplemented females
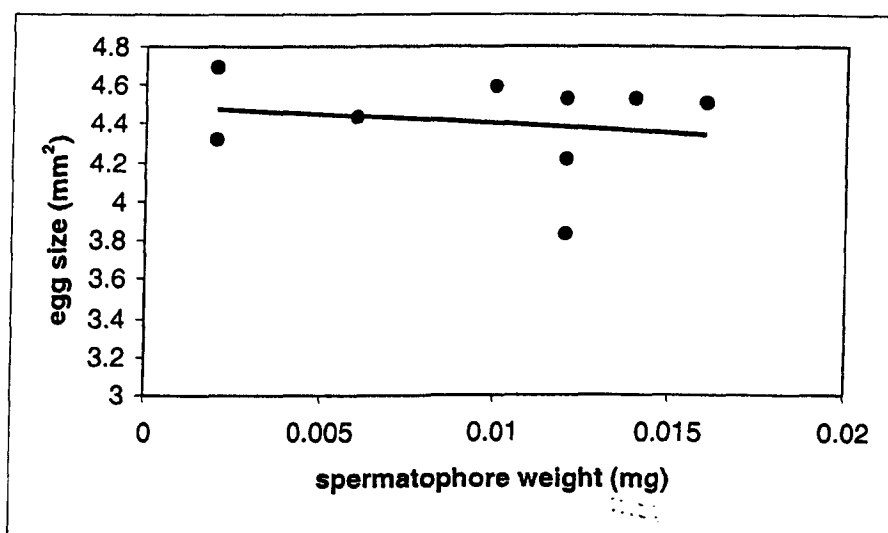
Figure 6.15 The relationship between spermatophore weight received and egg size for singly mated supplemented females

Nor was there any association between spermatophore weight received and egg weight (r_s = -0.146, N = 12, p>0.05; Fig. 6.16) or egg size (r_s = -0.208, N = 12, p>0.05; Fig. 6.17) for singly mated unsupplemented females.
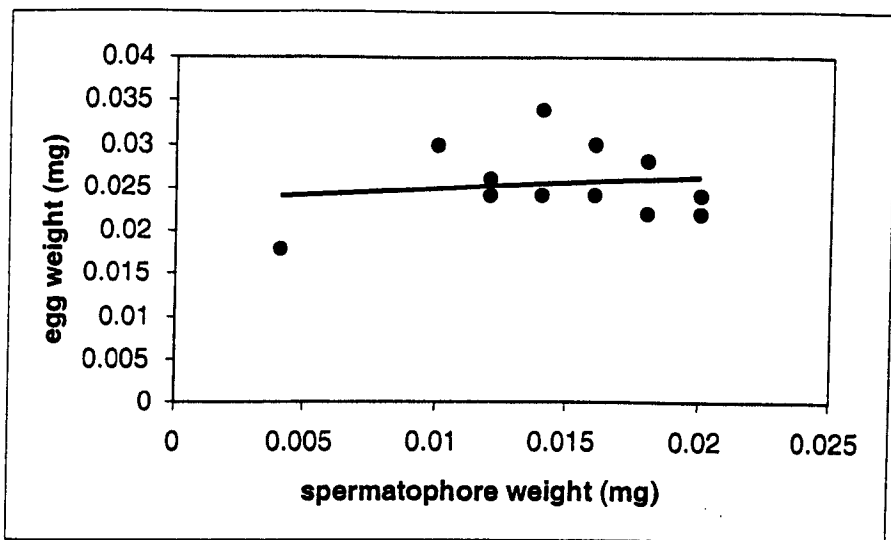
Figure 6.16 The relationship between spermatophore weight received and egg weight for singly mated unsupplemented females
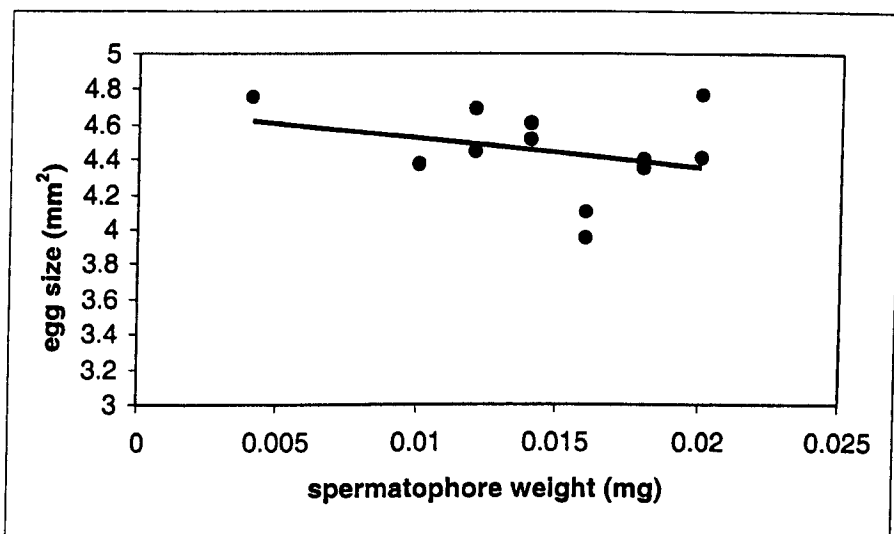
Figure 6.17 The relationship between spermatophore weight received and egg size for singly mated unsupplemented females

The total amount of spermatophore material received by multiply mated supplemented females was not correlated with egg weight ($r_s = 0.207$, N = 12, p>0.05; Fig. 6.18) or egg size ($r_s = 0.089$, N = 12, p>0.05; Fig. 6.19).
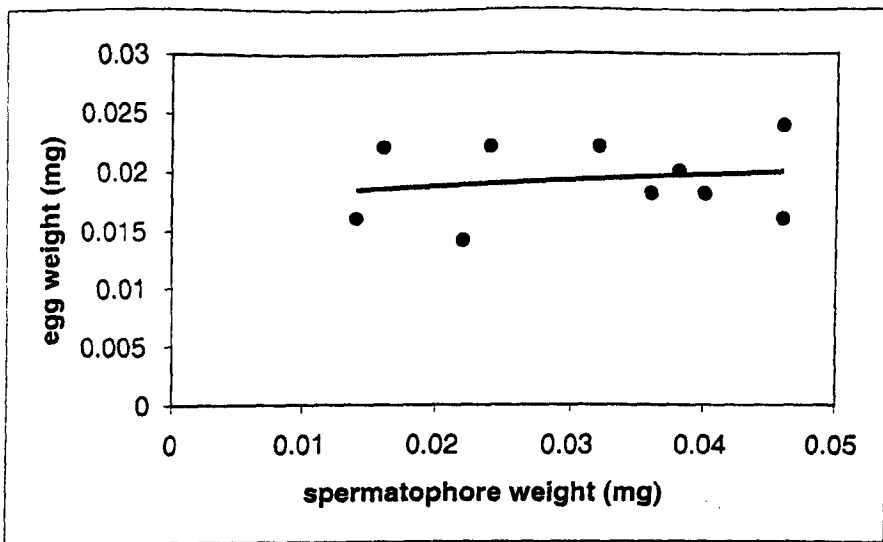
Figure 6.18 The relationship between the total weight of spermatophore material received and egg weight for multiply mated supplemented females
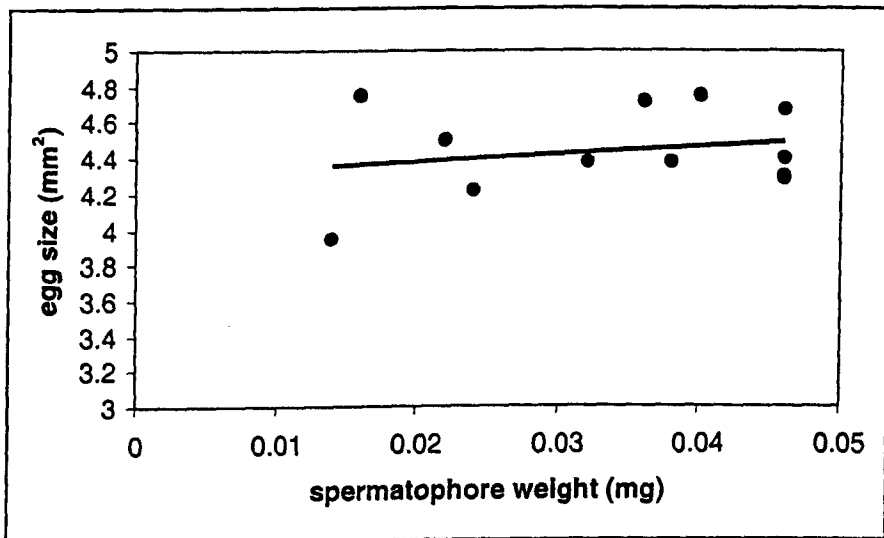
Figure 6.19 The relationship between the total weight of spermatophore material received and egg size for multiply mated supplemented females

There was no correlation between the total amount of spermatophore material received by multiply mated unsupplemented females and egg weight ($r_s = 0.182$, $N = 13$, $p>0.05$; Fig. 6.20), or egg size ($r_s = 0.050$, $N = 13$, $p>0.05$; Fig. 6.21).
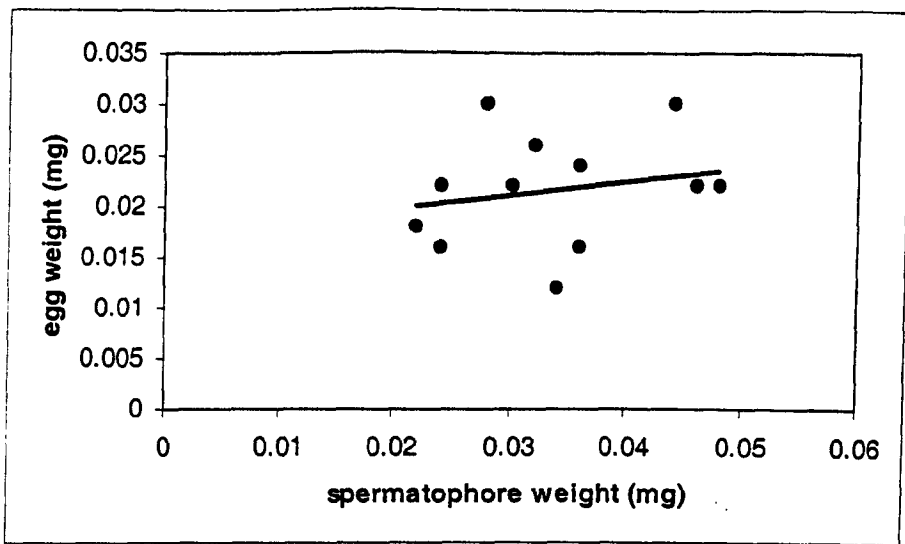
Figure 6.20 The relationship between the total weight of spermatophore material received and egg weight for multiply mated unsupplemented females
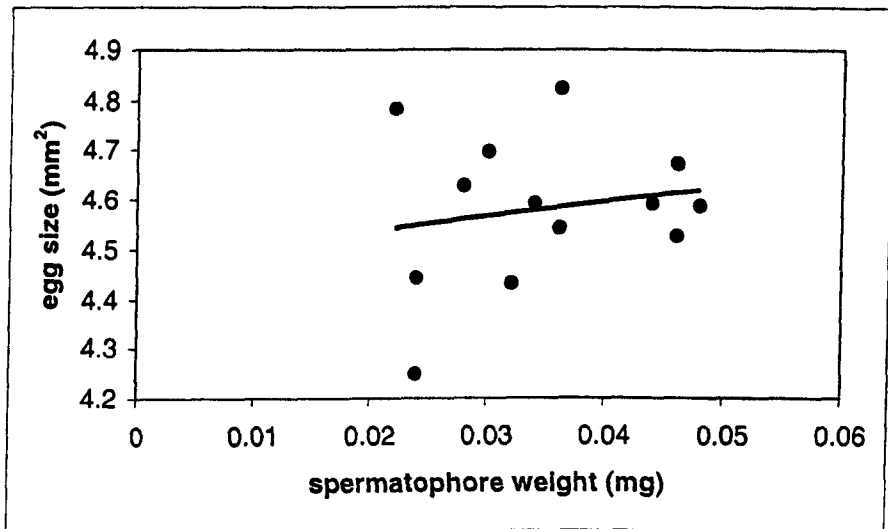
Figure 6.21 The relationship between the total weight of spermatophore material received and egg size for multiply mated unsupplemented females

6.2.11 The combined effects of male and female diet on fecundity, egg weight and egg size

The combination of the diets of male and female had no effect on fecundity either in singly mated females (one-way ANOVA, F = 1.195, df = 3,17, p>0.05), or multiply mated females (F = 2.783, df = 3,21, p>0.05).

The combination of male and female diets had no effect in singly mated females, either on egg weight (one-way ANOVA, F = 1.121, df = 3,17, p>0.05) or egg size (F = 0.063, df =

3,17, p >0.05). There was no effect of the dietary combination of the male and female in a
pair in multiply mated females, either on egg weight (F = 1.852, df = 3,21, p>0.05) or on
egg size (F = 2.560, df = 3,21, p >0.05).

6.2.12 Spermatophore size and female diet

There was a significant difference between supplemented and unsupplemented singly
mated females in the size of the spermatophore they received, with unsupplemented
females receiving larger spermatophores than supplemented females (t-test, t = -2.124, df =
27, p<0.05; Fig. 6.22). Among multiply mated females there was no difference between
supplemented and unsupplemented females in the total amount of spermatophore material
they received (t = -0.495, df = 23, p>0.05; Fig. 6.23). In both cases, data from the single
versus multiple mating experiment were used in the analyses.
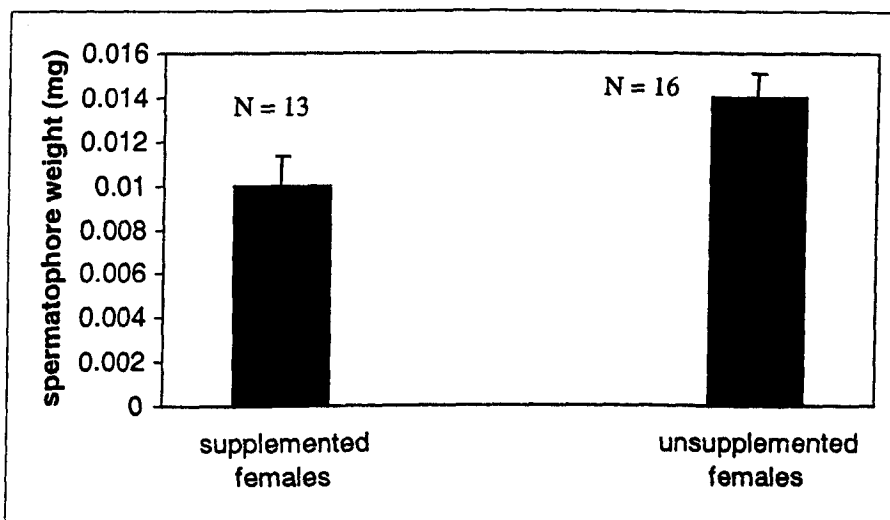
Figure 6.22 The weight of spermatophore passed to supplemented and unsupplemented singly mated females
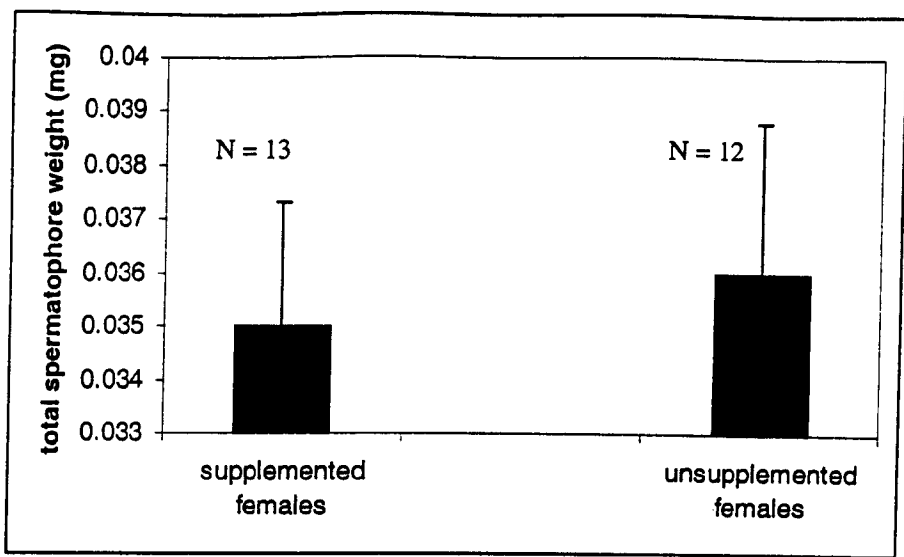
Figure 6.23 The total weight of spermatophore material passed to supplemented and unsupplemented multiply mated females

6.2.13 Diet and female longevity

There was no significant difference in lifespan between supplemented and unsupplemented females (t-test, $t = -1.297$, df $= 47$, $p > 0.05$; Fig. 6.24).
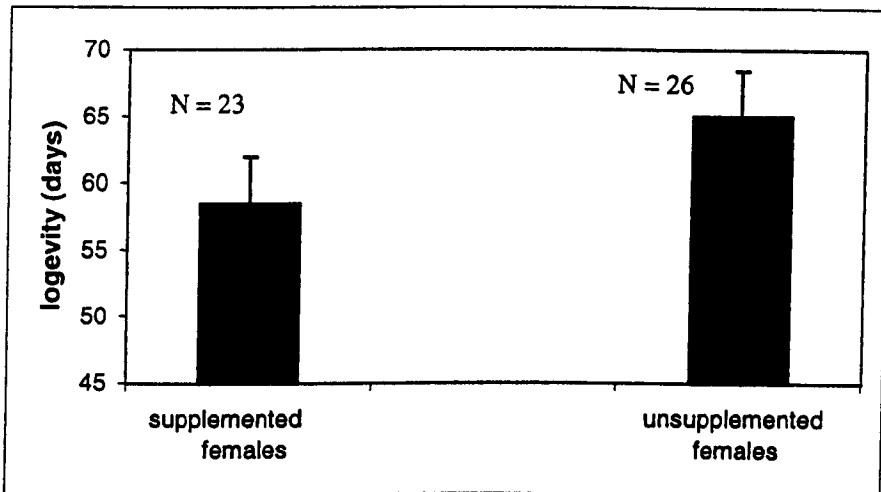
Figure 6.24 The longevity of supplemented and unsupplemented females

6.3 Discussion

Diet is an important factor in the mating behaviour of *L. punctatissima*. Supplemented males mated almost twice as often as unsupplemented ones, whereas the reverse was true

for females. Variation in mating success as a consequence of differences in diet quality is common in several species of bushcricket (Gwynne, 1981; 1985; Gwynne & Simmons, 1990; Gwynne, 1993; Schatral, 1993). This variation may arise because animals on a better quality diet are chosen more often as mates. For instance males may be able to invest more resources into energetically expensive calling behaviours (Simmons, 1994), which will increase the chance of getting a female response. Increased calling rates by males on high quality diets has been demonstrated in some species of bushcricket (Ritchie *et al.*, 1998) but not in others (Schatral, 1993). In my study, the reverese was true: more unsupplemented males than supplemented males were observed calling during each group session. This is due, however, to the fact that supplemented males mate more often and that, after mating, males cease calling for several hours. Unsupplemented males are therefore more likely to be calling because they are less likely to have mated recently.

There may also be differences between animals in the amount of energy they can dedicate to other activities. If males on a better diet can move around more they may be more likely to encounter females. Alternatively males on a higher quality diet may have more resources to dedicate to reproduction.

In this study there is no evidence for assortative mating based on diet. In the half and half breeding group, supplemented males mated more often than unsupplemented males and unsupplemented females mated more often than supplemented females. This could be the result of males choosing to mate with unsupplemented females, or females choosing to mate with supplemented males, or a combination of the two. Unsupplemented females lay more eggs than supplemented females following their first mating so males may gain an advantage by mating with these females, especially early in the season when they are more likely to be virgin. The results could also be due to unsupplemented females being more highly motivated to mate than supplemented females because of their restricted diet; this

assumes that the spermatophore can provide some nutrition. A third possibility is that supplemented males are able, as a result of their better diet, to produce spermatophores more quickly than unsupplemented males.

The restriction of spermatophore production under conditions of nutrient stress has been demonstrated in several species of tettigoniids (Gwynne, 1985; Gwynne & Simmons, 1990; Simmons & Bailey, 1990; 1993; Schatral, 1993). The scarcity of reproductively active males under these conditions skews the operational sex ratio (Emlen & Oring, 1977) towards females, and is probably the main contributory factor in the facultative reversal of conventional sex roles (Gwynne, 1985; 1990). Role reversal under these conditions is typified by heightened male discrimination of females and an increase in inter-female competition for males (Gwynne & Simmons, 1990). In my study, unsupplemented females in the half and half group did mate about twice as often as supplemented females, which suggests that they may have been foraging for extra nutrients contained within the spermatophore. However, there is no evidence from my study that, under poor diet conditions, male discrimination is heightened or females compete for males: there was no difference in the proportion of rejections of males by females or females by males in the unsupplemented and supplemented breeding groups, and I did not observe any female-female fights over males in any group. Unsupplemented males, if anything, show less discrimination than supplemented males since larger females obtain more matings than smaller females in the supplemented breeding group but not in the unsupplemented breeding group (Chapter 5).

There was no evidence that males altered the size of spermatophores under conditions of nutrient stress in this study: spermatophore size did not differ significantly between supplemented and unsupplemented males. Gwynne (1985) argues that producing smaller spermatophores as a response to poor diet would be unlikely to evolve as it increases the

chances that females will remove the ampulla before complete sperm transfer has been achieved. Consequently males may reduce their mating frequency rather than the size of their spermatophore as a response to diet stress, which is what appears to have happened in this study.

It might be expected that females on a poor diet would have fewer resources to dedicate to reproduction, and consequently they would lay fewer or smaller eggs than females on a better diet. A decrease in fecundity with a decrease in diet quality is common in insects (Wheeler, 1996). In multiply mated females in this study, there was no effect of diet on any measure of reproductive output: neither fecundity, egg weight or egg size differed significantly between supplemented and unsupplemented females. Unsupplemented singly mated females, however, laid more eggs than supplemented ones, the reverse of what might be expected.

If this is a real result and not just a statistical anomaly, one possible explanation is that females whose diet is poor may 'expect' to die sooner, perhaps as a result of disease or predation, and so concentrate their egg laying efforts into their early oviposition bouts, whereas females whose diet is of a better quality are able to spread their investment in egg laying across the whole season. In the laboratory population, there was no difference between supplemented and unsupplemented females in the length of time they lived. In the natural situation, however, the insects are subject to predators and diseases not present in the laboratory. It is possible therefore that the laboratory population may have been protected from the adverse effects of poor diet on longevity.

Males passed larger spermatophores to unsupplemented females than they did to supplemented females. There may be two explanations for this observation. First, males may have needed to provide unsupplemented females with a larger spermatophore because

they eat them more quickly and they have to last long enough to protect the ampulla while sperm transfer is completed. This is supported by the fact that there is no difference between supplemented and unsupplemented females in the time it takes to consume the spermatophore, despite the spermatophore the unsupplemented female receives being larger. Second, as larger spermatophores contain more sperm (Vahed & Gilbert, 1996a) males may have been providing these females with a greater quantity of sperm to reflect the fact that they lay more eggs in their first oviposition bout.

Generally, spermatophores had no effect on the reproductive success of the females irrespective of whether the female was supplemented or unsupplemented: neither fecundity, egg weight or egg size were correlated with the total weight of spermatophore material females received. The one exception was in supplemented singly mated females in which increased spermatophore weight led to a decrease in fecundity. This may be an aberrant result since it seems unlikely that the spermatophore would contain any substances that would adversely affect a female's ability to lay eggs.

Even so, it is possible that unsupplemented females may actively seek matings. Such spermatophore feeding under conditions of diet stress might benefit females because specific nutrients contained within the spermatophore compensate for their poor diet. Spermatophore consumption increased the number and weight of eggs of the bushcricket *K. nartee* when females were maintained on a poor quality diet (Simmons, 1990; Simmons & Bailey, 1990), but had no effect on reproductive output in *D. verrucivorus* (Wedell & Arak, 1989), *Leptophyes laticauda* (Vahed & Gilbert, 1996b) or *Gryllodes sigillatus* (Will & Sakaluk, 1994). As no attempt was made to deprive females of spermatophores in this study, the effects of nuptial feeding on female reproductive success were not tested directly. Vahed (1995), showed that female *L. punctatissima* that were denied access to spermatophylaces were no less successful in any measure of reproductive output than

females that were allowed to consume them, although he did not restrict the diet of the females in any other way.

6.4 Summary

Diet plays an important role in the mating behaviour of *Leptophyes punctatissima*, with supplemented males mating more often than unsupplemented males and unsupplemented females mating more often than supplemented females. These differences may be due to mate choice of supplemented males by unsupplemented females and/or supplemented males preferring to mate with unsupplemented females. On the other hand the results may be a consequence of males on a better diet being able to produce spermatophores at an increased rate and females whose diet is restricted being more highly motivated to mate to supplement their diet with spermatophores. There is no evidence to suggest a reversal of conventional sex roles as the rejection of males by females and females by males is not significantly different. Diet does not affect the size of spermatophores produced by males, and the amount of spermatophore material received has no observable effect on female reproductive success. Diet has no effect on female reproductive output but singly mated unsupplemented females lay more eggs than singly mated supplemented females, which may be due to females on a poor diet laying more eggs early on because they expect to die sooner. Males pass larger spermatophores to unsupplemented females which may reflect the fact that these females lay more eggs early in the season. There is no evidence to suggest that restricting the diet of females under experimental conditions adversely affects their lifespan.

7 Effects of Fluctuating asymmetry on mate choice and reproductive success

7.1 Introduction

If the level of asymmetry an organism displays is an honest signal of phenotypic or genetic quality the choosy sex may use the amount of asymmetry as a criterion of choice between prospective mates. The relationship between FA and mate choice has been documented for many species. Moller (1992) demonstrated a positive correlation between the level of tail symmetry and male mating success in the barn swallow *Hirundo rustica* Manning & Hartley (1991) and Petrie *et al.* (1991) showed that female peafowl (*Pavo cristatus*) mate preferentially with males who have symmetrical eyespot distributions in their tails. Female zebra finches (*Taeniopygia guttata*) choose to mate with males who have symmetrical leg bands more often than would be expected by chance alone (Burley, 1981; Swaddle & Cuthill, 1994). In insects the role of FA as a correlate of mating success has been demonstrated in several species. In the field cricket, *Gryllus campestris*, more symmetrical males are more successful in gaining mates because females are preferentially attracted to their calls (Simmons, 1995). More symmetrical male dung flies *Scatophaga stecoraria* gain more mates than less symmetrical males because they are more successful in contests over females (Ligget *et al.*, 1993). The association between increased FA and reduced mating success is not ubiquitous, however. Markow & Ricker (1992) showed that in *Drosophila simulans* mating males were larger than unmated males but showed increased FA and in *Drosophila pseudoobscura*, mating males and unmated males show equivalent levels of FA (Markow & Ricker, 1992).

There may be implications for the reproductive success of asymmetrical individuals beyond simply acquiring mates. Increased FA is associated with increased testicular dysfunction and hence sperm quality in the Florida panther *Felis concolor* (Roelke *et al.*,

1993), FA in forewing length is negatively correlated with fertilisation success in the fly *Dryomyza analis* (Otronen, 1998).

The reproductive success of asymmetric males which managed to secure a mating may also be compromised by post-copulatory female choice (Eberhard, 1996). For example a difference in investment in reproduction by females mated to symmetrical and asymmetrical males has been demonstrated in several species, including domestic hens (*Gallus gallus*), which lay more eggs if the mating male has more symmetrical wattles (Forkman & Corr, 1996).

If asymmetrical individuals invest disproportionately in basic maintenance there should be less energy available for other physiological processes such as reproduction (Moller & Swaddle, 1997). In a review of 17 studies investigating the association between FA and fecundity, Moller & Swaddle (1997) reported 16 in which increased FA negatively correlated with some aspect of reproductive success. For example, in many insect species female fecundity is correlated with the number of matings achieved (Ridley, 1988) and less symmetric females may suffer reduced fecundity because they mate less often than more symmetrical females.

The level of FA exhibited by an individual may also affect its ability to gather or utilise the resources required for successful reproduction (Moller, 1997; Moller & Swaddle, 1997); either because maintenance costs are high (e.g. asymmetric individuals may have a higher basal metabolic rate) (Mitton & Koehn, 1985; Ozernyuk, 1989; Moller & Swaddle, 1997), or because increased FA negatively correlates with ability to compete for limited resources (Moller *et al.*, 1996).

The size and number of clutches are important components of overall fecundity. Moller (1994b) showed that outer tail asymmetry in the barn swallow is directly related to the

onset of breeding: symmetric females begin breeding earlier and produce more offspring than less symmetric females. In this species, recruitment rate diminishes as the season progresses (Moller, 1994a) hence the offspring of asymmetric females are numerically underrepresented in the following generation.

A crucial element of overall reproductive success is offspring survival; individuals who survive and reproduce will increase the inclusive fitness of the parents. Parental selection has been observed in many organisms. Spontaneous abortion of embryos with inferior phenotypes has been reported in humans (Boue *et al.*, 1975; Simpson, 1982; Wolf *et al.*, 1984) and plants (Moller, 1996). Infanticide of asymmetric offspring has been noted in mice, *Mus musculus* (Ehret, 1975), scorpions, *Pandinus imperator* (Ehret, 1975) and humans (Ford, 1964; Montag & Montag, 1979; Daly & Wilson, 1984).

In this chapter I investigate the effects of asymmetry on both mate choice and reproductive success and seek to answer the following questions. Does the level of asymmetry affect mate choice and reproductive success in males and females? Do more symmetrical females lay more or better quality eggs and do more symmetrical males produce larger spermatophores? Does the level of asymmetry of the male affect the length of time he needs to recover between matings, or the reproductive success of the female he mates with? And finally, does asymmetry affect reproductive success by affecting longevity?

7.2 Results

The mean asymmetry score for each individual was used as a measure of its level of asymmetry in all analyses. Data on mating success and mating behaviour were analysed separately for the supplemented and unsupplemented breeding groups and were not analysed at all for the half and half breeding group for the reasons given in Section 5.2.

7.2.1 Asymmetry and male mating success

Mean FA was not correlated with the number of matings achieved for males in either the supplemented or the unsupplemented breeding groups, (Spearman rank correlation, S group (Fig. 7.1): $r_s = 0.083$, N = 10, p>0.05; US group (Figure 7.2): $r_s = -0.367$, N = 10, p>0.05).
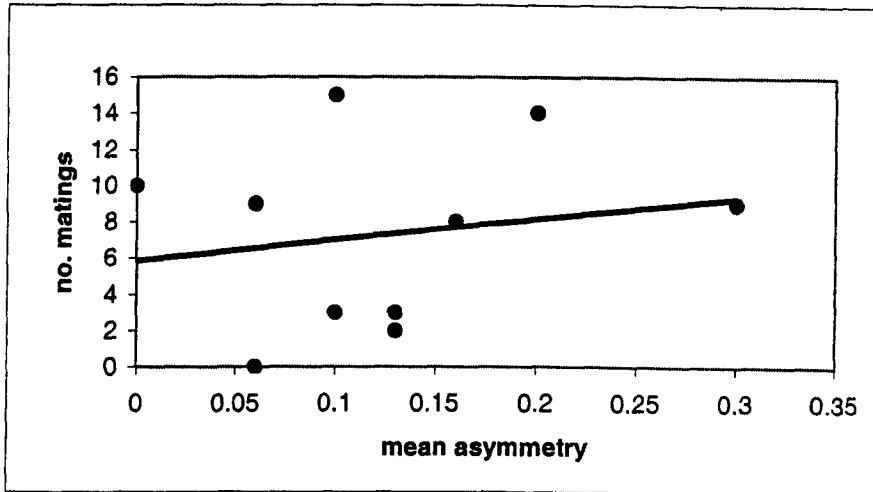
Figure 7.1 The relationship between mean FA and the number of matings achieved for males in the supplemented breeding group
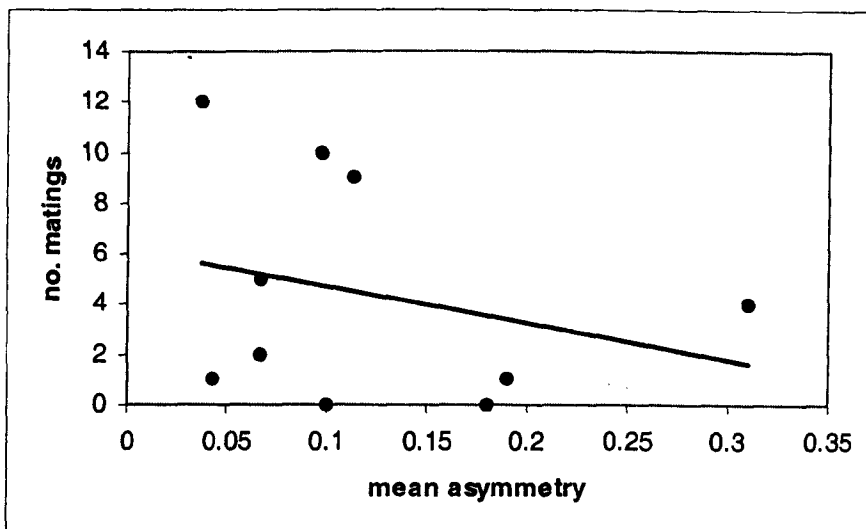
Figure 7.2 The relationship between mean FA and the total number of matings achieved for males in the unsupplemented breeding group

Nor was there any correlation between mean FA and the number of different partners males mated with either in the supplemented or the unsupplemented breeding group (Spearman rank correlation, S group (Fig. 7.3): $r_s = 0.000$, N = 10, p>0.05;. US group (Fig. 7.4): $r_s = -0.258$, N= 10, p>0.05).
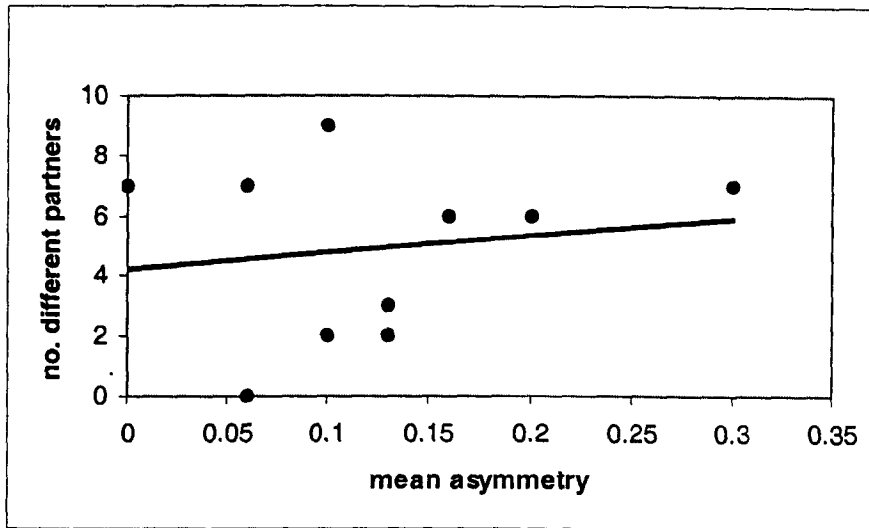
Figure 7.3 The relationship between mean FA and number of different partners males mated with in the supplemented breeding group
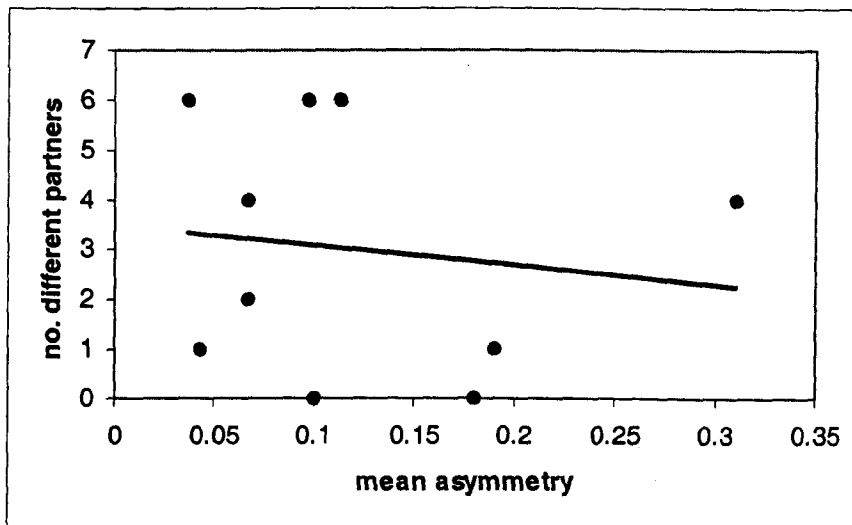
Figure 7.4 The relationship between mean FA and the number of different partners mated with for males in the unsupplemented breeding group

7.2.2 Asymmetry and female mating success

There was no correlation between mean FA and the total number of matings achieved by females in either the supplemented or the unsupplemented breeding group (Spearman rank correlation, S group (Fig. 7.5): $r_s = 0.465$, $N = 10$, $p>0.05$; US group (Fig. 7.6): $r_s = 0.105$, $N = 10$, $p>0.05$).
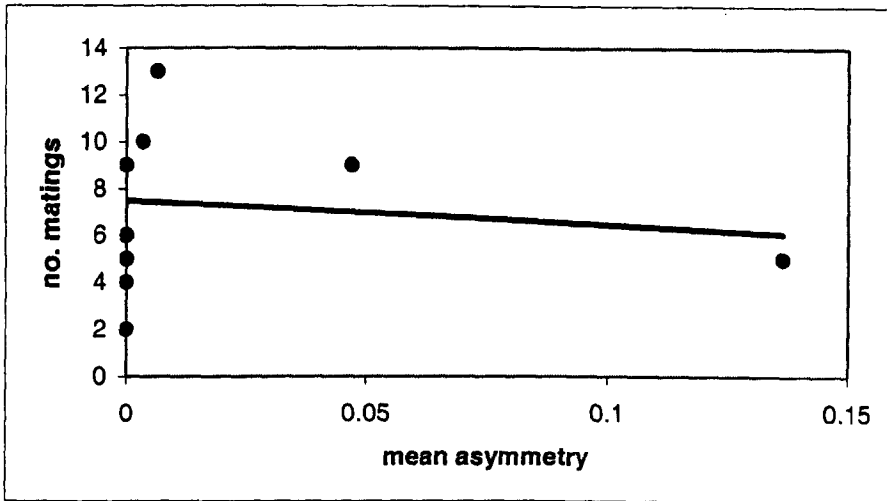
Figure 7.5 The relationship between mean FA and the total number of matings achieved by females in the supplemented breeding group
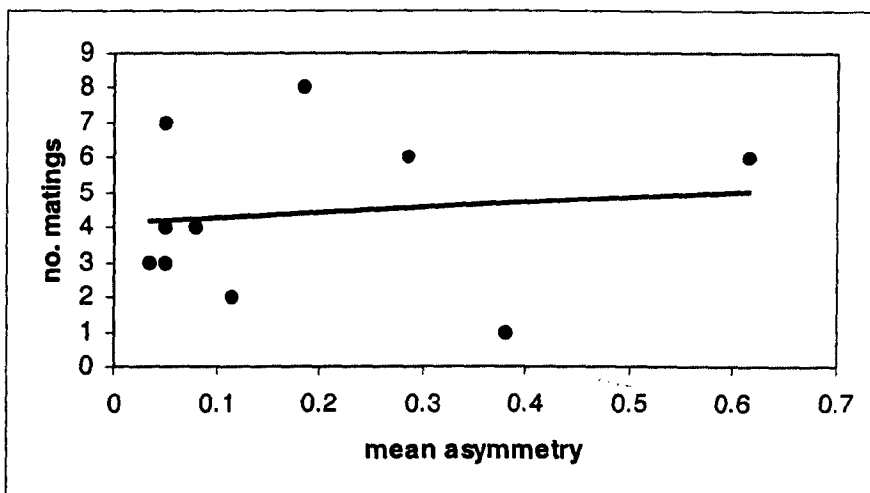
Figure 7.6 The relationship between mean FA and the total number of matings achieved by females in the unsupplemented breeding group

Nor was mean FA correlated with the number of different partners females mated with in either the supplemented or the unsupplemented breeding group (Spearman rank correlation, S group (Fig. 7.7): $r_s = 0.545$, N= 10, p>0.05; US group (Fig. 7.8):$r_s = 0.334$, N = 10, p>0.05).
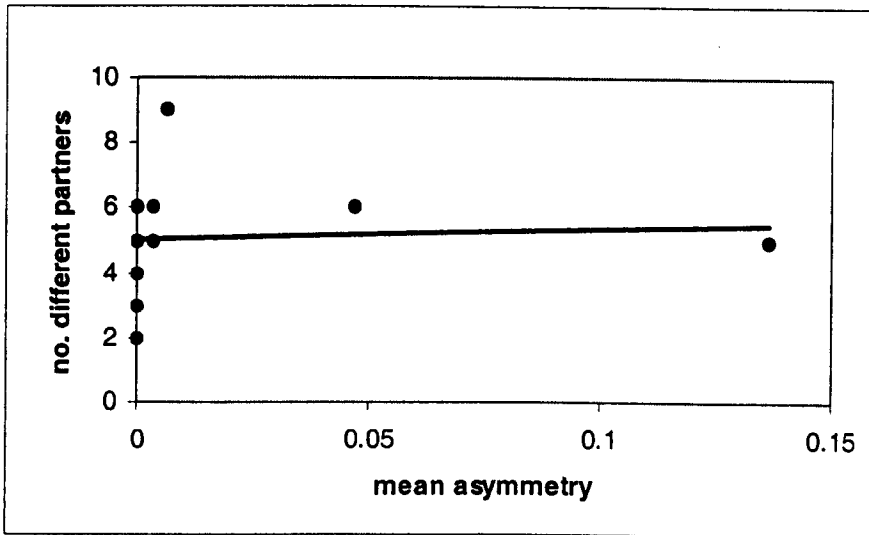
Figure 7.7 The relationship between mean FA and the number of different partners females mated within the supplemented breeding group
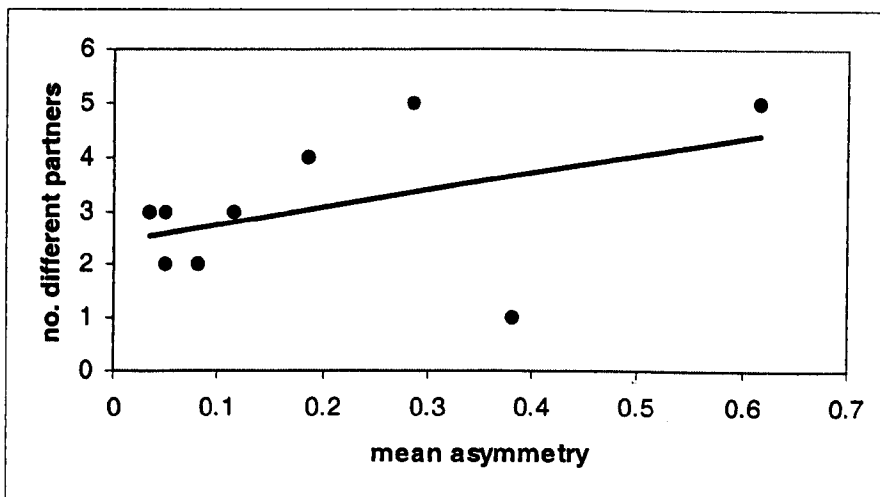
Figure 7.8 The relationship between mean FA and the number of different partners females mated with in the unsupplemented breeding group

7.2.3 Assortative mating with respect to asymmetry

There was no assortative mating with respect to FA in either the supplemented breeding group (Spearman rank correlation, $r_s = -0.020$, N = 72, p>0.05; Fig. 7.9) or the unsupplemented breeding group ($r_s = 0.098$, N = 44, p>0. 05; Fig. 7.10).
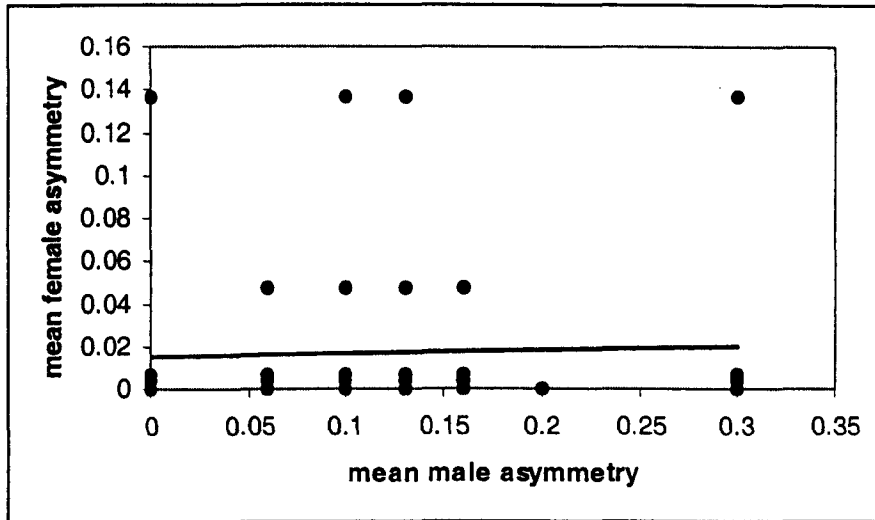
Figure 7.9 The relationship between female asymmetry and male asymmetry in mated pairs in the supplemented breeding group
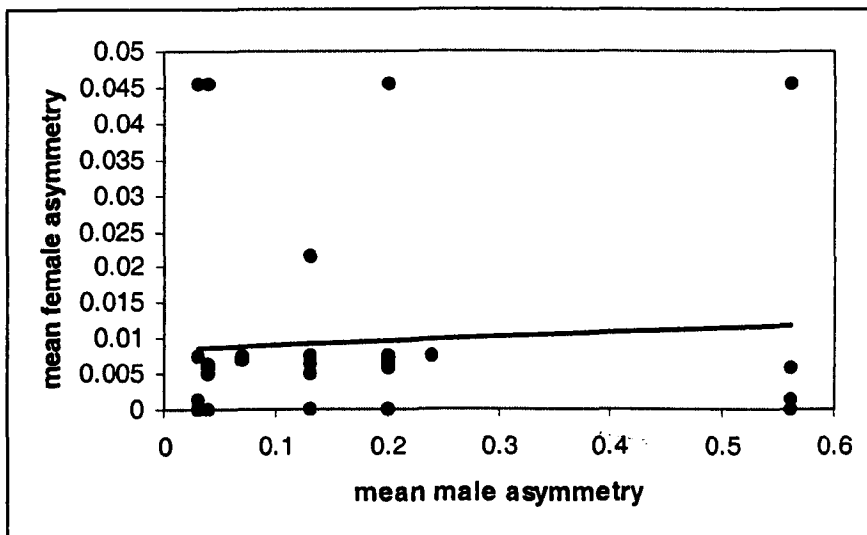
Figure 7.10 The relationship between female asymmetry and male asymmetry in mated pairs in the unsupplemented breeding group

7.2.4 Asymmetry and mating behaviour

There was no correlation between FA and the length of time males spent copulating in either the supplemented breeding group (Spearman rank correlation, $r_s = 0.005$, N = 72, p>0.05; Fig. 7.11) or the unsupplemented breeding group ($r_s = -0.133$, N = 44, p>0.05; Fig. 7.12).
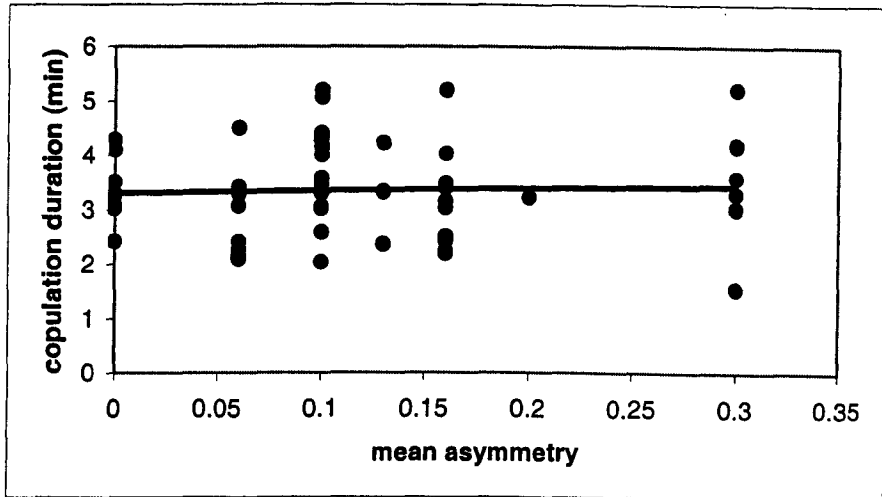
Figure 7.11 The relationship between mean FA and copulation duration for males in the supplemented breeding group
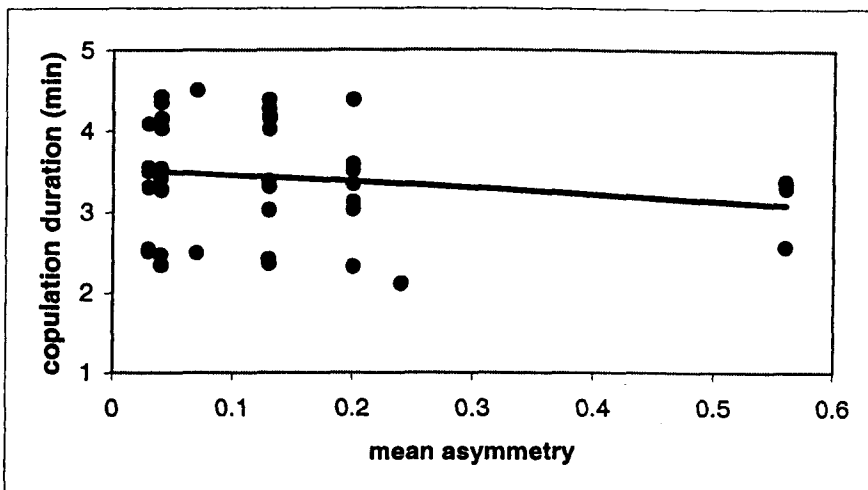
Figure 7.12 The relationship between mean FA and copulation duration for males in the unsupplemented breeding group

The time lag from the end of copulation until the female began to eat the spermatophore was not related to male FA in the either the supplemented breeding group (Spearman rank

correlation, $r_s = -0.091$, N = 65, p>0.05; Fig. 7.13); or the unsupplemented breeding group ($r_s = 0.128$, N = 40, p>0.05; Fig. 7.14).
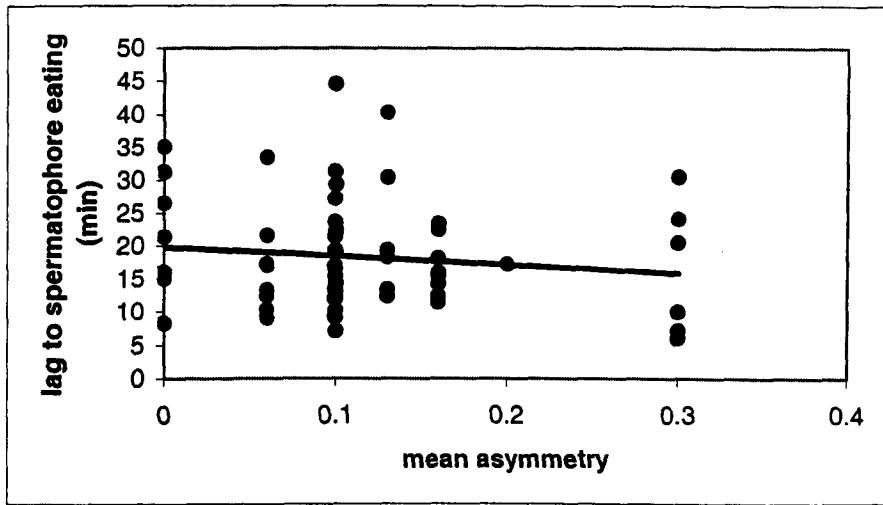
Figure 7.13 The relationship between male FA and the time between the end of copulation and the onset of spermatophore consumption by the female he mates with in the supplemented breeding group
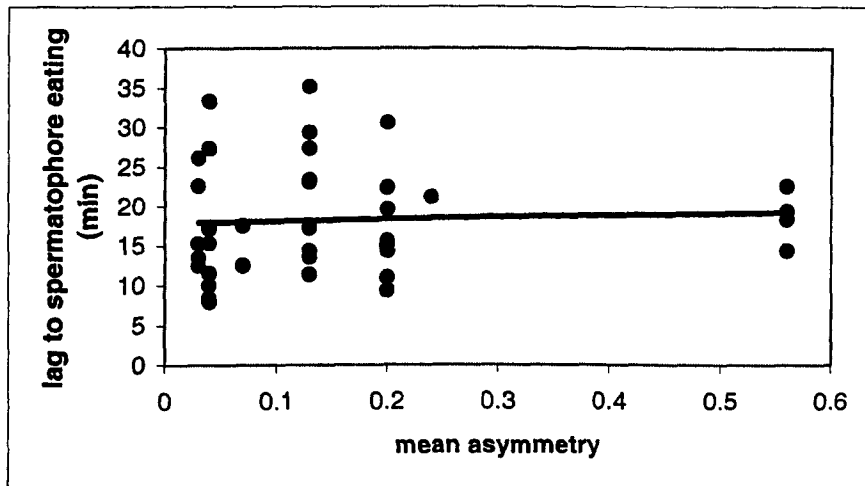
Figure 7.14 The relationship between male FA and the time between the end of copulation and the onset of spermatophore consumption by the female he mates in the unsupplemented breeding group

There was no correlation between male FA and the time taken for females to consume the spermatophore fully in either the supplemented breeding group (Spearman rank correlation, $r_s = 0.084$, N = 59, P > 0.05; Fig. 7.15), or the unsupplemented breeding group ($r_s = -0.086$, N = 36, p>0.05; Fig. 7.16).
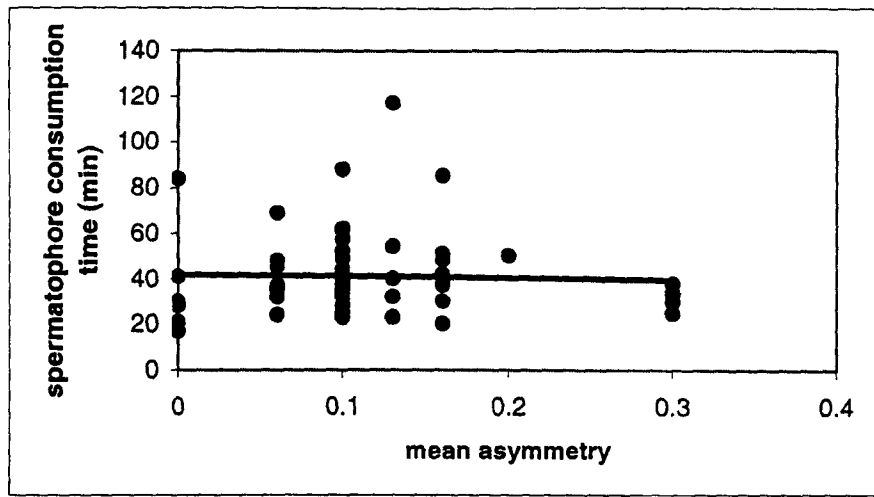
Figure 7.15 The relationship between a male's FA and the time taken for the female he mates with to eat the spermatophore for males in the supplemented breeding group
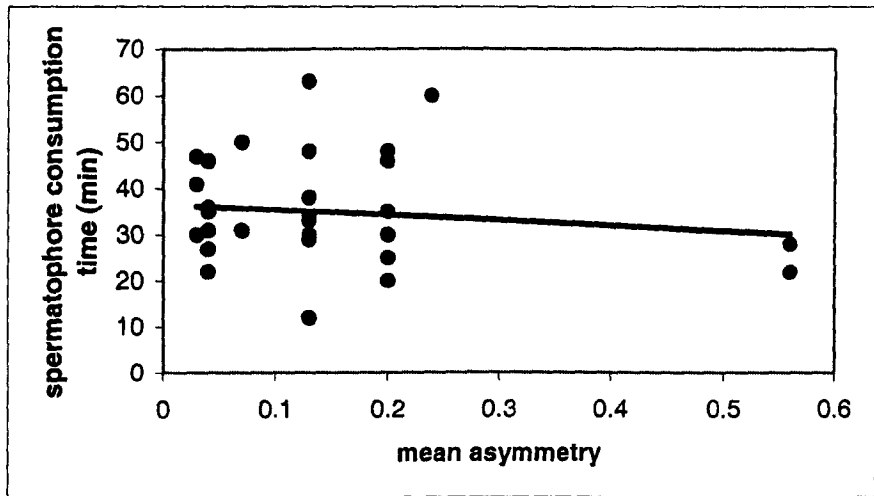
Figure 7.16 The relationship between a male's FA and the time taken for the female he mates with to eat the spermatophore in the unsupplemented breeding group

There was no correlation between FA and the length of time females spent copulating in either the supplemented breeding group (Spearman rank correlation $r_s = -0.177$, $N = 72$, $p > 0.05$; Fig. 7.17); or in the unsupplemented breeding group ($r_s = -0.222$, $N = 44$, $p > 0.05$; Fig. 7.18).
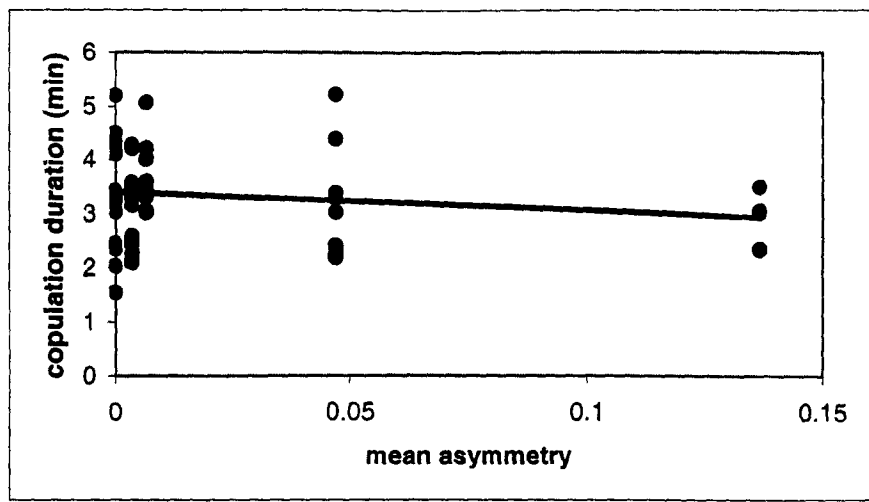
Figure 7.17 The relationship between FA and copulation duration for females in the supplemented breeding group
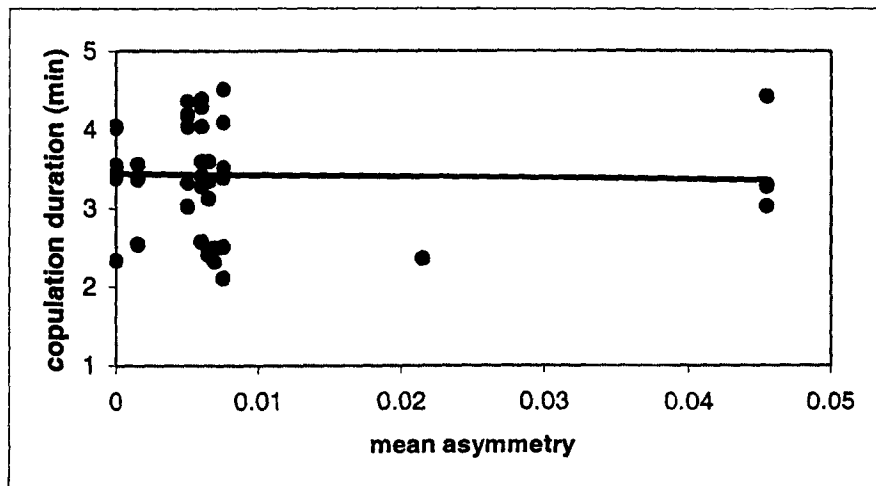
Figure 7.18 The relationship between FA and copulation duration for females in the unsupplemented breeding group

There was no correlation between female FA and time lag from the end of copulation until the female began to consume the spermatophore in either the supplemented breeding group (Spearman rank correlation, $r_s = 0.050$, N = 65, p>0.05; Fig. 7.19); or the unsupplemented breeding group ($r_s = 0.128$, N = 40, p>0.05; Fig. 7.20).
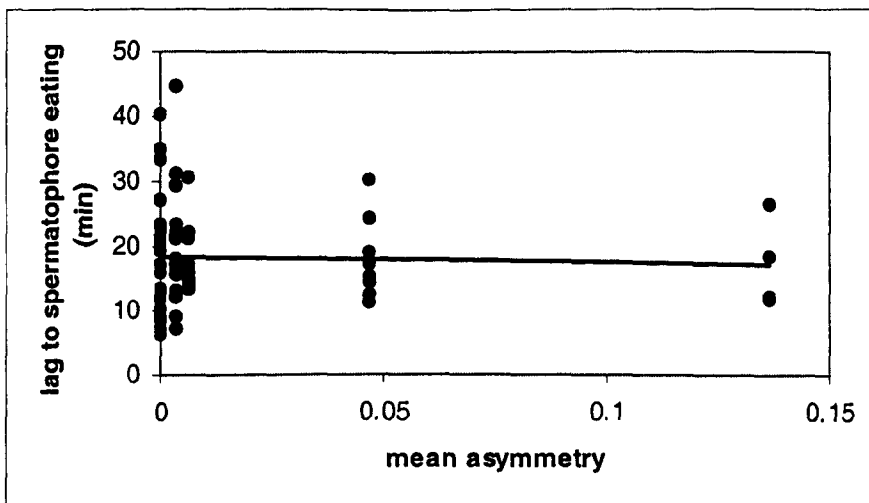
Figure 7.19 The relationship between FA and the time lag from the end of copulation until spermatophore consumption begins for females in the supplemented breeding group
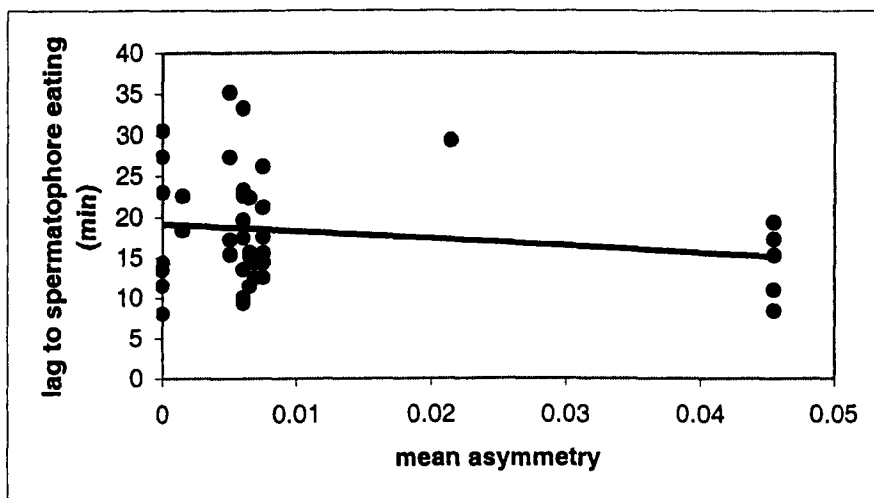
Figure 7.20 The relationship between FA and the time lag from the end of copulation until spermatophore consumption begins for females in the unsupplemented breeding group

There was no correlation between FA and the time taken for females to consume the spermatophore fully in either the supplemented breeding group ($r_s = 0.155$, N = 65, p>0.05: Fig. 7.21); or in unsupplemented breeding group ($r_s = -0.086$, N = 40, p>0.05; Fig. 7.22).
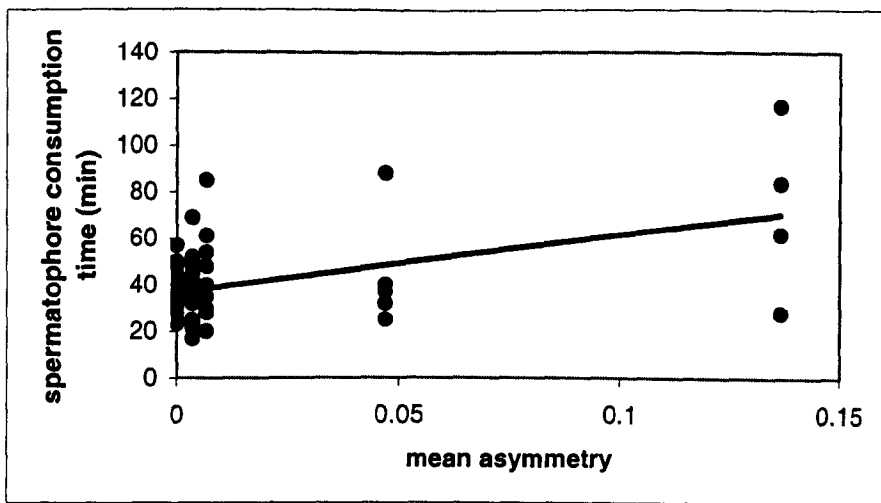
Figure 7.21 The relationship between FA and the time taken to consume the spermatophore fully for females in the supplemented breeding group
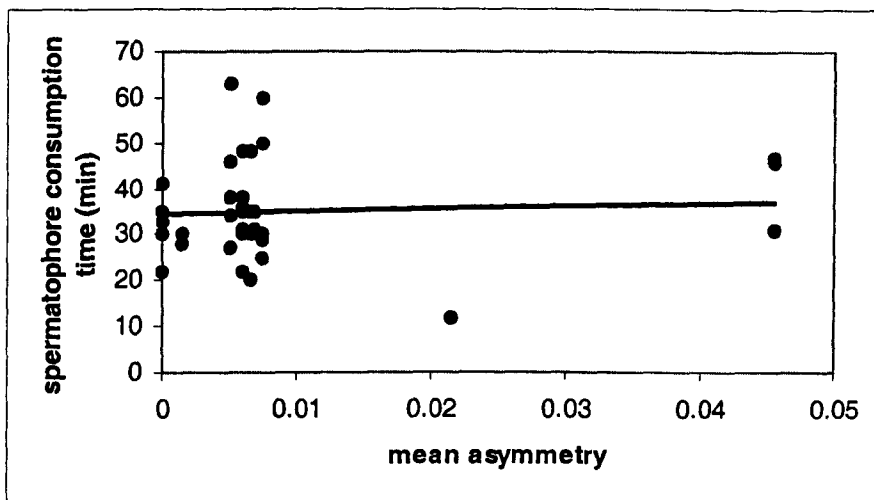
Figure 7.22 The relationship between FA and the time taken to consume the spermatophore fully for females in the unsupplemented breeding group

7.2.5 Asymmetry and spermatophore size

There was no correlation between FA and the mean weight of spermatophores produced by males (data from the single versus multiple mating experiment, Spearman rank correlation, $r_s = -0.053$, N =53, p>0.05; Fig. 7.23), nor was there any evidence that asymmetry influenced the relative investment in spermatophores (data from the single versus multiple mating experiment, ANOVA relative spermatophore weight (calculated as spermatophore weight as a percentage of body weight arcsine transformed), F = 1.077, df = 1,97, p >0.05).

194
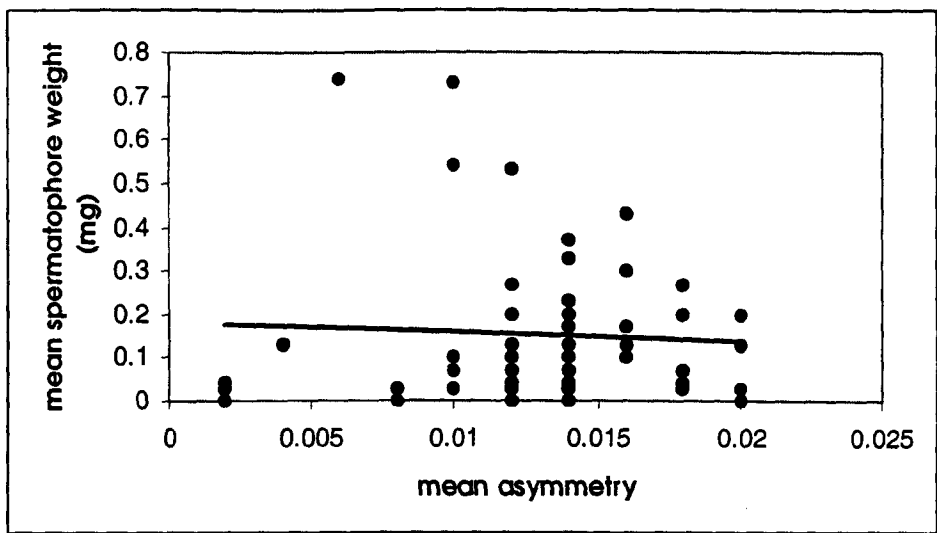
Figure 7.23 The relationship between mean FA and male spermatophore size

7.2.6 Asymmetry and female fecundity, egg weight and size

There was no correlation between mean FA and the number of eggs females laid (combined data from breeding group experiment and single versus multiple mating experiment, Spearman rank correlation, $r_s = 0.079$, N= 80, p>0.05; Fig. 7.24), nor was there any correlation between FA and either egg weight (data from single versus multiple mating experiment, $r_s = 0.072$, N = 46, p>0.05; Fig. 7.25) or egg size (data from single versus multiple mating experiment, $r_s = 0.128$, N = 46, p>0.05; Fig. 7.26).
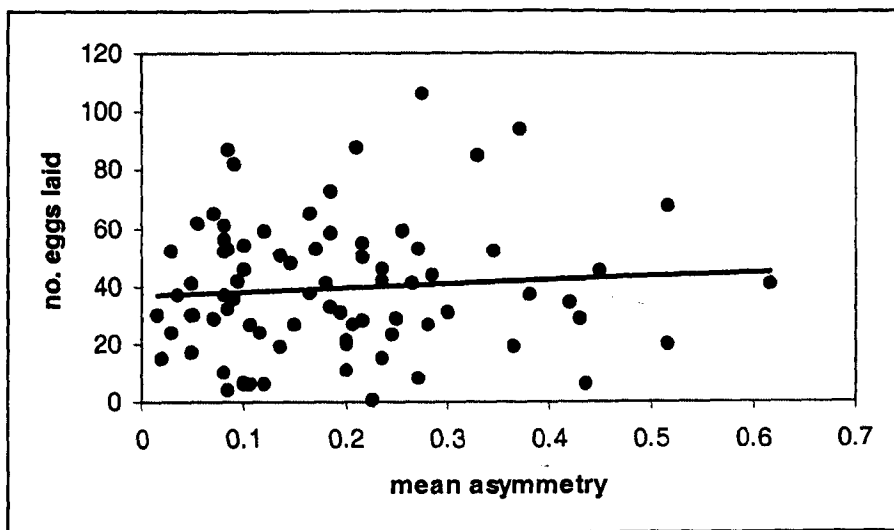
Figure 7.24 The relationship between mean FA and female fecundity
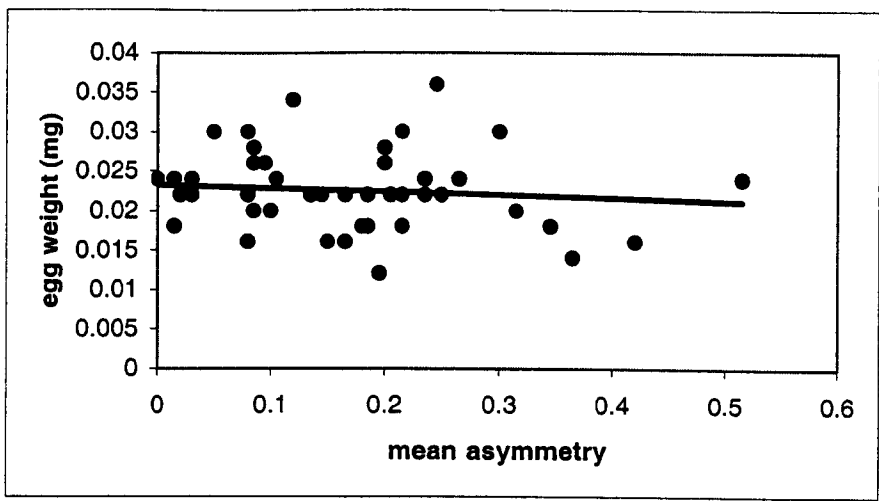
Figure 7.25 The relationship between mean FA and egg weight
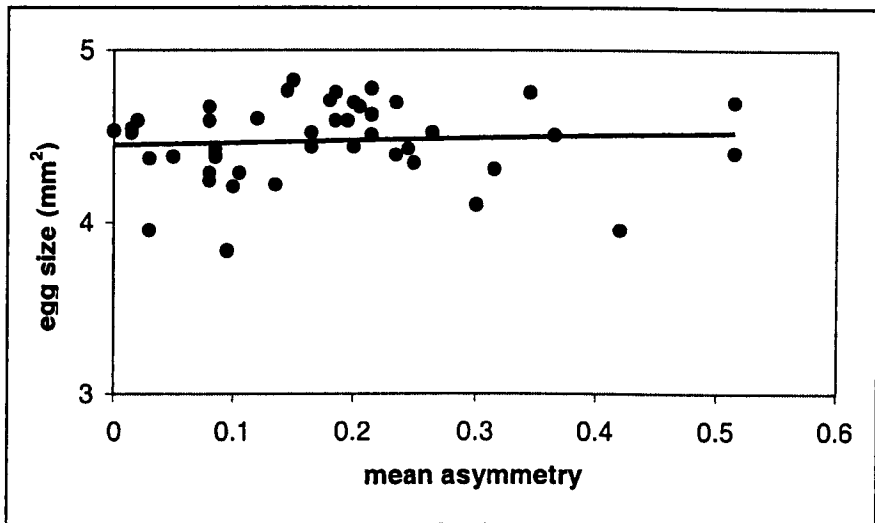
Figure 7.26 The relationship between FA and egg size

7.2.7 Asymmetry and female longevity

There was no correlation between FA and female longevity (data from single versus multiple mating experiment, Spearman rank correlation, $r_s = 0.120$, N = 49, p>0.05; Fig. 7.27).
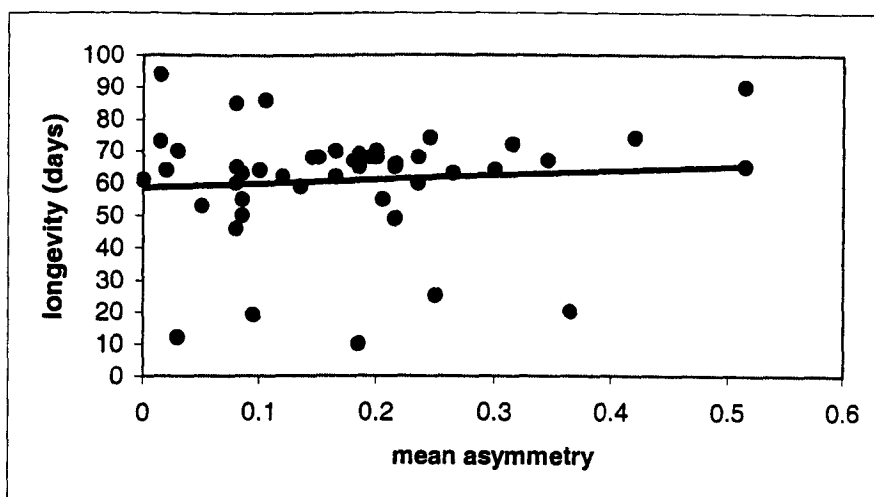
Figure 7.27 The relationship between FA and female longevity

7.3 Discussion

Mating success was not affected by an individual's asymmetry for either males or females. As I showed in Chapter 4, the level of FA in the traits examined as a percentage of trait size is low and they may be too minor to provide a basis for mate choice. In many studies FA is measured in secondary sexual characteristics, since these traits often show more phenotypic variation than other morphological characters, such that only animals in the best current condition can invest sufficient energy to produce an ideal form in the face of prevailing stress. Functional traits are probably subject to greater canalizing selection (Waddington, 1940) which may explain the low levels of asymmetry in the femur and tibia of the insects in this study.

The level of symmetry in functional traits may influence mating success if it directly affects an individual's ability to gain access to members of the opposite sex. Thornhill (1992) demonstrated a negative relationship between FA in a functional trait and mating success in the Japanese scorpionfly. Males with more symmetrical forewings did better in contests over dead arthropod prey than less symmetric rivals. This relationship has been reported for other insect species. In the damselfly (*Coenagrion puella*) males with higher forewing FA suffered reduced mating success (Harvey, 1993), because less symmetric

197

males were less successful in capturing females that crossed their territory. Males of the yellow dungfly (*Scatophaga stercoraria*) had lower mating success because they competed less well in contests over females at dung pats than more symmetric males (Ligget *et al.*, 1993). However in both the Harvey & Walsh and the Liggett *et al.* studies it is unclear to what extent the level of FA in the population is confounded by measurement error. Harvey & Walsh (1993) for example only took one measurement of the traits under examination; so the actual between sides variation may be due in part to measurement error. In my study the confounding effects of measurement error were controlled for, yet FA was still a poor predictor of mating success. In a study of the damselfly *Enellagma ebrium*, in which the effects of measurement error were controlled for, Leung & Forbes (1997) also found that the FA of functional characteristics was a poor predictor of individual quality.

As described in Section 3.10, I have observed male *L. punctatissima* physically interacting with each other in contests which may be fights over access to the best calling positions. In the natural situation, if more symmetrical individuals win these contests more often, then access to the best calling sites may translate into a greater mating success because these males are likely to be heard by more females (Walker, 1983). In the laboratory, however, any effect of gaining the best calling site would probably be negligible because all individuals were within calling range of all the others.

The songs of Orthoptera are often subject to sexual selection (Simmons, 1988b; Simmons & Ritchie, 1996). Increased FA in the stridulatory apparatus has been linked to decreased male mating success in the field cricket *Gryllus campestris* (Simmons, 1995; Simmons & Ritchie, 1996). In *L punctatissima*, males and females duet, and as such both sexes may be subject to changes in song parameters as a result of increased FA, which may affect mating success. I found significant levels of directional asymmetry in tegmina length for both males and females in this study (Section 4.2.2), consequently I was not able to investigate

the relationship between tegmina FA and mating success. This does not exclude the possibility, however, that mating success could be related to asymmetry in actual harp length (Eggert & Sakaluk, 1994; Simmons & Ritchie, 1996). Interestingly the amount of FA as a percentage of trait was greater for the tegmina than for either the femur or tibia (Section 4.2.2) which may indicate that this structure is less highly canalized (Waddington, 1940) and hence may be subject to greater variation.

There was no association between spermatophore size and asymmetry for males in this study either for absolute spermatophore size or the spermatophore size relative to the male's body weight. Spermatophore size is an important component of fitness in many orthopteran species (Sakaluk, 1984; Eggert & Sakaluk, 1994; Vahed & Gilbert, 1996a) because larger spermatophores contain a greater sperm number of sperm and are related to greater fertilization success (Eggert & Sakaluk, 1994). Eggert & Sakaluk (1994), however, reported a similar lack of correlation between FA and spermatophore size in the decorated cricket, spermatophore size co-varied with male size but was not related to level of FA.

Asymmetry was not related to any measure of the reproductive output of females in this study. Neither fecundity, egg size or egg weight was related the level of asymmetry expressed by females. If individuals with greater developmental instability have less resources available after basic maintenance to invest in other physiological processes (Moller & Swaddle, 1997), then it might be expected that these individuals would show a reduction in fitness when compared to individuals which had been better able to buffer their development. Lower fecundity as a result of increased FA has been reported for species as diverse as insects (Parsons, 1962), birds (Moller, 1992) and mammals (Moller et al., 1996). Another component of a females reproductive fitness is longevity, females with a greater lifespan will have a longer period in which to lay eggs. There was however, was no relationship between asymmetry and the longevity of females in this study.

The lack of correlation between the level FA and these measures of fitness may have a number of explanations. First, FA may not be a sensitive measure of fitness in this species: I have found no relationship between any measure of fitness and FA. Second, the asymmetry of the structures I measured may not be important. The asymmetry of the functional morphological traits I examined was very small when compared to mean trait size and hence may not signal quality reliably. Finally, the importance of FA as an indicator of developmental stability and individual quality may have been overestimated in the published literature. Palmer (1999) has suggested that there may be a tendency for authors to submit only those studies that demonstrate a relationship between FA and measures of fitness; this would lead to a publication bias which could artificially inflate the relative importance of FA as a measure of developmental stability. Simmons *et al.* (1999) have also pointed out that there may be a tendency for researchers to embrace the latest popular ideas with less scientific rigour, the result being that there is an initial rise in positive results, which then decline as the results of studies are investigated more thoroughly

7.4 Summary

There was no association between the level of FA displayed and the mating success of either males or females in this study. Nor was spermatophore size or any measure of female reproductive output related to the amount of FA individuals displayed. It may be that FA is not a sensitive measure of fitness in this species, or that the FA of the traits measured was not large enough to signal reliably the level of developmental stability of individuals.

8 The role of calling in mate choice and reproductive success

8.1 Introduction

There is considerable evidence that within species variation in the characteristics of mating calls may be shaped by sexual selection. The association between elaborate non-acoustic characters and increased mating success in males is well documented (Darwin, 1859; Fisher, 1915; Andersson, 1994). The acoustic displays of birds, frogs, toads and orthopteran insects are analagous to the secondary sexual characters found in other species and consequently may be subject to modification through sexual selection. In the tungara frog, for example, females prefer to mate with males that produce more complex calls (Rand & Ryan, 1981).

Females have been shown to respond differentially to the calls of males in many orthopteran species. For example, in *Acheta domestica* females prefer the calls of dominant males over those of subordinate males in playback experiments (Crankshaw, 1979), while in *Conocephalus nigropleurum*, females are more attracted to the songs of larger males (Gwynne, 1982). The criteria on which females base their choice is, however, less clear. The songs of larger males are often of a greater amplitude, hence females may simply find them easier to locate: female *C. nigropleurum* for example orient towards recordings of multi-male chorusing in preference to those of a single male call (Morris *et al.*, 1978). In the field, females in the katydid *Orchelimum nigripes* often move between several calling males before actually selecting a mating partner (Feaver, 1977), the females may thus be using the call of the males simply to locate them, with their choice of mate based on some other criteria.

If females are using song as the sole criterion of choice between prospective partners then it might be expected that some aspect of the call other than mere amplitude or quantity will act as the cue. Female *Gryllus integer* have been shown to discriminate between males based on their song bout duration, preferring to mate with males that had longer uninterrupted periods of calling (Hedrick, 1986), in *Gryllus lineaticeps* females prefer males with a higher chirp rate (Wagner & Reiser, 2000). Such calling strategies are more energetically expensive and may be governed by environmental factors such as the nutritional condition of the male (Wagner & Hoback, 1999). It could also indicate a male of superior genetic quality, for instance males that have been better able to stave off parasitic infection (Hamilton & Zuk, 1982). Female *Gryllus bimaculatus* are sensitive to variations in syllable repetition rate (Doherty, 1985; Schildberger, 1985) and prefer to mate with larger males whose songs contain a greater syllable repetition rate within the natural range for the species (Shuvalov & Popov, 1973; Simmons, 1988a). Female *Gryllus veletis* and *Gryllus pennsylvannicus* (Zuk, 1988) are often found paired with older males in the field. Older males have significantly lower gut parasite loads (Zuk, 1988). Simmons & Zuk (1992), showed that chirp variability varied with age in *G bimaculatus* possibly as a result of wear and tear on the stridulatory apparatus. It is possible that this information may be used by females to gauge the age and therefore the level of parasitic infection of a prospective mate (Simmons & Zuk, 1992).

Interestingly Ritchie (1995), found the opposite effect in the bushcricket *Ephippiger ephippiger*. In this species females discriminated strongly against the calls of older males in the laboratory. Choice of younger males would be adaptive if females gain some material benefit. Males of this species produce relatively large spermatophores and one possibility is that spermatophore quality declines with age.

In many species there is an inverse relationship between the size of a signaller and the frequency of the signal. This offers females the opportunity to select larger males on the basis of their call. Evidence that females actually use frequency as a basis of choice is, however, equivocal. Female black-horned tree crickets (*Oecanthus nigricornis*) orient preferentially to the lower frequency calls of larger males when the calls of several males were played simultaneously but showed no such preference in response to individual male calls (Brown *et al.*, 1996). The songs of *O. nigricornis* travel long distances and females are usually relatively close to several singing males, however, so they are normally in a situation where they can exercise choice. Females can also move among males without incurring any great costs (Brown *et al.*, 1996). In other species of Orthoptera the opposite pattern has been observed. Females in *Kawanaphilia nartee* (Gwynne & Bailey, 1988) and *Tettigonia cantans* (Latimer & Sippel, 1987) perform phonotaxis preferentially towards the higher frequency calls of smaller males. Higher frequency signals suffer greater attenuation by the environment as they travel through the air and consequently do not travel as far as lower frequency signals, consequently females may have been choosing these calls because they perceived the male as being nearer (Ritchie *et al.*, 1995). This preference would act to reduce the travelling distance towards a mate and so reduce the risk of predation.

Because it is a duetting species *L. punctatissima* has the opportunity for mate choice on the basis of call characteristics in both males and females. The extreme brevity of the male call may, however, limit the ability of the female to process its structure. Robinson *et al.* (1986) also argue that the short latency of the female reply to a male call in *L. punctatissima* may preclude detailed neuronal processing of the male call by the female brain. The response of a female to a male call is likely to be the result of an acoustic motor reflex: the time required for processing via interneurons would preclude the female replying to the male call within the strict phonotactic time window (Boyan, 1980). However, this may not completely eliminate the possibility of female choice based on the song characteristics of

males. As shown in Chapter 4, there is considerable variation both in overall calling behaviour (proportion of time spent calling, calling bout length and inter-call interval) and in the characteristics of individual calls (call length, syllable number, syllable length and inter-syllable interval) which provides at least the potential for mate choice based on the song characteristics of males. Females may be able to sample the calls of several males before they actually raise their tegmina and respond to what they perceive to be the signal of a high quality individual. Conversely, they may break off communication with a male by closing their tegmina if they decide that the male they are responding to is of poor quality.

I was unable to analyse the characteristics of the female call. However, since it is considerably briefer than the male's call and consists usually of only a single syllable, the amount of information it contains is likely to be extremely small. Even so, it would be possible for a male to exert choice on the basis of the female response by performing phonotaxis to, for example, the fastest response. This is likely to be the female nearest to him and such a choice would reduce his predation risk by reducing the distance he has to travel to find the female. Experiments in the laboratory have suggested that, given a choice of two female replies to the same male call, both falling within the response window, the male responds to the first one he hears, regardless of the characteristics of the second call such as loudness (J. Rhinelaender, pers. comm.). This may be analagous to the female preference for high frequency calls in some other species of bushcrickets (Latimer & Sippel, 1987; Gwynne & Bailey, 1988).

In this chapter I investigate whether there is any relationship between a male's mating success and his calling behaviour or certain characteristics of his call.

8.2 Results

For the reasons given in Section 5.2, the data for the supplemented and unsupplemented breeding groups was analysed separately and data from the half and half breeding group was not included in the analyses. There was no correlation between call length and the total number of matings achieved for males in either the supplemented or the unsupplemented breeding group (Spearman rank correlation, S group (Fig. 8.1): r_s = -0.467, N = 8, p>0.05; US group (Fig. 8.2): r_s = -0.252, N =7, p>0.05).
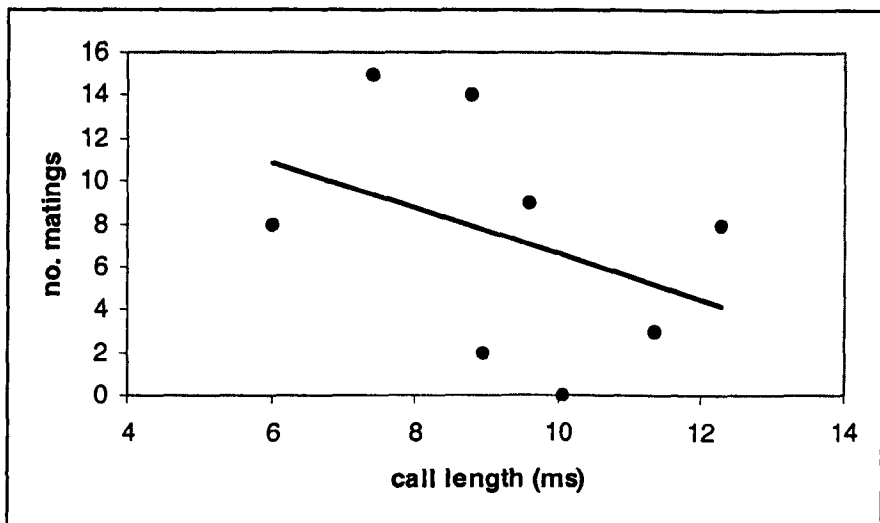
Figure 8.1 The relationship between call length and the total number of matings achieved by males in the supplemented breeding group
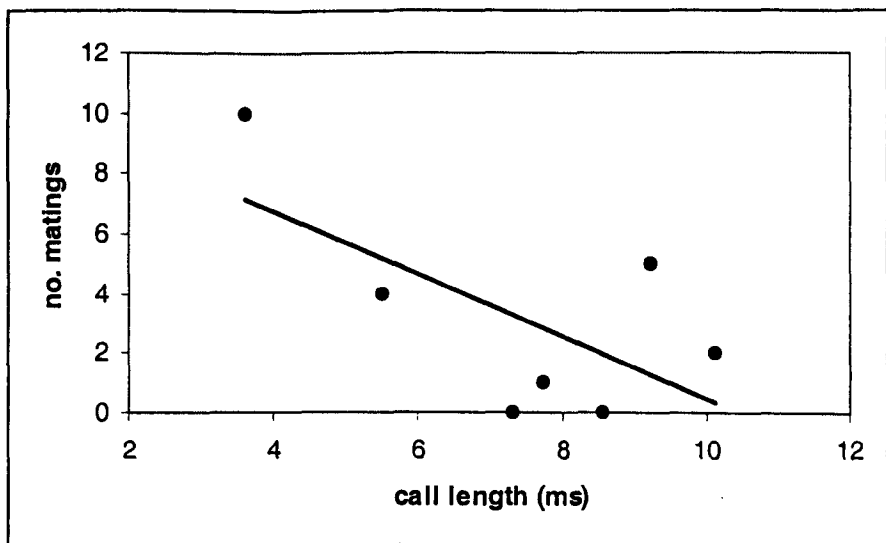
Figure 8.2 The relationship between call length and the total number of matings achieved by males in the unsupplemented breeding group

Nor was there any correlation between call length and the number of different partners males mated with in either the supplemented or the unsupplemented group (Spearman rank correlation, S group (Fig. 8.3): $r_s = -0.256$ N = 8 p>0.05; US group (Fig. 8.4): $r_s = -0.327$ N = 7 p>0.05).
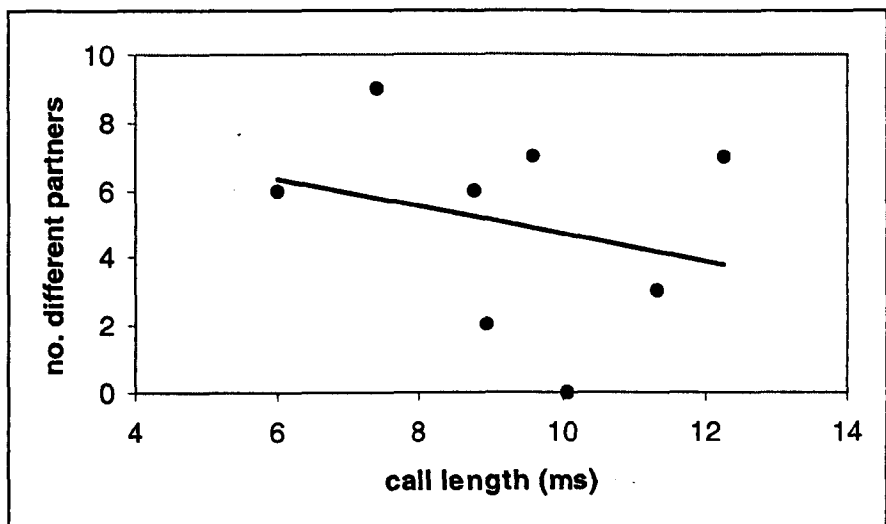
Figure 8.3 The relationship between call length and the number of different partners mated with for males in the supplemented breeding group
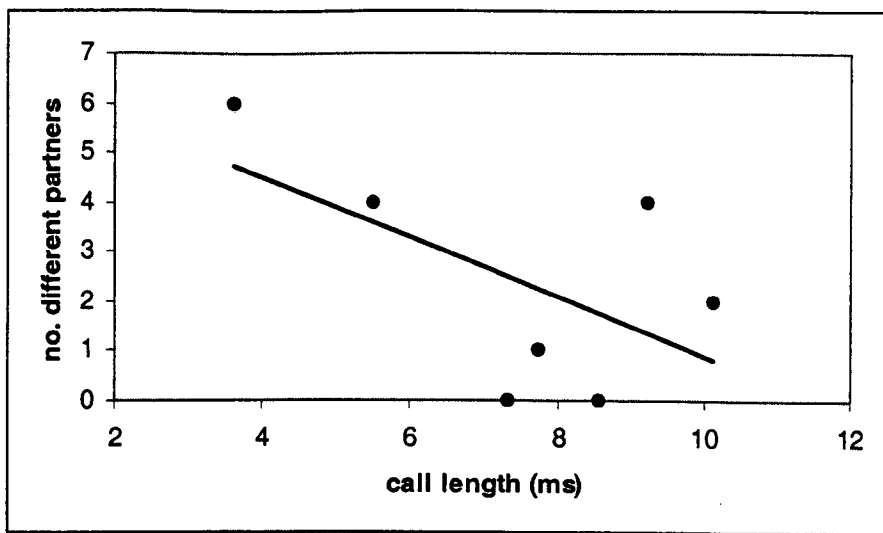
Figure 8.4 The relationship between call length and the number of different partners mated with for males in the unsupplemented breeding group

There was no correlation between inter-call interval and the number of matings achieved by males in either the supplemented or the unsupplemented group (Spearman rank correlation, S group (Fig. 8.5): $r_s = -0.054$, N = 8, p >0.05; US group (Fig. 8.6): $r_s = -0.018$, N = 7, p>0.05).
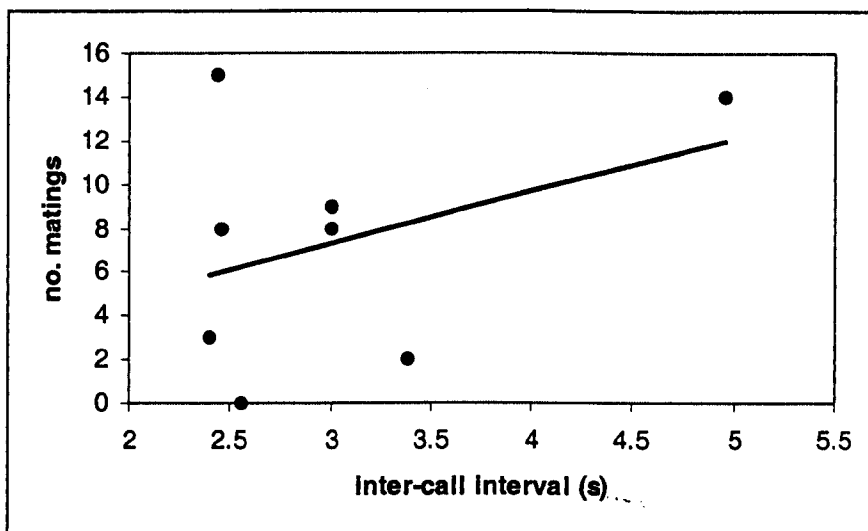
Figure 8.5 The relationship between inter-call interval and the total number of matings achieved by males in the supplemented breeding group
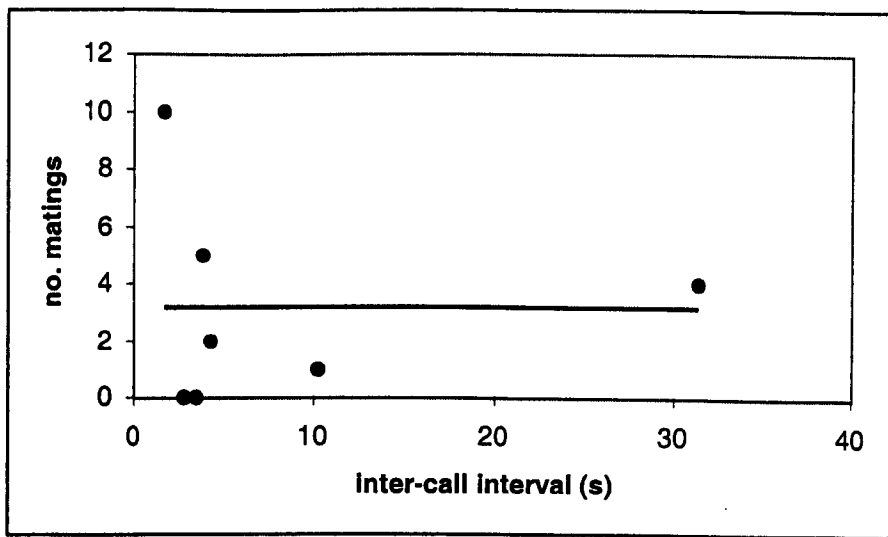
Figure 8.6 The relationship between inter-call interval and the total number of matings achieved by males in the unsupplemented breeding group

There was no correlation between inter-call interval and the number of different partners males mated with in either the supplemented or the unsupplemented group (Spearman rank correlation, S group (Fig. 8.7): $r_s = -0.218$, N = 8, p >0.05; US group (Fig. 8.8): $r_s = 0.036$, N = 7, p>0.05).
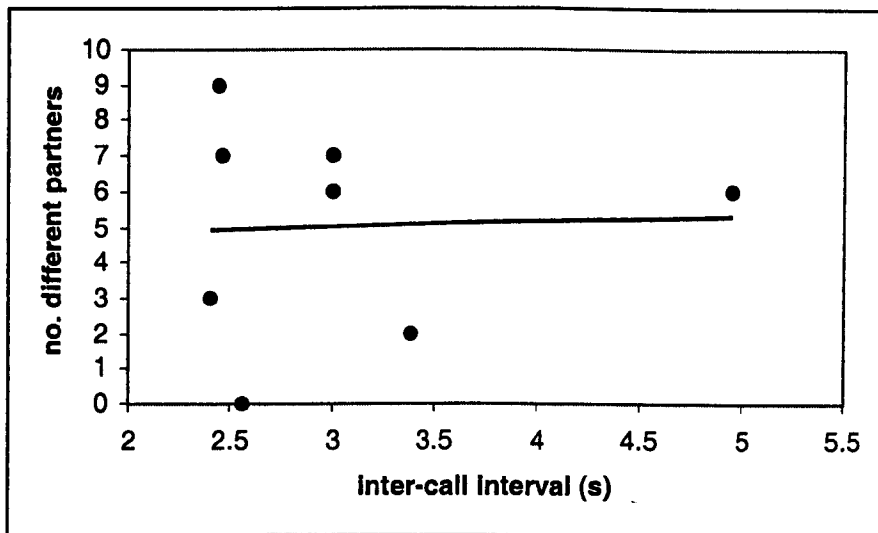
Figure 8.7 The relationship between inter-call interval and the number of different partners mated with for males in the supplemented breeding group
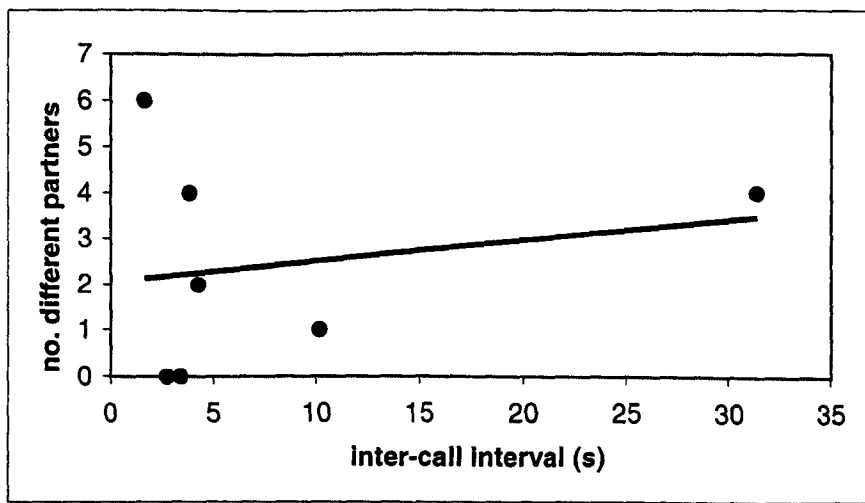
Figure 8.8 The relationship between inter-call interval and the number of different partners mated with for males in the unsupplemented breeding group

There was no correlation between mean bout length and the total number of matings achieved by males in either the supplemented or the unsupplemented group (Spearman rank correlation, S group: (Fig. 8.8): $r_s = 0.421$, N = 10, p>0.05; US group (Fig. 8.10): $r_s = -0.366$, N = 10, p>0.05.
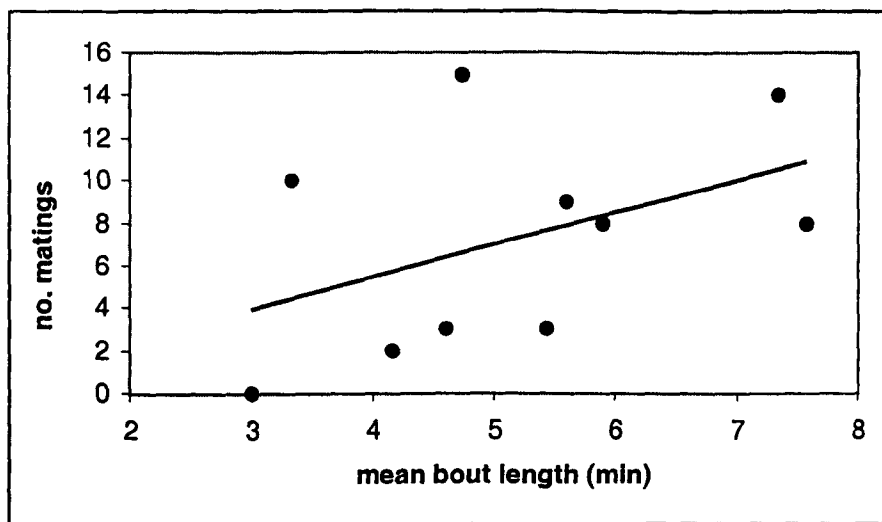
Figure 8.9 The relationship between mean bout length and the total number of matings achieved by males in the supplemented breeding group
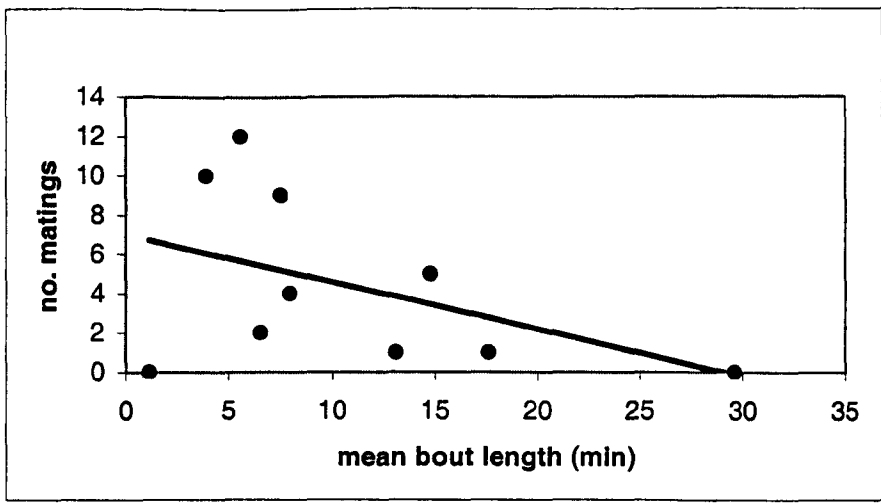
Figure 8.10 The relationship between mean bout length and the total number of matings achieved by males in the unsupplemented breeding group

There was no correlation between calling bout length and the number of different partners males mated with in either the supplemented or the unsupplemented group (Spearman rank correlation, S group (Fig. 8.11): $r_s = 0.438$, N = 10, p>0.05; US group (Fig. 8.12): $r_s = -0.359$, N = 10, p>0.05).
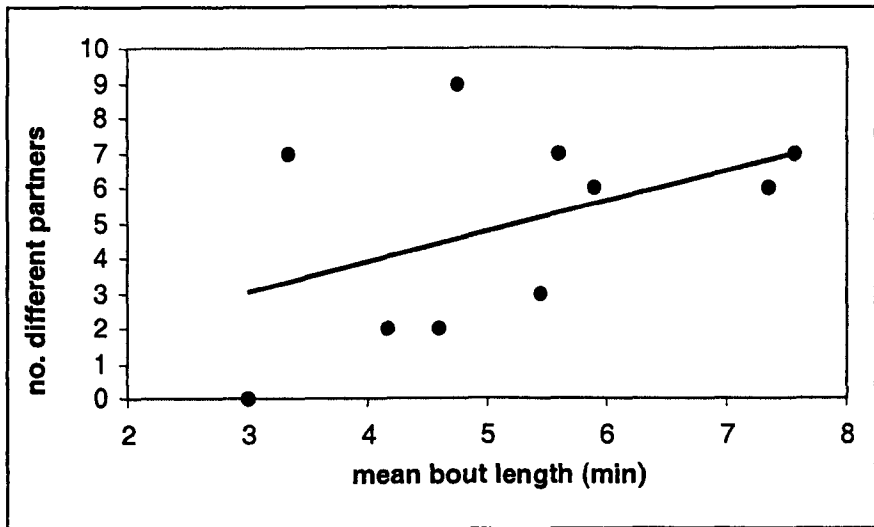
Figure 8.11 The relationship between mean bout length and the number of different partners mated with for males in the supplemented breeding group
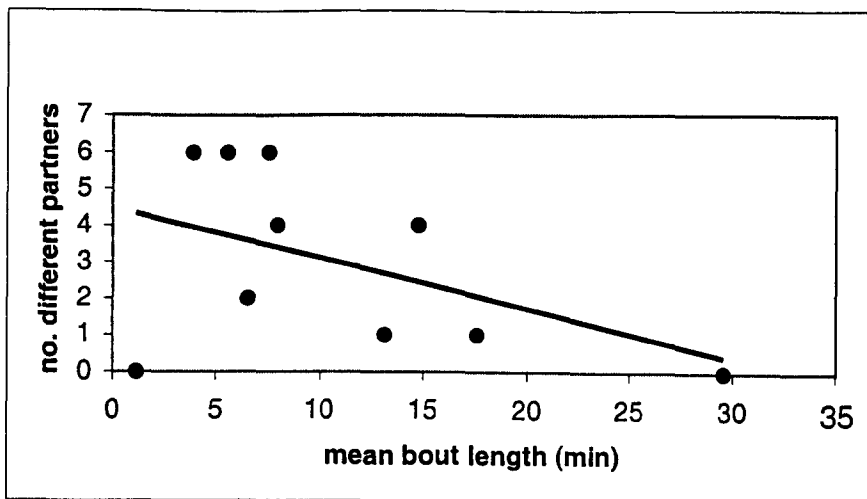
Figure 8.12 The relationship between mean bout length and the number of different partners mated with for males in the unsupplemented breeding group

There was a significant correlation between the percentage amount of time males spent calling and the total number of matings they achieved in the unsupplemented group, with males who called for longer gaining more matings (Spearman rank correlation, $r_s = 0.713$, $N = 10$, $p<0.05$; Fig. 8.13). There was no correlation between the percentage amount of time spent calling and number of matings achieved in the supplemented breeding group (Fig. 8.14: $r_s = -0.085$, $N = 10$, $p>0.050$).
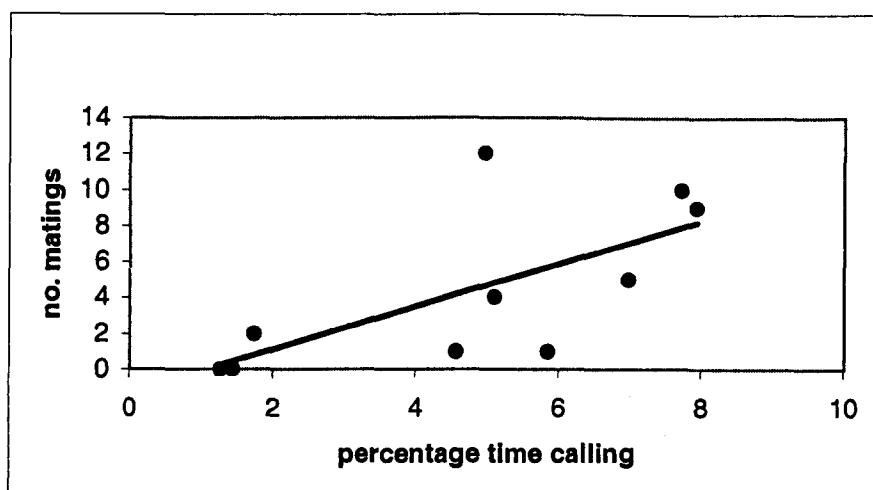
Figure 8.13 The relationship between percentage time calling and the total number of matings for males in the unsupplemented breeding group
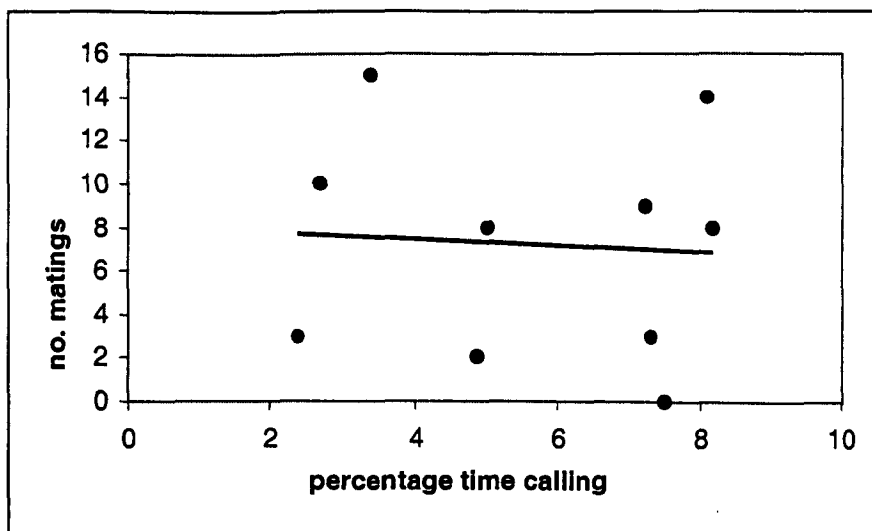
Figure 8.14 The association between percentage time spent calling and the total number of matings achieved for males in the supplemented breeding group

There was a significant correlation between the percentage time males spent calling and the number of different partners mated with in the unsupplemented group, with males who called for longer mating with significantly more different partners (Spearman rank correlation, $r_s = 0.774$, N = 10, p<0.05; Fig. 8.15). There was no correlation between the percentage time spent calling and the number of different partners males mated with in the supplemented group (Fig. 8.16: $r_s = -0.148$, N =10, p>0.05).
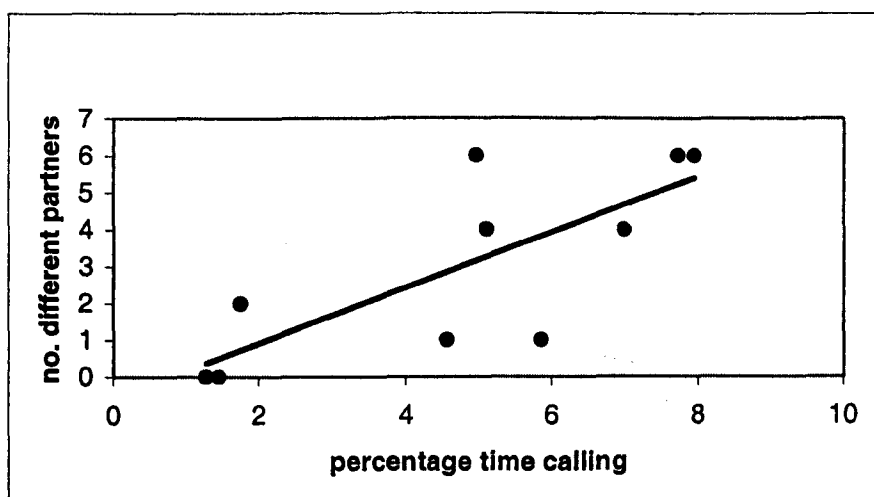
Figure 8.15 The relationship between percentage time spent calling and the number of different partners males mated with in the unsupplemented breeding group
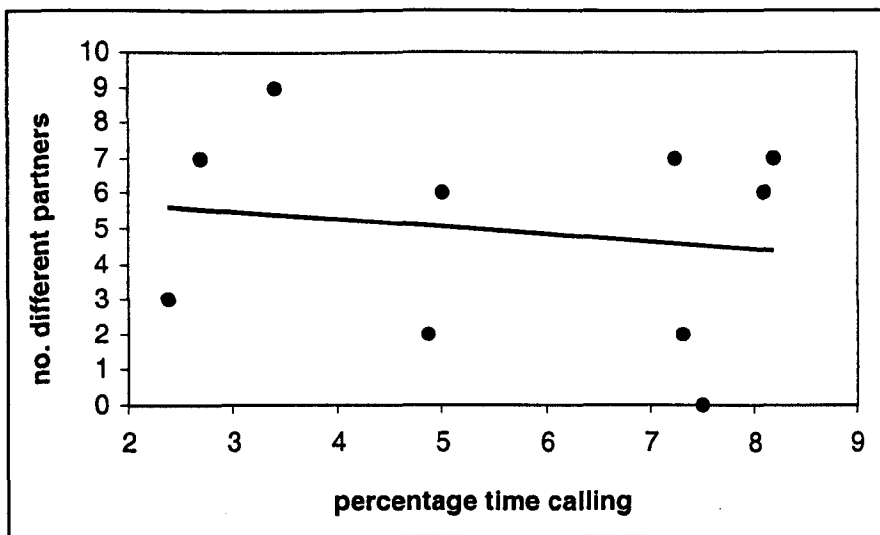
Figure 8.16 The relationship between percentage time spent calling and the number of different partners males mated with in the supplemented breeding group

8.3 Discussion

Males who called for a greater proportion of their time gained more matings and mated with a greater number of different partners, though this relationship was significant only for males in the unsupplemented breeding group. By increasing their amount of calling relative to others, males increase the chance that they will be heard by a receptive female, which may translate into greater mating success.

There may be disadvantages to calling for a greater proportion of the time, however, since calling may attract predators or increase the chance of attack by any parasitoid flies that orient to the calls of stridulating males (Cade, 1975; Zuk *et al.*, 1995; Cade *et al.*, 1996; Zuk *et al.*, 1996).

There was no evidence that any other aspects of calling behaviour or any characteristics of the call itself affected male mating success. Since multiple mating increases fecundity (Section 5.2.9), it appears to be to the female's advantage simply to mate with as many males as possible, so it is perhaps not surprising that there is no evidence that females choose mates on the basis of their calls. As I showed in Chapter 4, the characteristics of the

call and the calling behaviour of the male provide little or no information to the female, at least about the size, level of asymmetry or diet of the male concerned.

As discussed in Section 4.3.3, there may be selection pressure on call length in *L. punctatissima* because of the requirement for the female response to fall in a narrow time window after the of the male call. The briefness of the call also limits complexity, e.g. the number of syllables and the time between them. The nature of short signals places limits on the amount of information they can contain (Robinson, 1980).

One possible factor which may have shaped the unusual signalling system in *L. punctatissima* is predation (Robinson, 1980). *L. punctatissima* is a well-camouflaged animal but is more likely to be located by visually hunting predators as it stridulates; by limiting the amount of tegminal movement individuals may lessen the risk of being eaten. The ultrasonic frequency of the call is out of the detectable range for many predatory species, but would give positional information to any predator capable of detecting ultrasounds (Robinson, 1980). During the night *L. punctatissima* is at risk from echo-locating bats. Four species of British bats have been shown to take insects from vegetation (Blackmore, 1964); all these species produce cries which overlap in frequency with the call of *L. punctatissima*. By keeping its call brief, *L. punctatissima* reduces the chance of being located by a bat.

Ultrasonic frequencies are more susceptible than sonic frequencies to environmental attenuation as they travel through the air (Michelsen & Larson, 1983); this coupled with the short response window in this species places a limit on the distances over which individuals can communicate. Consequently, the maximum distance for phonotaxis in *L. punctatissima* is only a few metres (Zimmerman *et al.*, 1989). The short response window may have been the result of predation pressure, since a short communication distance

reduces the distance a male has to travel to mate with a female and, therefore, the period during which he is at higher risk of being located by a visually-hunting predator. Since adults tend to be thinly distributed in the wild, a possible consequence of the short communication distance is that males and females that are able to mate should respond to the first signal they hear; if they do not, they may not get another opportunity to mate.

8.4 Summary

There was some evidence that an increase in the total amount of time spent calling increases male mating success. No other characteristics of the male call were correlated with mating success, however. The brevity of the male call limits the amount of information it can contain and it is therefore unlikely to offer females much opportunity to discriminate between prospective mates. Both males and females probably respond to and mate with respectively the first female or the first male they hear, which is likely to be the closest one.

9   General Discussion

As discussed in the introduction to this thesis, opportunities for mate choice by both males and females in *L. punctatissima* occur at two stages of the mating system. First, females could choose on the basis of the male call (Crankshaw, 1979; Gwynne, 1982) and males on the basis of the female response. Second, once the male has approached the female both sexes could choose on the basis of proximate cues such as size, asymmetry, smell, vibration or sexual behaviour.

However, there is no evidence from this study that females were choosing males on the basis of their call characteristics. It may be that females choose on the basis of some call characteristic that I have not measured, but it seems likely that, as predicted by Robinson (1986) the amount of information that can be contained in such a brief call is so small that mate choice is not possible. I did not analyse the call characteristics of females so it is not possible to say whether or not males showed choice on the basis of the female response but, since the female response is even briefer than the male call, male mate choice on the basis of the female response is even more unlikely.

The observation that larger males mate more often than smaller males in this study may be as a consequence of female choice for larger males or because larger males are more successful in male-male contests over females. It could also be due to larger males having more resources at their disposal which allows them to produce spermatophores at a faster rate than smaller males. Although larger males produce larger spermatophores, females who mate with larger males do not appear to benefit in terms of their reproductive success, since the number or weight of eggs they lay is not related to the total amount of spermatophore material they receive.

There was a tendency for larger females to mate more than smaller females. This could be the result of male choice for larger females or an increased motivation to mate in larger females. Males who mate with larger females do not appear to benefit in terms of their reproductive success, since the number or weight of eggs females lay is not related to their size.

There was no assortative mating based on size, which suggests that male choice for large females and female choice for large males cannot both be operating. There is also some evidence against mate choice on the basis of size, in that the chance of being rejected as a mate does not appear to be related to size, either for males or females.

The level of body asymmetry had no effect on mating success, either in males or females.

There was no assortative mating with respect to diet. In the half and half group supplemented males mated more often than unsupplemented males with the converse being true for females. This could be the result of males choosing to mate with unsupplemented females, or females choosing to mate with supplemented males, or a combination of the two. Unsupplemented females lay more eggs than supplemented females following their first mating so males may gain an advantage by mating with these females, especially early in the season when they are more likely to be virgin. There is some evidence against mate choice on the basis of diet, however, in that the diet of the partner had no effect on whether they were accepted or rejected as mates, and alternative explanations may be more likely. Females could be more highly motivated to mate when their diet is restricted if nutrients in the spermatophore can compensate for their poor nutrition. Supplemented males are likely to have greater energy reserves as a result of their better diet, and can probably produce spermatophores more quickly than unsupplemented males; they may also be better able to compete with other males than unsupplemented males.

There is considerable variation in the number of eggs laid by females so it would be advantageous to males if they could mate with females that are the most fecund. Most of the variation in the fecundity of females in this study was due to the number of times they mated, and female fecundity may depend on the amount of sperm they can acquire. There was no indication that size, diet or asymmetry had any effect on female fecundity. However, it is possible that factors which were not investigated, such as genetic compatibility or the level of parasitic infection, could influence the number of eggs a female lays and could be the basis of choice.

Male reproductive success is highly dependent on the number of matings achieved. The greater mating success of large/supplemented males could be a result in part of male-male competition, but the evidence suggests that an important factor affecting mating success is how quickly the male can produce spermatophores. The increased energy reserves which may result from both larger size and a better quality diet are likely to increase the rate at which males can produce spermatophores. There may be pressure on males to produce spermatophores as quickly as possible early in the season so as to maximize the chance of mating with a virgin female and fertilizing her possibly high quality eggs. If males are spermatophore limited, pseudocopulatory behaviour and interference, which may both lead to a another male 'wasting' a spermatophore, could be useful strategies in reducing competition from other males.

The increase in female reproductive success with the number of matings achieved does not seem to be due to the number of spermatophores eaten. Unsupplemented females did mate more often than supplemented females, however, so the spermatophore may be able to compensate for a poor diet in some way. Some evidence to support this comes from the fact that males give larger spermatophores to unsupplemented females than they do to supplemented females. It is possible that the quality of eggs varies with diet irrespective of

their size or weight. I was unable to measure the hatching success of eggs, however, so it was not possible to test this in the laboratory.

It is difficult to determine whether mate choice operates in *L. punctatissima* based on the results of this study, as the data did not allow me to discrimination between various alternatives. However data from a wild population of *L.punctatissma* indicate that sexually mature adults appear to be thinly distributed in the wild such that mate choice may be a luxury they cannot afford. Females need to mate as often as possible to maximise the number of eggs they can lay and females that are too choosy of prospective mates may suffer as a result of reduced fecundity. Also from both a male and female perspective, there appears to be little advantage in choosing one mate over another, at least in terms of the factors investigated in this study. Males do not benefit from mating with larger or less asymmetric females, though there is some advantage in mating with a virgin female, especially if she is unsupplemented, because females lay heavier eggs after their first mating and unsupplemented females also lay more eggs. Although larger males did produce larger spermatophores, these did not seem to increase female reproductive success, so choosing a larger male would not benefit a female in that respect. These results indicate that mate choice probably plays a relatively unimportant role in this species.

References

Alatalo, R. V., Hoglund, J. & Lundberg, A. (1988) Sexual selection models and patterns of variation in tail ornaments in birds. *Biological Journal of the Linnean Society*, **34**, 363-374.

Alexander, R. D. (1961) Aggressiveness, territoriality and sexual behaviour in field crickets. *Behaviour*, **17**, 130-223.

Andersson, M. (1994) *Sexual Selection*. Princeton, New Jersey: Princeton University Press.

Andersson, M. & Iwasa, Y. (1996) Sexual selection. *Trends in Ecology & Evolution*, **11**, 53-58.

Ankey, C. D. (1977) Male size and mate selection in lesser snow geese. *Evolutionary Theory*, **3**, 143-148.

Arak, A. (1988) Female mate selection in the natterjack toad: active choice or passive attraction? *Behavioral Ecology & Sociobiology*, **22**, 317-327.

Arnqvist, G. & Thornhill, R. (1998) Evolution of animal genitalia: patterns of phenotypic and genotypic variation and condition of dependence of genital and non-genital morphology in water striders (Heteroptera: Gerridae: Insecta). *Genetical Research*, **71**, 193-212.

Bailey, W. J. (1985) Acoustic cues for female choice in bushcrickets (Tettigoniidae). In: *Acoustic and Vibrational Communication in Insects* (Ed. by Kalmring, K. & Elsner, N.), pp. 101-110. Berlin and Hamburg: Paul Parey.

Bailey, W. J., Withers, P. C., Endersby, M. & Gaull, K. (1993) The energetic costs of calling in the bush-cricket *Requena verticalis* (Orthoptera, Tettigoniidae, Listroscelidinae). *Journal of Experimental Biology*, **178**, 21-37.

Bateman, A. J. (1948) Intrasexual selection in *Drosophila. Heredity*, **2**, 349-368.

Bateman, P. W. (1998) Assortative mating by both sexes in the cricket *Platygryllus primiformis. Transactions of the American Entomological Society*, **124**, 63-68.

Bell, P. D. (1980) Opportunistic feeding by the female tree cricket, *Oecanthus nigricornis. Canadian Entomologist*, **112**, 431-432.

Belovsky, G. E., Slade, J. B. & Chase, J. M. (1996) Mating strategies based on foraging ability: an experiment with grasshoppers. *Behavioral Ecology*, **7**, 438-444.

Berglund, A., Rosenqvist, G. & Svensson, I. (1989) Reproductive success of females limited by males in two pipefish species. *American Naturalist*, **133**, 506-516.

Bjorksten, T., David, P., Pomiankowski, A. & Fowler, K. (1999) Fluctuating asymmetry of sexual and nonsexual traits in stalk-eyed flies: a poor indicator of developmental stress and genetic quality. *Journal of Evolutionary Biology*, **13**, 89-97.

Boldyrev, B. T. (1915) Contributions a l'etude de la structure des spermatophores et des particularites de la copulation chez Locustidae et Grylloidea. *Horae Societatis Entomologicae Rossicae*, **41**, 1-245.

Borgia, G. (1979) Sexual selection and the evolution of mating systems. In: *Sexual Selection and Reproductive Competition in Insects* (Ed. by Blum, M. S. & Blum, N. A.), pp. 19-80: Academic Press.

Boue, J., Boue, A. & Lazar, P. (1975) Retrospective and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions. *Teratology*, **12**, 11-25.

Bowen, B. J., Codd, C. G. & Gwynne, D. T. (1984) The katydid spermatophore (Orthoptera: Tettigoniidae): male investment and its fate in the mated female. *Australian Journal of Zoology*, **32**, 23-31.

Boyan, G. S. (1980) Auditory neurones in the brain of the cricket *Gryllus bimaculatus* (De Geer). *Journal of Comparative Physiology*, **140**, 81-93.

Bradley, B. P. (1980) Developmental stability of *Drosophila melanogaster* under artificial and natural selection in constant and fluctuating environments. *Genetics*, **95**, 1033-1042.

Brown, W. D., Wideman, J., Andrade, M. C. B., Mason, A. C. & Gwynne, D. T. (1996) Female choice for an indicator of male size in the song of the black-horned tree cricket, *Oecanthus nigricornis* (Orthoptera: Gryllidae: Oecanthinae). *Evolution*, **50**, 2400-2411.

Bruckner, D. (1976) The influence of genetic variability on wing symmetry in honeybees (*Apis mellifera*). *Evolution*, **30**, 100-108.

Burley, N. (1981) Sex-ratio manipulation and selection for attractiveness. *Science, New York*, **211**, 721-722.

Cade, W. (1975) Acoustically orientating parasitoids: fly phonotaxis to cricket song. *Science, New York*, **190**, 1312-1313.

Cade, W. H., Ciceran, M. & Murray, A.-M. (1996) Temporal patterns of parasitoid fly

(*Ormia ochracea*) attraction to field cricket song (*Gryllus integer*). *Canadian*

Journal of Zoology, **74,** 393-395.

Calos, J. B. & Sakaluk, S. K. (1998) Paternity of offspring in multiply-mated female

crickets: the effect of nuptial food gifts and the advantage of mating first.

Proceedings of the Royal Society of London, Series B: Biological Sciences, **265,**

2191-2195.

Campanella, P. J. & Wolf, L. L. (1974) Temporal leks as a mating system in a temperate

zone dragonfly (Odonata: Anisoptera). I. *Plathemis lydia* (Drury). *Behaviour*, **51,**

233-261.

Clarke, G. M. & McKenzie, J. A. (1992) Fluctuating asymmetry as a quality control

indicator for insect mass rearing projects. *Journal of Ecological Entomology*, **85,**

2045-2050.

Clutton-Brock, T. H. & G.A., P. (1992) Potential reproductive rates and the operation of

sexual selection. *The Quarterly Review of Biology*, **67,** 437-456.

Clutton-Brock, T. H., Hiraiwa-Hasegawa, M. & Robertson, A. (1989) Mate choice on

fallow deer leks. *Nature, London*, **340,** 463-465.

Cole, C. J. & Townsend, C. R. (1983) Sexual behaviour in unisexual lizards. *Animal*

Behaviour, **31,** 724-728.

Cotton, D. C. F. (1980) Distribution records of Orthoptera (Insecta) from Ireland. *Bulletin*

of the Irish Biogeographical Society, **4,** 13-22.

Coulson, J. C. (1966) The influence of the pair bond and age on the breeding biology of the kittiwake gull *Rissa tridactyla*. *Journal of Animal Ecology*, **35**, 269-279.

Crankshaw, O. S. (1979) Female choice in relation to calling and courtship songs in *Acheta domesticus*. *Animal Behaviour*, **27**, 1274-1275.

Crespi, B. J. (1989) Causes of size assortative mating in arthropods. *Animal Behaviour*, **38**, 980-100.

Dale, S. & Slagsvold, T. (1996) Mate choice on multiple cues decision rules and sampling strategy in female pied flycatchers. *Behaviour*, **133**, 903-944.

Daly, M. & Wilson, M. (1984) A sociobiological analysis of human infanticide. In: *Infanticide: Comparative and Evolutionary Perspectives* (Ed. by Hausfater, G. & Hrdy, S. B.), pp. 487-502. New York: Aldine.

Darwin, C. (1859) *On the Origin of Species by Means of Natural Selection*. London: Murray.

Darwin, C. (1874) *The Descent of Man and Selection in Relation to Sex*. London: Murray.

Del Castillo, R. C., Nunez-Farfan, J. & Cano-Santana, Z. (1999) The role of body size in mating success of *Sphenarium purparescens* in central Mexico. *Ecological Entomology*, **24**, 146-155.

Deura, K. & Hartley, J. C. (1982) Initial diapause and embryonic development in the speckled bush-cricket, *Leptophyes punctatissima*. *Physiological Entomology*, **7**, 253-262.

Dewsbury, D. A. (1982) Ejaculate cost and male choice. *American Naturalist*, **119**, 601-610.

Dobler, S., Heller, K. G. & von Helversen, O. (1994) Song pattern-recognition and an auditory time window in the female bush-cricket *Ancistrura nigrovittata* (Orthoptera, Phaneropteridae). *Journal of Comparative Physiology, A: Sensory, Neural and Behavioral Physiology*, **175**, 67-74.

Dobzhansky, T. (1937) *Genetics and the Origin of Species*. New York: Columbia University Press.

Doherty, J. A. (1985) Trade-off phenomena in calling song recognition and phonotaxis in the cricket *Gryllus bimaculatus* Dee Geer. *Journal of Comparative Physiology, A: Sensory, Neural and Behavioral Physiology*, **156**, 787-801.

Downhower, J. F. & Lank, D. B. (1994) Effects of previous experience on mate choice by female mottled sculpins. *Animal Behaviour*, **47**, 369-372.

Duncan, C. J. (1960) The biology of *Leptophyes punctatissima* (Bosc.) (Orthoptera: Tettigoniidae). *Entomologist*, **93**, 76-78.

Eberhard, W. G. (1996) *Female Control: Sexual Selection by Cryptic Female Choice.* Princeton: Princeton University Press.

Eggert, A. K. & Sakaluk, S. K. (1994) Fluctuating asymmetry and variation in the size of courtship food gifts in decorated crickets. *American Naturalist*, **144**, 708-716.

Ehret, G. (1975) Schallsignalle der Hausmaus (*Mus musculus*). *Behaviour*, **52**, 38-55.

Emlen, S. T. & Oring, L. W. (1977) Ecology, sexual selection and evolution of mating systems. *Science, New York*, **197**, 215-222.

Erway, L., Hurley, L. S. & Fraser, A. S. (1970) Congenital ataxia and otolith defects due to manganese deficiency in mice. *Journal of Nutrition*, **100**, 643-654.

Feaver, M. (1977). Aspects of the Behavioral Ecology of Three Species of *Orchelimum*. Ph.D. thesis, University of Michigan.

Field, L. H. & Sandlandt, G. R. (1983) Aggression and mating behaviour in the Stenopelmatidae (Orthoptera: Ensifera) with reference to the New Zealand wetas. In: *Orthopteran Mating Systems: Sexual Competition in a Diverse Group of Insects* (Ed. by Gwynne, D. T. & Morris, G. K.), pp. 87-102. Boulder: Westview Press.

Fisher, R. A. (1915) The Evolution of Sexual Preferences. *Eugenics Review*, **7**, 184-192.

Ford, C. S. (1964) *A Comparative Study of Human Reproduction*. New Haven: Yale University Press.

Forkman, B. & Corr, S. (1996) Influence of size and asymmetry of sexual characteristics in the rooster and hen on the number of eggs laid. *Applied Animal Behavior Science*, **49**, 285-291.

Fox, C. W. (1993a) The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia*, **96**, 146.

Fox, C. W. (1993b) Multiple mating, lifetime fecundity and female mortality of the bruchid beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Functional Ecology*, **7**, 203-208.

Fox, S. F. (1975) Natural selection on morphological phenotypes of the lizard *Uta stansburiana. Evolution*, **29**, 95-107.

Friedel, T. & Gillot, C. (1977). Contribution of male produced proteins to vitellogenesis in Melanoplus sanguinipes. Journal of Insect Physiology, 23, 489-151.

Funk, D. H. (1989). The mating of tree crickets. *Scientific American*, **261**, 42-49.

Gerhardt, H. C. (1982) Sound pattern recognition in some North American treefrogs (Anura: Hylidae): implications for mate choice. *American Zoologist*, **22**, 581-595.

Graham, J. H., Emlen, J. M., Freeman, D. C., Leamy, L. J. & Kieser, J. A. (1998) Directional asymmetry and the measurement of developmental instability. *Biological Journal of the Linnean Society*, **64**, 1-16.

Grubbs, F. E. (1969) Procedures for detecting outlying observations in samples. *Technometrics*, **11**, 1-21.

Gwynne, D. T. (1981) Sexual difference theory: mormon crickets show role reversal in mate choice. *Science, New York*, **213**, 779-780.

Gwynne, D. T. (1982) Mate selection by female katydids (Orthoptera: Tettigoniidae, *Conocephalus nigropleurum*). *Animal Behaviour*, **30**, 734-738.

Gwynne, D. T. (1983) Male nutritional investment and the evolution of sexual differences in Tettigoniidae and other Orthoptera. In: *Orthopteran Mating Systems: Sexual*

Competition in a Diverse Group of Insects (Ed. by Gwynne, D. T. & Morris, G. K.), pp. 35-52. Boulder, Colorado: Westview Press.

Gwynne, D. T. (1984a) Courtship feeding increases female reproductive success in bushcrickets. *Nature, London,* **307,** 361-363.

Gwynne, D. T. (1984b) Male mating effort, confidence of paternity, and insect sperm composition. In: *Sperm Competition and the Evolution of Animal Mating Systems* (Ed. by Smith, R. L.), pp. 117-149. New York: Academic Press.

Gwynne, D. T. (1985) Role reversal in katydids: habitat influences reproductive behaviour (Orthoptera: Tettigoniidae, *Metaballus* sp.). *Behavioral Ecology & Sociobiology,* **16,** 355-361.

Gwynne, D. T. (1986) Courtship feeding in katydids: investment in offspring or in obtaining fertilizations? *American Naturalist,* **128,** 342-352.

Gwynne, D. T. (1988) Courtship feeding in katydids benefits the mating male's offspring. *Behavioral Ecology & Sociobiology,* **22,** 373-377.

Gwynne, D. T. (1990) The katydid spermatophore: evolution of a parental investment. In: *The Tettigoniidae: Biology, Systematics and Evolution* (Ed. by Bailey, W. J. & Rentz, D. C. F.), pp. 27-40. Bathurst, Australia: Crawford House Press.

Gwynne, D. T. (1993) Food quality controls sexual selection in mormon crickets by altering male mating investment. *Ecology,* **74,** 1406-1413.

228

Gwynne, D. T. (1995) Variation in bush-cricket nuptial gifts may be due to common ancestry rather than ecology as taxonomy and diet are almost perfectly confounded. *Behavioral Ecology*, **6**, 458.

Gwynne, D. T. & Bailey, W. J. (1988) Mating system, mate choice and ultrasonic calling in a zaprochiline katydid (Orthoptera: Tettigoniidae). *Behaviour*, **105**, 202-223.

Gwynne, D. T., Bowen, B. J. & Codd, C. G. (1984) The function of the katydid spermatophore and its role in fecundity and insemination (Orthoptera: Tettigoniidae). *Australian Journal of Zoology*, **32**, 15-22.

Gwynne, D. T. & Simmons, L. W. (1990) Experimental reversal of courtship roles in an insect. *Nature, London*, **346**, 172-174.

Haldane, J. B. S. (1949) Disease and evolution. *Richercha Scientifica*, **19**, 68-76.

Hall, M. J. (1983) Social organisation in an enclosed group of red deer (*Cervus elaphus* L.) on Rhum. II. Social grooming, mounting behaviour, spatial organisation and their relationship to dominance rank. *Zeitschrift fur Tierpsychologie*, **61**, 250-262.

Halliday, T. R. (1974) Sexual behaviour of the smooth newt (*Triturus vulgaris*). *Journal of Herpetology*, **8**, 277-292.

Halliday, T. R. (1983) The study of mate choice. In: *Mate Choice* (Ed. by Bateson, P.), pp. 3-32. Cambridge: Cambridge University Press.

Hamilton, W. D. & Zuk, M. (1982) Heritable true fitness and bright birds: a role for parasites? *Science, New York*, **218**, 384-387.

Harris, E. F. & Nweeia, M. T. (1980) Dental asymmetry as a measure of environmental stress in the Ticuna Indians of Columbia. *American Journal of Physical Anthropology*, **53**, 133-142.

Hartley, J. C. (1993) Acoustic behaviour and phonotaxis in the duetting ephippigerines, *Steropleurus nobrei* and *Steropleurus stali* (Tettigoniidae). *Zoological Journal of the Linnean Society*, **107**, 155-167.

Hartley, J. C. & Robinson, D. J. (1976) Acoustic behaviour of both sexes of the speckled bush cricket *Leptophyes punctatissima*. *Physiological Entomology*, **1**, 21-25.

Hartley, J. C., Robinson, D. J. & Warne, A. C. (1974) Female response song in the ephippigerines *Steropleurus stali* and *Platystolus obvius* (Orthoptera, Tettigoniidae). *Animal Behaviour*, **22**, 382-389.

Hartley, J. C. & Warne, A. C. (1972) The developmental biology of the egg stage of Western European Tettigoniidae (Orthoptera). *Journal of Zoology, London*, **68**, 267-298.

Harvey, I. F. & Walsh, K. J. (1993) Fluctuating asymmetry and lifetime mating success are correlated in males of the damselfly *Coenagrion puella* (Odonata: Coenagrionidae). *Ecological Entomology*, **18**, 198-202.

Haug, M., Brain, P. F. & Aron, C. (1990) *Heterotypical Behaviour in Man and Animals*. London: Chapman & Hall.

Hay, D. E. & McPhail, J. D. (1975) Mate selection in three spined sticklebacks. *Canadian Journal of Zoology*, **53**, 441-450.

Hedrick, A. V. (1986) Female preferences for male calling bout duration in a field cricket. *Behavioral Ecology & Sociobiology*, **19**, 73-77.

Hedwig, B. & Elsner, N. (1985) Sound production and sound detection in a stridulating acridid grasshopper. In: *Acoustic and Vibrational Communication in Insects* (Ed. by Kalmring, K. & Elsner, N.), pp. 61-72. Berlin and Hamburg: Paul Parey.

Heller, K. G. (1984) Zur Bioacustik und Phylogenie der Gattung *Poecilimon* (Orthoptera: Tettigoniidae: Phaneropterinae). *Zool Jahrb (syst)*, **111**, 69-117.

Heller, K. G. & von Helversen, D. (1986) Acoustic communication in phaneropterid bushcrickets: species specific delay of female stridulatory response and matching male sensory time window. *Behavioral Ecology & Sociobiology*, **18**, 189-198.

Heller, K.-G. & von Helversen, D. (1991) Operational sex ratio and individual mating frequencies in two bushcricket species (Orthoptera, Tettigoniidae, *Poecilimon*). *Ethology*, **89**, 221-228.

Heller, K.-G. & Reinhold, K. (1994) Mating effort function of the spermatophore in the bush-cricket *Poecilimon veluchianus* (Orthoptera, Phaneropteridae): support from a comparison of the mating behaviour of two subspecies. *Biological Journal of the Linnean Society*, **53**, 153-163.

Huxley, J. S. (1942) *Evolution, The Modern Synthesis*. London: Allen & Unwin.

Janetos, A. C. (1980) Strategies of female choice: a theoretical analysis. *Behavioral Ecology & Sociobiology*, **7**, 107-112.

Karino, K. (1997) Female mate preference for males having large symmetric fins in the bower-holding cichlid *Cyathopharynx furcifer. Ethology*, **103**, 883-892.

Kindvall, O. (1995) The impact of extreme weather on habitat preference and survival in a metapopulation of the bushcricket *Metrioptera bicolor* in Sweden. *Biological Conservation*, **73**, 51-58.

Kirpichnikov, V. S. (1981) *Genetic Basis of Fish Selection*. Berlin: Springer Verlag.

Lande, R. (1980) Sexual dimorphism, sexual selection and adaptation in polygenic characters. *Evolution*, **34**, 292-305.

Lande, R. (1981) Models of speciation by sexual selection on polygenic characters. *Proceedings of the National Academy of Sciences, USA*, **78**, 3721-3725.

Latimer, W. & Sippel, M. (1987) Acoustic cues for female choice and male competition in *Tettigonia cantans. Animal Behaviour*, **35**, 887-900.

Leamy, L. (1984) Morphometric studies in inbred and hybrid house mice. V. Directional and fluctuating asymmetry. *American Naturalist*, **123**, 579-593.

Leary, R. F., Allendorf, F. W. & Knudson, K. L. (1992) Genetic, environmental and developmental causes of meristic variation in rainbow trout. *Acta Zoologica Fennica*, **191**, 79-95.

Leung, B. & Forbes, M. R. (1997) Fluctuating asymmetry in relation to indices of quality and fitness in the damselfly, *Enallagma ebrium* (Hagen). *Oecologia*, **110**, 472-477.

Ligget, A. C., Harvey, I. F. & Manning, J. T. (1993) Fluctuating asymmetry in *Scatophaga stercoraria* L: successful males are more symmetrical. *Animal Behaviour*, **45**, 1041-1043.

Ludwig, W. (1932) *Das Rechts-Links Problem im Tierreich und beim.* Berlin: Springer Verlag.

Manning, J. T. & Hartley, M. A. (1991) Symmetry and ornamentation are correlated in the peacock's train. *Animal Behaviour*, **42**, 1020-1021.

Markow, T. A. & Ricker, J. P. (1992) Male size, developmental stability and mating success in natural populations of three *Drosophila* species. *Heredity*, **69**, 122-127.

Marshall, J. A. & Haes, E. C. M. (1988) *The Grasshoppers and Allied Insects of Great Britain and Ireland.* Colchester: Harley Books.

Masaki, S., Kataoka, M., Shirato, K. & Nakagahara, M. (1987) The evolutionary differentiation of right and left tegmina in crickets. In: *Evolutionary Biology of Orthopteroid Insects* (Ed. by Baccetti, B.), pp. 347-357. Chichester: Horwood.

Mayr, E. (1942) *Systematics and the Origin of Species.* New York: Columbia University Press.

Mays, D. L. (1971) Mating behaviour of nemobiine crickets: *Hygronemobius, Nemobius* and *Pternemobius. Florida Entomologist*, **54**, 113-126.

McKenzie, J. A. & Yen, J. L. (1995) Genotype, environment and the asymmetry phenotype. Dieldrin-resistance in *Lucilia cuprina* (the Australian sheep blowfly). *Heredity*, **75**, 181-187.

Michelsen, A. & Larson, O. N. (1983) Strategies for acoustic communication in complex environments. In: *Neuroethology and Behavioral Physiology* (Ed. by Huber, F. & Markl, H.), pp. 321-331. Berlin, Heidelberg, New York: Springer Verlag.

Mitton, J. B. & Koehn, R. K. (1985) Shell shape variation in the blue mussel, *Mytilus edulis* L., and its association with enzyme heterozygosity. *Journal of Experimental Marine Biology and Ecology*, **90**, 73-80.

Moller, A. P. (1990) Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality. *Animal Behaviour*, **40**, 1185-1187.

Moller, A. P. (1992) Female swallow preference for symmetrical male sexual ornaments. *Nature, London*, **357**, 238-240.

Moller, A. P. (1994a) *Sexual Selection and the Barn Swallow*. Oxford: Oxford University press.

Moller, A. P. (1994b) Sexual selection in the barn swallow (*Hirundo rustica*). IV. Patterns of fluctuating asymmetry and selection against asymmetry. *Evolution*, **48**, 658-670.

Moller, A. P. (1996) Floral asymmetry, embryo abortion, and developmental selection in plants. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **263**, 53-56.

Moller, A. P. (1997) Developmental stability and fitness: a review. *American Naturalist*, **149**, 916-932.

Moller, A. P., Cuervo, J. J., Soler, J. J. & Zamora-Munoz, C. (1996) Horn asymmetry and fitness in gemsbok *Oryx g. gazella. Behavioral Ecology*, **7**, 247-253.

Moller, A. P. & Hoglund, J. (1991) Patterns of fluctuating asymmetry in avian feather ornaments: implications for models for sexual selection. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **245**, 1-5.

Moller, A. P. & Swaddle, J. P. (1997) *Asymmetry, Developmental Stability, and Evolution*. Oxford: Oxford University Press.

Montag, B. A. & Montag, T. W. (1979) Infanticide: a historical perspective. *Minnesota Medicine*, **May,** 368-372.

Moore, M. C., Whittler, J. M., Billy, A. J. & Crews, D. (1985) Male-like behaviour in an all female lizard: relationship to ovarian cycle. *Animal Behaviour*, **33,** 284-289.

Morris, G. K. (1979) Mating systems, paternal investment and aggressive behaviour of acoustic Orthoptera. *Florida Entomologist*, **62,** 9-17.

Morris, G. K., Kerr, G. E. & Fullard, G. H. (1978) Phonotactic preferences of meadow katydids (Orthoptera: Tettigoniidae, *Conocephalus nigropleurum*). *Canadian Journal of Zoology*, **56,** 1479-1487.

Nickle, A. D. (1976) Interspecific differences in frequency and other parameters of pair forming sounds of bush katydids. *Annals of the Entomological Society of America*, **69,** 1136-1144.

Nisbet, I. C. T. (1977) Courtship feeding and clutch size in common terns. In: *Evolutionary Ecology* (Ed. by Stonehouse, B. & Perrins, C.), pp. 101-109. London: Macmillan.

O'Donald, P. (1962) The theory of sexual selection. *Heredity*, **17,** 541-552.

O'Donald, P. (1967) A general model of sexual and natural selection. *Heredity*, **22**, 499-518.

Olsson, M. (1993) Mate preference for large females and assortative mating for body size in the sand lizard *Lacerta agilis. Behavioral Ecology & Sociobiology*, **32**, 337-341.

Otronen, M. (1993) Size assortative mating in the yellow dung fly *Scatophaga stercoraria. Behaviour*, **126**, 63-76.

Otronen, M. (1998) Male asymmetry and postcopulatory sexual selection in the fly *Dryomyza anilis. Behavioral Ecology & Sociobiology*, **42**, 185-191.

Ozernyuk, N. D. (1989) The principle of minimum of energy in ontogenesis and canalisation of developmental processes. *Ontogenesis*, **20**, 117-127.

Palmer, A. P. (1990) Fluctuating asymmetry: a primer. In: *Developmental Instability: Its Origins and Evolutionary Implications* (Ed. by Markow, T. A.), pp. 335-364. Netherlands: Kluwer Academic Press.

Palmer, A. R. (1999) Detecting publication bias in meta-analyses: a case study of fluctuating asymmetry and sexual selection. *American Naturalist*, **154**, 220-233.

Palmer, A. R. & Strobeck, C. (1986) Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology and Systematics*, **17**, 391-421.

Parker, G. A. (1970) Sperm competition and its evolutionary significance in the insects. *Biological Reviews*, **45**, 525-567.

Parker, G. A. (1983) Mate quality and mating decisions. In: *Mate Choice* (Ed. by Bateson, P.), pp. 141-166. Cambridge: Cambridge University Press.

Parsons, P. A. (1962) Maternal age and developmental viability. *Journal of Experimental Biology*, **39**, 251-260.

Parsons, P. A. (1964) Parental age and the offspring. *Quarterly Review of Biology*, **39**, 258-275.

Parsons, P. A. (1992) Fluctuating asymmetry: a biological monitor of environmental and genomic stress. *Heredity*, **68**, 361-364.

Partridge, L. & Farquhar, M. (1983) Lifetime mating success of male fruitflies (*Drosophila melanogaster*) is related to their size. *Animal Behaviour*, **31**, 871-877.

Petrie, M., Halliday, T. & Sanders, C. (1991) Peahens prefer peacocks with elaborate trains. *Animal Behaviour*, **41**, 323-331.

Phelan, J. P. & Austad, S. N. (1994) Selecting animal models of human aging: inbred strains often display less biological uniformity than F1 hybrids. *Journal of Gerontology*, **49**, B1-B11.

Pickford, R. & Gillott, C. (1971) Insemination in the migratory grasshopper *Melanoplus sanguinipes*. *Canadian Journal of Zoology*, **49**, 1583-1588.

Picton, H. D., Palmisciano, D. & Nelson, G. (1990) Fluctuating asymmetry and testing isolation of Montana grizzly bear populations. *International Conference on Bear Research and Management*, **8**, 421-424.

Pusey, A. E. (1980) Inbreeding avoidance in chimpanzees. *Animal Behaviour*, **28**, 243-252.

Rand, A. S. & Ryan, M. J. (1981) The adaptive significance of a complex vocal repertoire in a neotropical frog. *Zeitschrift fur Tierpsychologie*, **57**, 209-214.

Real, L. (1990) Search theory and mate choice. 1. Models of single-sex discrimination. *American Naturalist*, **136**, 376-404.

Reinhold, K. (1999) Paternal investment in *Poecilimon veluchianus* bushcrickets: beneficial effects of nuptial feeding on offspring viability. *Behavioral Ecology & Sociobiology*, **45**, 293-299.

Reinhold, K. & Heller, K. G. (1993) The ultimate function of nuptial feeding in the bush-cricket *Poecilimon veluchianus* (Orthoptera, Tettigoniidae, Phaneropterinae). *Behavioral Ecology & Sociobiology*, **32**, 55-60.

Reynolds, J. D. & Cote, I. M. (1995) Direct selection on mate choice - female red lipped blennies pay more for better mates. *Behavioral Ecology*, **6**, 175-181.

Ridley, M. (1988) Mating frequency and fecundity in insects. *Biological Reviews*, **63**, 509-549.

Ritchie, M. G., Couzin, I. D. & Snedden, W. A. (1995) What's in a song? Female bushcrickets discriminate against the song of older males. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **262**, 21-27.

Ritchie, M. G., Sunter, D. & Hockham, L. R. (1998) Behavioral components of sex role reversal in the tettigoniid bushcricket *Ephippiger ephippiger*. *Journal of Insect Behavior*, **11**, 481-491.

Rivero, A., Alatalo, R. V., Kotiaho, J. S., Mappes, J. & Parri, S. (2000) Acoustic signalling in a wolf spider: can call characteristics predict male quality? *Animal Behaviour*, **60**, 187-194.

Robinson, D. J. (1980) Acoustic communication between the sexes of the bush cricket, *Leptophyes punctatissima*. *Physiological Entomology*, **5**, 183-189.

Robinson, D. (1990) Acoustic communication between the sexes in bushcrickets. In: *The Tettigoniidae: Biology, Systematics and Evolution* (Ed. by Bailey, W. J. & Rentz, D. C. F.), pp. 112-129. Bathurst, Australia: Crawford House Press.

Robinson, D., Rheinlaender, J. & Hartley, J. C. (1986) Temporal parameters of male-female sound communication in *Leptophyes punctatissima*. *Physiological Entomology*, **11**, 317-323.

Roelke, M. E., Martenson, J. S. & O'Brien, S. J. (1993) The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Current Biology*, **3**, 340-350.

Ruban, G. I. (1992) Plasticity of development in natural and experimental populations of Siberian sturgeon (*Acipenser baeri*) Brandt. *Acta Zoologica Fennica*, **191**, 43-46.

Rutowski, R. L. (1982) Epigametic selection by males as evidenced by courtship partner preference in the checkered white butterfly. *Animal Behaviour*, **30**, 108-112.

Ryan, M. J. (1985) *The Tungara Frog*. Chicago: University of Chicago Press.

Ryan, M. J. (1991) *Sexual Selection, Sensory Systems and Sensory Exploitation*. Oxford: Oxford University Press.

Sakaluk, S. K. (1984) Male crickets feed females to ensure complete sperm transfer. *Science, New York*, **223**, 609-610.

Sakaluk, S. K. & Smith, R. L. (1988) Inheritance of male parental investment in an insect. *American Naturalist*, **132**, 594-601.

Schatral, A. (1993) Diet influences male-female interactions in the bush-cricket *Requena verticalis* (Orthoptera, Tettigoniidae). *Journal of Insect Behavior*, **6**, 379-388.

Schildberger, K. (1985) Recognition of auditory patterns by identified auditory neurones in the cricket brain. In: *Acoustic and Vibrational Communication in Insects* (Ed. by Kalmring, K. & Elsner, N.), pp. 41-49. Berlin and Hamburg: Paul Parey.

Schneiderman, H. A. & Gilbert, L. I. (1964) Control of growth and development in insects. *Science, New York*, **143**, 325-333.

Sciulli, P. W., Doyle, W. J., Kelly, C., Siegal, P. & Siegal, M. I. (1979) The interaction of stressors in the induction of increased levels of fluctuating asymmetry in the laboratory rat. *American Journal of Physical Anthropology*, **50**, 279-284.

Shuvalov, V. F. & Popov, A. V. (1973) Significance of some parameters of the calling songs of male crickets *Gryllus bimaculatus* for phonotaxis of females. *Journal of Evolutionary Biochemistry and Physiology*, **9**, 177-182.

Siegel, M. I. & Doyle, W. J. (1975) The effects of cold stress on fluctuating asymmetry in the dentition of the mouse. *Journal of Experimental Zoology*, **193**, 385-391.

Simmons, L. W. (1986) Intermale competition and mating success in the field cricket, *Gryllus bimaculatus* (De Geer). *Animal Behaviour*, **34**, 567-579.

Simmons, L. W. (1987) Heritability of a male character chosen by females of the field cricket *Gryllus bimaculatus. Behavioral Ecology & Sociobiology*, **21**, 129-133.

Simmons, L. W. (1988a) The calling song of the field cricket *Gryllus bimaculatus* (De Geer): constraints on transmission and its role in intermale competition and female choice. *Animal Behaviour*, **36**, 380-394.

Simmons, L. W. (1988b) Male size, mating potential and lifetime reproductive success in the field cricket, *Gryllus bimaculatus* (De Geer). *Animal Behaviour*, **36**, 372-379.

Simmons, L. W. (1990) Nuptial feeding in tettigoniids: male costs and the rates of fecundity increase. *Behavioral Ecology & Sociobiology*, **27**, 43-47.

Simmons, L. W. (1991) Female choice and the relatedness of mates in the field cricket *Gryllus bimaculatus*. *Animal Behaviour*, **41**, 493-501.

Simmons, L. W. (1994) Reproductive energetics of the role reversing bush-cricket, *Kawanaphila nartee* (Orthoptera, Tettigoniidae, Zaprochilinae). *Journal of Evolutionary Biology*, **7**, 189-200.

Simmons, L. W. (1995) Correlates of male quality in the field cricket, *Gryllus campestris* L.: age, size, and symmetry determine pairing success in field populations. *Behavioral Ecology*, **6**, 376-381.

Simmons, L. W. (1996). Tegmen asymmetry and its influence on frequency modulation and song preferences of field crickets (*Gryllus campestris* L.). Proceedings of the *XX International Congress of Entomology, Firenze, Italy, August 25-31, 1995*, p. 365.

Simmons, L. W. & Bailey, W. J. (1990) Resource influenced sex roles of zaprochiline tettigoniids (Orthoptera: Tettigoniidae). *Evolution*, **44**, 1853-1868.

Simmons, L. W. & Gwynne, D. T. (1991) The refractory period of female katydids (Orthoptera: Tettigoniidae): sexual conflict over the remating interval? *Behavioral Ecology*, **2**, 276-282.

Simmons, L. W. & Gwynne, D. T. (1993) Reproductive investment in bushcrickets: the allocation of male and female nutrients to the offspring. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **252**, 1-5.

Simmons, L. W. & Ritchie, M. G. (1996) Symmetry in the songs of crickets. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **263**, 1305-1311.

Simmons, L. W., Tomkins, J. L., Kotiaho, J. S. & Hunt, J. (1999) Fluctuating paradigm. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **266**, 593-595.

Simmons, L. W. & Zuk, M. (1992) Variability in call structure and pairing success of male field crickets, *Gryllus bimaculatus*: the effects of age, size and parasite load. *Animal Behaviour*, **44**, 1145-1152.

Simpson, G. G., Golbus, M. S., Martin, A. O. & Sarto, G. E. (1982) *Genetics in Obstetrics and Gynaecology*. New York: Grune & Stratton.

Siva-Jothy, M. T. (1987) Variation in copulation duration and the resultant degree of sperm removal in *Orthetrum cancellatum. Behavioral Ecology & Sociobiology*, **20**, 147-151.

Siva-Jothy, M. T. & Tsubaki, Y. (1989) Variation in copulation duration in *Mnaia pruinosa pruinosa* Selys (Odonata: Calopterygidae) 1. Alternative mate-securing tactics and sperm precedence. *Behavioral Ecology & Sociobiology*, **24**, 39-45.

Smith, R. L. (1979) Paternity assurance and altered roles in the mating behaviour of giant

waterbugs *Abedus herberti. Animal Behaviour*, **27**, 716-725.

Sokal, R. & Rohlf, F. J. (1995) *Biometry*. San Francisco: W. H. Freeman.

Sorenson, L. G. & Derrickson, S. R. (1994) Sexual selection in the northern pintail (*Anas*

acuta): the importance of female choice versus male-male competition in the

evolution of sexually selected traits. *Behavioral Ecology & Sociobiology*, **35**, 389-

400.

Soule, M. E. (1979) Heterozygosity and developmental stability: another look. *Evolution*,

33, 396-401.

Spooner, J. D. (1968) Pair-forming acoustic systems of phaneropterine katydids

(Orthoptera Tettigoniidae). *Animal Behaviour*, **16**, 197-212.

Stebbings, R. E. (1977) Order Chiroptera: bats. In: *The Handbook of British Mammals* (Ed.

by Corbet, G. B. & Southern, H. N.), pp. 68-128. Oxford: Blackwell Scientific

Publications.

Swaddle, J. P. & Cuthill, I. C. (1994) Preference for symmetric males by female zebra

finches. *Nature, London*, **367**, 165-166.

Swaddle, J. P. & Witter, M. S. (1994) Food, feathers and fluctuating asymmetry.

Proceedings of the Royal Society of London, Series B: Biological Sciences, **225**, 147-

152.

Thornhill, R. (1976a) Sexual selection and nuptial feeding behaviour in *Bittacus apicalis*

(Insecta: Mecoptera). *American Naturalist*, **110**, 529-548.

Thornhill, R. (1976b) Sexual selection and paternal investment in insects. *American Naturalist*, **110**, 153-163.

Thornhill, R. (1992) Fluctuating asymmetry and the mating system of the Japanese scorpionfly, *Panorpa japonica*. *Animal Behaviour*, **44**, 867-879.

Tomkins, J. L. & Simmons, L. W. (1995) Patterns of fluctuating asymmetry in earwig forceps: no evidence for reliable signalling. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **259**, 89-96.

Trivers, R. L. (1972) Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man, 1871-1971* (Ed. by Campbell, B.), pp. 137-179. Chicago: Aldine.

Vahed, K. (1995) The evolution and function of the spermatophylax in bushcrickets (Orthoptera: Tettigoniidae). Ph.D. thesis, University of Nottingham.

Vahed, K. (1996) Prolonged copulation in oak bushcrickets (Tettigoniidae: Meconematinae: *Meconema thalassinum* and *M. meridionale*). *Journal of Orthopteran Research*, **5**, 199-204.

Vahed, K. (1998) The function of nuptial feeding in insects: a review of empirical studies. *Biological Reviews*, **73**, 43-78.

Vahed, K. & Gilbert, F. (1996a) Differences across taxa in nuptial gift size correlate with differences in sperm number and ejaculate volume in bushcrickets (Orthoptera: Tettigoniidae). *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **263**, 1257-1265.

Vahed, K. & Gilbert, F. S. (1996b) No effect of nuptial gift consumption on female reproductive output in the bushcricket *Leptophyes laticauda* Friv. *Ecological Entomology*, **22**, 479-482.

van Staaden, M. J. (1997) Sexual signalling in bladder grasshoppers: tactical design for maximizing calling range. *Journal of Experimental Biology*, **200**, 2597-2608.

Van Valen, L. (1962) A study of fluctuating asymmetry. *Evolution*, **16**, 125-142.

Verrel, P. A. (1995) Males choose larger females as mates in the salamander *Desmognathus santeelah*. *Ethology*, **99**, 162-171.

Waage, J. K. (1979) Dual function of the damselfly penis: sperm removal and transfer. *Science, New York*, **203**, 916-918.

Waddington, C. H. (1940) *Organisers and Genes*. Cambridge: Cambridge University Press.

Wagner, W.E. & Hoback, W.W. (1999) Nutritional effects on male calling behaviour in the variable field cricket. *Animal Behaviour*, **57**, 89-95.

Wagner, W.E. & Reiser, M.G. (2000) The importance of calling song and courtship song in female mate choice in the variable field cricket. *Animal Behaviour*, **59**, 1219-1226.

Walker, T. J. (1978) Post-copulatory behaviour of the two spotted tree cricket, *Neoxabea bipunctata*. *Florida Entomologist*, **61**, 39-40.

Walker, T. J. (1983) Diel calling patterns in nocturnal Orthoptera. In: *Orthopteran Mating Systems: Sexual Competition in a Diverse Group of Insects* (Ed. by Gwynne, D. T. & Morris, G. K.), pp. 67-92. Boulder: Westview Press.

Wallace. A. R. (1889) *Darwinism*. London: Macmillan.

Warne. A. C. (1972) Embryonic development and the systematics of the Tettigoniidae (Orthoptera: Saltatoria). *International Journal of Insect Morphology and Embryology*, **1**, 267-287.

Warne, A. C. & Hartley, J. C. (1975) Response strigilation by female *Leptophyes punctatissima* (Bosc) (Orth. Phaneropteridae). *Entomologist's Monthly Magazine*, **111**, 188.

Wedell. N. (1991) Sperm competition selects for nuptial feeding in a bushcricket. *Evolution*, **45**, 11975-1978.

Wedell, N. (1992) Protandry and mate assessment in the wartbiter *Decticus verrucivorus* (Orthoptera: Tettigoniidae). *Behavioral Ecology & Sociobiology*, **31**, 301-309.

Wedell, N. (1993a) Mating effort or paternal investment? Incorporation rate and cost of male donations in the wartbiter. *Behavioral Ecology & Sociobiology*, **32**, 239-246.

Wedell. N. (1993b) Spermatophore size in bushcrickets: comparative evidence for nuptial gifts as a sperm protection device. *Evolution*, **47**, 1203-1212.

Wedell, N. (1994) Variation in nuptial gift quality in bush crickets (Orthoptera, Tettigoniidae). *Behavioral Ecology*, **5**, 418-425.

Wedell, N. & Arak, A. (1989) The wartbiter spermatophore and its effect on female reproductive output (Orthoptera: Tettigoniidae, *Decticus verrucivorus*). *Behavioral Ecology & Sociobiology*, **24**, 117-125.

Wedell, N. & Sandberg, T. (1995) Female preference for large males in the bush-cricket *Requena* Sp-5 (Orthoptera, Tettigoniidae). *Journal of Insect Behavior*, **8**, 513-522.

Weigensberg, I., Carriere, Y. & Roff, D. A. (1998) Effects of male genetic contribution and paternal investment to egg and hatchling size in the cricket, *Gryllus firmus*. *Journal of Evolutionary Biology*, **11**, 135-146.

Wells, K. D. (1977) The social behaviour of anuran amphibians. *Animal Behaviour*, **25**, 666-693.

Wheeler, D. (1996) The role of nourishment in oogenesis. *Annual Review of Entomology*, **41**, 407-431.

Wigglesworth, V. B. (1972) *The Principles of Insect Physiology*. London: Chapman & Hall.

Will, M. W. & Sakaluk, S. K. (1994) Courtship feeding in decorated crickets: is the spermatophylax a sham? *Animal Behaviour*, **48**, 1309-1315.

Williams, G. C. (1966) *Adaptation and Natural selection*. Princeton, N.J.: Princeton University Press.

Wolf, D. P., Byrd, W., Dandekar, P. & Quigley, M. M. (1984) Sperm concentration and the fertilization of human eggs in vitro. *Biology of Reproduction*, **31**, 837-848.

Wolf, H. (1985) Monitoring the activity of auditory interneurone in a free-moving grasshopper. In: *Acoustic and Vibrational Communication in Insects* (Ed. by Kalmring, K. & Elsner, N.), pp. 51-60. Berlin and Hamburg: Paul Parey.

Zahavi, A. (1975) Mate selection - a selection for a handicap. *Journal of Theoretical Biology*, **53**, 205-214.

Zakharov, V. M. (1992) Population phenogenetics: analysis of developmental stability in natural populations. *Acta Zoologica Fennica*, **191**, 7-30.

Zantiev, R. D. & Korunovskaya, O. S. (1986) Sound communication in bushcrickets (Tettigoniidae: Phaneropterinae) of the European part of the USSR. *Zool. J.*, **65**, 1151-1163.

Zimmermann, U., Rheinlaender, J. & Robinson, D. (1989) Cues for male phonotaxis in the duetting bushcricket *Leptophyes punctatissima*. *Journal of Comparative Physiology, A: Sensory, Neural and Behavioral Physiology*, **164**, 621-628.

Zuk, M. (1988) Parasite load, body size, and age of wild caught male field crickets (Orthoptera: Gryllidae): effects on sexual selection. *Evolution*, **42**, 969-976.

Zuk, M., Simmons, L. W. & Rotenberry, J. T. (1995) Acoustically orienting parasitoids in calling and silent males of the field cricket *Teleogryllus oceanicus*. *Ecological Entomology*, **20**, 380-383.

Zuk, M., Simmons, L. & Rotenberry, J. T. (1996). Phonotactic parasitoids and calling behavior in the field cricket *Teleogryllus oceanicus*. Proceedings of the *XX International Congress of Entomology, Firenze, Italy, August 25-31, 1996*, p. 367.