The Evolution and Function of the Spermatophylax in Bushcrickets (Orthoptera: Tettigoniidae).

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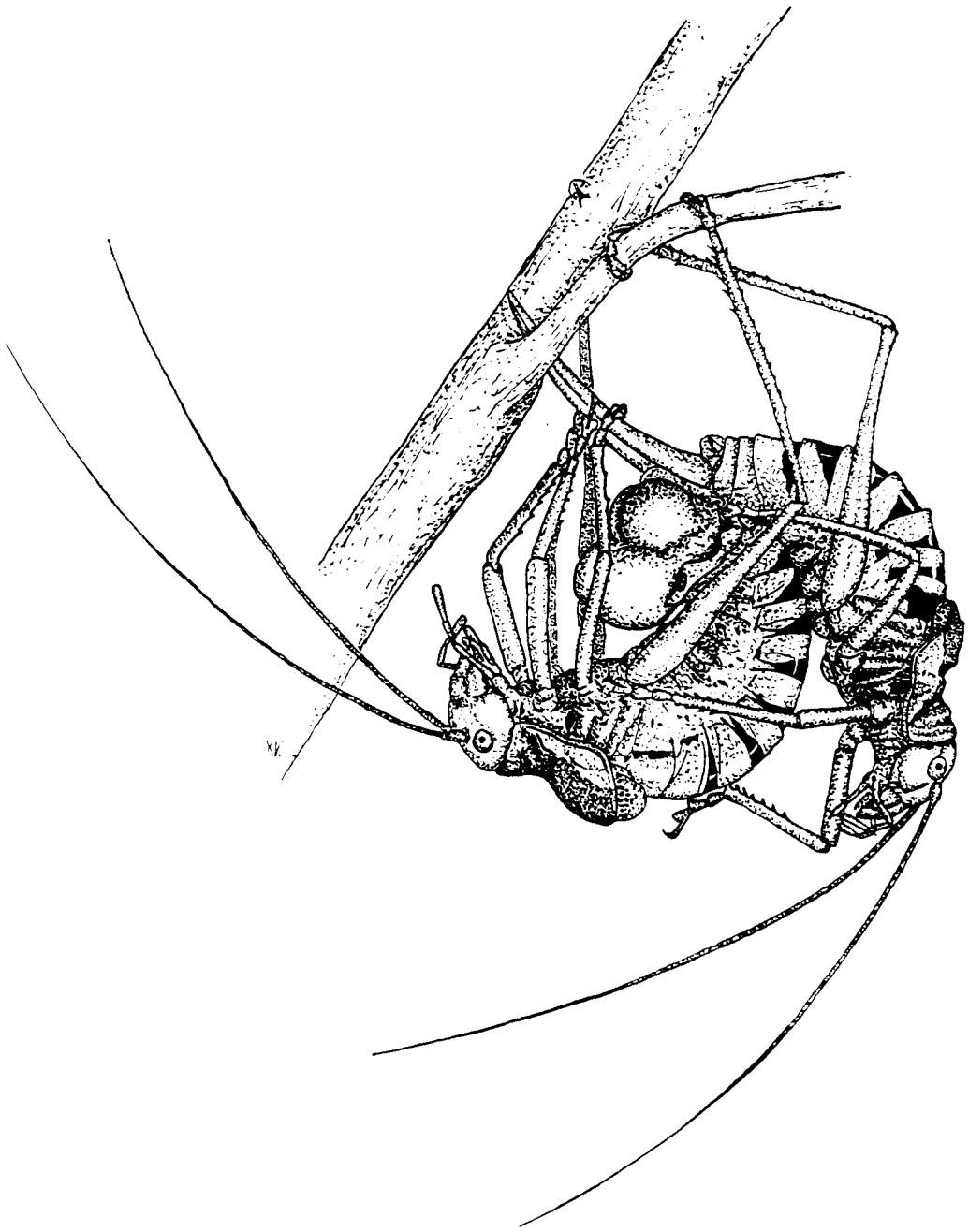

"...and soon from the male's convulsive loins there is seen to issue, in painful labour, something monstrous and unheard of, as though the creature were expelling its entrails in a lump".

J.H.Fabre (1899).

To my Father.

Abstract

In certain species of cricket and bushcricket (Orthoptera; Ensifera), the male transfers an elaborate spermatophore to the female at mating. This consists of a sperm-containing ampulla and an often substantial, sperm-free, gelatinous mass known as the spermatophylax. After mating, the female eats the spermatophylax before consuming the ampulla. The spermatophylax is particularly well developed in the bushcrickets (Tettigoniidae) and can contribute to a loss of as much as 40% of male body weigh at mating in some species. Recently, there has been considerable debate over the selective pressures responsible for the evolution and maintenance of the spermatophylax and other forms of nuptial feeding in insects. Two different, though not mutually exclusive, functions have been suggested for the spermatophylax: 1) nutrients from the spermatophylax may function to increase the weight and\or number of eggs laid by the female, i.e. may function as paternal investment; 2) the spermatophylax may function to prevent the female from eating the ampulla before complete ejaculate transfer, i.e. may be regarded as a form of mating effort. In this study, a comparative approach combined with laboratory manipulations were used in an attempt to elucidate the selective pressures responsible for the origin, evolutionary enlargement and maintenance (= function) of the spermatophylax in bushcrickets.

The results suggest that the spermatophylax originated as an adaptation to maximise ejaculate transfer by countering the tendency of females to eat the ampulla prematurely. The spermatophylax appears to be analogous to a range of adaptations found in males of the sub-order Ensifera, which may be interpreted as functioning to maximise ejaculate transfer. These adaptations include prolonged copulation following spermatophore transfer, feeding the female with glandular secretions following spermatophore transfer, post-copulatory mate guarding and multiple copulations with the same female. The occurrence of prolonged copulation following spermatophore transfer appears to be associated with the total loss of the spermatophylax in the meconematine bushcricket *Meconema* and with the considerable reduction in spermatophylax size in the ephippigerine bushcricket *Uromenus rugiscollis*. This supports the hypothesis that prolonged copulation and the spermatophylax are analogous in function.

The subsequent evolutionary enlargement of the spermatophylax appears to have accompanied the evolutionary enlargement of ejaculate volume and sperm number, i.e. appears to have proceeded to facilitate the transfer of larger ejaculates. A comparative study of 43 species of bushcricket revealed a positive relationship, across taxa, between evolutionary changes in spermatophylax size and changes in ampulla size (i.e. ejaculate volume) and sperm number, with male body weight controlled for. The current function of the large spermatophylax appears to be the same as that of the small spermatophylax, i.e. to ensure complete sperm \ ejaculate transfer. No significant difference in the shape of the sperm transfer curve relative to the mean duration of spermatophylax consumption was found between *Leptophyes punctatissima* (small spermatophylax) and *L. laticauda* (large spermatophylax). Furthermore, in *L. laticauda*, males appear to adjust the size of the spermatophylax in relation to the amount of sperm or volume of ejaculate they are able to produce: a positive relationship was found between spermatophylax mass and sperm number and between spermatophylax mass and ampulla mass (i.e. ejaculate volume).

The possibility that the spermatophylax additionally functions as paternal investment cannot, however, be ruled out on this basis. In order for male-donated nutrients to function as paternal investment they must 1) have a positive effect on offspring

fitness and\or number and 2) the nutrient donating male must stand to fertilise most or all of the offspring which benefit from his nutrients. A positive effect of spermatophylax consumption on egg weight and\or number has previously been documented in some species of bushcricket, though has not been found in others. In this study, no effect of spermatophylax consumption on female reproductive output was found in *L.punctatissima*, *L.laticauda*, or *Steropleurus*, even when, in the latter two cases, females were maintained on a restricted diet. Furthermore, in *L.punctatissima* and *Steropleurus stali* (though not in *L.laticauda*) it appears that the spermatophylax-donating male is unlikely to fertilise eggs in which his nutrients might be incorporated, in light of the short female re-mating interval, the pattern of last-male sperm precedence and the pattern of oviposition. The enormous spermatophylax of *S.stali* is unlikely, therefore, to function as paternal investment. Recent studies suggest that in a number of other bushcricket species, including some with very large spermatophylaxes, the spermatophylax is also unlikely to function as paternal investment for the above reasons. In conclusion, while the paternal investment hypothesis lacks generality, the ejaculate-protection hypothesis seems to be more widely applicable and appears to successfully account for the origin, evolutionary enlargement and current function of the spermatophylax in bushcrickets.

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1. The Spermatophylax and Other Forms of Nuptial Feeding in Insects.

1.1 *The Spermatophylax.*

At mating, male crickets and bushcrickets (Orthoptera; Ensifera) transfer their ejaculate in a spermatophore which remains at least partly external to the female's reproductive tract (Boldyrev 1915; Alexander & Otte 1967a). In certain species, the sperm-containing portion of the spermatophore (the ampulla) bears a large, sperm-free, gelatinous mass called the spermatophylax (meaning sperm-protector; Boldyrev 1915) (fig.1.1a). After the spermatophore has been transferred, the female bends double and eats the spermatophylax before consuming the ampulla (fig.1.1b). In species with a large spermatophylax, this process may take several hours, during which time sperm are transferred from the ampulla to the female's sperm-storage organ (Boldyrev 1915).

The spermatophylax is particularly well developed in the bushcrickets (Tettigoniidae), though interestingly there is considerable interspecific variation in the relative size of this character throughout the family (Boldyrev 1915; Gwynne 1983a, 1990a; see fig 4.1 in chapter 4). At one extreme, the spermatophylax is more or less absent in some species and only about 2% of the male's body weight is lost at mating (Gwynne 1983a). At the other extreme, certain species produce a very substantial spermatophylax which contributes to a loss of up to 40% of male body weight (Busnel & Dumortier 1955).

Fig. 1.1 a) A female *Steropleurus catalaunicus* (Tettigoniidae: Ephippigerinae) bearing a spermatophore with a large spermatophylax (a = ampulla, s = spermatophylax). The spermatophore represented over 40% of the male's body weight in this case. **b)** The same female beginning to eat the spermatophylax.


a
s
a


b

Within the Ensifera, the spermatophylax is also found in certain members of the Haglidae (Dodson et al 1983), the Gryllidae (Boldyrev 1927; Alexander & Otte 1967a), the Stenopelmatidae (Field & Sandlant 1983) and the Rhaphidophoridae (Boldyrev 1912, 1915). Interestingly, a similar structure has evolved independently in certain lacewings and dobson-flies (orders Neuroptera and Megaloptera; Withycombe 1922; Hungerford 1936; David 1936; Toschi 1965; Principi 1985; Hayashi 1992, 1993). As in the Ensifera, the spermatophylax is eaten by the female after spermatophore deposition (see Hayashi 1992 for excellent photographs of females of the megalopteran *Protohermes* eating the spermatophylax). There is also interspecific variation in spermatophylax size in these groups: the megalopteran *Protohermes grandis* produces a large spermatophylax which contributes to a loss of up to 20% of male body weight at mating, while in *P.immaculatus* up to 10% of male body weight is lost in spermatophore production (Hayashi 1993).

One cost of producing a large spermatophylax is manifest in a male refractory period between matings, decreasing a male's life-time mating potential (Sakaluk et al 1987; Simmons 1990a; Gwynne 1990b; Heller & Helversen 1991; Hayashi 1993). In tettigoniid species which produce proportionally large spermatophylaxes, males may require as much as 5 days before they are ready to mate again (Busnel et al 1956; Simmons 1990a). This contrasts with the male refractory period of as little as 5 minutes in certain species with minute spermatophylaxes (Meixner & Shaw 1986).

Recently, there has been considerable debate over the selective pressures responsible for the evolution and maintenance of the spermatophylax and other forms of nuptial feeding in insects (Wickler 1985, 1986; Gwynne 1986a; Sakaluk 1986a; Quinn & Sakaluk 1986; Simmons & Parker 1989). Three main selective pressures have been put forward as having potentially shaped the evolution and maintenance of the

spermatophylax. These are: a) female choice (female selection of males able to produce larger spermatophylaxes), b) selection on males to maximise ejaculate transfer and c) selection for male parental investment.

It is important to note two things at this stage. Firstly, the selective pressures responsible for the evolution of a character need not be the same as those responsible for its maintenance (see Simmons & Parker 1989); and second, none of the selective pressures listed above need be mutually exclusive - all could theoretically contribute to the evolution and maintenance of a character. Despite these points, it is convenient to consider the evidence to support the role of each selective pressure separately.

1.1.1 *Female choice and the evolution\ maintenance of the spermatophylax.*

1.1.1.a *Pre-mating discrimination*

The role of female choice for males able to supply larger, more nutritive spermatophores in the evolution and maintenance of this form of nuptial feeding has been stressed by Thornhill (1976a) and Gwynne (1984a). Some support for this was provided by Gwynne (1982). He showed that in the tettigoniid *Conocephalus nigropleurum* (Conocephalinae), heavier males produce heavier spermatophores (as in other tettigoniids, see Gwynne et al 1984; Gwynne & Bailey 1988; Wedell & Arak 1989; Galliart & Shaw 1991). When given a choice between two singing males of different weight, females always mated with the heavier individual.

However, because the spermatophylax is produced during mating in tettigoniids, females cannot directly assess the size of the spermatophylax a male will produce before mating. If females use male body weight to assess spermatophylax size, males could be "dishonest" and produce a spermatophylax smaller than predicted by male body weight, unless the production of a large spermatophylax benefitted the male in some other way (Gwynne 1986b).

Furthermore, it is possible that female *C.nigropleurum* prefer heavier males for other reasons, such as their superior competitive ability. Male conocephaline bushcrickets, including *C.nigropleurum*, are aggressive towards conspecific males (Morris 1971). A dominant male will maintain an area around it that is free of conspecific singers. In at least one conocephaline genus (*Orchelimum*), heavier males are dominant in aggressive encounters (Morris 1979; Faever 1983).

Because females may benefit from the consumption of a greater amount of spermatophylax (Gwynne 1984a, 1988a, Simmons 1990a), they may well benefit by choosing to mate with heavier males as these males tend to produce heavier spermatophores (Gwynne 1982; Gwynne et al 1984; Gwynne & Bailey 1988; Wedell & Arak 1989; Galliart & Shaw 1991). However, my point is that while such a preference might lead to the evolution of heavier males, it is unlikely to lead to the evolution of proportionately larger spermatophylaxes. Unless, that is, spermatophylax production were a cheap way of increasing male body weight, which seems improbable given that spermatophylax production is known to be costly (see introduction to section 1.1).

There is evidence that, although within species heavier males produce heavier spermatophores, they invest proportionately less weight in spermatophores than small males (Gwynne et al 1984; Simmons 1990a). Therefore, selection for heavier

males within a population might actually result in the evolution of proportionally smaller spermatophores.

While it seems that pre-mating female choice is unlikely to have played a significant role in the evolution of the large spermatophylax, it may be an important factor in the evolution and maintenance of nuptial feeding in groups where females can assess the size of the food gift before mating (eg. the Mecoptera and the Empididae, see Thornhill & Alcock 1983).

1.1.1.b *Post-mating discrimination (cryptic female choice).*

Sakaluk (1986a) suggested that female choice via post-mating discrimination may have been important in the evolution of the large spermatophylax. He observed that in the cricket *Gryllodes supplicans*, females leave the spermatophores of males which have produced a larger spermatophylax attached for longer periods. Sakaluk (1986a) proposed that by spermatophore removal, females may reduce the number of sperm transferred and eggs fertilised by males who provide inadequate nuptial gifts (ie. resources), and reward more generous males by permitting fuller insemination. He reasoned that discrimination of this sort would accord females a means of extracting greater nutritional investment from males. However, this argument would appear to be flawed in that it suggests that an individual female is able to benefit from facilitating the evolution of male behaviour in a direction that ultimately benefits female interests (see Simmons & Parker 1989). Obviously, natural selection does not work in this way.

After a female has mated and undergone all its associated costs (see Daly 1978), it is hard to see how she would benefit from discriminating against the use of sperm from

a male on the grounds that the spermatophylax (ie. resource) he produced was inadequate. The only way a female could benefit from doing so would be if the size of the spermatophylax correlated with an aspect of male genetic quality, such as ability to gather food or degree of resistance to parasites (incidentally, Simmons 1993 has found no significant influence of the degree of infection by a common protozoan gut-parasite on the size of the spermatophylax produced in the bushcricket *R. verticalis*).

Post-mating female choice for male characters reflecting "good genes" seems perfectly feasible: for example, Simmons 1986, 1987 showed that females of the gryllid *Gryllus bimaculatus* (a species which does not produce a spermatophylax) leave spermatophores of larger males attached for longer. However, post-mating choice for "good resources" (ie. immediate non-genetic benefits) seems unlikely.

Before invoking "good genes" models of female choice to account for the evolution of the large spermatophylax, however, it is necessary to question whether the female is actively discriminating against the use of sperm from a male on the basis of the size of his spermatophylax. Rather than constituting a form of active female choice, it would seem more logical to regard the positive relationship between spermatophylax size and ampulla attachment duration in *Gryllodes supplicans* (see Sakaluk 1985, 1986a) as a passive consequence of the fact that larger spermatophylaxes simply take longer for the female to eat (the female eats the ampulla within minutes of having finished the spermatophylax) (see Sakaluk 1984, 1985). Because the spermatophylax is produced by the male, it could be argued that it is the male, rather than the female, who is influencing the duration of spermatophore attachment in this case.

1.1.2 *Selection to maximise ejaculate transfer and the evolution\ maintenance of the spermatophylax.*

1.1.2.a *The spermatophylax, sperm transfer and sperm competition.*

The spermatophylax has been viewed as a sperm-protecting device, functioning to prevent the female from eating the ampulla before complete sperm transfer (Gerhardt 1913, 1914, 1921; Boldyrev 1915, 1928a; Alexander & Otte 1967a; Sakaluk 1984; Wedell & Arak 1989; Reinhold & Heller 1993). It has been suggested that, in performing this function, the spermatophylax may be analogous to male post-copulatory behaviours found in the Ensifera such as prolonged copulation, multiple mating with the same female, feeding the female with glandular secretions (Boldyrev 1915) and mate-guarding (Alexander & Otte 1967a).

Gwynne et al (1984) were amongst the first to provide empirical support for the sperm-protection hypothesis. They demonstrated that, when the spermatophylax was experimentally removed, females of the bushcricket *Requena verticalis* would eat the ampulla within 3 minutes after spermatophore deposition. This was found to prevent the transfer of any ejaculate.

Further support for this hypothesis was provided by Sakaluk (1984). He demonstrated that, in the gryllid *Gryllodes supplicans*, the mean time taken by a female to eat the spermatophylax and remove the ampulla corresponded to the mean time for complete sperm transfer to the spermatheca. Males producing smaller spermatophylaxes would therefore transfer less sperm. A similar correspondence between the mean spermatophylax-eating time and the time taken for complete sperm transfer has subsequently been found in an un-named species of zaprochiline

bushcricket (Simmons & Gwynne 1991), in the bushcricket *Poecilimon veluchianus* (Reinhold & Heller 1993) and also appears to occur in *Decticus verrucivorus* (Wedell & Arak 1989).

In the bushcricket *R. verticalis*, however, a correspondence between the spermatophylax-eating time and the time for complete sperm transfer is less certain. Gwynne et al (1984) found a negative exponential relationship between the number of sperm remaining in the ampulla and time from the end of mating in this species. They found that 75% of sperm appears to leave the ampulla in half the mean spermatophylax-eating time. However, from the data presented, it could be argued that the time taken for the sperm to be *completely* drained from the ampulla corresponds fairly well with the mean spermatophylax-eating time. In another experiment, Gwynne (1986b) examined the rate of sperm transfer to the spermatheca (the female's sperm-storage organ). He demonstrated that the spermatophylax of *R. verticalis* appears to be twice as large as is necessary to ensure the transfer of a full complement of sperm and substances in the ejaculate which induce a refractory period in the female (this is a non-receptive period following mating, see below). Consequently, Gwynne (1986b) rejected the hypothesis that the large spermatophylax of this species functions to protect the ejaculate.

The transfer of a full complement of sperm may be particularly important in the face of sperm competition resulting from female multiple-mating. Sperm competition occurs when the ejaculates of two or more males overlap in time within the reproductive tract of the same female and consequently compete for the fertilisation of the female's eggs (Parker 1970). This phenomenon is prevalent in insects, where females may store sperm in a viable condition for long periods of time in the spermatheca and frequently mate with more than one male (Parker 1970; Ridley

1988; 1990). When sperm competition occurs, the male that has inseminated most sperm is likely to have the greater probability of obtaining fertilisations, especially where there is some degree of sperm mixing (Woodhead 1985; Parker 1990a; Parker et al 1990; see chapter 2, section 2.1.1). Sperm competition is likely, therefore, to select for males to maximise sperm transfer.

Evidence to support the role of sperm competition in the evolution and maintenance of the spermatophylax is provided by studies of spermatophylax function in the gryllid *G.supplicans* and the tettigoniid *D.verrucivorus*. Both these species show overall sperm mixing (ie. fertilisation is by a lottery: Sakaluk 1986b; Wedell 1991). In *G.supplicans* and *D.verrucivorus*, the size of the spermatophylax influences the duration of ampulla attachment, females taking longer to consume larger spermatophylaxes (Sakaluk 1985; Wedell & Arak 1989). The duration of ampulla attachment determines the number of sperm transferred to the spermatheca in *G.supplicans* (Sakaluk 1984) and probably also in *D.verrucivorus* (Wedell & Arak 1989). Sakaluk (1986b) found a significant positive correlation between the duration of ampulla attachment for the first male and the percentage of eggs later fertilised by that male, but for only one of two experimental groups. Similarly, in *D.verrucivorus* the proportion of eggs fertilised by either the first or second male in a mating sequence is dependent upon the relative size of the spermatophylaxes produced: the male which has produced the largest spermatophylax fertilises a greater proportion of the female's eggs (Wedell 1991).

1.1.2.b *Ejaculate transfer and female refractory periods.*

The production of a larger spermatophylax and the consequent transfer of a greater volume of ejaculate may also benefit the male by inducing a longer sexual refractory period in the female (for tettigoniids see Gwynne 1986b; Wedell & Arak 1989;

Simmons & Gwynne 1991). This is a period following mating in which females are non-receptive to sexually active males. Female refractory periods have been documented in a number of insect groups and, in many cases, appear to be triggered by materials passed in the ejaculate such as accessory gland substances (see reviews of Leopold 1976; Thornhill & Alcock 1983; Chen 1984).

A male stands to benefit from inducing a longer refractory period in the female because this increases the chance that she will lay eggs before mating with another male. This lowers the risk of sperm competition (see Parker 1970). The induction of a refractory period in females would be particularly important for males of species with last-male sperm precedence (where the female uses sperm from the most recent mating to fertilises the majority of her eggs). In such species, a male may fail to fertilise any of a female's eggs unless she oviposits before re-mating (in the majority of insects, females use stored reserves of sperm to fertilise eggs as they are laid).

An increase in the duration of ampulla attachment, independent of the amount of spermatophylax consumed, has been demonstrated to result in a longer female refractory period in the tettigoniids *R. verticalis* (Gwynne 1986b), *D. verrucivorus* (Wedell & Arak 1989) and a zaprochiline tettigoniid (Simmons & Gwynne 1991). In *R. verticalis*, the full refractory period was induced by half the ampulla attachment time that results from spermatophylax feeding; the spermatophylax appears to be twice as large as is necessary to ensure the induction of a full refractory period in the female (Gwynne 1986b). In *D. verrucivorus* and the zaprochiline, however, the ampulla attachment time required to induce a full refractory period in the female corresponds with the mean time taken for females to eat the spermatophylax (Wedell & Arak 1989; Simmons & Gwynne 1991).

Simmons and Gwynne (1991) proposed that there is likely to be sexual conflict over the duration of the refractory period in bushcrickets: females may be selected to obtain spermatophore nutrients from additional matings, while males should be selected to prevent or delay female re-mating. They demonstrated that in the zaprochiline, females allowed to consume the spermatophylax showed an increase in the length of the refractory period (up to 19 days long) with increasing ampulla attachment time, when maintained on both high and low-food diets. However, females prevented from consuming the spermatophylax only responded to increasing ampulla attachment time with an increase in the duration of the refractory period when maintained on a high-food diet. Females maintained on a low-food diet and prevented from consuming the spermatophylax failed to respond to the duration of ampulla attachment. These females re-mated within 3-5 days regardless of ampulla attachment duration. This suggests that when nutritionally limited, female bushcrickets have a higher motivation to re-mate in order to consume extra spermatophylaxes (see also Gwynne 1990a who found that in *R.verticalis*, females on low-quality diets have shorter refractory periods than those on high-quality diets). Simmons and Gwynne (1991) suggested that when nutrients are limited, males able to satiate the female's hunger by producing a large spermatophylax may benefit by ensuring that the female responds to refractory-inducing substances in the ejaculate. They propose that sexual conflict over the duration of the refractory period could be instrumental in the evolution of larger spermatophylaxes.

1.1.2.c *Ejaculate transfer and rates of oviposition.*

Males of a number of insect groups are known to transfer substances in the ejaculate which stimulate oviposition (see reviews of Leopold 1976; Chen 1984). For example, male *Acheta domesticus* (Gryllidae) transfer prostaglandin synthetase in the ejaculate (Destephano et al 1974,1976). This enzyme converts the precursor

arachidonic acid into prostaglandins, among them PGE_1 and PGE_2 (Loher 1981). These compounds have been shown to both induce vitellogenesis and stimulate oviposition in the gryllids *A.domesticus* and *Teleogryllus commodus* (Destephano & Brady 1977; Loher 1979; Stanley-Samuelson et al 1986). This accounts for the increased egg output following mating in these and other gryllids (Loher & Edson 1973; Bentur et al 1977; Simmons 1988a).

A third way, therefore, in which males may benefit from the production of a larger spermatophylax and the consequent transfer of a larger amount of ejaculate is by an increase in the rate of oviposition following mating. This was demonstrated in *D.verrucivorus* by Wedell and Arak (1989). They showed that an increase in the duration of ampulla attachment results in a more rapid onset of oviposition and an increased oviposition rate. This leads to an increase in the number of eggs laid by females in their refractory periods.

1.1.3 *Paternal investment and the evolution\ maintenance of the spermatophylax.*

A third factor that has been put forward as being important in the evolution and maintenance of the spermatophylax, and other forms of nuptial feeding in insects, is male parental investment (Thornhill 1976a; Morris 1979; Gwynne 1984a; Gwynne 1988a; 1988b; Gwynne 1990a; Simmons 1990a). Gwynne (1986b, 1988b, 1990a) proposed that while the spermatophylax appears to have originated in the context of intrasexual selection as a protective device to avoid premature removal of the ampulla, elaboration of spermatophylax size may have proceeded through natural selection for male parental investment.

Trivers (1972) defined male parental investment (paternal investment) as "any investment by the parent in an offspring that increases the offspring's chance of surviving at the cost of the parent's ability to invest in other offspring". Simmons & Parker (1989) extend the scope of this definition and term "any increase in a given male's total surviving progeny by increasing the reproductive output of a given female" as paternal investment. Where a male gains via increasing an individual female's gametic output (i.e. egg weight and/or number) through the donation of a nuptial gift, he is thus investing paternally (Simmons & Parker 1989).

Paternal investment is distinct from mating effort, which is defined as "that proportion of reproductive effort expended in finding a member of the opposite sex or in subduing members of the same sex in order to mate" (Low 1978). Where a male gains from the donation of a nuptial gift by increasing the proportion of eggs he fertilises from a given female or by increased mating opportunities, this is considered as mating effort (Simmons & Parker 1989, cf. section 1.1.2)

On theoretical grounds, males are unlikely to have been selected to provide parental investment before fertilisation because of the uncertainty of parentage (Alexander & Borgia 1979; Gwynne 1984b; Wickler 1985). While a female is always certain to be the parent of offspring she produces, a male may never be certain that he will father the female's offspring that stand to benefit from his "paternal" investment. Therefore, donations made by males to their mates such as nuptial food gifts are more likely to represent a form of mating effort than parental investment (Alexander & Borgia 1979; Gwynne 1984b; Wickler 1985).

A demonstration that the nuptial food gifts of male insects represent paternal investment requires that 1) the gift results in an increase in offspring fitness and\ or

number, and 2) that the donating male has a genetic representation in the offspring that are nutritionally benefitted (Wickler 1985, 1986; Gwynne 1986a; Sakaluk 1986a; Simmons & Parker 1989).

1.1.3.a *The spermatophylax and offspring fitness\ number.*

The effects of spermatophylax consumption on offspring fitness and number have been examined so far in the tettigoniids *R.verticalis* (Bowen et al 1984; Gwynne 1984a, 1988a, 1988b; Gwynne et al 1984), *D.verrucivorus* (Wedell & Arak 1989), an un-named species of zaprochiline (Simmons 1990a) and *Poecilimon veluchianus* (Reinhold & Heller 1993).

In *R.verticalis*, the spermatophylax is reasonably large, contributing to a mean loss of 12.5% (Gwynne 1990b) to 19% (Gwynne 1986b) of male body weight at mating. Bowen et al (1984) found that the spermatophylax of this species consists of 13.5 % protein (wet weight) and used radio-labelling techniques to investigate the fate of spermatophore proteins in mated females. Results indicated that nutrients from the male become more concentrated in the ovaries and are incorporated into the next batch of eggs developed after mating, a process which takes 9-13 days.

In the same species, Gwynne et al (1984) investigated the effect of spermatophylax consumption on female reproductive output. The results failed to demonstrate any effect: females receiving 0, 1 or 2 spermatophylaxes (with insemination held constant) did not differ in the number of eggs produced or in the weights of these eggs. Gwynne et al (1984) proposed that the effects of spermatophylax consumption might have been masked by the high-protein laboratory diet.

In a further study, Gwynne (1984a) found that when females of this species were maintained on a lower-protein diet and given a wide range of male nutrient contribution (0,1,3 or 7 spermatophylaxes, with insemination held constant), a positive effect of the number of spermatophylaxes consumed on both egg number and egg weight resulted. This demonstrates that females may benefit from multiple-mating when resources are scarce.

In further experiments with *R. verticalis*, Gwynne (1988a) found a positive effect of the number of spermatophylaxes eaten (0,1, or 3 spermatophylaxes) on both egg weight and number in females on both low and high-protein diets. He also found that the amount of dietary protein and the number of spermatophylaxes consumed appeared to affect female fecundity differently: an increase in either dietary protein or the number of spermatophylaxes consumed (0,1, or 3 spermatophylaxes) resulted in a significant increase in the number of eggs laid, but only spermatophylax feeding led to a significant increase in egg weight. This led Gwynne (1988a) to propose that males provide nutritional components in the spermatophylax not available from other sources.

The benefits to be gained by a female through the consumption of several spermatophylaxes, however, have little bearing on the benefits of spermatophylax production for an individual male. What is important, in this respect, is differences in female gametic output arising from the consumption of a smaller or larger spermatophylax. Gwynne (1984a) found a significant difference in the number of eggs laid between females receiving 0 or 1 spermatophylax (on a low-protein diet), but no significant difference in egg weight. Gwynne (1988a), on the other hand, found a positive effect of spermatophylax size (0.5, 1, or 1.5 spermatophylaxes, also on a low-protein diet) on the mean weight of pairs of eggs (but not the mean weight of lots of 5 eggs) and no effect on egg number.

Gwynne (1988a) examined the effect of the number of spermatophylaxes consumed by a female on several aspects of offspring fitness such as over-winter survival of embryos, maturation time of nymphs and adult size. Although the number of spermatophylaxes eaten had no effect on the percentage over-winter survival of offspring, offspring of females producing larger eggs had a greater probability of surviving the winter. Nor did the number of spermatophylaxes eaten have any direct effect on the mean adult size of progeny, but it did significantly increase the mean time taken for sons to mature to adulthood. Gwynne (1988a) proposed that this might lead to an increase in adult size and thus fitness of sons.

In the light of these indirect effects of spermatophylax feeding on possible components of offspring fitness in *R. verticalis*, Gwynne (1988a) suggested that the spermatophylax may have evolved as parental investment to enhance the fitness of the mating male's offspring. He conceded, however, that this could only be the case if the males father the offspring that receive the benefits of their courtship feeding.

Positive effects of spermatophylax feeding on female fecundity have also been demonstrated in an un-named species of zaprochiline bushcricket (Simmons 1990a; Simmons & Bailey 1990). The spermatophylax of this species is large, contributing to a loss of 16-20% of male body weight (Simmons & Gwynne 1991). Simmons (1990a) took mating pairs (which had been feeding on a relatively poor food source) from the field and divided them into two groups: in one, females were deprived of the spermatophylax, though insemination was assured; in the other, females were allowed to eat the spermatophylax. Females in the latter group had a significantly greater gametic mass and an increase in the number of developing eggs evident after only 24 hours. Furthermore, a difference in the weight of mature ovarian eggs

occurred 48 hours after mating. Subsequent radio-labelling studies have confirmed that this increase in female reproductive output results from the incorporation of spermatophylax proteins into developing eggs (Simmons & Gwynne 1993).

Wedell & Arak (1989), however, demonstrated that positive effects of spermatophylax feeding on female fecundity do not occur in all bushcricket species. They examined the effect of spermatophylax consumption on various parameters of female reproductive output in the Wartbiter bushcricket, *D.verrucivorus*. The spermatophylax of this species contributes to a loss of 9% of the male's body weight at mating (Wedell & Arak 1989). Wedell & Arak (1989) found that female egg weight and lifetime fecundity were not influenced by the amount of spermatophylax consumed, even when adults were maintained on a low-protein diet. The amount of protein in the diet, however, did have a positive effect on these variables. Wedell & Arak (1989) concluded that the spermatophylax of *D.verrucivorus* functions primarily as a sperm-protection device (= mating effort) rather than as a form of paternal investment.

In the bushcricket *Poecilimon veluchianus*, Reinhold and Heller (1993) have similarly found no effect of spermatophylax consumption on female fecundity, even though the spermatophylax of this species is very large, contributing to a mean loss of 26% of male body weight (Heller & Helversen 1991). Reinhold and Heller (1993) found that females deprived of the spermatophylax, with insemination held constant, and females allowed to eat the spermatophylax showed no difference in the number of eggs subsequently laid, the weight of eggs or the absolute wet or dry weights of hatched larvae. The relative dry weight of hatched larvae, however, was increased as a result of spermatophylax consumption.

1.1.3.b *Does the male father the offspring that stand to benefit from his spermatophylax investment?*

In order for the spermatophylax, and other nuptial gifts, to function as paternal investment, it is essential that the nutrient-donating male fathers most or all of the offspring that benefit from his nutrition. A recent debate has centred on whether this can be the case (Wickler 1985, 1986; Gwynne 1986a; Sakaluk 1986a, Simmons & Parker 1989).

Wickler (1985) stated that "there is no good evidence for male nutrient gifts being turned into paternal investment in any insect studied so far". He argued that the prevalence of female re-mating together with the time delay for male nutrients to be incorporated into mature eggs and the sperm precedence characteristics of most insects seem to leave only minor chances for actual paternal investment. Wickler (1985, 1986) used the bushcricket *R.verticalis* to illustrate his point: the time span for spermatophylax proteins from a mating male to be incorporated into the next batch of mature eggs is 9-13 days (Bowen et al 1984), while the female becomes sexually receptive again only 4 days after a previous mating (Gwynne 1986b). Thus, Wickler (1985,1986) argued, with either first- or last-male sperm-precedence, males could easily exploit their later or earlier rivals' nutrient donations to the female.

Sakaluk (1986a) presented evidence for the alternative view, that male courtship gifts do, in fact, increase the survivorship of at least some of their own offspring and thereby constitute paternal investment. Citing recent evidence of sperm mixing in the spermatophylax-producing gryllid *G.supplicans* (see Sakaluk 1986b), he argued that mixed sperm utilisation strategies are more prevalent in insects than Wickler (1985,1986) supposed. With mixed sperm use and subsequent numerical sperm-

competition, males would be assured of a fertilisation success directly related to the size of their nuptial gift. Furthermore, Sakaluk argued, they would be assured of genetic representation in at least some of the offspring which might benefit from their nuptial food gifts, regardless of the females past, or future, mating activity.

In light of recent data revealing comparatively rapid incorporation rates of male-donated nutrients into eggs of several dipterans and lepidopterans, Simmons and Parker (1989) suggested that even in species with high last-male sperm precedence, the nutrient-donating male may fertilise eggs to which he contributes (see also Parker & Simmons 1989, who propose that males will be selected to ensure rapid incorporation of their donations into eggs which they can fertilise.).

To summarise, whether the nutrient-donating male fathers the offspring which stand to benefit from his nutrition depends, in part, upon: 1) the pattern of sperm use by multiply-mated females, 2) the length of the female refractory period, 3) the rate of incorporation of male-donated nutrients into eggs and 4) the pattern of oviposition - ie. the time taken for the female to lay eggs which benefit from the male nutrition (see Wickler 1985, 1986; Gwynne 1986a; Sakaluk 1986a; Simmons & Parker 1989).

Using a genetic marker and radio-labels, Gwynne (1988b) determined the paternity of offspring from doubly-mated female *R. verticalis* and detected which of the potential fathers donated most nutrients to individual offspring. He found that virtually all nymphs from the first batch of eggs were fertilised by the first male to mate, while nutrients from both males were found in the progeny. Even after the second batch, the first male continued to have complete paternity. Gwynne (1988b) concluded that the investment of the first-mating male appears to function as parental investment. However, Gwynne (1988b) noted that this left the problem that a male

mating with a female at the end of her refractory period will be cuckolded: although he would contribute to offspring nutritionally, his chances of fathering them would be extremely low. Males would therefore be expected to discriminate against non-virgins. However, no evidence of male discrimination against non-virgins has been revealed in this species (Lynam et al 1992; Simmons et al, in press). Gwynne (1988b) cited results indicating that mated female *R. verticalis* are less successful in inter-female competition than virgins (subsequently published as Lynam et al 1992). Thus, he proposed, a female at the end of her refractory period may rarely gain access to males in nature. However, once they have oviposited, non-virgins no longer appear to be at a disadvantage in competition with other females (Lynam et al 1992). These females would still fertilise the majority of their eggs with sperm from the first male (Gwynne 1988b), so the problem of subsequently-mating males being cuckolded remains.

Recently, evidence has been provided that male *R. verticalis* reduce the size of the spermatophylax produced when mating older females (but not non-virgins per se.) (Simmons et al 1993). This would fit with the hypothesis that the spermatophylax has a paternal investment function in this species.

In an un-named species of zaprochiline, Simmons (1990a) found that an increase in the number of developing eggs as a result of spermatophylax feeding was evident only 24 hours after mating. An increase in the weight of mature ovarian eggs occurred after 48 hours. Simmons (1990a) argued that due to this rapid incorporation of male-derived nutrition into eggs, together with a female refractory period of more than 5 days (later discovered to be up to 19 days long, Simmons & Gwynne 1991) and an interval between egg batches of 3 days, the nutrient-donating male is likely to fertilise eggs to which he contributes even if last-male sperm-precedence is high (the sperm precedence pattern of this species is currently unknown, Simmons 1992). In

light of Gwynne's (1988a,1988b) work with *R.verticalis*, Simmons (1990a) proposed that tettigoniid males may provide nutrients to their own offspring and consequently the spermatophylax can be considered as paternal investment. In the zaprochiline, however, a mating-effort function for the spermatophylax has also been demonstrated (see Simmons & Gwynne 1991).

Wedell (1993a) used radio-labels to investigate the rate of incorporation of spermatophylax proteins into eggs in *D.verrucivorus*. She found that females started to lay eggs containing radioactivity from the spermatophore at 6 or more days following mating. The levels of radioactivity in eggs peaked at 10 days following mating, though the isotope could be detected in eggs until 30 days after mating. The mean female refractory period in this species is only 4.6 days (Wedell & Arak 1989), therefore a female is likely to have re-mated before laying eggs in which the previous male's nutrients are incorporated. Given that the radio-label was traceable in eggs up to about 30 days after mating, a female is likely to mate many times during the period in which the first male's spermatophylax material is incorporated into the eggs (Wedell 1993a). A pattern of sperm-mixing has been found in *D.verrucivorus* (i.e. the female uses sperm from all males in proportion to their numerical representation within the spermatheca) (Wedell 1991). Therefore, spermatophore material from the female's earlier matings will be incorporated into eggs that are fertilised by later-mating males. In other words, one male's investment is likely to benefit other males' offspring (Wedell 1993a). This, together with the lack of evidence for an effect of spermatophylax feeding on fecundity in this species (see Wedell & Arak 1989) led Wedell (1993a) to conclude that the spermatophylax of *D.verrucivorus* is unlikely to function as paternal investment.

In the bushcricket *Poecilimon veluchianus*, Achmann et al (1992) and Reinhold and Heller (1993) propose that the investing male will only rarely be the father of the benefitting progeny. In this species, the female inter-mating interval most frequently

observed in the field is 2 days (Heller & Helversen 1991). This is probably too short a time to allow the incorporation of spermatophore nutrients into developing eggs: in a congeneric species, such nutrient incorporation takes 7 days (Helversen, in Reinhold & Heller 1993). Furthermore, females often do not lay eggs at all on the day following copulation (Achmann et al 1992). Another point is that the next batch of eggs is fully developed (with a chorion) within the ovary after a previous batch has been laid (Heller & Helversen 1991). This is important because such eggs can have no further nutrients added to them. As eggs which mature first are laid first, and fertilisation occurs at oviposition, there will be a further time lag before the newly matured eggs, which may contain spermatophylax nutrients, can be laid and fertilised. A high degree of last-male sperm precedence has been revealed in this species (Achmann et al 1992). Therefore, subsequently-mating males are likely to fertilise eggs which benefit from the previous male's nutrients. In light of this, together with evidence that the spermatophylax of *P.veluchianus* functions to ensure sperm-transfer (see Reinhold & Heller 1993), Achmann et al (1992) and Reinhold and Heller (1993) concluded that the large spermatophylax of this species (which represents about 26% of male body weight) is best considered as mating effort rather than paternal investment.

In the bushcricket *Metaplastes ornatus*, the female re-mating interval is also short, lasting for only 2-4 days (Helversen & Helversen 1991). Furthermore, there is also pronounced last-male sperm precedence in this species (Helversen & Helversen 1991). Consequently, Helversen & Helversen (1991) argued that nutrients from one male's spermatophylax are likely to be incorporated into eggs which will predominantly be fertilised by other males. Therefore, the large spermatophylax of this species (which contributes to a mean loss of 22% of male body weight at mating) is unlikely to be maintained by selection for paternal investment (Helversen & Helversen 1991).

1.1.3.c *Paternal investment and sex-role reversal.*

Incidental evidence that the spermatophylax may be important to females arises from observations that in certain bushcrickets which produce large spermatophores, a reversal in typical sex-roles occurs. In nutrient-limited environments, sexually active males (ie. males able to produce spermatophores) become a limiting resource for which females compete, while males are able to choose larger, more fecund females as mates (Gwynne 1981, 1983a, 1983b, 1984c, 1985, 1990b; Thornhill & Gwynne 1986; Gwynne & Simmons 1990; Simmons & Bailey 1990; Simmons 1992). However, evidence that male investment in the spermatophylax may control sexual selection and sexual differences cannot be used as indirect support for the paternal investment hypothesis for spermatophylax function (Gwynne 1986b; Reinhold & Heller 1993). This is because male mating effort that incidently increases female fitness while reducing the male's opportunity for further matings (= non-promiscuous mating effort, Gwynne 1984b) is expected to control sexual selection (via its effect on the operational sex ratio) in a similar manner as true parental investment (Gwynne 1984a; Gwynne 1991).

Zeh & Smith (1985) and Quinn & Sakaluk (1986) suggested that regardless of the adaptive significance of male-provided benefits, they should be classed as paternal investment because of their likely "effect" on the degree to which one sex limits the other's reproduction (and thus the intensity of sexual selection on the sexes). Un-questioningly classifying all male-provided benefits as paternal investment may be useful when addressing questions concerning the occurrence of sex-role reversal, or the benefits of multiple-mating for females. However, it is clearly misleading when the questions are concerned with the evolution and function of these male donations.

1.2 *Other Forms of Nuptial-Feeding in Insects.*

In addition to the spermatophylax, there are numerous ways in which males of a variety of insects feed females during or after mating (see reviews of Thornhill 1976a; Thornhill & Alcock 1983; Gwynne 1983a; Zeh & Smith 1985; Parker & Simmons 1989). In the following section, I will review these other forms of nuptial feeding in insects and discuss the empirical evidence concerned with their function. The classification used is based on that of Thornhill (1976a).

A rather diverse array of behaviours are included within the scope of nuptial feeding in insects. One thing that most of these behaviours have in common, however, is that they have been considered as forms of paternal investment by, for example, Thornhill (1976a, 1979).

1.2.1 *Food captured or collected by the male.*

1.2.1.a *Nuptial prey.*

The presentation of a prey arthropod on which the female feeds during copulation occurs in the Bittacidae and Panorpidae (Mecoptera: Bornemisoza 1964, Thornhill 1976b, 1979) and certain members of the Empididae (Diptera: reviewed by Kessel 1955; Engelmann 1970; see also Downes 1970; Svensson et al 1990).

In the black-tipped hangingfly, *Hylobittacus apicalis* (Bittacidae), Thornhill (1976b) provided evidence that nuptial feeding functions to ensure ejaculate transfer (ie. = mating effort). The duration of copulation is positively correlated with the size of the

nuptial prey (as it is in the empidid *Empis borealis*, see Svensson et al 1990). Consequently, males tend to discard small prey and use only larger prey items as nuptial gifts (Thornhill 1976b). Females receiving large prey feed while copulating for about 20 minutes, after which time the male pulls away, usually taking the partly-eaten prey with him. Twenty minutes corresponds with the time taken for the complete transfer of both sperm and substances that induce a full refractory period in the female and stimulate oviposition (Thornhill 1976b).

Another way in a which male of this species may gain by feeding females is by reducing the potentially risk-taking foraging activity of his mate during her refractory period, during which time she presumably lays eggs fertilised by his sperm (Thornhill 1979; cf. Boggs, 1990, who proposed that male-derived nutrients may increase female fitness through their effects on foraging as well as fecundity, and produced evidence that multiple mating and the consequent receipt of more male-derived nutrients allows decreased foraging expenditure in female *Heliconius* butterflies).

That female choice may be instrumental in shaping the male gift-giving behaviour in *H.apicalis* is also apparent: females sometimes refuse to copulate with males that possess small or unpalatable prey (Thornhill 1976b, 1979). A paternal investment function, on the other hand, seems unlikely for nuptial feeding in this species (Wickler 1985). Female *H.apicalis* have a high mating frequency, mating about 4 times per day (Thornhill 1976b). The female refractory period lasts 3-4 hours during which about 3 eggs are laid (Thornhill 1976b, 1979). Thornhill (1976b) proposed that last-male sperm-precedence is likely to be high in this species. If this is the case, males are unlikely to fertilise eggs which benefit from their nuptial gift nutrients, unless developing eggs, in which male nutrients may be incorporated, can be

matured and laid within the space of the 3-4 hour refractory period. Furthermore, virgin female *H.apicalis* apparently have mature eggs in their oviducts when sexually receptive (Thornhill 1976b). These mature eggs can have no further nutrients added to them, and will be laid first. This further decreases the chance that males will fertilise eggs containing their nutrients.

1.2.1.b *Nuptial food other than prey.*

Examples of insects in which the male feeds the female with a food item other than prey include thynnine wasps, a lygaeid bug and a drosophilid fly. Male thynnine wasps collect nectar on which they feed their mates during copulation (Given 1954; Alcock 1981a, 1981b). In the lygaeid bug *Stilbocoris natalensis*, males present their mates with a pre-digested fig seed on which they feed during copulation (Carayon 1964).

Male *Drosophila subobscura*, and other members of the *obscura* species group, re-gurgitate a drop of food on which the female feeds prior to copulation (Steele 1986a, 1986b). Steele (1986b) presented evidence that this behaviour functions to slow the female down, making it easier for the male to complete a frontal display, and to circle and attempt to mount before the female moves away. The effectiveness of the drop in this role depends upon its attractiveness to the female, which is influenced by its size and content and by the female's nutritional status (Steele 1986b). Steele (1986a) demonstrated that feeding on these male offerings can benefit the females: females that take the drop have a higher fecundity on a low-nutrient medium than females denied access to the drop. What is not clear is whether males fertilise these eggs, ie. whether the re-gurgitated food can additionally function as paternal investment. In another species of *Drosophila*, male-induced vitellogenesis and female unreceptivity occur for 1-2 days following mating (Baumann 1974). Parker &

Simmons (1989) argue that male *D.subobscura* are therefore likely to benefit from the increase in female fecundity resulting from drop-feeding, as this occurs over a similar period of time.

1.2.2 *The male's body.*

1.2.2.a *Parts of the male's body.*

Female consumption of parts of the male's wings during mating occurs in the orthopterans *Hapithus agitator* (Gryllidae; Alexander & Otte 1967b) and *Cyphoderris* (Haglidae; Morris 1979; Dodson et al 1983). In *H.agitator*, pairs remain in copula after spermatophore transfer while the female eats the males tegmina. Alexander and Otte (1967b) interpret this behaviour as functioning to keep the female in place during insemination and prevent the female from eating the spermatophore before it is emptied of sperm (see chapter two).

In *Cyphoderris*, the female feeds on the male's unsclerotised, fleshy hindwings and the resultant flow of haemolymph prior to and during copulation (Morris 1979; Dodson et al 1983). This behaviour might be interpreted as functioning to maintain the female's position while the male engages his genitalia .

1.2.2.b *The whole of the male's body.*

Thornhill (1976a) included cases of cannibalism of the male by the female during or after copulation as paternal investment. This behaviour has been observed in praying mantises (Roeder 1935; Edmunds 1975), the orthopteran *Stenopelmatus*

(Stenopelmatidae; Field & Sandlant 1983) and certain ceratopogonid midges (Goetghebuer 1914; Edwards 1920; Downes 1978).

In the praying mantis *Mantis religiosa*, Roeder (1935, 1967) proposed that sexual cannibalism may be promoted by the conditions of captivity and may be rare in nature because it is counteracted by the courtship of the male. Liske and Davis (1984, 1987) support this view. They describe the elaborate male courtship behaviour of the Chinese praying mantis, *Tenodera aridifoia sinesis* which they interpret as functioning to reduce the likelihood of cannibalism. In 69 mating sequences, Liske and Davis (1987) observed only a single case of sexual cannibalism in this species.

It would seem unlikely that sexual cannibalism could be adaptive from the male's point of view, unless 1) the male has a very high probability of fertilising a large portion of the female's eggs, 2) the male's prospects of finding a second mate are poor and 3) the food offered to the female in this way results in a substantial increase in offspring fitness and\or number (see Buskirk et al 1984; Simmons & Parker 1989).

1.2.3 *Glandular products of the male.*

1.2.3.a *External glandular secretions.*

Dorsal glands at which the female nibbles or palpates are widespread in male orthopteroid insects in which the female mounts upon the male's back during copulation (Alexander & Brown 1963). Male cockroaches (Blattidae: Roth 1969; Breed 1983) and bushcrickets (Tettigoniidae: Gerhardt 1914; Engelhardt 1915;

Boldyrev 1915; Rentz 1972) produce secretions from glands situated on their dorsal tergites which the females palpate prior to copulation. In *Oecanthus* (Gryllidae; Oecanthinae) females feed on secretions from the male's metanotal glands prior to, during, and after copulation (eg. Hohorst 1937; Alexander & Otte 1967a; Funk 1989). Feeding from male metanotal glands also occurs in the gryllid *Discoptila* (Gryllinae) during the prolonged copulation (Boldyrev 1928a). In *Allonemobius* (Gryllidae; Nemobiinae) females feed on a glandular secretion from the male's tibial spurs during the prolonged copulation (Mays 1971; Bidochka & Snedden 1985). I discuss the function of glandular feeding in *Oethanthus* and *Allonemobius* in chapter two.

Male malachiid beetles posess glandular structures which occur either on the elytral tips or on the frons (reviewed by Engelmann 1970). Females feed on these glands during courtship.

Female cockroaches are known to feed on uric acid secreted by males at mating (Mullins & Kiel 1980; Schal & Bell 1982; Mullins et al 1992). Males store this waste-product of metabolism in their accessory glands (see Roth & Dateo 1964, 1965; Roth 1967). The uric acid is either released onto spermatophores, which are subsequently digested by the female, or is secreted into the male's genital chamber and directly ingested by the female after copulation (Schal & Bell 1982). It has been demonstrated that urates consumed by females in this way are transferred to the next ootheca (egg-pod) in *Blattela germanica* (Mullins & Keil 1980; Mullins et al 1992) and *Xestoblatta hamata* (Schal & Bell 1982). Females on low-protein diets transfer more male-derived uric acid to their oothecae than females on high-protein diets (Mullins & Keil 1980; Mullins et al 1992; Schal & Bell 1982). Mullins & Keil (1980) suggested that the transfer of urates might represent a "paternal investment of a nitrogen resource from which the female and her progeny might benefit". In order

for this to be demonstrated, it must be shown, among other things, that uric acid derived from the male results in an increase in offspring fitness. As yet there appears to be no firm evidence of this, though Mullins et al (1992) cite evidence that cockroach fat-body endosymbionts (bacteriocytes) transmitted to oocytes during oogenesis may be involved in the metabolism of urates in embryos.

1.2.3.b *Salivary secretions.*

The secretion of drops of saliva on which the female feeds during copulation occurs in the dipterans *Rivellia boscii* (Otitidae; Piersol 1907), *Cardiacephala myrmex* (Calobatidae; Wheeler 1924) and *Rioxa pornia* (Tephritidae; Pritchard 1967; cf section 1.2.1.b above, in which I discuss re-gurgitated food offered by male *Drosophila*) and in the mecopteran *Panorpa* (Mercier 1914; Steiner 1930; Thornhill 1979, Thornhill & Sauer 1991).

Male *Panorpa* secrete salivary masses when they are unable to offer the female a dead arthropod (Thornhill 1979). Females discriminate against males which cannot produce salivary masses and do not possess an arthropod (Thornhill 1979), suggesting that female choice may be an important selective pressure in the evolution and\or maintenance of nuptial feeding in *Panorpa*. The duration of copulation in *Panorpa* is positively related to the number of salivary masses provided by a male (Thornhill 1979, Thornhill & Sauer 1991). The duration of copulation is important in determining male reproductive success in *Panorpa*. In *P.vulgaris*, over the range of copulation durations naturally encountered, the number of sperm transferred to the female is linearly related to mating duration (Hoster T., Sauer K.P., cited in Thornhill & Sauer 1991). When two males mate with the same female in this species, the male that copulates the longest (ie. introduces the most sperm) fertilises

a greater proportion of the female's eggs (Thornhill & Sauer 1991). This suggests that sperm competition is also likely to be an important selective pressure in the evolution and\or maintenance of nuptial feeding in *Panorpa*.

Interestingly, in North American *Panorpa* species, males sometimes adopt an alternative strategy to secure matings. Instead of offering a dead arthropod or a salivary mass, they obtain matings by force (Thornhill 1979, 1980, 1981, 1984). Males rush at passing females, grasp a leg or wing with their genital claspers then attempt to re-position the struggling female and secure the anterior edge of the female's forewing in the clamp-like notal organ situated on the dorsum of the male's abdomen. When the female's wings are secured, the male attempts to copulate. The male retains hold of the female's wing with his notal organ during copulation (Thornhill 1980). Thornhill (1980) found that male *Panorpa* whose notal organs are rendered non-functional by the application of beeswax are unable to obtain forced matings. Thornhill and Sauer (1991) found that the duration of copulation is shorter for males with inoperative notal organs in situations where there is likely to be sexual conflict over the optimal mating duration. Thus the notal organ appears to function both to allow males to obtain matings by force and to extend mating duration with unwilling females (Thornhill 1980, Thornhill & Sauer 1991). Given that salivary masses or captured arthropods offered to female's are likely to be costly (Thornhill 1980), why do *Panorpa* males not always adopt the forced copulation strategy? The answer probably lies in the observation that males using dead arthropods or salivary masses have a considerably greater success rate in securing copulations than "rapists" (Thornhill 1979, 1980).

1.2.3.c *Spermatophores and substances in the ejaculate.*

In a variety of insect orders, males transfer their ejaculate in a spermatophore (see reviews of Davey 1965; Mann 1984). Because females of many species either digest or remove and eat the spermatophore at some point after its transfer (reviewed by Engelmann 1970), Thornhill (1976a) considered spermatophores as a potential form of paternal investment. Subsequently, there has been a considerable amount published concerning the potential of spermatophores and other seminal nutrients to act as paternal investment. The spermatophores referred to in this section consist of a simple sperm-capsule as opposed to a sperm capsule plus a gelatinous spermatophylax (see section 1.1).

It is important to note at this stage that evidence that females exhibit an increase in reproductive output from multiple mating or through receiving larger spermatophores (and larger ejaculates) should not be used as evidence that spermatophores act as paternal investment. This is because males of a number of insect groups are known to transfer substances in the ejaculate which stimulate oviposition (see reviews of Leopold 1976; Chen 1984; see section 1.1.2.c). Ridley (1988) demonstrated that an increase in fecundity resulting from multiple mating is widespread in insects, even in groups with no apparent nuptial gifts or spermatophores. Increases in fecundity resulting from multiple-mating, therefore, may be due to the receipt of more oviposition stimulants, rather than extra male-provided nutrients. Furthermore, even if increases in fecundity following multiple-mating are likely to be caused by the accumulation of male-derived nutrients, this does not provide evidence that the level of nutrients provided by an individual male can have positive effects on female reproductive output.

1.2.3.c.i. *Orthoptera.*

In the acridid *Melanoplus sanguinipes*, Friedel & Gillott (1977) showed that radio-labelled accessory gland proteins transferred in the spermatophore enter the female's haemolymph and accumulate in the ovary within 24-72 h. During copulation, male *M.sanguinipes* transfer several spermatophores (on average 7; Pickford & Gillott 1971). Because the amount of sperm in each spermatophore is sufficient to fertilise the eggs in several egg batches (see Pickford & Gillott 1976), Friedel and Gillott (1977) argued that "clearly, therefore, the sperm *per se* are not the reason for multiple spermatophore transfer". They proposed that the multiple spermatophore transfer may function to assist in vitellogenesis by providing proteins which could be taken up by developing oocytes (ie. function as paternal investment). However, the implication that it is not adaptive for males to transfer more sperm than is necessary to fertilise a female's eggs is clearly erroneous. When sperm from two or more males are in competition for the fertilisation of a females eggs, the relative number of sperm transferred by a male may determine his probability of obtaining fertilisations (see chapter 2, section 2.2). Sperm competition might therefore be expected to select for the transfer of several spermatophores to each female (or one extra large spermatophore).

Another point is that radio-labelling techniques are very sensitive and the amount of male-derived material transferred may be very small. Substances transferred to the oocytes by male *M.sanguinipes* may be transferred in too small a quantity to have a significant effect on female fecundity (Gillott, pers.comm). Furthermore, some of the accessory-gland proteins transferred by male *M.sanguinipes* have been demonstrated to function primarily as oviposition stimulants (Friedel & Gillott 1976), though this does not rule out the possibility that they might also function as

paternal investment.

Reigert (1965) demonstrated that multiple-mating leads to an increase in fecundity in *Melanoplus*. However, as previously stated, this does not constitute evidence of paternal investment because the increase in fecundity might well be due to the effects of oviposition stimulants in the ejaculate.

In another species of acridid, *Chorthippus brunneus*, Butlin et al (1987) found that radio-labelled amino acids injected into the male were transferred after mating to the female's haemolymph and to eggs. Butlin et al (1987) also found that the rate of egg production and the number of eggs per pod were significantly greater in multiply-mated females than in singly-mated females. This effect was enhanced when female access to food was restricted, suggesting that male-derived nutrients might have contributed to the difference. While this demonstrates that female *C.brunneus* benefit from multiple mating, it does not provide good evidence that amino acids transferred by the male function as paternal investment. This is because the females in the multiple-mating group are likely to have mated up to 25 times (see Butlin et al 1986). Consequently, it is unclear whether nutrients donated by an individual male have a positive effect on female reproductive output. Furthermore, because there is pronounced last-male sperm precedence in *Chorthippus* (Ritchie et al 1989), a knowledge of the time taken for the female to begin to lay eggs containing spermatophore nutrients from her most recent mate, together with the female re-mating interval, are crucial in determining the potential of the male-derived proteins to function as paternal investment (see section 1.1.3.c). Such data appears to be lacking at present.

In the gryllid *Gryllus bimaculatus*, Simmons (1988a) demonstrated that the consumption of spermatophores from multiple mating led to an increase in egg

weight and hatching success. While multiple mating appeared to provide females with reproductive benefits, females had to mate multiply throughout life in order to accrue them. Therefore, they are unlikely to constitute a paternal investment from individual males (Simmons 1988a).

1.2.3.c.ii *Coleoptera.*

In *Caryedon serratus* and *Acanthoscelides obtectus* (Coleoptera; Bruchidae), immuno-blotting and autoradiographic studies have revealed that some male secretions are transferred from the spermatophore to the haemolymph of females within 24h after mating (Huignard 1983; Boucher & Huignard 1987). Some of these secretions may function primarily as a chemical cue, acting to stimulate oogenesis, oviposition, or regulate female receptivity (Huignard 1969; Huignard et al 1977; Huignard 1983; Boucher & Huignard 1987). In *C.serratus*, Boucher & Huignard (1987) discovered that some of these secretions or their derivatives are found in mature oocytes collected 6 days after mating. Similarly, in *A.obtectus*, male-derived amino-acids were detected in eggs laid 24-36h after mating (Huignard 1983). Consequently, Boucher & Huignard (1987) suggested that secretions from the large spermatophore of *C.serratus*, which represents 15% of male body weight, may function as a trophic source for the female, used during vitellogenesis. They demonstrated that when females *C.serratus* are deprived of food through adult life, the number of matings influences female fecundity: females mating 4-5 times laid more eggs than females allowed only a single copulation. When females were fed, there was no effect of the number of copulations on female fecundity (Boucher & Huignard 1987). While this may demonstrate that females benefit from multiple-mating during periods of starvation, it does not constitute firm evidence that secretions transferred by a male function as paternal investment. This is because, as

in studies of *C.brunneus* (Butlin et al 1987) and *G.bimaculatus* (Simmons 1988a), females had to mate a number of times in order to accrue the nutritional benefits. Consequently, it is unclear whether the level of nutrients transferred by an individual male can have a positive effect on female reproductive output, or whether the spermatophore-donating male stands to fertilise the eggs in which his nutrients are incorporated.

Landa (1960) observed that in the coleopteran *Melolontha melolontha*, egg maturation was more rapid in females with the most spermatophores (ie. which had had the higher number of matings). Landa (1960) reasoned that this effect might be due to nutrients transferred in the spermatophore. However, this effect might equally be due to substances in the ejaculate which stimulate oviposition and\or oogenesis (see reviews of Chen 1984; Leopold 1980).

1.2.3.c.iii *Diptera.*

In *Drosophila* (Diptera), there is no spermatophore (Mann 1984), but radio-labelling experiments have revealed that males transfer amino acids in their ejaculates, and that these appear in female somatic tissue and ovaries within 24 hours in *D.mojavensis*, *D.melanogaster*, *D.pseudoobscura* and *D.mulleri* (Markow & Ankney 1984; Bownes & Partridge 1987; Pitnick et al 1991). These substances are transferred in greater quantities in *D.mojavensis* than in *D.melanogaster* (Markow & Ankney 1984).

In *D.mojavensis*, Markow et al (1990) demonstrated that females appear to benefit from the male donations. When nutritionally deprived, females that received large ejaculates exhibited higher levels of fecundity in the week following mating than females mated with ejaculate-depleted males. However, this difference might be due

to females receiving different amounts of oviposition stimulant, which is known to be transferred in the ejaculate in *Drosophila* (see Chen 1984).

Whether this nutrient contribution can function as paternal investment depends upon whether the donating male can fertilise eggs which benefit from his nutrient donation. Female *D.mojavensis* re-mate daily and mate several times (Markow & Ankney 1984; Markow 1988). The female sexual refractory period is about ten hours, though females are unable to oviposit in this period due to the presence of a copulatory plug (Markow 1988). Consequently, whether the nutrient-donating male can father the offspring that benefit from his nutrition will largely depend upon the sperm precedence pattern (ie. the proportion of offspring fertilised by a subsequently-mating rival). Using genetic markers and radio-labels, Markow (1988) determined the paternity of eggs produced by doubly-mated female *D.mojavensis* and detected which of the potential fathers donated nutrients to the eggs. The results revealed that when a female re-mates, the last male fertilises the majority of eggs subsequently laid (mean proportion of offspring fertilised by the last male = P2 = 0.79). These eggs contain nutrients from both males. Thus males mating first donate material to progeny sired by males mating later (Markow 1988). It was the probability of such a situation occuring that led Wickler (1985) to propose that male nutrient donations are unlikely to function as paternal investment in insects.

In *D.melanogaster* females are known to mate less frequently than in *D.mojavensis* (Markow & Ankney 1984). Little is known of the function of the accessory-gland proteins which Bownes & Partridge (1987) demonstated to be transferred by *D.melanogaster* at mating. They might well function to affect female reproductive behaviour (Bownes & Partridge 1987), i.e. they might act as oviposition stimulants (see for example Hihara 1981; Chen 1984). If they can be demonstrated to have a

positive effect on the fitness of offspring of the donating male, then they may additionally function as paternal investment.

1.2.3.c.iv *Lepidoptera.*

In the Lepidoptera, the spermatophore can be large (Rutowski et al 1983), though there is considerable interspecific variation in relative spermatophore mass (Svärd & Wiklund 1989). Spermatophore mass as a percentage of male body mass ranges from 1.4% in *Pararge aegeria* to 15.5% in *Colias nastes* (Svärd & Wicklund 1989; Rutowski et al 1983). Individual males of *Pieris napi* have been observed to transfer a spermatophore corresponding to 23% of their body weight (Fosberg & Wicklund 1989). Thornhill (1976a) proposed that the large size of the lepidopteran spermatophore suggests a paternal investment function. Subsequently there have been a number empirical studies examining this possibility.

Radio-labelled amino acids from the spermatophore have been demonstrated to be incorporated into female somatic tissue and eggs in *Danaus plexippus* (Danaidae; Boggs & Gilbert 1979), *Heliconius hecale*, *H.erato*, *H.chartonius* and *Dryas julia* (Nymphalidae; Boggs & Gilbert 1979; Boggs 1981; Boggs 1990), *Colias eurytheme* and *Pieris napi* (Pieridae; Boggs & Watt 1981; Wiklund et al 1993), *Lymire edwardsii* (Ctenuchidae; Goss 1977, cited in Boggs & Gilbert 1979) and *Plodia interpunctella* (Phycitidae; Greenfield 1982). Other substances which have been traced from the spermatophore to the female soma and eggs include ^{65}Zn in *Heliothis virescens* (Noctuidae; Engebreston & Mason 1980), ^{32}P in *Leucania separata* (Noctuidae; Chao 1981), sodium ions in *Thymelicus lineola* (Hesperiidae; Pivnick & McNeil 1987) and pyrrolizidine alkaloids in *Utetheisa ornatrix* and *Ithomia agnosia* (Eisner & Meinwald 1987; Dussourd et al 1991; Brown 1984).

Several studies have examined the effect of the size of the spermatophore received by females on fecundity. In a number of lepidopteran species, recently mated males produce spermatophores and ejaculates about half the size of those produced by males that have not recently mated (Boggs 1981; Rutowski 1979, 1984, Rutowski & Gilchrist 1986; Rutowski et al 1987; Sims 1979; Svärd 1985; Svärd & Wicklund 1986, 1989; Oberhauser 1988; He & Tsubaki 1992; Royer & McNeil 1993). Consequently, a number of studies have compared the fecundities of females receiving either large or small spermatophores. Of these, a positive effect of spermatophore size on female fecundity has been found in *C.eurytheme* (Rutowski et al 1987), while no effect has been found in *P.interpunctella* (Greenfield 1982), *Agriphla plumbifimbriella* and *Parapedsia teterrella* (Pyralidae; Marshall 1986, cited in Marshall & McNeil 1989), *Pseudaletia unipuncta* (Noctuidae; Fitzpatrick & McNeil 1989), *Euphydras editha* and *E.chalcedona* (Nymphalidae; Jones et al 1986) *D.plexippus* (Oberhauser 1989) and *Papilio machaon* (Papilionidae; Svärd & Wicklund 1991). These species in which no effect has been found include both those with relatively small spermatophores (3.5% of male body weight in *P.machaon*, Svard & Wicklund 1989) and those with relatively large spermatophores (10.8% of male body weight in *E.chalcedona*, Rutowski et al 1983).

Other studies have examined the effect of multiple mating on female fecundity. A positive effect has been found in *Papilio xuthus* (Watanabe 1988) and *Pieris napi* (Wiklund et al 1993). In *D.plexippus* the effect is disputed: Svärd & Wicklund (1988) found no effect of the number of times a female had mated on fecundity, while Oberhauser (1989) found a positive effect.

The experiments comparing fecundities of females mated to males producing smaller or larger spermatophores may be criticised for two separate reasons. Firstly, an

increase in fecundity resulting from the receipt of a larger spermatophore (or a larger number of spermatophores, through multiple matings) might be due to the effects of extra sperm or oviposition stimulants in the ejaculate as opposed to extra nutrients in the spermatophore (see Leopold 1976; Chen 1984). For example, Royer & McNeil (1993) found that the lower fecundity of female *Ostrinia nubilalis* (Pyralidae) which had been mated with previously-mated males (which produce smaller spermatophores) was due to the fact that such females retained a greater proportion of their egg complement. Royer & McNeil (1993) suggested that males which had mated previously were unable to transfer sufficient sperm, meaning that it is in the female's interests to hold back a larger proportion of their eggs when mating with such males.

If the effect of the receipt of more spermatophore nutrients on female fecundity is to be examined, ejaculate volume and sperm number should ideally be held constant. However, this may prove to be difficult in the Lepidoptera as the spermatophore is broken down in the female's reproductive tract (as opposed to the Ensifera, where females can be prevented from eating the spermatophore and still receive a full ejaculate). If it is the effect of nutrients in the ejaculate on female fecundity which is to be examined, controlling for the effect of oviposition stimulants may be very difficult indeed.

Marshall (1982) put forward a second reason why experiments examining the effect of smaller or larger spermatophores on female fecundity may be criticised. He questioned the underlying assumption of such studies - that spermatophore mass is an accurate measurement of spermatophore value to females. Marshall & McNeil (1989) found that the spermatophore of *Pseudaletia unipuncta* contains hydrocarbons, amongst other things. They suggested that as hydrocarbons are found in eggs, spermatophore hydrocarbons might be important for egg production. Marshall &

McNeil (1989) found no relationship between the total hydrocarbon content of spermatophores and spermatophore mass in this species. They suggested that this might account for the failure of Fitzpatrick and McNeil (1989) to find any effect of spermatophore mass on fecundity in *P.unipunctata*.

The question of whether males are likely to fertilise eggs which benefit from their nutrients arises once again. In the Lepidoptera, female multiple mating (polyandry) is widespread, though certain species are considered to be largely monandrous (see Erlich & Erlich 1978; Gwynne 1984b; Drummond 1984; Svärd & Wiklund 1989). The majority of lepidopterans studied so far exhibit pronounced last-male sperm precedence (Gwynne 1984b; Drummond 1984; Ridley 1989). With this pattern of sperm precedence, the time taken for nutrients to be incorporated into eggs and the time taken for these eggs to be laid (ie fertilised), together with the female re-mating interval, become crucial in determining whether the spermatophore can act as paternal investment.

In *Colias eurytheme*, a species which exhibits both last-male sperm precedence and polyandry (Boggs & Watt 1981), incorporation of male-derived nutrients into eggs is rapid: male-derived nutrients can be traced in eggs laid the day after mating (Boggs & Watt 1981). Egg production peaks on the second or third day following the start of oviposition (Boggs & Watt 1981). Females in the field typically re-mate about 4-8 days after a previous mating (Rutowski & Gilchrist 1986). Thus males are likely to fertilise eggs containing their nutrients (Rutowski et al 1987).

However, in *Plodia interpunctella*, a species which also exhibits pronounced last-male sperm precedence (Brower 1975), incorporation of male-derived nutrients into eggs takes 24-48 hours (Greenfield 1982). The female re-mating interval in this

species is only one day (Brower 1975). Therefore, subsequently-mating males are likely to benefit from the nutrient donations of previous males (if females re-mate at the end of their refractory periods in the field).

In *Pieris napi*, Wiklund et al (1993) found that the rate of incorporation of male-derived nutrients into eggs peaked at 3-4 days following mating and subsequently levelled off to stabilise at 40% of the maximum. They also found that, if given the opportunity, females of this species would re-mate after 3-5 days. Given that there is last-male sperm precedence in this species (Kaitala & Wiklund, in prep, cited in Wiklund et al 1993), the chance of a male fertilising a significant proportion of the eggs in which his nutrients are incorporated would appear to be slim (if females do tend to re-mate at the end of their refractory periods in the field). As Wiklund et al (1993) put it: "the nutrient investment of the first male to mate with a female 'subsidizes' the progeny of later-mating males". It would thus seem unlikely that nutrients in the spermatophore function as paternal investment in this species (but see Wiklund et al 1993, who propose that despite these points, the spermatophore qualifies as both mating effort and paternal investment in *P.napi*).

The rate of incorporation of male-derived nutrients into eggs can be less rapid in other species. The peak of incorporation of male derived amino acids into eggs varied from 3 to as many as 14 days after mating in *Heliconius hecale* and *H.erato* (Boggs & Gilbert 1979). Oberhauser (1992) examined the rate of breakdown of spermatophores in the bursa copulatrix of *Danaus plexippus* females. She found that the time required for the breakdown of large spermatophores is longer than the female re-mating interval: females re-mated after 3-4 days, at which point only half of the mass of larger spermatophores had been broken down. Oberhauser (1992) concluded that in this species, "a significant portion of a male's investment could be used to benefit offspring from other males".

Svard & Wicklund (1991) proposed that the timing of eventual use of male-derived nutrients for female reproductive output may be subject to substantial variation between species, being dependent, among other things, on the number of mature eggs that females have in the abdomen at the time of mating.

Boggs (1981) proposed that in addition to selection for paternal investment, spermatophore size in the lepidoptera may be maintained by selection on males to delay female re-mating. Several studies of lepidopterans have demonstrated that the duration of the female refractory period is dependent upon the size of the ejaculate received (Labine 1964; Obara et al 1975; Sugawara 1979; Rutowski 1980; Rutowski et al 1981; Rutowski 1984; Oberhauser 1989; He & Tsubaki 1991, Oberhauser 1992). The induction of a refractory period in females is likely to be important for males of most lepidopteran species due to the prevalence of last-male sperm precedence in this group (see Gwynne 1984b; Drummond 1984; Ridley 1989). By delaying female re-mating, a male may increase the chance that the female he has mated with will lay eggs which he can fertilise before mating with another male. That is, males can decrease the risk of sperm competition. Selection on males to induce refractory periods in their mates is likely to be stronger in species with a greater degree of polyandry (i.e. where the probability of sperm competition is greater: Svärd & Wicklund 1989)

In a comparative study of 20 species of butterfly belonging to the families Pieridae and Satyridae, Svärd and Wiklund (1989) provided strong evidence for the role of sperm competition in the evolution of spermatophore size in lepidopterans. They demonstrated that spermatophore size relative to male body weight is greater in the polyandrous Pieridae than in the largely monandrous Satyridae. More importantly,

within the Pieridae, the authors found a positive correlation between spermatophore size (relative to male body weight) and degree of polyandry (ie. probability of sperm competition). Interestingly, this pattern is opposite to that predicted by the paternal investment hypothesis (see Boggs 1981).

Recently, evidence has been provided that, within species, male lepidopterans appear to respond to an increased risk of sperm competition by producing larger spermatophores (He & Tsubaki 1991, 1992; He 1992). He and Tsubaki (1992) found that male *Pseudaletia separata* (Noctuidae) which had been reared in crowded conditions produced larger spermatophores than those reared in solitary conditions, although adult body weight did not differ between the two groups. Compared with females that received small spermatophores, females receiving larger ones showed neither higher fecundity nor longer lifespan but did have longer refractory periods (He & Tsubaki 1991; He 1992). He (1992) concluded that the production of large spermatophores may function to increase the proportion of eggs fertilised by a male by inducing longer refractory periods in females - a function which is particularly important when competition between males for females or the probability of female re-mating are high.

These studies suggest that sperm competition is likely to be a significant selective pressure in the evolution and maintenance of large spermatophores in lepidopterans. However, this does not preclude the possibility of large spermatophores incidently benefitting females and\or their offspring. Large spermatophores might even additionally function as paternal investment where circumstances (eg. the female re-mating interval, sperm-precedence pattern, rate of incorporation of male derived nutrients into eggs, the time taken for these eggs to be laid and the effects on offspring fitness or number) are favourable.

1.3 *Summary*

In certain species of cricket and bushcricket (Orthoptera; Ensifera), males transfer an elaborate spermatophore to the female at mating. This consists of a sperm-containing ampulla and an often substantial, sperm-free, gelatinous mass, the spermatophylax. After mating, the female eats the spermatophylax before consuming the ampulla (Boldyrev 1915). The spermatophylax is particularly well developed in the bushcrickets (Tettigoniidae), contributing to a loss of as much as 40% of male body weight at mating in some species (Busnel & Dumortier 1955). Interestingly, though, there is a large degree of variation in spermatophylax size between species (Gwynne 1983a). There has been considerable debate over the selective pressures responsible for the evolution and maintenance of the spermatophylax and other forms of nuptial feeding in insects (Wickler 1985, 1986; Gwynne 1986a; Sakaluk 1986a; Simmons & Parker 1989). On the one hand, empirical evidence has been provided that the spermatophylax functions as a form of male nutritional investment in offspring (= paternal investment). Spermatophylax proteins can be incorporated into developing eggs (Bowen et al 1984; Simmons & Gwynne 1993; Wedell 1993a) and have been demonstrated to increase the weight and number of eggs in some cases (*Requena verticalis*, Gwynne 1984a, 1988a; in a zaprochiline, Simmons 1990a) but not in others (*R. verticalis*, Gwynne et al 1984; *Decticus verrucivorus*, Wedell & Arak 1989; *Poecilimon veluchianus*, Reinhold & Heller 1993). Furthermore, in some species the male appears to stand a chance of fertilising a significant proportion of the eggs in which his spermatophylax nutrients are incorporated (in *R. verticalis*, Gwynne 1988b; in the zaprochiline, Simmons 1990a; Simmons & Gwynne 1993), though this does not appear to be the case in others (*Metaplastes ornatus*, Helversen & Helversen 1991; *P. veluchianus*, Achmann et al 1992; Heller & Helversen 1991; *D. verrucivorus*, Wedell 1993a).

On the other hand, there is evidence that the spermatophylax functions to increase the proportion of a female's eggs fertilised by a male (= mating effort) by preventing the female from eating the ampulla before complete sperm transfer (Sakaluk 1984; 1986b; Wedell & Arak 1989; Wedell 1991; Simmons & Gwynne 1991; Reinhold & Heller 1993; Wedell, in press). Empirical evidence concerning the function of other forms of nuptial feeding in insects is equally inconclusive. It is important to note, however, that the paternal investment and mating effort hypotheses for spermatophylax function need not be mutually exclusive. Furthermore, the selective pressures responsible for the evolutionary origin of a character need not be the same as those responsible for its maintenance (see Simmons & Parker 1989). Gwynne (1986b, 1988b, 1990a) proposed that while the spermatophylax appears to have originated in the context of intrasexual selection as a sperm-protection device, evolutionary enlargement of the spermatophylax may have proceeded through natural selection for paternal investment.

1.4 *Aims and outline of the thesis.*

Using a comparative approach combined with laboratory manipulations, this study aims to contibute to an appreciation of the selective pressures responsible for the origin, evolutionary enlargement and maintenance of the spermatophylax in bushcrickets.

In chapter two, I review the mating and post-mating behaviour of the Ensifera (crickets and bushcrickets) and present previously unpublished data on the mating behaviour and spermatophore size (as a percentage of male body weight) of 60 species of tettigoniid. I describe and evaluate the behaviours which Boldyrev (1915)

and Alexander and Otte (1967a) considered to be analogous to the spermatophylax in function.

In chapter three, I describe the unusual prolonged copulation which appears to replace the spermatophylax in certain members of the tettigoniid sub-families Ephippigerinae and Meconematinae. I contrast this with the copulatory behaviour and spermatophores of other members of these sub-families, and discuss the significance of prolonged copulation in revealing selective pressures responsible for the evolution of the spermatophylax.

In chapter four, I present the results of a comparative study of 43 tettigoniid species from 8 subfamilies, designed to test the ejaculate-protection hypothesis for the evolution of the large spermatophylax. I test the prediction that species with proportionately larger spermatophylaxes should have proportionately larger ampullae (i.e. ejaculate volume) which should contain more sperm.

In chapter five, I examine the time taken for complete sperm transfer and the spermatophylax-eating time in two *Leptophyes* species (Tettigoniidae; Phaneropterinae; Barbitistini) which differ in spermatophylax size. I test the prediction that if the evolutionary enlargement of the spermatophylax has occurred through the protection of greater volumes of ejaculate (as opposed to through paternal investment), the shape of the sperm-depletion curve in relation to the mean spermatophylax-eating time should not be significantly different for the two species.

In chapter six, I examine the relationship between ampulla size, sperm number and spermatophylax size in *Leptophyes laticauda* Friv., a species in which males produce a large spermatophylax. I test a prediction of the ejaculate-protection hypothesis, namely that within species, males should adjust spermatophylax size in relation to

the number of sperm and\or volume of ejaculate they are able to produce. In this chapter, I also examine an assumption of the ejaculate-protection hypothesis, namely that the production of a larger spermatophylax should result in a longer duration of ampulla attachment.

In chapter seven, I examine the pattern of sperm-precedence and female refractory periods in two *Leptophyes* species which differ greatly in spermatophylax size, and in the ephippigerine *Steropleurus stali* Bol. which produces a very substantial spermatophylax. Such data are important in determining the potential of the spermatophylax to function as a form of paternal investment.

In chapter eight, I examine the effects of spermatophylax-feeding on female fecundity in the two *Leptophyes* species and present preliminary results for the ephippigerines *S.stali* and *S.asturiensis* Bol.. I also examine the effect of double-mating *per se* on female fecundity in *L.punctatissima* Bosc..

[illegible] and Post-Mating Behaviour of Male Ensiferans (Crickets etc.): Adaptations to Maximise Sperm Transfer?

2.1 *Introduction.*

In order to understand the selective pressures responsible for the evolutionary origin of the spermatophylax in tettigoniids, it is necessary to place spermatophylax production in the broader context of the mating and post-mating behaviour of members of the sub-order Ensifera.

The Ensifera is a sub-order of the Orthoptera and may be divided into three principal superfamilies (Beier 1955; Key 1970) though, needless to say, there are numerous different classification schemes for this group (see, for example, Kevan 1982). The principal superfamilies are: the Tettigonioidea (bushcrickets or Katydids), the Grylloidea (field-crickets, tree-crickets and mole-crickets) and the Gryllacridoidea (cave- or camel-crickets, wetas and leaf-rolling crickets). Three different views of the phylogenetic relationships of these principal groups are summarised in Hennig (1981).

Male ensiferans exhibit a range of behaviours which have been interpreted as strategies to maximise sperm transfer by deterring the female from eating the externally-attached spermatophores before sperm transfer is complete (Boldyrev 1915; Gerhardt 1913, 1914, 1921; Sakaluk 1986a and references therein; Loher & Dambach 1989). Boldyrev (1915) included spermatophylax production in his

classification of these male "counter measures". Boldyrev's (1915) classification is as follows (direct translation; my sub-headings):

a) **Multiple matings with the same female.** The female immediately or a little while after copulation (4 sec - 1.5 mins) eats the "simple" spermatophore (eg. *Arachnocephalus*); a considerable proportion of sperm is lost in this case but thanks to very frequent matings (and probably the speed of sperm transfer) sperm enter the female; this is a very primitive method;

b) **Prolonged copulation**. Having deposited the spermatophore, the male remains in copulation with the female for a long time (56 min - 2h,12 min) and his presence itself protects the spermatophore from destruction by the female (eg.*Dolichopoda*);

c) **Feeding the female with glandular secretions**. For some time after copulation (7.5 - 33 min) the male entices the female with a secretion from a special gland on his metanotum to draw her attention from the premature destruction of the spermatophore (eg. *Oecanthus*);

d) **Spermatophylax production**. The spermatophore is supplied with an enormous, sperm - free, mucoid section (**spermatophylax**) which the female has to chew for some hours while sperm pass from the ampulla to the receptaculum seminis [spermatheca]; having eaten the mucoid barrier the female finally eats the sperm ampulla which is now empty (eg. the majority of the Tettigonioidea).

The additional category of **post-copulatory mate guarding** may be added to this list (Gerhardt 1913; Khalifa 1950a; Huber 1955; Alexander 1961; Alexander & Otte 1967a; Loher & Rence 1978). This behaviour is widespread amongst the Gryllinae

(Alexander & Otte 1967a). After spermatophore transfer the male maintains close contact with the female and behaves aggressively towards other males. Female movements towards spermatophore-eating are apparently prevented by the male (Alexander & Otte 1967a; Loher & Dambach 1989). Guarding may help maximise sperm transfer both by preventing premature spermatophore removal by the female and by allowing the male to monopolise her for repeated matings (Alexander 1961; Loher & Rence 1978).

2.1.1 *Why should males maximise sperm transfer?*

At one level, males may be selected to deliver sufficient sperm to fertilise all the eggs a female is likely to produce. At another level, in a group such as the Ensifera where polyandry (female mating with more than one male) is widespread (see section 2.1.2), sperm competition is likely to be a potent force influencing male reproductive success (see chapter 1, section 1.1.2.a, for a definition of sperm-competition).

When the sperm of two or more males are in competition for the fertilisation of a female's eggs, the male that has inseminated most sperm is likely to have a greater probability of obtaining fertilisations, especially where there is some degree of sperm mixing (Woodhead 1985; Parker 1990a; Parker et al 1990). Simmons (1987) demonstrated this in the field cricket, *Gryllus bimaculatus*. The proportion of a multiply-mated female's offspring fertilised by a male increased with the duration of spermatophore attachment and, therefore, the number of sperm transferred by that male. In addition, the proportion of a female's offspring fertilised by a male increased in proportion to the number of times he mated with her, such that second males mating three times after an initial double mating had the advantage at fertilisation.

Amongst the insects, a positive relationship between the probable amount of sperm transferred by a male, relative to a competing male, and the proportion of eggs fertilised by that male has also been demonstrated in the gryllid *Gryllodes supplicans* (Sakaluk 1986b), in the tettigoniid *Decticus verrucivorus* (Wedell 1991), in a chrysomelid beetle (Dickinson 1986, 1988), a species of burying beetle (Muller & Eggert 1989), a scorpion-fly (Thornhill & Sauer 1991), yellow dung flies (Parker et al 1990; Simmons & Parker 1992) and water striders (Rubenstein 1989). A similar phenomenon has also been documented in domestic hens (Martin et al 1974), golden hamsters (Ginsberg & Huck 1989) and muroid rodents (Dewsbury 1984). It seems, therefore, that the occurrence of sperm competition is likely to select for males to maximise sperm-transfer (cf chapter 4, section 4.4).

A longer duration of spermatophore attachment and the consequent transfer of a greater volume of ejaculate may also benefit the male by inducing a longer nonreceptive refractory period in the female (for tettigoniids see Gwynne 1986b; Wedell & Arak 1989; Simmons & Gwynne 1991), though there may be sexual conflict over the duration of this period (Simmons & Gwynne 1991). An increase in the amount of ejaculate transferred may also hasten the onset, and increase the rate, of oviposition (Sakaluk & Cade 1980; Wedell & Arak 1989). These effects stand to increase the likelihood that the female will lay eggs before mating with another male; they reduce the risk of sperm-competition.

2.1.2 *Polyandry in the Ensifera.*

Polyandry (hence sperm-competition) appears to be widespread in the Ensifera, occurring in the Grylloidea (eg. Boldyrev 1915; Alexander & Otte 1967a; Sakaluk & Cade 1983; Boak 1984; Walker 1984; Sakaluk 1987; Rost & Honegger 1987; Simmons 1988a; Solymar & Cade 1990), the Tettigonioidea (eg. Boldyrev 1915; Rentz 1963; Eluwa 1979; Gwynne 1984c, 1990b; Gwynne & Simmons 1990; Heller & Helversen 1991; Wedell 1993a) and the Gryllacridoidea (Richards 1961; Eades 1964; Field & Sandlant 1983; Barrett 1991).

One of the benefits of multiple-mating for female ensiferans probably arises from the consumption of extra spermatophores (see Simmons 1988a; Loher & Dambach 1989 for a discussion of the benefits of multiple-mating for female gryllids). Females of the gryllid *Gryllus bimaculatus* allowed to consume spermatophores from multiple matings were found to produce heavier eggs (Simmons 1988a). Similarly, in the tettigoniid *Requena verticalis*, an increase in the number of spermatophylaxes eaten by females was found to result in an increase in the weight and number of eggs produced (Gwynne 1984a, 1988a; but see Gwynne et al 1984, where no effect of an increase in number of spermatophylaxes consumed on egg weight or number was found in this species). In the gryllids *Gryllodes sigillatus* and *Gryllus veletis*, females given unlimited mating opportunities were found to live significantly longer than females given limited mating opportunities (Burpee & Sakaluk 1993). This was probably a result of the extra spermatophores consumed by females in the former category (Burpee & Sakaluk 1993). In *R. verticalis* and a zaprochiline bushcricket, females have been found to increase their mating frequency when food-limited (Gwynne 1990b; Gwynne & Simmons 1990; Gwynne 1991). This is

further evidence that female ensiferans benefit nutritionally from multiple matings.

2.1.3 *Why should females remove spermatophores before complete sperm-transfer?*

If females are re-mating simply to obtain additional spermatophores (see 2.1.2 above), there would be no need for them to wait until sperm transfer is completed before eating the spermatophore. This might partly explain why female ensiferans have a tendency to eat spermatophores before complete sperm transfer unless deterred from doing so by the male (see Hohorst 1937; Huber 1955; Loher & Rence 1978; Gwynne et al 1984; Sakaluk 1984; Evans 1988)

It is possible that the optimal degree of insemination is different from male and female perspectives: while sperm competition may select for males to deliver large quantities of sperm (see section 2.1.1), females may only require sufficient sperm to enable them to fertilise their supply of eggs (storage of sperm could involve costs to the female, see Thornhill & Alcock 1983). This would lead to sexual conflict over the duration of spermatophore attachment.

Simmons (1986, 1987) proposed that female *G.bimaculatus* use spermatophore-removal as a subtle form of mate-choice. Simmons (1986) demonstrated that un-guarded females remove spermatophores of smaller males sooner - often before complete insemination is achieved. As sperm-mixing occurs in this species, the paternity of offspring is dependent upon the numerical representation of sperm in the spermatheca (Simmons 1987; see section 2.1.2). Females may therefore effectively select the paternity of their offspring by manipulating the duration of spermatophore

attachment (Simmons 1986,1987). By mating with all available males and exercising post-copulatory mate choice, females may also accrue potential nutritional benefits associated with the consumption of spermatophores (Simmons 1986, 1988a).

Zuk (1987) found that in the gryllid *G.veletis*, the number of spermatophores a male is able to produce in 24 hours (0-10 spermatophores) is negatively correlated with levels of gregarine parasite infection. Therefore, females mating repeatedly with the same male may have been favoured by natural selection because healthy males are more likely to be able to provide several spermatophores in quick succession (Zuk 1987). More to the point, by removing spermatophores before complete sperm transfer, females may "test" the ability of the guarding male to produce additional spermatophores. This may ensure that the female fertilises more of her eggs with sperm from a male that is vigorous and relatively parasite-free (Zuk 1987, see also Simmons 1990b).

2.1.4 *Aims of this chapter.*

Since Boldyrev's (1915) review, a great deal has been published on the mating behaviour of Ensiferans. Many new species have been studied in this respect and an array of male mating and post-mating behaviours have been described. Furthermore, a growing interest in the possibility of male nutritional investment in offspring has led to new interpretations of the function of male post-copulatory behaviours which potentially involve transfer of nutrients to the female (see Gwynne 1983a). In the following sections of this chapter, I review the literature describing the mating and post-mating behaviour of male ensiferans. I then discuss evidence concerning the function of each category of male post-mating behaviour described.

2.2 *The Mating and Post-mating Behaviour of Male Ensiferans.*

For clarity and succinctness, the review of male mating and post-mating behaviour in the Ensifera is presented here in the form of a table (table 2.1, in appendix 2).

The higher classification adopted here follows that of Key (1970). This in turn was based on that of Beier (1955), from which it differs in the elevation of the Myrmecophilinae (Grylloidea) to family rank, and in regarding the Haglidae (= Prophalangopsinae) as belonging to the Tettigonioidea rather than the Gryllacridoidea.

Though primarily a review of the literature, personal observations on the mating behaviour and spermatophores of 60 species of tettigoniid are included. Methods used during these observations are outlined in chapter four, while collecting and rearing information is outlined in appendix 1.

2.3 *The Function of Different Male Post-Mating Behaviours in the Ensifera.*

2.3.1 *Post-copulatory mate guarding.*

During post-copulatory mate guarding in the Ensifera, the male maintains close contact with the female, behaves aggressively towards other males and makes movements directed at the female (antennation, jerking the body backwards and forwards and, in some cases, head-butting) should she attempt to remove the spermatophore or to leave (Khalifa 1950a; Huber 1955; Alexander 1961; Alexander & Otte 1967a; Loher & Rence 1978; Loher & Dambach 1989; Simmons 1990b; Sakaluk 1991; Simmons 1991).

This behaviour is found in *Gryllus*, *Acheta domesticus*, *Gryllodes supplicans*, *Modicogryllus conspersus*, *Teleogryllus commodus*, *Miogryllus verticalis* (Grylloidea; Gryllidae; Gryllinae), *Nemobius sylvestris* (Grylloidea; Gryllidae; Nemobiinae), *Balamara gidya* (Grylloidea; Gryllidae; Trigonidiinae) and *Cycloptilum antillarum* (Grylloidea; Gryllidae; Mogoplistinae) (see table 2.1, in appendix 2). Mate guarding in which the male maintains contact with the female after mating, but in which males apparently do not attempt to prevent the female from eating the spermatophore occurs in *Neonemobius*, *Allonemobius*, *Eunemobius*, *Hygronemobius alleni*, *Bobilla victorae* (Grylloidea; Gryllidae; Nemobiinae), *Hapithus agitator* (Grylloidea; Gryllidae; Eneopterinae), *Amphiacusta* spp (Grylloidea; Gryllidae; Phalangopsinae) and *Deinacrida* (Gryllacridoidea; Stenopelmatidae; Deinacridinae) (see table 2.1). A slightly different kind of mate-guarding occurs in *Hemideina* (Gryllacridoidea;

Stenopelmatidae; Henicinae): the male aggressively defends a hole in a tree containing his harem of females (see table 2.1).

Post-copulatory mate-guarding in the Ensifera may function both to deter the female from removing the spermatophore prematurely and to monopolise the female for repeated matings (Khalifa 1950a; Alexander 1961; Loher & Rence 1978; Evans 1988; Loher & Dambach 1989, Loher 1989). The latter function is obviously the most important in species in which males do not attempt to prevent the female from eating the spermatophore during guarding. Another function may be to prevent copulation attempts by rival males which can dislodge the spermatophore before complete sperm transfer (Sakaluk 1991). This appears to be the most important function of postcopulatory mate-guarding in *Gryllodes supplicans* (Sakaluk 1991).

Some support for the hypothesis that guarding may function to prevent the female from eating the spermatophore prematurely is provided by evidence that the time taken for complete sperm transfer roughly corresponds to guarding duration in certain species. For example, in *Acheta domesticus*, guarding and complete sperm transfer both take about 60 min (Khalifa 1949a, 1950a). In *Teleogryllus commodus*, the mean time taken for complete sperm transfer is about 70 min (Loher & Rence 1978) and guarding lasts an average of 53 min (Evans 1988) or 83 min (Loher & Rence 1978). In *Gryllus bimaculatus*, there is a positive relationship between the number of sperm transferred and spermatophore attachment duration over a period of 60 min (Simmons 1986) and guarding lasts for 40-60 min (Simmons 1990b). In *Gryllodes supplicans*, however, the mean duration of guarding (32 min) is shorter than the mean time taken for complete sperm transfer (50 min) (Sakaluk 1991). Unlike the previous species, *Gryllodes supplicans* males produce a spermatophylax which keeps the

female occupied during sperm transfer (Sakaluk 1984, 1985). Alexander & Otte (1967a) observed that in this species, guarding behaviour is "definitely less intense" than in *Gryllus* and *Acheta* and suggested that the spermatophylax "carries much of the function of this interaction".

Male actions during guarding suggest that they are attempting to control female spermatophore removal behaviour. In *Gryllus*, *Acheta domesticus* and *Teleogryllus commodus*, if the female starts to move away from the male or attempts to remove the spermatophore, the male jerks his body backwards and forwards and antennates her until she becomes motionless once more (Khalifa 1950a; Alexander 1961; Alexander & Otte 1967a; Loher & Rence 1978; Simmons 1990b). Similar behaviour, consisting of antennation of the female and a series of "push-ups", occurs in *Gryllodes supplicans* in response to female movement (Sakaluk 1991). According to Loher & Dambach (1989), male *T.commodus* employ, in addition, a less subtle behaviour to deter the female from eating the spermatophore: "if she makes conspicuous movements, such as bending back to pluck off the spermatophore with the mandibles, the male butts her with his head and she ceases at once". Similar "head-butting" behaviour, in response to female attempts to remove the spermatophore, has also been observed in *G.supplicans* (Sakaluk 1991).

Experiments designed to test the hypothesis that mate-guarding deters the female from prematurely removing the ampulla have produced conflicting results. In *A.domesticus* and *G.supplicans*, no difference in ampulla attachment duration has been found between females isolated from their mates after copulation and females who remained with guarding males (Sakaluk & Cade 1980; Sakaluk 1991), though in *G.supplicans*, Sakaluk (1991) did find a significant positive correlation between the duration of mate guarding and

ampulla attachment duration. The absence of an effect of a lack of guarding on ampulla attachment duration in *G.supplicans*, however, is not surprising as males of this species produce a spermatophylax. The duration of ampulla attachment in *G.supplicans* is governed by the time taken by females to consume the entire spermatophylax (Sakaluk 1984), which, in turn, is governed by spermatophylax size (Sakaluk 1985).

Other studies have supported the ampulla-retention hypothesis: females have been demonstrated to remove their spermatophores significantly sooner when males were prevented from guarding after mating, than when males were allowed to guard in *Gryllus campestris* (Huber 1955), *T.commodus* (Loher & Rence 1978, Evans 1988) and *Balamara gidya* (Evans 1988). In *T.commodus*, for example, females left their spermatophores attached for an average duration of only 7.3 min if guarding was interrupted and for 71 min if males were allowed to guard (Evans 1988). As complete sperm transfer takes an average of 69 min in this species (Loher & Rence 1978), females in the un-guarded group are likely to have interfered with sperm transfer. Un-guarded females of *T.commodus* and *B.gidya* also leave their mates significantly sooner than guarded females (Evans 1988).

Simmons (1986), however, questioned the ability of male post-copulatory mate guarding to control female behaviour. He criticised experiments conducted by Loher & Rence (1978) on *T.commodus*, pointing out that they were performed in confined observation cells which allowed little opportunity for females to move away from their mates. Simmons (1986) noted that in his experiments with *G.bimaculatus* in an open arena, where females had the opportunity to escape from unwanted suitors, males were never seen to successfully retain females that attempted to leave after mating. Loher & Dambach (1989), in turn, criticised Simmons' (1986) experiments,

suggesting that the open, exposed arena used by Simmons (1986) represented an unnatural environment, and that an urge to find shelter probably accounted for the tendency of females to leave their mates in his experiments (see Simmons 1991 for his response). Experiments by Evans (1988) on *T.commodus* were conducted in large arenas containing natural-habitat simulations, so neither Simmons' (1986) nor Loher & Dambach's (1989) criticisms apply. Evans' results, like those of Loher & Rence (1978), suggested that guarding influenced female spermatophore-removal behaviour and the tendency of females to stay with males after mating, though some cases of females successfully leaving guarding males were noted.

Guarding of females does not necessarily imply male control of females against their best interests. It may be in the female's interest to stay with, and receive sperm from, a male who is able to guard, if guarding ability is correlated with an aspect of male quality. Simmons (1990b) showed that guarding ability in *G.bimaculatus* is influenced by levels of infection by a protozoan gut-parasite: heavily infected males guard for a much shorter duration than mildly infected males.

2.3.2 *Multiple-mating with the same female.*

In its extreme form, multiple mating with the same female is characterised by the successive production of several spermatophores in a single mating encounter, each being eaten by the female shortly after its transfer. This behaviour in its most extreme is exemplified by *Orocharis* (Grylloidea; Gryllidae; Eneopterinae): the female immediately rubs off the first spermatophore and begins to eat it. She then climbs back on the male who transfers a second spermatophore while she feeds on the first. When she has finished eating, the female dismounts again and removes the second spermatophore, which she also begins to eat. Meanwhile, the male, who has

produced a third spermatophore, initiates yet another coupling. The entire sequence is repeated many times, and the male may produce up to 20 spermatophores in a 3.5 hour mating session (T.J Walker & R.Love in Alexander & Otte 1967a; Funk 1989).

Other species in which the transfer of more than one spermatophore per mating encounter appears to be the norm include: *Arachnocephalus vestitus* (Grylloidea; Gryllidae; Mogoplistinae), *Bobilla victorae*, *Hygronemobius alleni*, *Nemobius sylvestris* (Grylloidea; Gryllidae; Nemobiinae), *Amphiacusta* (Grylloidea; Gryllidae; Phalangopsinae), an undescribed trigoniidine (Grylloidea; Gryllidae; Trigonidiinae), *Scapteriscus* (Grylloidea; Gryllotalpidae; Scapteriscinae), *Udeopsylla robusta* (Gryllacridoidea; Rhaphidophoridae) and *Deinacrida* (Gryllacridoidea; Stenopelmatidae; Deinacridinae) (see table 2.1).

In other species, multiple mating would appear to be more opportunistic and to depend upon whether the female stays with the male after the first mating. These species include: *Gryllus*, *Acheta domesticus*, *Teleogryllus commodus*, *Teleogryllus* spp, *Miogryllus verticalis* (Grylloidea; Gryllidae; Gryllinae), *Neonemobius*, *Allonemobius*, *Eunemobius* (Grylloidea; Gryllidae; Nemobiinae) and possibly *Oecanthus* (Grylloidea; Gryllidae; Oecanthinae), *Hapithus agitator* (Grylloidea; Gryllidae; Eneopterinae) and *Hemideina* (Gryllacridoidea; Stenopelmatidae; Henicinae) (see table 2.1).

Multiple-mating with the same female is, in most cases, associated with mate-guarding, at least in the sense that males attempt to remain in contact with the female after mating. Apart from *Teleogryllus* spp (from South Africa, see Alexander & Otte 1967a), none of the species listed above produce a spermatophylax.

Multiple mating with the same female is clearly one way in which males can counteract the female tendency to eat spermatophores prematurely. In *Orocharis*, multiple spermatophore transfer not only counteracts female spermatophore-removal behaviour, but it also helps prolong spermatophore attachment duration. Females take 9 min to consume a spermatophore fully. During this period the male transfers the next spermatophore and complete sperm transfer occurs, a process which takes only 4 min (Funk 1989).

The benefit to males of multiple matings with the same female has been demonstrated by Simmons (1987). He showed that in the field cricket *G.bimaculatus*, when two males mate with the same female (ie. when their sperm are in competition), the proportion of the female's eggs fertilised by a given male increase in proportion to the number of times he mates with her relative to the competing male (see also Muller & Eggert 1989, who demonstrated the same thing in burying beetles).

Gwynne (1983a) suggested another benefit to the male of transferring several spermatophores to the same female: the spermatophores, which are generally eaten by the female, may represent paternal investment, ie. male nutritional investment in their own offspring (cf chapter 1 section 1.1.3). Gwynne (1983a) cited results by R.Rice, who studied an undescribed genus of trigonidiine which transfers several spermatophores in a single bout of mating. Using radiolabelled amino acids, Rice (cited in Gwynne 1983a) found that spermatophore nutrients were incorporated into the female's eggs. Whether these nutrients can act as paternal investment depends upon whether they have a positive effect on offspring fitness or number, and whether the donating male fertilises eggs which benefit from his nutrition (see chapter 1, section 1.1.3.b). This, in turn, depends upon the time taken for nutrients

to be incorporated into eggs, the time taken for the female to lay these eggs, the time taken before the female is likely to mate with another male, and the sperm-precedence pattern. As none of these variables appear to be known for the trigonidiine, it would be premature to consider multiple spermatophore production as a form of paternal investment in this species.

In *G.bimaculatus*, Simmons (1988a) found that females allowed to consume spermatophores from multiple matings produced heavier eggs which had a greater hatching success. However, as females must mate continually throughout their lives to accrue these benefits, an individual male is unlikely to benefit from his spermatophore donations. Consequently, Simmons (1988a) stated that the spermatophores of *G.bimaculatus* are best considered mating effort rather than paternal investment.

While it is uncertain whether a male can benefit from nutrients donated to the female following the transfer of several spermatophores, it seems clear that the female stands to benefit. The transfer of more than one spermatophore in a mating association is unlikely therefore to involve a conflict of interests between the sexes.

Another benefit to females of staying to receive more than one spermatophore from the same male was suggested by Zuk (1987). She demonstrated that in *G.veletis*, the number of spermatophores a male is able to produce in 24 h is negatively correlated with levels of gregarine (a protozoan gut-parasite) infection. Therefore females may benefit from staying to receive several spermatophores from the same male as they are likely to fertilise a greater proportion of their eggs with sperm from males with parasite-resistant genotypes.

2.3.3 *Feeding the female with a glandular secretion.*

In a different form of post-mating behaviour, the male produces a glandular secretion on which the female feeds following spermatophore transfer. Glandular-feeding is found in: *Discoptila fragosoi* (Grylloidea; Gryllidae; Gryllinae), *Neonemobius*, *Allonemobius* and *Eunemobius* (Grylloidea; Gryllidae; Nemobiinae), *Oecanthus* and *Neoxabea* (Grylloidea; Gryllidae; Oecanthinae) and, possibly, *Nemobius sylvestris* (Grylloidea; Gryllidae; Nemobiinae) (see table 2.1).

A similar type of post-mating behaviour is found in *Hapithus agitator* (Grylloidea; Gryllidae; Eneopterinae). In this species, the female feeds on the male's tegmina following spermatophore transfer (see table 2.1).

In *D.fragosoi*, *Oecanthus* and *N.bipunctata*, the secretion is produced by glands situated on the male's metanotum (Boldyrev 1915; Fulton 1915; Boldyrev 1928a, Hohorst 1937; Alexander & Otte 1967a; Walker & Gurney 1967; Walker 1978; Bell 1980; Funk 1989). In *Neonemobius*, *Allonemobius* and *Eunemobius* (all previously classified as *Pteronemobius*), the secretion is produced by glandular spurs situated on the male's hind tibia. The tips of these spurs, along with the resulting secretion, are eaten by the female following spermatophore transfer (Mays 1971). In *Nemobius sylvestris*, the male's tegmina are sometimes palpated by the female following spermatophore transfer, suggesting that secretory glands may be associated with this region (Gabutt 1954).

In *Neoxabea bipunctata*, males exhibit an interesting post-copulatory behaviour which accompanies the glandular feeding: after spermatophore transfer, while

the female is mounted upon the male and is feeding on his metanotal gland secretion, the male raises his hind legs and moves them back and forth along the body of the female for as long as 45 min (Walker 1978; Funk 1989). This behaviour has been interpreted as functioning to deter the female from dismounting and ending metanotal-gland feeding before complete sperm transfer (in the Oecanthinae, females tend to remove and eat their spermatophores shortly after dismounting) (Walker 1978; Funk 1989).

Glandular areas situated on the male's upper dorsal tergites are found in a number of tettigoniids (see Engelhardt 1915, Boldyrev 1915; Gwynne 1983). However, these are exclusively associated with pre-copulatory behaviour: the female mounts the male and palpates these areas, while her mate endeavours to grasp the base of her ovipositor or the sides of her sub-genital plate with his cerci and commence copulation (Boldyrev 1915; Rentz 1972; pers. obs.)

Hancock (1905) and Houghton (1909) suggested that the metanotal gland of *Oecanthus* may function to attract and hold the attention of the female while copulation takes place. Boldyrev (1915) proposed that the chief function of this gland in *Oecanthus* is to hold the attention of the female after spermatophore transfer to prevent her from eating the spermatophore before complete sperm transfer. Fulton (1915) pointed out that the gland may serve both purposes equally well as, in his observations of *O.fultoni*, the female fed from the gland both before and after spermatophore transfer. Another function of glandular feeding may be to maintain contact with the female for repeated matings. Males of *O.argentinus* have been observed to transfer a second spermatophore within 70 min of the first, and post-copulatory feeding in some *Oecanthus* species may last for up to 65 min (Walker & Gurney 1967).

Empirical evidence suggests that the primary function of glandular feeding in *O.pellucens* is to prevent the female from removing the spermatophore before sperm transfer is complete (Hohorst 1937). In this species, females were observed to feed from the male's secretion-filled thoracic cavity before spermatophore transfer in only 8% of 500 copulations, while in all cases females fed following spermatophore transfer (Hohorst 1937). Furthermore, when the secretion was removed or the cavity closed with tape, mating still occurred. However, after spermatophore transfer, females prevented from feeding on the males' glands dismounted immediately and ate the full spermatophore (Hohorst 1937). In normal matings of this species, females feed upon the male's metanotal gland secretions for 12-18 min following spermatophore transfer and eat the spermatophore within 1 min of dismounting (Hohorst 1937). The duration of feeding corresponds with the mean time taken for complete sperm transfer in this species (15 min) (Hohorst 1937).

Experiments conducted by Bell (1979, cited in Gwynne 1983a) on *O.nigricornis*, however, were less conclusive. Like Hohorst (1937), he showed that females denied access to male glands after spermatophore transfer left their spermatophores attached for a shorter duration than females allowed to feed on these glands. However, this period of time was apparently still sufficient to allow complete sperm transfer.

Bidochka & Snedden (1985) investigated the function of glandular feeding in *Allonemobius fasciatus*. Males of this species produce secretions from spurs situated on their hind tibia, upon which females feed during the extended copulation following spermatophore transfer. They found that females remained

in copulation following spermatophore transfer for significantly less time when mated to males whose tibial spurs were covered with paint (mean copulation duration = 4.5 min) than when mated to males whose tibial spurs were exposed (mean copulation duration = 22 min). However, the mean duration of spermatophore attachment did not differ significantly between the two experimental groups. Bidochka & Snedden (1985) suggested that the failure to find a difference in the duration of spermatophore attachment might have been due to the effects of "male harassment" of females in the close confines of the observation cells during post-copulatory mate guarding which occurs in this species.

Gwynne (1983a) proposed that glandular feeding in the Orthoptera may function as paternal investment (cf chapter 1, section 1.1.3). In support of this hypothesis, he cited results by Bell (1979, cited in Gwynne 1983a) who found a positive relationship between the time females spent feeding on male glands and their subsequent oviposition rate in *O.nigricornis*. However, it is not clear whether this study controlled for spermatophore attachment duration, which can have a positive effect on female fecundity: Sakaluk & Cade (1980, 1983) demonstrated that a longer duration of spermatophore attachment led to an increase in daily offspring production in the house cricket *Acheta domesticus* (see also chapter 1, section 1.1.2.c).

2.3.4 *Prolonged copulation.*

Boldyrev (1915) observed prolonged coupling, lasting 56-132 min, following the transfer of a simple spermatophore in the rhaphidophorid *Dolichopoda euxina*. He contrasted this with the brief copulation (lasting 3-4 min) and the spermatophylax - bearing spermatophores of the rhaphidophorid *Tachycines asynamorus*. Boldyrev

was amongst the first to propose that prolonged copulation following spermatophore transfer functions to prevent the female from removing the spermatophore before it is emptied of sperm.

Prolonged copulation following spermatophore transfer has now been recorded in: *Discoptila fragosoi* (Grylloidea; Gryllidae; Gryllinae), *Neonemobius*, *Allonemobius* and *Eunemobius* (Grylloidea; Gryllidae; Nemobiinae), *Anurogryllus arboreus* (Grylloidea; Gryllidae; Brachytrupinae), *Hapithus agitator* (Grylloidea; Gryllidae; Eneopterinae), *Amphiacusta* spp and *Phaeophilacris spectrum* (Grylloidea; Gryllidae; Phalangopsinae), *Neocurtilla hexadactyla* (Grylloidea; Gryllotalpidae; Gryllotalpinae), *Dolichopoda* and *Hadenoecus* (Gryllacridoidea; Rhaphidophoridae; Dolichopodinae), *Gymnoplectron longipes* (Gryllacridoidea; Rhaphidophoridae; Macropathinae), *Meconema* (Tettigonioidea; Tettigoniidae; Meconematinae), *Uromenus rugiscollis* (Tettigonioidea; Tettigoniidae; Ephippigerinae) and *Pterophylla beltrani* (Tettigonioidea; Tettigoniidae; Pseudophyllinae) (see table 2.1). This behaviour may also occur in *Ceuthophilus* (Gryllacridoidea; Rhaphidophoridae; Ceuthophilinae), *Zealandrosandrus gracilis* (Gryllacridoidea; Stenopelmatidae; Henicinae), *Deinacrida* (Gryllacridoidea; Stenopelmatidae; Deinacridinae), *Gymnoproctus sculpturatus* (Tettigonioidea; Tettigoniidae; Hetrodinae), *Dichopetala emarginata* (Tettigonioidea; Tettigoniidae; Phaneropterinae) and *Decticita brevicauda* (Tettigonioidea; Tettigoniidae; Decticinae) (see table 2.1). In the last five species, it is not entirely clear at which point during the prolonged copulation the spermatophore is transferred.

Apart from *Uromenus rugiscollis*, none of the above species produce a spermatophylax. In *U.rugiscollis*, the spermatophylax is considerably reduced in size compared to other members of the same subfamily (Ephippigerinae) in

which copulation following spermatophore transfer is brief (see chapter 3)

In *Amphiacusta* spp, *Neonemobius*, *Allonemobius*, *Eunemobius*, *Deinacrida* and *Hapithus agitator*, in addition to prolonged coupling, males may mate more than once with the same female in a mating association (see section 2.3.2).

Alexander & Otte (1967a) pointed out that "a longer copulatory act, whatever its advantages, could change selection in several ways - for example by giving a stronger advantage to more elaborate female-attracting dorsal glands... or to a greater ability of the male to grasp and hold the female."

In *Discoptila fragosoi*, *Neonemobius*, *Allonemobius* and *Eunemobius*, males produce glandular secretions upon which the females feed during the prolonged copulation (Boldyrev 1928a, Mays 1971, Bidochka & Snedden 1985, see section 2.3.3). In *Hapithus agitator*, females feed upon the male's tegmina during the prolonged copulation (Alexander & Otte 1967b).

Particularly firm coupling devices are found in, for example, *Hadenoecus* (the males "eversible organs" act as claspers and enclose the end of the female's abdomen; Hubbel & Norton 1978), *Meconema* (the elongated cerci of the male wrap around the end of the female's abdomen; see chapter 3) and *Neocurtilla* ("the abdomens are tightly held together by hooks", Baumgartner 1910). The specialised sub-genital plate of *Pterophylla beltrani* males, which has a slit which fits over the female's ovipositor during copulation (Shaw & Galliart 1987) may be an adaptation to maintain hold on the female during the prolonged coupling.

It is important to distinguish prolonged copulation which occurs before

spermatophore transfer from that which occurs following spermatophore transfer. Obviously only the latter can be interpreted as a means of ensuring complete sperm transfer. The amount of time spent in copulation before spermatophore transfer can be considerable - for example *Copiphora rhinoceros* (Tettigoniidae) pairs spend up to 4 hours in *copula* before the spermatophore is transferred, and separate 1 min later (Morris 1980). Interestingly, though, there is considerable variation between species in the amount of time spent in copulation before spermatophore transfer, particularly amongst the tettigoniids (see table 2.1). I discuss interspecific variation in copulation duration before spermatophore transfer further in chapter 3, part 1.

Observations on *Meconema* lend some support to the hypothesis that prolonged copulation following spermatophore transfer functions to increase the duration of spermatophore attachment by preventing the female from eating the spermatophore before complete sperm transfer (see chapter 3). In *M.meridionale*, pairs remain in copulation for 35-105 mins following spermatophore transfer (chapter 3). Females ate spermatophores (or at least made movements toward spermatophore-eating before being prevented from doing so) about 1 min after the end of copulation in all cases (n=7). Copulation duration, therefore, is likely to determine the duration of ampulla attachment. When males were experimentally removed shortly after spermatophore transfer, females made movements towards spermatophore-eating about 1 min later in all cases (n=4). Furthermore, preliminary data suggest that there is a positive relationship between the size of the spermatophore produced by a male and the duration of copulation following spermatophore transfer. This suggests that males may adjust copulation duration in relation to the amount of sperm they are able to produce (see chapter 3).

It is important to note that although time spent in copulation after spermatophore transfer can be lengthy in some of the species in which prolonged copulation occurs (about 7 hours in *Gymnoplectron longipes*, several hours in *Hadenoecus*, 1-4 hours in *Dolichopoda*, 1 hour - 2 hours,21 min in *Uromenus*, 35 min - 1 hour,45 min in *Meconema meridionale*, 15 min - 1 hour,30 min in *Discoptila fragosoi*, 30 min - 1 hour,15 min *Phaophilacris spectrum*, 20-45 min in *Allonemobius* and 24-37 min in *Pterophylla beltrani*, see table 1), it is comparatively short in others (7 min in *Amphiacusta*, 7-13 min in *Hapithus agitator*, over 10 min in *Neocurtilla hexadactyla* , 10-16 min in *Anurogryllus arboreus* and 13-24 min in *Meconema thalassinum*). The species in which the "prolonged" copulation is comparatively short qualify for this category because the time spent in copulation is likely to constitute a significant proportion of the time available for sperm transfer. This is either because the female eats the spermatophore shortly after the end of copulation (eg. *H.agitator* and *M.thalassinum*) or because the male retains the spermatophore upon the termination of copulation (eg. *Amphiacusta* and *Anurogryllus arboreus*).

Alexander & Otte (1967a) and Dambach & Lichtenstein (1989) suggested that in species in which sperm transfer occurs during copulation, the rate of sperm transfer may be comparatively rapid, as these species often have spermatophores with comparatively short, stout sperm tubes (based on observations of the spermatophores of *N.hexadactyla*, *A.arboreus*, *Amphiacusta* spp and *H.agitator*).

Differences between species in the duration of copulation following spermatophore transfer, where this is the only strategy used to prevent premature spermatophore

removal, may be due to differences in the amount of sperm produced and therefore the time taken for complete sperm transfer. For example: copulation following spermatophore transfer in *Meconema thalassinum* lasts 17 min, on average, while in *M.meridionale*, copulation continues for an average of 81 min following spermatophore transfer (ie. approx. 5 x longer) (see chapter 3). The mean number of sperm per spermatophore in *M.meridionale* is 3.6 x greater than that of *M.thalassinum*, even though these species do not differ significantly in male body weight (see chapter 3).

Greater efficiency of male coupling devices, which may be associated with prolonged copulation (Alexander & Otte 1967a), might allow males to remain in copulation for longer than would be in the female's best interests. Interestingly, observations of the behaviour of *M.meridionale* and *Decticita brevicauda* females during copulation suggest sexual conflict over the mating duration. In *D.brevicauda*, Rentz (1963) observed that on several occasions females apparently "tried to pull off mating males by dragging them against branches". In all copulations of *M.meridionale* which I have observed (n=7), from shortly after spermatophore transfer to the end of copulation, the female pushes at the male with her hind legs, runs backwards and forwards, and shakes as if trying to dislodge the male; she sometimes even bends round to bite at the male's abdomen (see chapter 3).

2.3.5 *The spermatophylax.*

The spermatophylax (see chapter 1) is found in 12% (4/34) of the genera of the Grylloidea studied, 23% (3/13) of the genera of the Gryllacridoidea studied and 90% (62/69) of the genera of the Tettigonioidea studied.

Within the Grylloidea, the spermatophylax has so far been recorded only in the subfamily Gryllinae of the Gryllidae and is known to occur in *Gryllodes supplicans*, *Gryllomorpha dalmatina*, *Valerifictorus shimba* and two *Teleogryllus* spp from South Africa (see table 2.1). Within the Gryllacridoidea, the spermatophylax has been recorded in *Tachycines asynamorus* (Rhaphidophoridae; Rhaphidophorinae), *Troglophilus cavicola* (Rhaphidophoridae; Troglophilinae) and *Stenopelmatus* (Stenopelmatidae: Stenopelmatinae) (see table 2.1).

Within the Tettigonioidea, the spermatophylax has been recorded in *Cyphoderris* (Haglidae) and occurs in the vast majority of the Tettigoniidae studied (90% of the 68 genera listed in table 2.1). The spermatophylax is found in members of all 15 subfamilies of the Tettigoniidae for which data are available. These are the Tettigoniinae, Bradyporinae, Saginae, Conocephalinae, Microtettigoniinae, Phasmodinae, Listroscelidinae, Meconematinae, Zaprochilinae, Hetrodinae, Ephippigerinae, Pycnogastrinae, Mecopodinae, Pseudophyllinae and Phaneropterinae. This suggests that the spermatophylax may have been present in the ancestral tettigoniid (Gwynne 1990a).

The occurrence of the spermatophylax in the Haglidae (= Prophalangopsinae) is interesting because both Ander (1939) and Ragge (1955) consider that members of the early Haglidae may have been ancestral to both the Tettigoniidae and the

Grylloidea (though Sharov, 1967, disagrees with this view, and considers the Haglidae to be ancestral to the Grylloidea and the Gryllacridoidea, but not the Tettigoniidae). The possibility exists, therefore, that the spermatophylax of the Tettigoniidae and Gryllidae is homologous (ie.inherited from a common ancestor). However, the fact that within the Gryllidae the spermatophylax is only found in one of the 8 subfamilies for which data are available, and only occurs in 4 of the 10 genera studied belonging to this subfamily, could indicate that the spermatophylax has evolved independently in this group. It is necessary to know the phylogenetic relationship of this subfamily (the Gryllinae) to the rest of the Gryllidae in order to solve this problem. Unfortunately, such information is currently unavailable (Walker & Masaki 1989). Similarly, without a phylogeny for the Gryllacridoidea, it is difficult to deduce whether the spermatophylax has evolved independently in this group. Hubbell & Norton (1978) give a phylogeny (at the subfamily level) for the Gryllacridoid family Rhaphidophoridae. Their phylogeny indicates that the Macropathinae and the Ceuthophilinae (in which the spermatophylax appears to be absent) diverged prior to the Rhaphidophoridae (in which the spermatophylax is present). This would suggest that the spermatophylax is a secondary modification in the Rhaphidophoridae and has, therefore, evolved independently in this group.

For a discussion of the empirical evidence concerned with the function of the spermatophylax, see chapter 1.

2.3.6 *No "counter-measures".*

There remains a final category, also noted by Boldyrev (1915), in which males exhibit no apparent method to deter the female from removing the spermatophore before complete sperm transfer.

Species in this category include: *Pachyramma waitomoensis* (Gryllacridoidea; Rhaphidophoridae; Macropathinae), *Mecopoda elongata* (Tettigonioidea; Tettigoniidae; Mecopodinae), *Saga ephippigera* (Tettigonioidea; Tettigoniidae; Saginae), *Ruspolia nitidula* and *Neoconocephalus* (Tettigonioidea; Tettigoniidae; Conocephalinae) and possibly *Zabalius apicalis* (Tettigonioidea; Tettigoniidae; Pseudophyllinae), *Hemideina* (Gryllacridoidea; Stenopelmatidae; Henicinae) and *Gryllotalpa gryllotalpa* (Grylloidea; Gryllotalpidae; Gryllotalpinae) (see table 2.1).

In *S.ephippigera*, *R.nitidula* and *Neoconocephalus*, a spermatophylax is present, though it is minute and vestigial. It does not appear to be replaced by any other method of preventing premature ampulla removal by females.

Why do males of these species not adopt "counter measures" against premature spermatophore removal by females? The answer may simply be that the females have, for some reason, lost the tendency to remove spermatophores before complete sperm transfer. Indeed, females of all of the above species do not, as a rule, attempt to remove or eat the spermatophore soon after its transfer, but either leave it in place for several hours before eating or removing it (*S.ephippigera*, *R.nitidula*, *Neoconocephalus*, *M.elongata*, *Z.apicalis*, *G.gryllotalpa*, see table 2.1), or simply leave it in place until it dries and falls off (*P.waitomoensis*, *Hemideina*, see table 2.1).

In *R.nitidula*, for example, Boldyrev (1915) observed that females tended to leave the spermatophore ampulla in place for 12 hours before eating it, while sperm transfer apparently takes only about 2 hours (Boldyrev 1915). I have also observed mating in this species and my observations confirm those of Boldyrev:

about 2 min after copulation, the female bends double and begins to groom her ovipositor from the tip down, her subgenital plate and the underside of her abdomen. During these grooming motions, part of the tiny spermatophylax is usually eaten. However, females leave the ampulla attached for an average of 15.8 $\pm$ 1.17 hours (range 1-29, n=31) (see table 2.1). Females removed the spermatophore within 2 hours (ie. before complete sperm transfer) in only one out of 31 cases (ie. 3% of cases) (pers.obs.).

It is open to speculation why females of these species should differ from other ensiferans in their spermatophore-eating behaviour in the absence of male hindrance.

2.4 *Conclusion.*

Boldyrev's (1915) observations on mating behaviour and spermatophores in the Ensifera were based on only 7 species of grylloid (belonging to 6 genera, 4 subfamilies and 2 families), 2 species of gryllacridoid (belonging to 2 genera, 2 subfamilies and 1 family) and 26 species of tettigonioid (belonging to 16 genera, 6 subfamilies and 1 family). Here I have summarised data for about 62 species of grylloid (belonging to 34 genera, 11 subfamilies and 3 families), 31 species of gryllacridoid (belonging to 13 genera, 10 subfamilies and 3 families) and 124 species of tettigonioid (belonging to 69 genera, 15 subfamilies and 2 families).

Despite Boldyrev's (1915) comparatively limited sample size, his classification of post-mating behaviours in the Ensifera still holds (though with the addition of post-copulatory mate-guarding), with many new species being added to each of Boldyrev's categories. Furthermore, there is at least some empirical evidence for each

category to support Boldyrev's hypothesis that these behaviours function to counteract the tendency of females to eat the spermatophore before complete ejaculate transfer. The occurrence of some of these behaviours in a number of distantly related species suggests that they may have evolved independently a number of times within the Ensifera.

In conclusion, the data suggest that the problem of females being able to manipulate spermatophores (which generally remain partly external to the female), and particularly the tendency of females to eat the spermatophore before complete ejaculate transfer, is widespread in the Ensifera. Males have consequently developed a range of adaptations to prevent premature spermatophore removal and thereby maximise ejaculate transfer (and probably, therefore, maximising the chances of success in the event of sperm competition). The production of a spermatophylax is just one of these adaptations. It should be noted, however, that this hypothesis, ie. that the spermatophylax originated as a form of mating-effort, does not exclude the possibility of the spermatophylax subsequently/additionally functioning as paternal investment (see Gwynne 1986b, 1988b, 1990a).

2.5 *Summary.*

Boldyrev, in 1915, proposed that the production of a spermatophylax is one of a number of strategies, adopted by males of the sub-order Ensifera, to ensure complete ejaculate transfer by countering the tendency of females to eat the spermatophore prematurely. The other "counter-measures" described by Boldyrev were prolonged copulation following spermatophore transfer, multiple mating with the same female and feeding the female with a glandular secretion following spermatophore transfer.

To this list, later authors added the category of post-copulatory mate guarding. Since Boldyrev's review, a great deal has been published on the mating behaviour ensiferans. Many new species have been studied in this respect and an array of male mating and post-mating behaviours have been described. Furthermore, behaviours described by Boldyrev which involve the transfer of nutrients to females have been interpreted as potentially functioning as forms of paternal investment in offspring. In this chapter, I review data on the mating and post-mating behaviour of over 200 species of ensiferan, belonging to 36 subfamilies and 8 families. I then discuss the empirical evidence concerning the function of each category of male post-mating behaviour described. Despite Boldyrev's comparatively limited sample size, his classification of post-mating behaviour in the ensifera still holds, with many new species being added to each of his categories. Furthermore, there is at least some empirical evidence for each category to support Boldyrev's hypothesis that these behaviours function to counteract the tendency of females to eat the spermatophore before complete ejaculate transfer. This supports the hypothesis that the spermatophylax originated as a form of mating effort, though it does not rule out the possibility of the spermatophylax subsequently or additionally functioning as paternal investment.

...on in Bushcrickets.

3.1 *Introduction.*

In chapter two, I described the range of different male post-mating behaviours found within the sub-order Ensifera which may be seen as alternative adaptations to ensure full ejaculate transfer by deterring females from eating the externally-attached sperm-ampulla before ejaculate transfer is complete (see also reviews of Boldyrev 1915; Loher & Dambach 1989). These behaviours include rapidly-repeated multiple mating with the same female, feeding the female with glandular secretions, post-copulatory mate guarding, spermatophylax production and prolonged copulation following spermatophore transfer (see chapter 2). Here, I describe the prolonged copulation following spermatophore transfer which appears to replace the spermatophylax in the bushcrickets *Uromenus rugiscollis* Serv. (Ephippigerinae), *Meconema meridionale* Costa and *M.thalassinum* DeGeer (Meconematinae). I contrast this with the copulatory behaviour and spermatophores of other members of these sub-families.

3.2 *Prolonged Copulation in* Uromenus rugiscollis.

3.2.1 *Introduction.*

Rigalleau (1936) observed that while copulation in *U.rugiscollis* lasts much longer than in the related ephippigerine *Ephippiger ephippiger*, the spermatophore is considerably smaller. However, he did not discuss the possible significance of this

observation. Here, I provide quantitative data on copulation and spermatophore size in *U.rugiscollis* and contrast this with similar data for 15 other species belonging to the sub-family Ephippigerinae.

3.2.2 *Methods.*

U.rugiscollis were collected as adults from the region of Vendee, Western France, in August 1991. A range of other ephippigerine species were collected as adults from numerous localities in Spain and France in August 1990 and 1991 (see appendix for collection localities for each species). At least three males and three females of each species were taken back to the laboratory at Nottingham, where sexes were maintained separately under conditions outlined by Hartley & Dean (1974). Observations of mating behaviour were conducted both on wild-caught individuals and offspring subsequently reared in captivity. Males were not used for mating until at least two weeks following collection or the final moult in order to ensure that spermatophore size was unlikely to be reduced as a result of male age or mating history (see chapter 6). For each species, individual stridulating males, and females which showed signs of receptivity (ie. showing phonotaxis to the male call or exhibiting a response-song, where present), were transferred to black nylon-mesh observation cages (measuring approx. 10cm x 10cm x 10cm), one pair per cage. The number of pairs set up depended upon the number of pairs available for each species. This varied from a single pair to 51 pairs (see table 3.1: the sample sizes correspond to the number of pairs used). Cages were observed intermittently until mating occurred. All stages of copulation were timed with a digital stopwatch. After mating, spermatophores were removed with forceps and these, together with recently mated males, were weighed separately on an electrobalance, to an accuracy of 1mg. For six species, including *U.rugiscollis*, the ampulla was then separated from the spermatophylax and weighed to the nearest 0.01mg on a Cahn-25

electrobalance.

For non-parametric comparisons, non-parametric one-way analysis of variance (Meddis 1984) was used. Means are cited ± standard error.

3.2.3 *Results.*

3.2.3.a *Copulatory behaviour of* U.rugiscollis.

The following account of the copulatory behaviour of *U.rugiscollis* is based upon observations of twelve separate pairs. Prior to copulation, pairs of *U.rugiscollis* in close proximity to one another were often seen to exchange bouts of tremulation (rapid vibration of the body). In a successful copulation, the female would mount the male, who would reach the tip of his abdomen backwards and upwards with his cerci raised. The male would then grasp the underside of the female's abdomen with his cerci. Interestingly, while in other ephippigerines, the sharp spurs on the inner tip of each cercus of the male engage with sockets situated either side of the sub-genital plate of the female (Hartley & Warne 1984), in *U.rugiscollis* these sharp spurs appeared to grip the soft underbelly of the female, just behind the sub-genital plate (eighth sternite). As noted by Chopard (1951), the male's cercal spurs in this species would leave a visible mark on the female's underside after mating. At this point, the female would generally walk a short distance and the male would bend underneath the female into a C-shape, grasping the ovipositor with his first and second pairs of legs. The male would partly invert his genitalia and would insert the long pair of titillators (which are notably longer than in other ephippigerines and visibly protrude from the end of the male's abdomen at rest) into the female's genital chamber, after which he would move them rhythmically in and out. There was generally little

change until the bi-lobed ampulla and relatively small spermatophylax were secreted, an average of 104 ± 8.6 min (range: 68 - 164 mins, n= 10) from the start of copulation. After spermatophore transfer, the pairs did not separate immediately, but would remain in copulation (see fig 3.1a) for, on average, a further 101 ± 10.2 min (range: 60 - 141 mins, n= 8). The mean total time spent in copulation was 218 ± 15 min (range: 167 - 278 mins, n= 6). The spermatophore as a whole represented an average of 11.5 ± 0.7 % (range: 8.7 - 15.7%, n = 12) of male body weight. The spermatophylax represented 6.5 ± 0.7% (range: 3.7-9.8%, n=9) of male body weight, while the ampulla represented 5.4 ± 0.3% (range: 4.3-7.3%, n=9). As in most other bushcrickets observed, the female would bend double shortly after the end of copulation and would begin to consume the spermatophylax. In the one case observed carefully, the female took 1 hour, 50 minutes to finish the spermatophylax before eating the ampulla. After copulation, the male appeared to pay the female no further attention, as in most other bushcrickets. In all cases observed (n=10), males resumed stridulation within 24 hours after mating. In one case, a male was observed to resume stridulation as little as 39 mins after the end of copulation.

3.2.3.b *Copulatory behaviour of other ephippigerines.*

The copulatory behaviour of the ephippigerine *Steropleurus stali* Bol. is described in detail in chapter 7, section 7.3.2.c. Copulation in the other ephippigerines observed, excluding *U.rugiscollis*, followed the same general pattern, apart from there being a distinct difference between species in the amount of time spent in copulation before spermatophore transfer. Details of mean copulation durations prior to and following spermatophore transfer, spermatophore weight as a percentage of male body weight and male body weight for each species are given in table 3.1. In this table, the values for spermatophore weight as a percentage of male body weight are the average of a single spermatophore per male as a percentage of his body weight. The

Fig. 3.1 Prolonged copulation in bushcrickets. **a)** *Uromenus rugiscollis* during the prolonged copulation which continues after spermatophore transfer. Note the fully-secreted spermatophylax which is relatively small compared with those of other members of the sub-family Ephippigerinae (compare with fig.1.1 & fig 4.1 p). **b)** *Meconema meridionale* during the prolonged copulation. The spermatophore is fully secreted at this point, though it is hidden by the male's sub-genital plate.


a


b

Table 3.1: mean spermatophore mass as a percentage of male body mass, mean male body mass and mean copulation durations prior to and following spermatophore transfer for species of ephippigerine (means are cited ± S.E.; range of values given in brackets).

Species	Spermatophore mass as % male body mass	Male body mass (g)	Copulation duration (min)	Start of copulation to ampulla transfer (min)	Ampulla transfer to end of copulation (min)
Ephippiger ephippiger (Fiebig)	28.1±1 (18.1-35.6, n=20)	2.55±0.1 (2.01-3.20, n=20)	26.1±2.7 (18-42, n=9)	13.6±1.2 (11.3-17, n=4)	6.1±1.2 (4-9.5, n=4)
E.perforatus (Rossi)	20.6±2.1 (10-26, n=7)	1.67±0.1 (1.37-1.90, n=7)	26.8±5.7 (15.5-34,n=3)	3.5±0.3 (2.8-3.9, n=4)	23.4±5.8 (12.0-30.2, n=3)
E.terrestris (Yers.)	30.6±0.3 (30.3-30.9, n=2)	0.99±0.1 (0.89-1.09, n=2)	<30 (n=1)	-	-
Ephippigerida taeniata (Sauss.)	28.0±0.9 (19.3-33.0, n=21)	4.10±0.12 (2.93-5.41, n=21)	3.0±0.6 (1.8-5.1, n=5)	1.1±0.3 (0.6-1.5, n=3)	1.5±0.4 (0.7-2.0, n=3)
E.zapateri (Bol.)	40.5 (n=1)	1.80 (n=1)	<30 (n=1)	-	-
E.saussureiana (Bol)	28.1 (n=1)	0.94 (n=1)	<26 (n=1)	-	-
Baetica ustulata (Ramb.)	29.1 (n=1)	1.86 (n=1)	25 (n=1)	23 (n=1)	2 (n=1)
Callicrania monticola (Serv.)	28.0±0.7 (26.8-29.2, n=3)	2.15±0.18 (1.80-2.40, n=3)	34.2±2.7 (26.8-29.2, n=2)	28.2±1.7 (26.5-29.9, n=2)	6.0±1 (5-7, n=2)
Steropleurus stali (Bol.)	27±0.7 (15.8-36.7, n=51)	1.29±0.03 (0.80-1.86, n=51)	16.2±0.9 (10-24, n=19)	13.9±1.0 (10-20, n=10)	1.7±0.2 (0.5-3.2, n=16)
S.asturiensis (Bol.)	27.0±1.6 (14.2-31.0, n=10)	1.21±0.04 (0.97-1.37, n=10)	18.7±2.7 (12-25, n=4)	13±3 (10-16, n=2)	2 (n=2)

Table 3.1 (continued).

Species	Spermatophore mass as % male body mass	Male body mass (g)	Copulation duration (min)	Start of copulation to ampulla transfer (min)	Ampulla transfer to end of copulation (min)
S.brunneri (Bol.)	30.6 (n=1)	1.69 (n=1)	2.3 (n=1)	0.9 (n=1)	1.4 (n=1)
S.parezi (Bol.)	24.7 (n=1)	1.13 (n=1)	5.0 (n=1)	2.0 (n=1)	3.0 (n=1)
S.martorelli (Bol.)	26.6±3.3 (20.8-35.3, n=3)	2.62±0.22 (2.28-3.04, n=3)	3.0 (n=1)	0.9 (n=1)	2.1 (n=1)
S.catalaunicus (Bol.)	40.3 (n=1)	1.60 (n=1)	18.2 (n=1)	15.0 (n=1)	3.2 (n=1)
S.andalusius (Ramb.)	29.0±1 (28-30, n=2)	6.3±0.4 (5.9-6.7, n=2)	<30 (n=1)	-	-
Uromenus rugiscollis (Serv.)	11.5±0.7 (8.7-15.7, n=12)	1.13±0.08 (0.80-1.65, n=12)	217.7±15.0 (167-278, n=6)	104.3±8.6 (68-164, n=10)	100.6±10.2 (60-141, n=8)

sample-sizes given in table 2.1 therefore correspond to the number of different males used. In some species (*Steropleurus brunneri*, *S.perezi*, *S.martorelli*, *Ephippiger perforatus* and *Ephippigerida taeniata*), copulation prior to spermatophore transfer was brief, ranging from an average of less than 1 min. to an average of about 4 mins.. In other species (*S.stali*, *S.asturiensis*,*S.catalaunicus*, *Ephippiger ephippiger*, *Baetica ustulata* and *Callicrania monticola*), copulation prior to spermatophore transfer was relatively prolonged, ranging from an average of 13 min. to an average of 28 min..

Shortly after the end of copulation, the female would bend double and begin to eat the enormous spermatophylax which is typical of members of this subfamily (see chapter 1, figs 1.1a & 1.1b; chapter 4, fig 4.1p). In *S.stali*, females took an average of 7.49 $\pm$ 0.74 hours (range 2-14 hours, n= 10) to consume the entire spermatophylax (chapter 7), after which they would eat the ampulla.

The duration of copulation following spermatophore transfer in *U.rugiscollis* was significantly greater than in the other ephippigerines observed (mean duration in *U.rugiscollis* = 100.6 $\pm$ 10.2 min, n= 8; mean duration in the other ephippigerines = 4.8 $\pm$ 1.93 min, n= 11; H= 13.2, p< 0.001), as was the mean duration of copulation prior to spermatophore transfer (mean duration in *U.rugiscollis* = 104.3 $\pm$ 8.6 min, n= 10; mean duration in the other ephippigerines = 10.5 $\pm$ 2.9 min, n= 11; H= 15.0, p< 0.001). Spermatophore (ie. spermatophylax plus ampulla) mass as a proportion of male body mass was significantly smaller in *U.rugiscollis* than in the other ephippigerines (mean spermatophore mass as a percentage of male body mass in *U.rugiscollis* = 11.5 $\pm$ 0.7%, n= 12; mean for the 15 other ephippigerines = 29.2 $\pm$ 1.3%, n= 15; H= 19.3, p< 0.001).

While there are good *a priori* reasons to suppose that the prolonged copulation

following spermatophore transfer in *U.rugiscollis* has led to a reduction in spermatophylax size in this species (see chapter 2), it is possible that other factors could be responsible for the reduced spermatophylax size. Because male body mass in *U.rugiscollis* is in the lower end of the range for an ephippigerine (table 3.1), one possibility is that the relatively small spermatophylax size in this species is due to the nature of the allometric relationship between male body size and spermatophore size as a proportion of male body size. However, this does not appear to be the case because no significant correlation was found between male body mass and spermatophore mass, as a percentage of body mass, across the ephippigerine species studied here ($r = 0.10$, 14 d.f., $p = 0.72$).

The hypothesis that the spermatophylax in bushcrickets has evolved as a mechanism to ensure complete ejaculate transfer predicts that, other things being equal, species with proportionately larger spermatophylaxes should produce proportionately larger ampullae (see Wedell, in press; chapter 4). As predicted, a positive relationship between ampulla mass, relative to male body mass, and spermatophylax mass, relative to male body mass has been found across taxa in bushcrickets (Wedell, in press; chapter 4). In *U.rugiscollis*, however, prolonged copulation following spermatophore transfer appears partly to replace the function of the spermatophylax in prolonging time available for sperm transfer. Therefore, despite the fact that the spermatophylax produced by male *U.rugiscollis* is smaller than in the other species of ephippigerine, no difference in the size of the ampulla relative to male body weight is necessarily expected. A comparison of the values of ampulla mass as a percentage of male body mass reveals no significant difference between *U.rugiscollis* and five other randomly-selected ephippigerines (*S.stali*, *E.ephippiger*, *E.terrestris*, *Ephippigerida saussureiana* and *E.taeniata*) (mean ampulla mass as a percentage of male body mass for *U.rugiscollis* $= 5.4 \pm 0.3$, $n = 9$; mean for five other ephippigerines $= 5.5 \pm 0.6$, $n = 5$; $H = 0.04$, $p > 0.05$). Spermatophylax mass as a

percentage of male body mass, on the other hand, is approximately four times greater in these other ephippigerines than in *U.rugiscollis* (mean spermatophylax mass as a percentage of male body mass for *U.rugiscollis* = 6.5 ± 0.7, n= 9; mean for the five other ephippigerines = 24.0 ± 1.2%, n= 5).

3.2.4 *Discussion.*

In *Uromenus rugiscollis*, a species with prolonged copulation following spermatophore transfer, the spermatophylax is considerably reduced in size compared to other members of the sub-family ephippigerinae in which copulation following spermatophore transfer is typically brief. This suggests that prolonged copulation following spermatophore transfer in this species has partly replaced the function of the large spermatophylax.

The benefit of adopting prolonged copulation following spermatophore transfer, as opposed to spermatophylax production, as a strategy to ensure complete sperm transfer may be that it enables males to have a relatively higher mating frequency. In *Steropleurus stali* and *Ephippiger ephippiger*, the cost of producing a large spermatophylax is manifest as a period following mating in which males do not stridulate or attempt another mating. This period lasts an average of 3 days (range: 2-4 days) in *S.stali* (chapter seven) and 3-5 days in *E.ephippiger* (Busnel et al 1956). Conversely, in *U.rugiscollis*, with its small spermatophylax, the male refractory period lasts less than 24 hours, and possibly as little as 40 minutes.

One cost of the prolonged-copulation strategy, on the other hand, might be a greater vulnerability to predation (see Gwynne 1989; Magnhagen 1991 for reviews of the recent literature on predation risks during copulation). Interestingly, most of the

ephippigerines in this study generally call diurnally (usually in the morning), apart from *U.rugiscollis* in which males begin to stridulate at dusk (pers.obs). Perhaps the habit of mating after nightfall in *U.rugiscollis* renders mating pairs less vulnerable to the attentions of certain predators (eg. passerine birds), meaning that prolonged copulation is not selected against to such an extent.

While prolonged copulation following spermatophore transfer in bushcrickets can be interpreted as a male adaptation to maximise sperm transfer by preventing females from eating the ampulla prematurely (see chapter two), prolonged copulation prior to spermatophore transfer obviously cannot perform this function. Interestingly, there is considerable variation between species in the duration of copulation prior to spermatophore transfer in the ephippigerines studied. In *U.rugiscollis*, the duration of this period is the longest, lasting about 100 min. The other ephippigerines studied fall into two main groups: in one, copulation prior to spermatophore transfer is also fairly long, lasting from an average of 13 min to about 30 min; in the other, the duration of this period is short, lasting from an average of less than 1 min to about 4 mins. The spermatophores of all of these species, apart from *U.rugiscollis*, are large (over 20% of male body weight). This suggests that differences between these species in the duration of copulation prior to spermatophore transfer are not related to the amount of time required to produce a large spermatophore.

Within the family Tettigoniidae as a whole, there is also a great deal of variation between species in the duration of copulation prior to spermatophore transfer (the details of copulation in a range of tettigoniids are given in chapter 2, table 2.1). In addition to the Ephippigerinae, prolonged copulation prior to spermatophore transfer is found in certain members of the tettigoniid subfamilies Pycnogastrinae, Hetrodinae, Bradyporinae, Tettigoniinae and Conocephalinae (see chapter 2, table 2.1).

The function of prolonged copulation prior to spermatophore transfer in the ephippigerines and in other bushcrickets is unclear. One possibility is that the male attempts to stimulate the female to release stored sperm during this period (cf Helversen & Helversen 1991). During the prolonged copulation prior to spermatophore transfer found in certain ephippigerines, males insert a pair of barbed titillators into the females genital chamber (see Hartley & Warne, 1984, for an illustration of the titillators in an ephippigerine) and appear to move them rhythmically in and out for a considerable period of time before the spermatophore is transferred. This behaviour has also been noted in members of other bushcricket subfamilies. For example, Boldyrev (1928a) described prolonged copulation prior to spermatophore transfer (lasting 1.5 hours) in the Bradyporine bushcricket *Bradyporus multituberculatus*. He noted that during this period, the titillators are inserted into the female's genital chamber and produce a "distinct scratching sound" as they rub against its inner surface. Boldyrev (1928a) proposed that the titillators "serve to irritate by scratching the walls of the genital chamber and perhaps to enlarge the chamber before the spermatophore is introduced". The titillators may therefore simply function to produce an opening into which the spermatophore is secreted (as was also suggested by Hartley & Warne 1984). Alternatively, or additionally, it is possible that movements of the titillators in the female's genital chamber during the prolonged copulation prior to spermatophore transfer might stimulate the release of previously stored sperm from the spermatheca, as do movements of the male's specialised sub-genital plate within the female's genital chamber in the phaneropterine bushcricket *Metaplastes ornatus* (Helversen & Helversen 1991).

In the ephippigerine *Steropleurus stali*, a species in which prolonged copulation prior

to spermatophore transfer (lasting for an average of 13 min) occurs, there appears to be a pattern of last-male sperm precedence (chapter 7). This would be expected if males stimulated females to release stored sperm prior to spermatophore transfer. However, in the tettigoniine bushcricket *Decticus verrucivorus*, a species in which copulation prior to spermatophore transfer is also reasonably prolonged (lasting 5 to 7 mins; Wedell 1992), a pattern of sperm-mixing occurs (Wedell 1991). This does not, therefore, support the hypothesis that males stimulate the release of previously stored sperm during this period.

Another possibility which has been suggested is that prolonged copulation prior to spermatophore transfer in bushcrickets might function as a period of mate assessment for males (Wedell 1992). Wedell (1992) found that in *D.verrucivorus*, the duration of copulation prior to spermatophore transfer was significantly shorter when males were mating with virgin females than when males were mating with older, previously mated, females. In addition, Wedell (1992) found a negative correlation between the duration of copulation prior to spermatophore and virgin-female weight. Wedell (1992) interpreted this as indicating that males use copulation prior to spermatophore transfer as a mate-assessment period and require a longer time in copulation when assessing lower quality females (ie. older females and lighter females).

Alexander & Otte (1967a) proposed that prolonged copulation will select for a greater ability of the male to grasp and hold the female. In *U.rugiscollis*, as in the other ephippigerines, the sharp barbs on the inner side of each cercus of the male are responsible for maintaining a grip on the female during copulation. Unlike the other ephippigerines, however, in which these barbs fit into a socket either side of the female's sub-genital plate (see Hartley & Warne 1984), in *U.rugiscollis* the cerci appear to grip the relatively soft underside of the female, the barbs sinking into the

cuticle and leaving visible wounds after mating (as noted by Chopard 1951). Copulating pairs of *U.rugiscollis* are almost impossible to separate without the risk of tearing the underside of the female (pers obs). This manner of coupling might be the product of sexual conflict over the optimal copulation duration, with prolonged copulation being primarily in the male's, rather than the female's, interests.

3.3 *Prolonged Copulation in* Meconema.

3.3.1 *Introduction.*

While the biology of *Meconema thalassinum* (De Geer), the oak bushcricket, is relatively well known (reviewed by Oschmann 1991), little is known of the biology of its congener *M.meridionale* (Costa) or their relative *Cyrtaspis scutata* (Charpentier). All that appears to have been documented for the latter two species is the call of the males (Lienhardt 1921; Heller 1988) and the fact that *C.scutata* can often be found late in the season (Gélin 1908; Tempère 1923; Chopard 1951). Here I describe the atypical spermatophores and unusual copulatory behaviour of *M.meridionale* and *M.thalassinum* and contrast this with the copulatory behaviour of *C.scutata*. The copulatory behaviour and spermatophores do not appear to have been described previously for any member of the sub-family Meconematinae other than *M.thalassinum* (the copulatory behaviour of which was described by Gerhardt 1914).

3.3.2 *Methods.*

3.3.2.a *The species.*

M.thalassinum is found throughout Europe, including Great Britain (Marshall & Haes 1988; Oschmann 1991). *M.meridionale* has a more southerly distribution, being found in Southern France, South-West Germany, Switzerland, Austria, Italy and Yugoslavia (Bellman 1988). *C.scutata* is found in the South and West of France, Portugal, Spain, the Azores, Italy, Dalmatia and Algeria (Chopard 1951). While

M.thalassinum is fully winged, both *M.meridionale* and *C.scutata* are micropterous. All three species are arboreal and nocturnal (Chopard 1951; Tempère 1923).

3.3.2.b *Collecting and rearing methods.*

A single gravid female *M.meridionale* was collected in August 1990 at Mercus, Ariège, in the foothills of the French Pyrenees, after searching young oak trees with a torch at 10pm. In the laboratory, 65 eggs were laid in cotton-wool. These were separated and placed on filter paper on damp cotton wool in a petri dish and exposed to an incubation cycle of 30 days at 15°c, followed by 70 days at 8°c, before raising to 15°c again. Hatching began 70 days later and continued sporadically over a period of 2 months. This treatment was based on that recommended for the eggs of *M.thalassinum* by Hartley & Warne (1972). The nymphs had five instars and took about six weeks to become adult.

Ten adult female *M.thalassinum* were collected in September 1991 from Wollaton Park, Nottingham, England by beating from oak trees and by directly collecting individuals found ovipositing on the lower trunks of oak trees in the evening. Females were taken back to the laboratory, where they laid a number of eggs in cotton wool. These were treated as above and incubated following the guidelines recommended for this species by Hartley & Warne (1972). The resulting nymphs were reared to maturity in the laboratory.

The *C.scutata* were collected in August 1991 from Landevieille, Vendée, Western France by beating from young oak trees. Six male and six female penultimate nymphs were collected in all. These were raised to adulthood in the laboratory.

Housing and feeding conditions were identical for all three species. Adults and

nymphs were housed in transparent plastic canisters (10cm x 10cm x 15cm) with nylon mesh inserted in the lids. Approximately four individuals were kept in each canister. On reaching maturity, all individuals were maintained in separate canisters. A leafy twig of oak or *Buddleia* in a stoppered vial of water was placed in each cage as a moulting platform and, most importantly, to maintain humidity. All three species appeared to be very susceptible to desiccation. A number of mortalities occurred when the vegetation dried out, even within the space of one day. To maintain humidity, cages were sprayed with water on a daily basis. Cages were kept at room temperature near a window, though out of direct sunlight.

All three species were frequently observed feeding on small insects in captivity and appeared to be almost entirely carnivorous. A number of aphids were introduced to each container twice a week. Fruit-flies (*Drosophila*) were also accepted by adults of these species. A pinch of wheat-germ was provided to supplement the diet and was observed being eaten in each species.

3.3.2.c *Observations of mating behaviour.*

For observations of mating behaviour, individuals of each species were kept under a reverse light\dark cycle from about a week after the imaginal moult, until death. A desk-lamp with a 40 watt bulb, attached to a time-switch, provided the light source. Individual pairs were temporarily placed in a black nylon mesh cage (10cm x 10cm x 10cm) and observed. All stages of mating were timed with a digital stopwatch. Observations of mating behaviour were based upon six separate pairs for *C.scutata*, eight pairs for *M.meridionale* and six pairs for *M.thalassinum*. After mating, spermatophores were removed with watch-maker's forceps and weighed to the nearest 0.01 mg on a Cahn-25 electrobalance. Males were weighed on an electrobalance accurate to 1 mg. In *M.thalassinum* and *M.meridionale*, the number of

sperm contained in the spermatophores were counted using the method outlined in chapter 4.

For non-parametric comparisons, non-parametric analysis of variance (Meddis 1984) was used. Means are cited $\pm$ standard error.

3.3.3 *Results.*

3.3.3.a *Meconema meridionale.*

Like the oak bushcricket, *M.thalassinum* (see Sismondo 1980), male *M.meridionale* called by drumming the hind foot on the substrate. Unlike *M.thalassinum* which produces rapid bursts of drumming reminiscent of machine-gun fire, male *M.meridionale* produced 4-10 distinct taps per bout of drumming. A sonogram of this call is given by Heller (1988).

In the presence of a female, the male would extend and lower his abdomen, forming a sinusoidal shape, and sit motionless with his hind femora raised and parted. Males would often remain in this position for some time. Should a female pass by, the male would turn rapidly so that his abdomen faced her. If the female paused to palpate the dorsal surface of the end of the male's abdomen, or even walked over the male in passing, he would move backwards under her body with great rapidity. The male would clasp the end of the female's abdomen with his anal cerci and would bend under her to adopt the copulatory position (fig 3.1b), holding the end of the female's abdomen with his front pairs of legs and grasping the tip of the ovipositor in his jaws. The long, curved cerci of the male would wrap around the end of the female's abdomen and cross over one another on the other side.

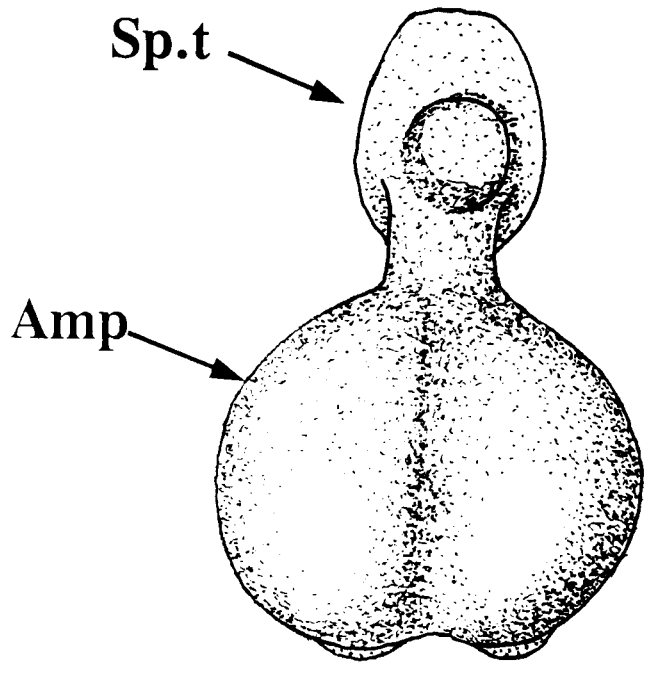
The spermatophore was transferred to the female on average 53 ± 2.8 seconds (range: 40 - 60 secs, n= 6) from the start of copulation. Unlike the spermatophores of the majority of bushcrickets studied (see chapter 2, section 2.3.5) that of *M.meridionale* completely lacked a spermatophylax (fig 3.2a). It consisted of a bilobed ampulla, containing the sperm, and a short sperm-tube which was inserted into the female's genital opening. The mean weight of the spermatophore was 1.69 ± 0.15 mg (range: 1.03 - 2.36 mg, n = 8), representing a mean loss of 1.77 ± 0.18 % (range: 0.98 - 2.49 %, n = 8) of the male's body weight. About two minutes later, the lobes of the ampulla were hidden by the male's subgenital plate. The pairs would remain in copulation for an average of 81 ± 9 minutes (range: 35 - 105 mins., n = 7) following spermatophore transfer. In all cases observed (n= 8), from shortly after spermatophore transfer to the end of copulation, the female intermittently kicked and pushed at the male with her hind legs, ran backwards and forwards and shook as if trying to dislodge the male. In three cases, females were even observed to bend round and bite at the male's abdomen.

After copulation had ended, the simple ampulla was visible protruding from the female's sub-genital plate. About one minute from the end of copulation, the female would bend double and begin to eat the ampulla. The male would groom his genitals at about five minutes after the end of copulation, and would then walks. Males were observed to resume drumming and abdomen-lowering to nearby females as little as ten minutes after copulation had ended.

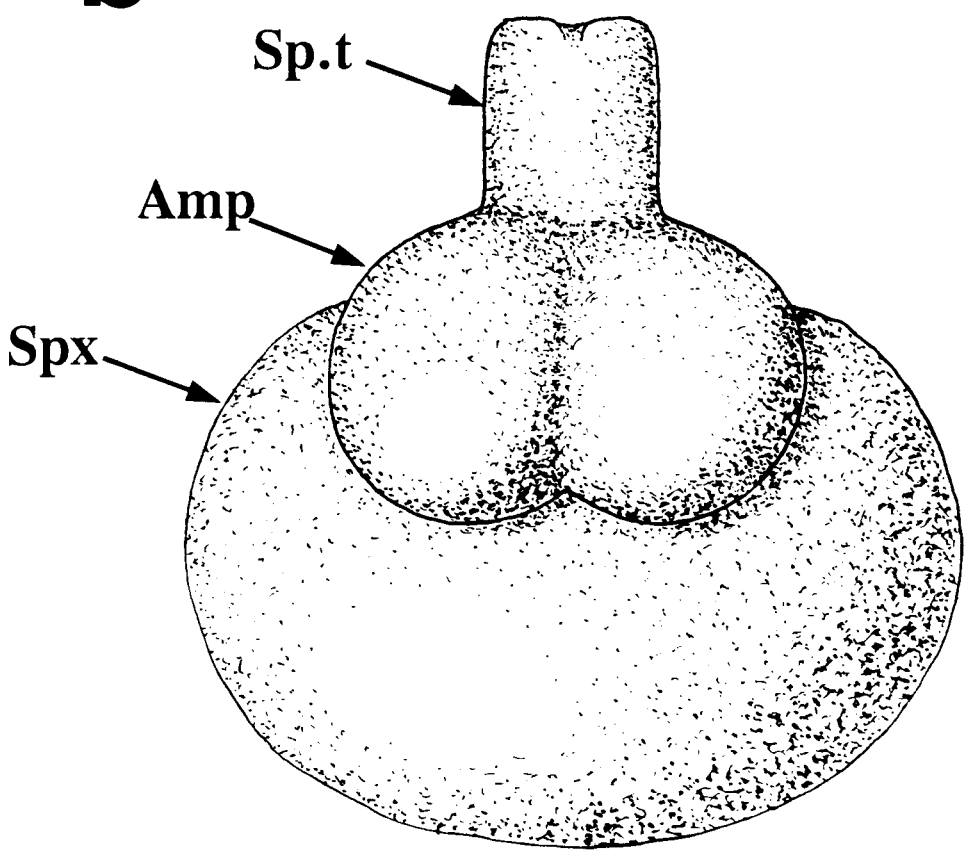
Sperm counts of spermatophores removed just after transfer and at the end of copulation in *M.meridionale* revealed that 70 - 99% of sperm (mean = 89 ± 5.41 %, n = 5) leaves the ampulla by the end of copulation. Since the female would eat the spermatophore around one minute after separating from the male, sperm transfer

Fig 3.2 a) Spermatophore of *Meconema meridionale*. **b)** Spermatophore of *Cyrtaspis scutata* (Sp.t= sperm-tube; Amp= ampulla; Spx= spermatophylax; the scale bars each represent 1mm).

a



b



must occur during the prolonged copulation. The female would also attempt to eat the spermatophore more or less immediately (within about 1 min) if the male was artificially removed soon after its deposition (based on 4 observations).

The above observations support the hypothesis that prolonged copulation following spermatophore transfer functions to prevent the female from eating the ampulla before complete sperm transfer (see Boldyrev 1913). This hypothesis would predict that males should remain in copulation for longer if they have produced a larger spermatophore which contains more sperm. When data on copulation duration after spermatophore transfer were plotted against spermatophore weight (after copulation) for the same male in *M.meridionale*, a positive correlation was found, as predicted ($r = 0.87$, 3 d.f., $p < 0.05$, one-tailed). Males, therefore, appear to adjust the duration of copulation in relation to the size of the ampulla and amount of sperm they have produced, though more data are needed to verify this relationship.

3.3.3.b *M.thalassinum.*

Copulation in *M.thalassinum* has previously been described by Gerhardt (1914) and is very similar in all stages to that of *M.meridionale*, described above, apart from the fact that the duration of copulation following spermatophore transfer is shorter.

As in *M.meridionale*, the male would lower his abdomen and bend it into an S-shape in the presence of a female. If the female approached, the male would straightens his abdomen, lowering and extending it, while bending his head and thorax downward. This would result in an angle of over 90° between the male's extended abdomen and his wings. Both of these male pre-copulatory positions are illustrated by Gerhardt (1914). If the female approached the tip of the male's abdomen, the male would rapidly move backwards under the female, grasping the end of her abdomen with his

cerci before bending under the female into a C-shape and holding the tip of the ovipositor in his mandibles. This copulatory position is illustrated by Gerhardt (1914). As in *M.meridionale*, the male's cerci encompassed the end of the females abdomen. They did not, however, cross over one another at the other side, but merely touched.

As in *M.meridionale*, the spermatophore, which was transferred about 1 minute after the start of copulation, completely lacked a spermatophylax. Following spermatophore transfer, copulation continued for an average of 17 $\pm$ 1.7 mins (range: 13-24 mins, n=6). The duration of copulation observed here closely corresponds with that given by Gerhardt (1914). The duration of copulation following spermatophore transfer was notably shorter in *M.thalassinum* than in *M.meridionale* (in which the mean duration of copulation following spermatophore transfer was 81 min, range: 35-105 min, see section 3.3.3.a above). As in *M.meridionale*, females were frequently seen to kick at the male with their hind legs during copulation. Within about a minute after the end of copulation, the female would bend double and begin to eat the spermatophore.

The hypothesis that prolonged copulation following spermatophore transfer functions to ensure complete sperm transfer would predict that the shorter duration of copulation following spermatophore transfer in *M.thalassinum*, compared to *M.meridionale*, is due to males producing less sperm per mating. A comparison of spermatophore weights reveals that *M.thalassinum* males produced significantly smaller spermatophores than *M.meridionale* males, both in absolute terms (mean spermatophore weight for *M.thalassinum* = 0.56 $\pm$ 0.06 mg, range: 0.45 - 0.71mg, n= 4; mean for *M.meridionale* = 1.69 $\pm$ 0.15 mg, range: 1.03 - 2.36 mg, n= 8; non-parametric ANOVA testing an *a priori* directional prediction (*M.thalassinum* spermatophore mass < *M.meridionale* spermatophore mass) Z = 2.72, p< 0.01)

and as a percentage of male body weight (mean spermatophore weight as a percentage of male body weight in *M.thalassinum* = 0.59 ± 0.07%, range: 0.47-0.79%, n= 4; mean for *M.meridionale*= 1.77 ± 0.18%, range: 0.98- 2.49%, n= 8; Z= 2.72, p<0.01). The spermatophores of *M.thalassinum* were found to contain significantly fewer sperm than those of *M.meridionale* (mean sperm number per spermatophore for *M.thalassinum* = 48.6 ± 4.9 x 10^3 sperm, range: 35 x 10^3 - 61 x 10^3 sperm, n= 5; mean for *M.meridionale* = 172.6 ± 6.8 x 10^3 sperm, range: 154 x 10^3 -189 x 10^3 sperm, n= 5; non-parametric ANOVA testing an *a priori* directional prediction (*M.meridionale* sperm number > *M.thalassinum* sperm number) Z= 2.61, p< 0.01). This difference did not appear to be due to differences in male body weight: no significant difference was found between the body weight of *M.thalassinum* and *M.meridionale* males (mean body weight for *M.thalassinum* males= 0.094 ± 0.002g, range: 0.09 - 0.1g, n= 4; mean for *M.meridionale*= 0.097 ± 0.003g, range: 0.086 - 0.1g, n= 8; non-parametric ANOVA testing whether there is any difference between species, H= 0.61, p> 0.05).

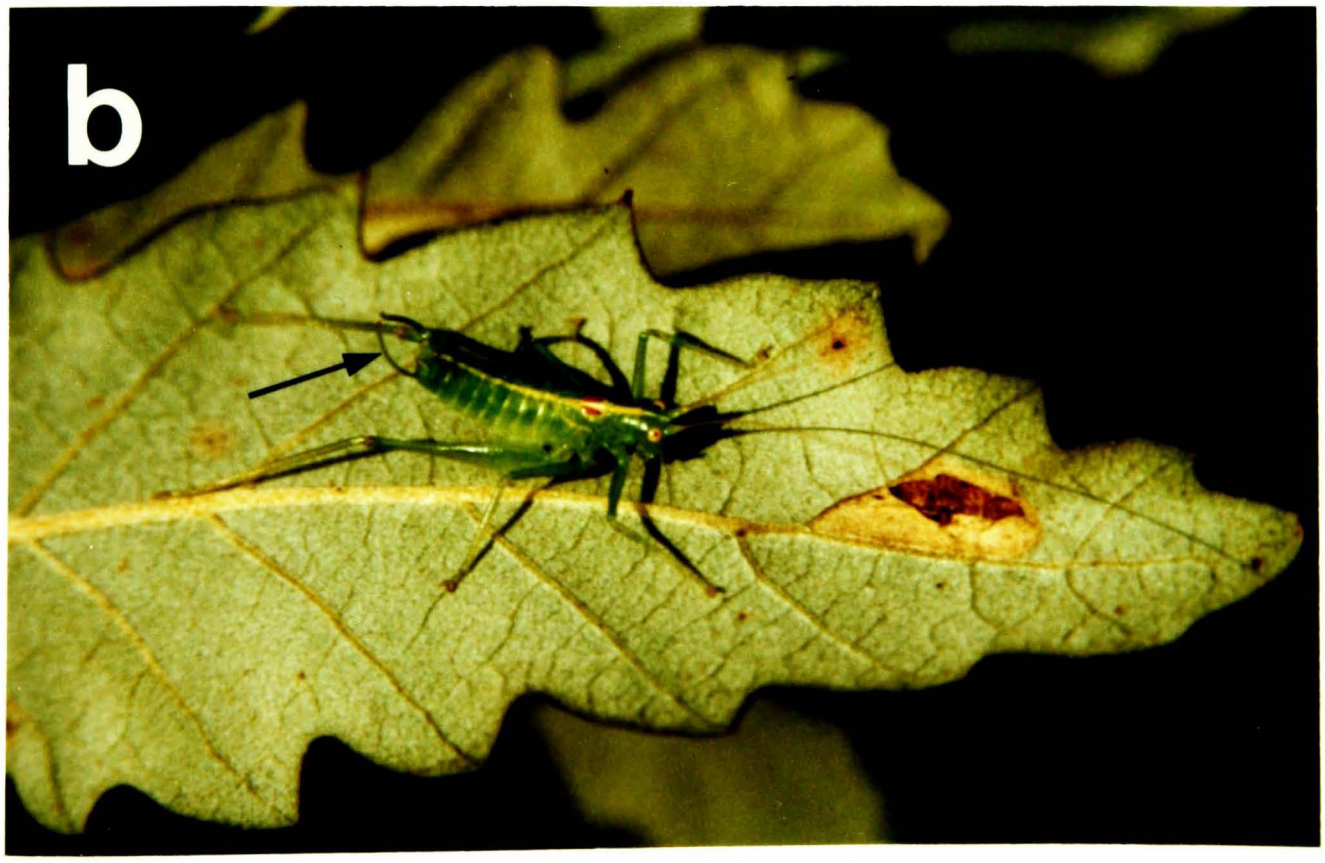
3.3.3.c *Cyrtaspis scutata.*

Male *C.scutata* call in the typical manner of most bushcrickets (ie. elytral stridulation). Heller (1988) gives a sonogram for this species along with an electronmicrograph of the somewhat unusual stridulatory apparatus.

If a receptive female approached, the male would briefly investigate her with his antennae before slowly turning so his abdomen faced her. In a successful copulation, the female would move forward onto the male's back, palpating the dorsal surface. When the female had advanced so that her head was just behind the male's pronotum, the male would reach the tip of his abdomen backwards and upwards, and positions his anal cerci either side of the base of the female's ovipositor. Pairs would

Fig 3.3 a) A female *Cyrtaspis scutata* (Tettigoniidae: Meconematinae) bearing a freshly-deposited spermatophore. Note the spermatophylax, which is absent in the related *Meconema*, a species with prolonged copulation following spermatophore transfer. **b)** A male *Meconema meridionale*, at rest. Note the characteristically long, curved cerci (marked by the arrow) which wrap around the end of the female's abdomen during the prolonged copulation and allow the male to maintain a firm hold on the female during this period.


a


b

remain in this position, the female on top of the male with both facing in the same direction, during the comparatively brief copulation.

The spermatophore was transferred, on average, just over two minutes (143 ± 41 s, range: 92-223 s, n = 3) from the start of copulation. This consisted of a short sperm tube inserted into the female and an external, white, bilobed sperm-ampulla bearing a translucent spermatophylax (fig 3.2b). The mean weight of the spermatophore was 17 ± 0.8 mg (range: 14.2 - 20.1 mg, n = 7), representing a mean loss of 9.2 ± 0.4 % (range: 7.5 - 10.5 %, n = 7) of the male's body weight. The sperm - containing ampulla represented 3.7 ± 0.2 % (range: 2.9 - 4.2% , n = 6) of the male's body weight and the gelatinous spermatophylax represented 5.4 ± 0.4 % (range: 3.6 - 6.6 %, n = 6).

Pairs would remain in copulation for an average of 91 ± 14 seconds (range: 70 - 118 s, n = 3) following spermatophore transfer. After the pairs had separated, the ampulla and spermatophylax were clearly visible at the base of the ovipositor (fig 3.3a). Shortly after the end of copulation, the female would bend double and begin to eat the spermatophylax, taking 1 - 2 hours to do so before eating the ampulla. The male would walk away and begin to groom his genitals at about five minutes after the end of copulation.

3.3.4 *Discussion.*

The observed prolonged copulation in *Meconema meridionale* and *M.thalassinum* highlights the adaptive significance of the unusually long, curved, pincer-like cerci characteristic of males of this genus (see fig 3.3b). Alexander & Otte (1967a) proposed that prolonged copulation, whatever its advantages, is likely to select for a greater ability of the male to grasp and hold the female. The cerci of male

Meconema appear to be well adapted for this purpose. They wrap around the end of the female's abdomen, crossing on the other side in the case of *M.meridionale*. Pairs are very difficult to separate: it may take five minutes prying at the male's cerci with forceps and pulling at the male before he relinquishes his grip. Interestingly, the cerci of *M.meridionale* are longer than those of *M.thalassinum* (4mm long as opposed to 3mm long, Bellman 1988) even though both species are of similar body size. It seems probable that the longer duration of copulation in the latter species has selected for longer cerci. The cerci of male *Cyrtaspis scutata*, on the other hand, are relatively short and are more like those of other bushcrickets. They do not encompass the end of the female's abdomen, but merely lie either side of it during the comparatively brief copulation.

The mean duration of copulation following spermatophore transfer was found to be significantly greater (by about five times) in *M.meridionale* than in *M.thalassinum*. As predicted by the hypothesis that prolonged copulation following spermatophore transfer functions to ensure complete sperm transfer, *M.meridionale* males were found typically to produce a significantly greater number of sperm per mating (about four times more) than *M.thalassinum* males. The hypothesis that the spermatophylax in bushcrickets also functions to ensure complete sperm transfer predicts that variation in spermatophylax size between species should similarly be positively related to the number of sperm produced per mating (this appears to be the case, see chapter 4).

C.scutata, unlike *Meconema*, was found to resemble most other bushcrickets in that males produce a spermatophylax and copulation following spermatophore transfer is relatively brief (see chapter 2, table 2.1 for a review of the mating behaviour of a number of tettigoniids). It appears that the spermatophylax has not previously been recorded for a member of the sub-family Meconematinae. The presence of the

spermatophylax in members of all tettigoniid sub-families studied so far (see chapter 2, section 2.3.5) including the Meconematinae suggests that the spermatophylax was probably present in the ancestral meconematine. The absence of the spermatophylax in *Meconema* would therefore appear to be a secondary character, associated with prolonged copulation following spermatophore transfer. This, together with the observation that the spermatophylax of the ephippigerine *U.rugiscollis*, in which prolonged copulation following spermatophore transfer also occurs, is considerably reduced in size compared to other members of the sub-family in which copulation following spermatophore transfer is brief (section 3.2), suggests that the spermatophylax and prolonged copulation following spermatophore transfer are analogous in function (as was proposed by Boldyrev, 1915). This supports the hypothesis that the spermatophylax in bushcrickets functions to prevent the female from eating the ampulla before complete sperm transfer, though it does not rule out the possibility that the spermatophylax might additionally function as a form of paternal investment in offspring.

3.4 *Summary.*

In this chapter, I describe prolonged copulation which appears to replace the spermatophylax in function in the bushcrickets *Uromenus rugiscollis* (Ephippigerinae), *Meconema meridionale* and *Meconema thalassinum* (Meconematinae). I contrast this with the copulatory behaviour of other members of the same subfamilies. In the ephippigerine *U.rugiscollis*, copulation following spermatophore transfer is long, lasting about 100 min, and the spermatophylax is small, representing about 7% of male body weight. This stands in contrast to 15 other species of ephippigerine, in which copulation following spermatophore transfer is typically brief, lasting an average of about 5 mins, and the spermatophylax is

large, representing over 20% of male body weight. In the meconematines *M.meridionale* and *M.thalassinum*, copulation following spermatophore transfer continues for an average of 81 and 17 mins, respectively, and the spermatophores, unlike those of the majority of bushcrickets, completely lack a spermatophylax. In the meconematine *Cyrtaspis scutata*, on the other hand, copulation following spermatophore transfer lasts only one minute, thirty seconds and males produce a spermatophylax. As predicted by the hypothesis that prolonged copulation following spermatophore transfer functions to ensure complete sperm transfer, the longer duration of copulation in *M.meridionale*, compared with *M.thalassinum*, was found to be associated with the production of a greater number of sperm per mating (about 4 times more sperm). The association of prolonged copulation following spermatophore transfer with the complete loss of the spermatophylax in *Meconema* and the considerable reduction in spermatophylax size in *U.rugiscollis* suggests that the spermatophylax and prolonged copulation following spermatophore transfer are analogous in function. This supports the hypothesis that the spermatophylax functions to prevent the female from eating the ampulla before complete sperm transfer, but does not rule out the possibility of the spermatophylax additionally functioning as a form of paternal investment in offspring.

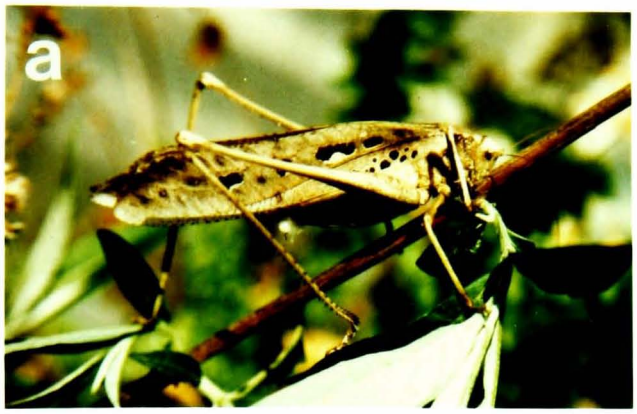
4. The Evolution of Spermatophylax Size in Bushcrickets.

4.1 *Introduction.*

Within the Tettigoniidae, there is a considerable degree of variation between species in the size of the spermatophylax relative to male body size (Boldyrev 1915; Gwynne 1983b, 1990a; Chapter 2, table 2.1; figs. 4.1a - 4.1p). At one extreme, the spermatophylax is absent in species such as *Meconema thalassinum* (chapter 3) and *Mecopoda elongata* (pers.obs; see fig 4.1a) and the spermatophore may represent as little as 0.6% of male body weight (chapter 3). At the other extreme, in species such as *Steropleurus catalaunicus* and *S.stali* (see fig 1.1a & 1.1b, in chapter 1; fig 4.1p), the spermatophylax is an enormous quadri-lobed structure and males may lose over 40% of their body weight at mating (chapter 3). Such differences in relative spermatophylax size may even be found between species belonging to the same genus. For example, in *Phaneroptera nana*, the spermatophylax contributes to a mean loss of 5.5% of male body weight, while in *P.falcata* it contributes to a mean loss of 16% of male body weight (chapter 2, table 2.1; figs 4.1d & 4.1j). Similarly, in *Leptophyes punctatissima*, males lose up to 8% of their body weight at mating, while in *L.laticauda* males lose up to 33% of their body weight (see chapter 7; figs 4.1c & 4.1o).

There are two main hypotheses concerning the selective pressures responsible for the evolutionary enlargement of the spermatophylax in the Tettigoniidae. Gwynne (1986b,1988b,1990a) proposed that while the spermatophylax may have originated

Fig. 4.1 Interspecific variation in spermatophylax size in the Tettigoniidae. a) *Mecopoda elongata*; b) *Ruspolia nitidula*; c) *Leptophyes punctatissima*; d) *Phaneroptera nana*; e) *Platycleis affinis*; f) *Conocephalus dorsalis*; g) *Leptophyes bosci*; h) *Metrioptera saussuriana*; i) *Pholidoptera griseoaptera*; j) *Phaneroptera falcata*; k) *Tettigonia viridissima*; l) *Poecilimon affinis*; m) *Polysarcus scutatus*; n) *Tylopsis lilifolia*; o) *Leptophyes laticauda*; p) *Steropleurus stali*.


a


b


c


d

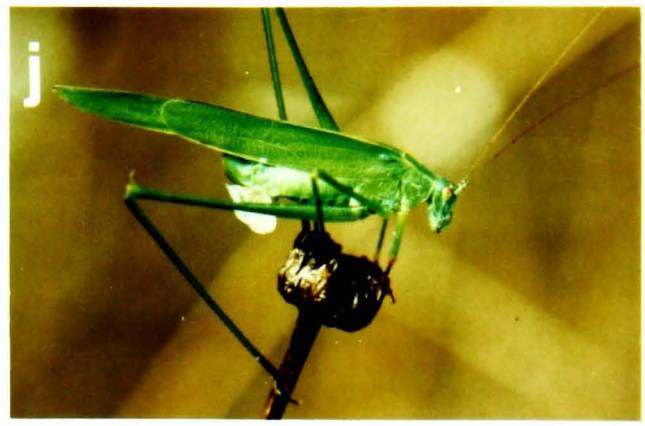

e


f


g


h


i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p

in the context of intrasexual selection as a means of ensuring complete sperm\ ejaculate transfer (see chapter 1, section 1.1.2; chapter 2), elaboration of spermatophylax size may subsequently have proceeded through natural selection for male parental investment (= paternal investment hypothesis) (see chapter 1, section 1.1.3). The alternative hypothesis is that elaboration of spermatophylax size occurred to facilitate the transfer of larger amounts of sperm\volumes of ejaculate, still within the context of intrasexual selection (= ejaculate-protection hypothesis).

The ejaculate-protection hypothesis predicts that species with proportionately larger spermatophylaxes should have proportionately larger ampullae (an estimate of ejaculate volume), which should contain proportionately more sperm. The paternal investment hypothesis, on the other hand, does not predict a relationship between these variables because selection is envisaged as acting on the spermatophylax alone, elaborating it beyond the size necessary to allow complete sperm\ejaculate transfer (see Gwynne 1986b, 1988b, 1990a).

Here, I present the results of a comparative study designed to test the ejaculate-protection hypothesis.

4.2 *Methods.*

A variety of species of bushcricket were collected as adults from Spain in August 1990, France in August 1990 and August 1991, Greece in July 1991 and England in September 1990 and 1991. Collecting methods and localities for each species are given in appendix 1. Bushcrickets were taken back to the laboratory at Nottingham, where sexes were separated and maintained under conditions outlined by Hartley & Dean (1974), as detailed in appendix 1. Stocks of *Mecopoda elongata*, which

originated from Malaysia, were purchased from an entomological dealer.

Spermatophores were obtained both from wild-caught individuals and from offspring subsequently reared in the laboratory (details of oviposition media used, treatment of eggs and rearing conditions for nymphs are outlined in appendix 1). Males were not used for mating until at least two weeks following collection or the final moult, in order to ensure that spermatophore size and sperm number were unlikely to be reduced due to male age or mating history (see chapter 6). For each species, individual stridulating males, and females which showed signs of receptivity (ie. showing phonotaxis to the male call or exhibiting a response-song, where present), were transferred to black nylon-mesh observation cages (measuring approx. 10cm x 10cm x 10cm), one pair per cage. Individuals were transported to these cages on a twig in order to minimise disturbance. Cages were observed intermittently until mating occurred. Directly after the end of copulation, the entire spermatophore was removed from the female using watchmakers' forceps. This was weighed on a Cahn-25 electrobalance to an accuracy of 0.01 mg. The ampulla was then separated from the spermatophylax and weighed separately. Ampulla weight was subtracted from the weight of the entire spermatophore to give the spermatophylax weight in each case. This minimised handling of the spermatophylax which, being mucoid in consistency, is somewhat prone to desiccation. The recently mated male in each case was weighed on an electrobalance to an accuracy of 0.1 mg. The weight of the spermatophore produced was added to male body weight to give male pre-mating body weight. In most cases, the ampulla was then placed in a plastic vial in a known volume of physiological locust saline (from 0.05 ml - 6ml, depending upon the size of the ampulla). The ampulla was crushed with watchmakers' forceps and its contents were suspended by thorough mixing with watchmakers' forceps for five mins. This was found to result in an even suspension of sperm. A portion of each sample was then transferred to a haemocytometer (Neubauer, improved). The

number of sperm in the centre grid was counted under a microscope. Two sub-samples were counted per sample and a mean value was taken. This value was multiplied by the appropriate dilution factor to give an estimate of the total sperm number in the original sample. Where possible, all measurements were conducted on a number of individuals of each species and a mean value was taken.

Measurements of male body weight, spermatophylax weight and ampulla weight were obtained for 43 species of bushcricket. Data for one additional species was taken from the literature, giving data for 44 species representing 27 genera and 8 sub-families, though most species were from the sub-families Phaneropterinae and Tettigoniinae. Sperm counts were conducted on 31 of these species and a further 4 were taken from the literature, giving data for 35 species representing 23 genera and 8 sub-families.

Analysis.

One problem the comparative approach faces is how to control for the effects of common ancestry. Closely related taxa are likely to resemble one another both in the character under investigation and its potential morphological and ecological correlates. Consequently, data for such taxa cannot be treated as independent points in statistical analysis (Harvey & Pagel 1991; Harvey & Purvis 1991).

I used the non-directional independent-comparisons method (see Harvey & Pagel 1991; Harvey & Purvis 1991 for details) to examine the relationship between evolutionary changes in spermatophylax size and changes in ampulla size and sperm number. This method allows for phylogenetic effects using the principle that differences in a character between two taxa which share an immediate common ancestor should not be confounded by phylogenetic differences (see Felsenstein

1985). This method involves calculating the difference in character x and the difference in character y between the species within each of the lowest-level clades of a given branching phylogeny, then again at the next highest level clades and so on, until the two highest nodes of the tree are compared (Harvey & Pagel 1991). The ancestral character state is estimated as the average of the two daughter values. Such an estimate is an inference made at an intermediate stage in the statistical analysis and should not be used outside this context (Harvey & Purvis 1991). The direction of the comparison at a given clade should be the same for each character. It can be convenient to choose the direction of the comparison so that the result for one of the values is always positive (Harvey & Purvis 1991).

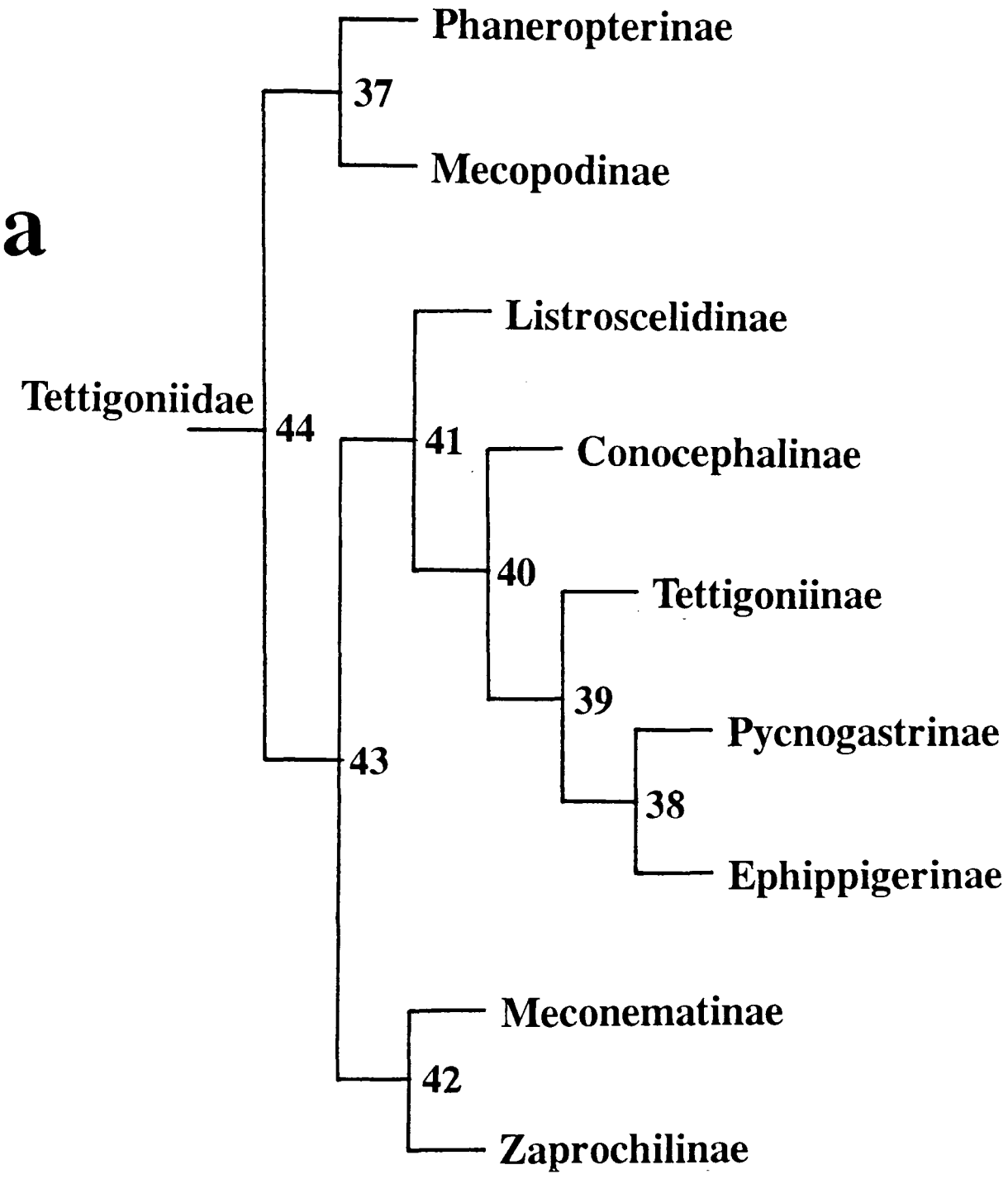
The set of differences (contrasts) for character x and for character y provide a way to test whether changes in x and y are correlated (see Harvey & Pagel 1991). Each of the relationships between contrasts for x and contrasts for y represents an independent incidence of the evolution of this relationship; the data can be treated as independent points in statistical analysis (Harvey & Pagel 1991).

Ideally, this method requires that the true branching phylogeny is known. However, in the absence of such, a taxonomy may be used to represent the branching of species (Harvey & Pagel 1991; Harvey & Purvis 1991). There is currently no detailed phylogeny for the family Tettigoniidae. However, there is a phylogeny at the level of the genus for the sub-family Tettigoniinae (Rentz & Coless 1990) and Gorochov (1988) gives the possible phylogenetic relationships between the sub-families of the Tettigoniidae. I have used these sources together with the taxonomies of the remaining groups to construct the diagrams shown in figs 4.2a-f, which were used to generate the comparisons.

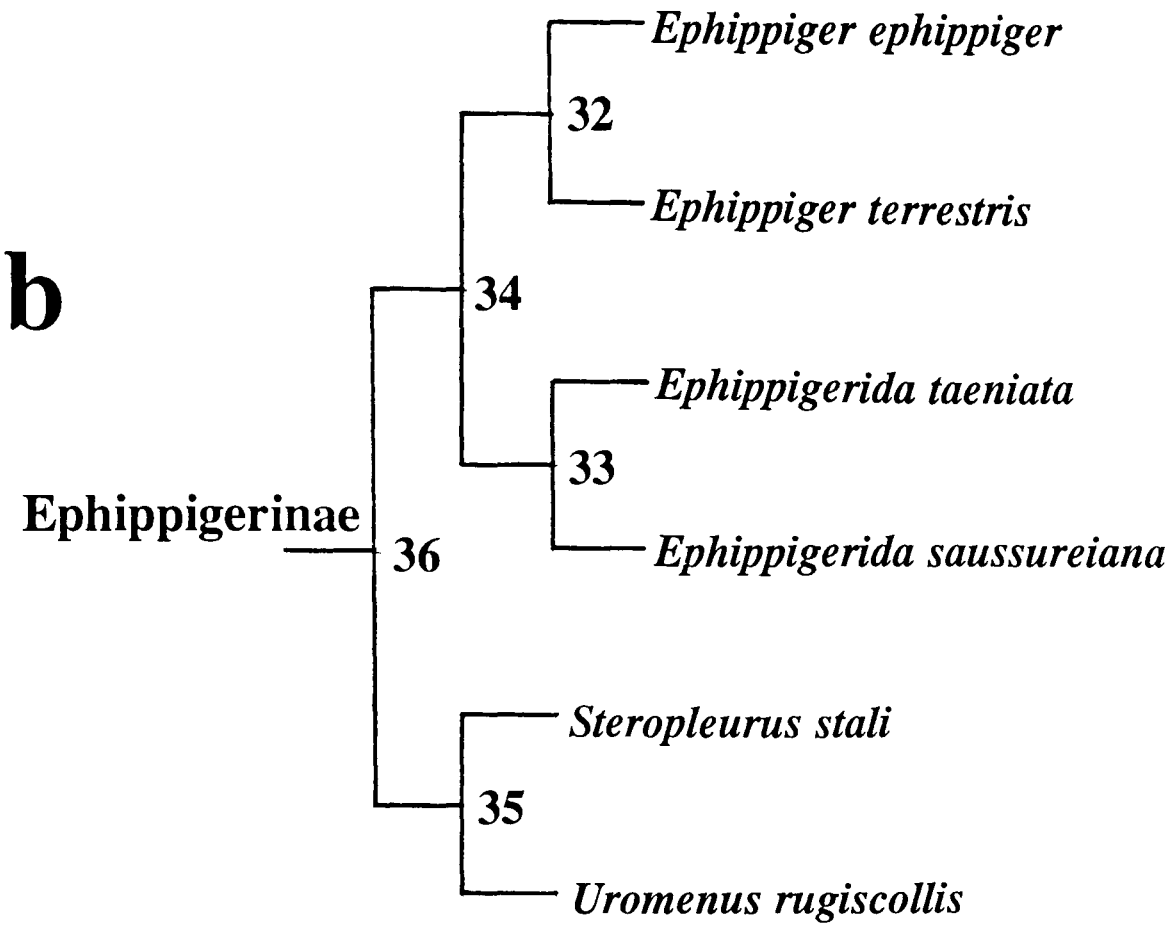
The overall classification for the sub-family Phaneropterinae (fig 4.2e) was based on

Fig 4.2 Branching diagrams reflecting the taxonomic and\or phylogenetic relationships between the different species of bushcricket studied. These diagrams were used in the calculation of the contrasts (see text). **a)** probable phylogenetic relationships between the different tettigoniid sub-families studied (from Gorochov 1988); **b)** taxonomic relationships between the different species of ephippigerine studied; **c)** taxonomic relationships between the conocephalines studied (con. = tribe Conocephalini; cop= tribe Copiphorini); **d)** taxonomic relationships between the different meconematines studied; **e)** taxonomic\ phylogenetic relationships between the phaneropterines studied (general taxonomy based on Bei-Bienko 1954 and Kevan 1982; relationships between members of the tribe Barbitistini, especially members of the genus *Poecilimon*, based on Heller 1984 & 1990) (Barb = Barbitistini; Phan = phaneropterini; Tyl = Tylopsini); **f)** phylogenetic relationships between members of the sub-family Tettigoniinae used in this study (from Rentz & Coless 1990) (Tett = Tettigoniini; Plat = Platycleidini).

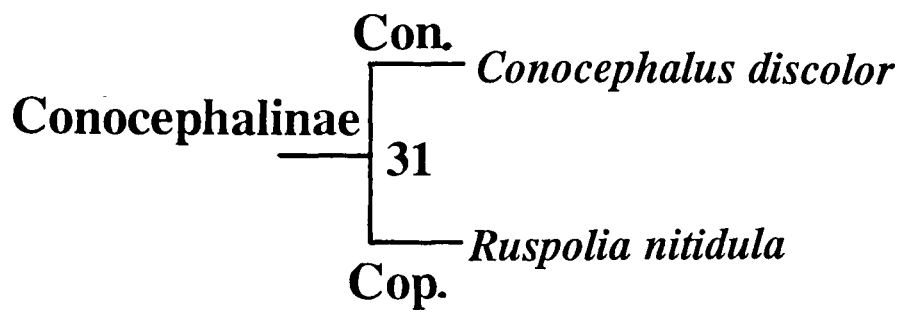
a


Phaneropterinae
37
Mecopodinae
Listroscelidinae
Tettigoniidae
44
41
Conocephalinae
40
Tettigoniinae
39
Pycnogastrinae
43
38
Ephippigerinae
Meconematinae
42
Zaprochilinae

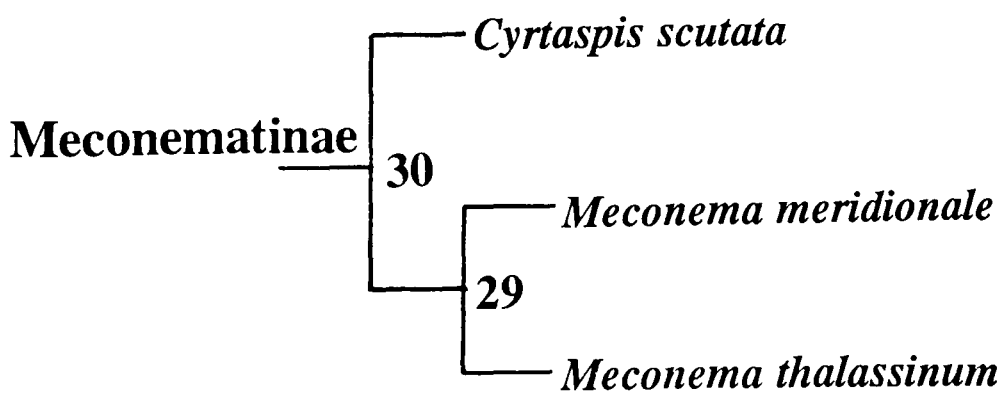
b


Ephippiger ephippiger
32
Ephippiger terrestris
34
Ephippigerida taeniata
33
Ephippigerinae
36
Ephippigerida saussureiana
Steropleurus stali
35
Uromenus rugiscollis

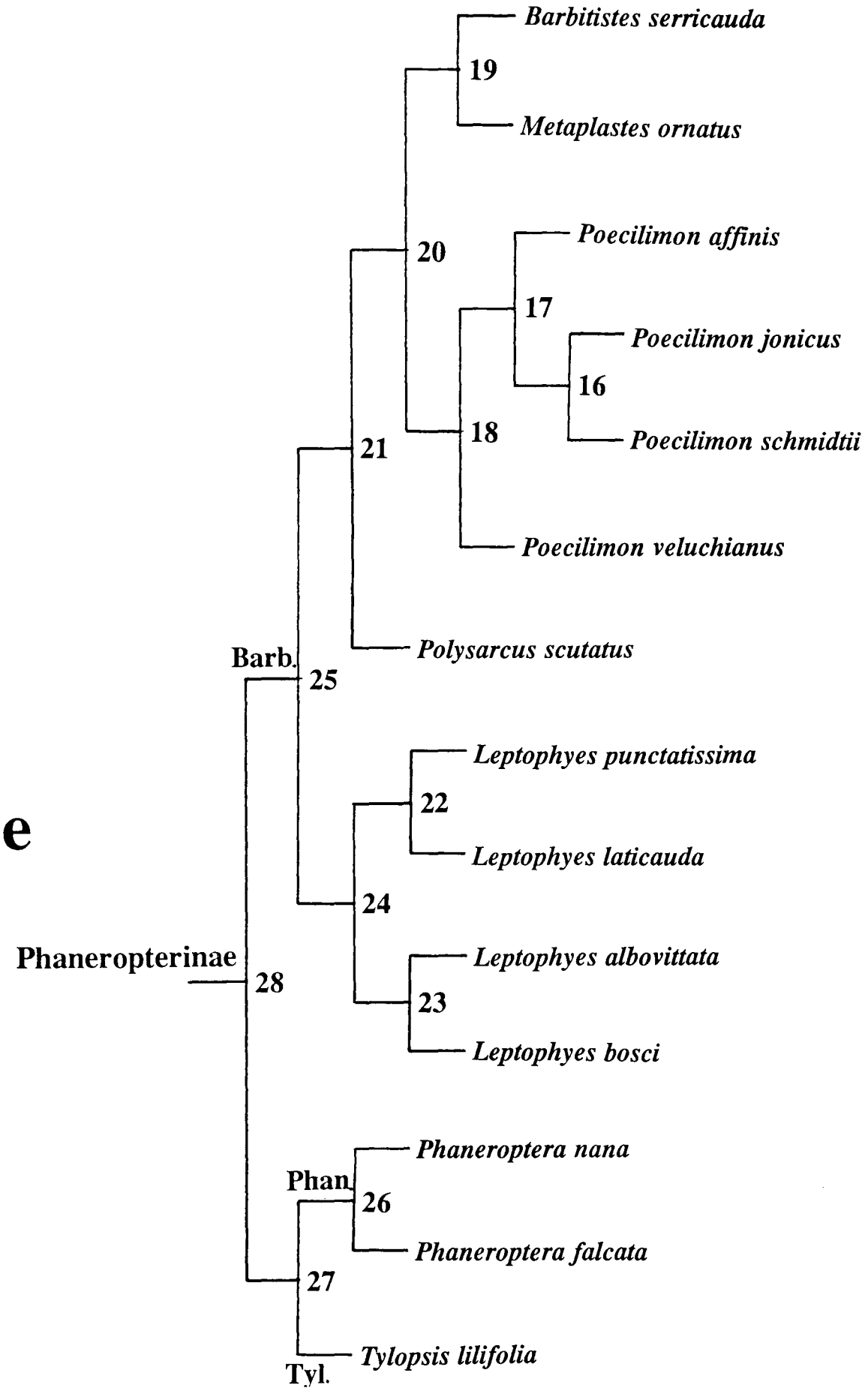
c

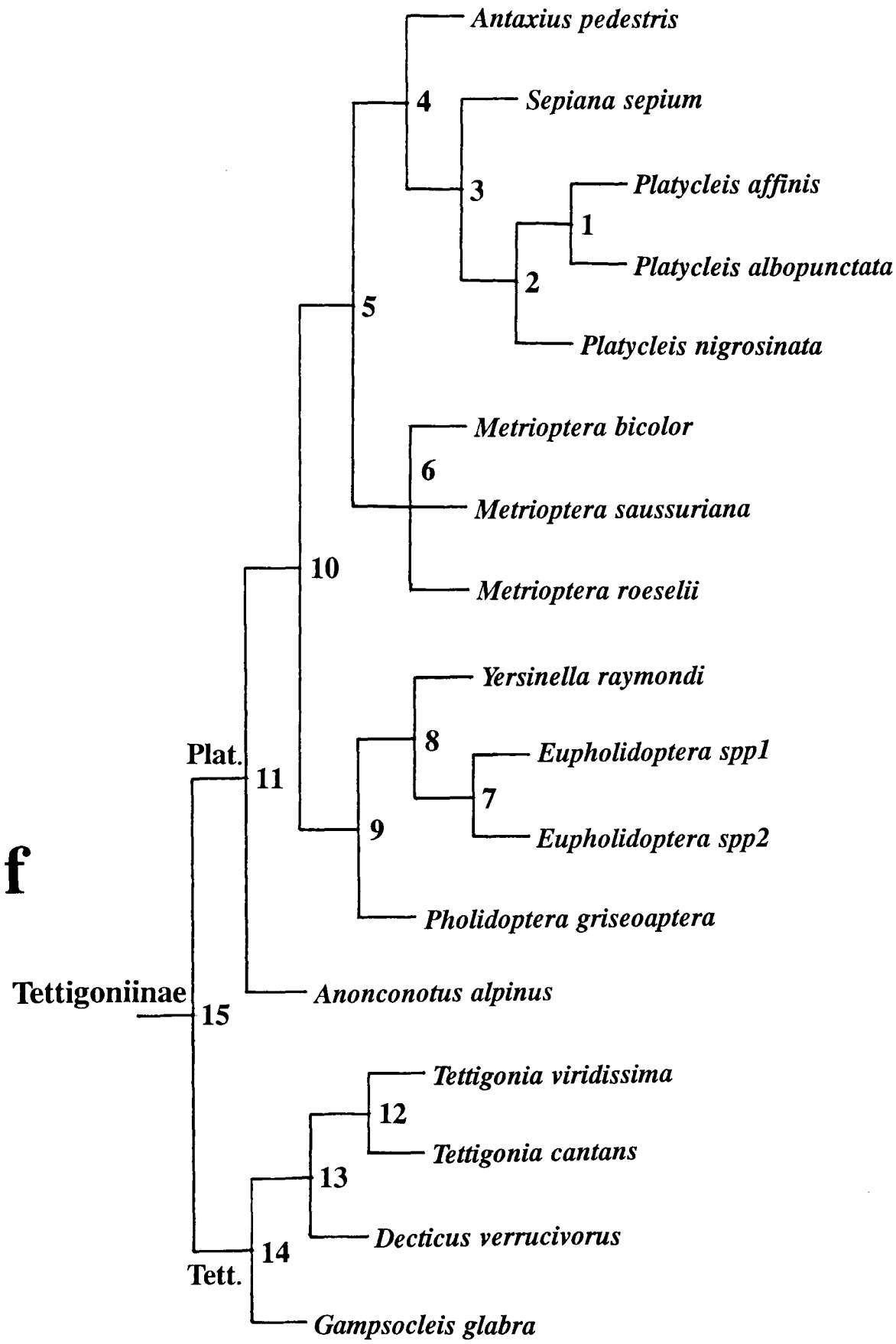

Con.
Conocephalus discolor
Conocephalinae
31
Ruspolia nitidula
Cop.

d


Cyrtaspis scutata
Meconematinae
30
Meconema meridionale
29
Meconema thalassinum

e


Barbitistes serricauda
19
Metaplastes ornatus
20
Poecilimon affinis
17
Poecilimon jonicus
16
Poecilimon schmidtii
18
21
Poecilimon veluchianus
Polysarcus scutatus
Barb.
25
Leptophyes punctatissima
22
Leptophyes laticauda
24
Phaneropterinae
28
Leptophyes albovittata
23
Leptophyes bosci
Phaneroptera nana
Phan.
26
Phaneroptera falcata
27
Tylopsis lilifolia
Tyl.


Antaxius pedestris
4
Sepiana sepium
3
Platycleis affinis
1
Platycleis albopunctata
2
Platycleis nigrosinata
5
Metrioptera bicolor
6
Metrioptera saussuriana
10
Metrioptera roeselii
Yersinella raymondi
8
Eupholidoptera spp1
7
Eupholidoptera spp2
9
Pholidoptera griseoaptera
Plat.
11
Anonconotus alpinus
Tettigoniinae
15
Tettigonia viridissima
12
Tettigonia cantans
13
Decticus verrucivorus
14
Tett.
Gampsocleis glabra

f

that given by Bei-Bienko (1954) and Kevan (1982), while the relationships between species in the tribe Barbitistini and genus *Poecilimon* were taken from Heller (1984; 1990). Within the sub-family Ephippigerinae (see fig 4.2b), I have grouped *Steropleurus stali* and *Uromenus rugiscollis* together because these species are placed within the same genus (*Uromenus*) by Harz (1969). Within the sub-family Tettigoniinae (see fig 4.2f), the relationships between the three members of the genus *Platycleis* for which data were obtained is based on the sub-generic groupings given by Harz (1969).

A problem for the independent comparisons method is the occurrence of multiple nodes, that is nodes from which more than two daughter taxa are represented as direct descendants. With more than two branches from a node, the logic of finding a simple difference breaks down (Pagel & Harvey 1991). Only one multiple node occurred in the branching diagram of the taxonomic relationships between the species used in this study. This was between the three *Metrioptera* species (sub-family Tettigoniinae). Each is placed in a separate sub-genus (Harz 1969), making further grouping of the species difficult. I have used the method given by Pagel & Harvey (1989, cited in Harvey & Pagel 1991: p 157) to generate the contrast in this case. That is, I have grouped two of the three species together because they show greater similarity in the character in question. I have then taken the contrast as the difference between the value of the character of the remaining species and the mean value of the characters of the grouped species.

Another problem faced by the independent comparisons method is that of scaling, that is correcting for unequal variance. Scaling is necessary because in a taxon full of long branches, a greater degree of variation in character values might be expected than in a taxon full of only short branches (Pagel & Harvey 1991; Harvey & Pagel 1991). In order to scale properly, it is necessary to know the branch lengths (though

these can be estimated; see Harvey & Pagel 1991 for a full discussion of this subject). Because the phylogenetic relationships between the species used in this study are not fully resolved and taxonomies are used as a substitute, it does not seem appropriate to attempt to estimate branch lengths. The comparisons used in this study have not therefore been scaled.

For each species, the obtained values for male body mass, spermatophylax mass, ampulla mass and sperm number were $\log_{10}$ transformed. Because sperm number data were available for fewer species than ampulla data, two sets of contrasts were calculated for body mass and spermatophylax mass: one set was calculated using species for which there was sperm number data and the other set was calculated using species for which ampulla mass data was available. The confounding effect of body mass was removed by calculating residuals from the linear regressions of a given variable (spermatophylax mass contrasts, ampulla mass contrasts or sperm number contrasts) on contrasts of body mass. Further regression analysis was performed on the residual contrasts of spermatophylax mass (dependent variable) against the residual contrasts of ampulla mass and on the residual contrasts of spermatophylax mass (dependent variable) against the residual contrasts of sperm number to test the prediction that changes in spermatophylax mass should be positively related to changes in ampulla mass and sperm number. In all cases, regressions were forced through the origin, as recommended by Harvey & Pagel (1991). For clarity, plots are presented using both raw species data and the contrasts. Means are cited $\pm$ standard error.

4.3 *Results.*

Mean values of male body mass, spermatophylax mass, ampulla mass and sperm number for each species are given in table 4.1. Log-log plots of the species data of spermatophylax mass, ampulla mass and sperm number on male body mass are presented in figs 4.3, 4.4 and 4.5 respectively. The relationship between residual spermatophylax mass (residuals from the linear regression of spermatophylax mass against male body mass; see fig. 4.3) against residual sperm number (residuals from the linear regression of sperm number against male body mass; see fig. 4.5) is presented in fig. 4.6. As predicted by the ejaculate-protection hypothesis, there appears to be a positive relationship between the two variables. However, because species values cannot be treated as independent data points in statistical analysis (Harvey & Pagel 1991), no statistical analysis was performed on this data. The relationship between residual spermatophylax mass and residual ampulla mass (residuals from the linear regression of ampulla mass against male body mass; see fig 4.4) is presented in fig. 4.7. As predicted by the ejaculate-protection hypothesis, there appears to be a positive relationship between these two variables. Once again, however, this relationship was not analysed statistically due to the reasons given above.

For the set of contrasts using species for which sperm number data were available, contrasts in sperm number were positively related to contrasts in male body mass (fig. 4.8; slope = 1.18 ± 0.21, $t_{31} = 5.52$, $p << 0.001$, $r^2 = 0.49$). The slope of this regression is not significantly different from 1 ($t_{31} = 0.86$, N.S.). For this data set, contrasts in spermatophylax mass were also positively related to contrasts in body mass (fig. 4.9; slope = 1.30 ± 0.22, $t_{31} = 5.9$, $p << 0.001$, $r^2 = 0.52$).

Table 4.1. Mean male weight, ampulla weight, ampulla as % male weight, spermatophylax (sp'lax) weight, spermatophylax as % male weight and sperm number for different bushcricket species (letters indicate source of reference; dashes indicate missing values).

	Sub-families and species	Male weight (mg)	(n)	Ampulla weight (mg)	(n)	% of male weight	Sp'lax weight (mg)	(n)	% of male weight	Sperm number ($x10^4$)	(n)
	Phaneropterinae										
1	*Phaneroptera nana*	289	7	5.24	7	1.8	9.16	7	3.2	3.8	5
2	*Phaneroptera falcata*	187	1	10.02	1	5.4	16.51	1	8.8	31.2	1
3	*Tylopsis lilifolia*	340	6	13.17	6	3.9	69.72	6	20.5	-	-
4	*Barbitistes serricauda*	721	1	47.5	1	6.6	158.6	1	22.0	369.0	1
5	*Leptophyes punctatissima*	175	15	1.05	10	0.6	5.97	14	3.4	11.5	16
6	*Leptophyes laticauda*	478	17	20.28	17	4.2	103.65	17	21.7	168.76	17
7	*Leptophyes albovittata*	112	2	1.78	2	1.6	6.75	2	6.0	26.35	2
8	*Leptophyes bosci*	235	4	3.08	4	1.3	13.53	4	5.8	71.11	4
9	*Poecilimon schmidtii*	525	8	9.17	6	1.8	63.39	6	12.1	84.5	2
10	*Poecilimon jonicus*	324	4	5.82	3	1.8	21.96	4	6.8	20.43	3
11	*Poecilimon veluchianus*	710	1	37.0	1	5.2	145.0	1	20.4	1040.0[a]	50
12	*Poecilimon affinis*	1328	4	30.89	3	2.3	170.27	4	12.8	438.0	3
13	*Polysarcus scutatus*	1688	4	48.6	2	2.9	221.3	2	13.0	362.0	1
14	*Metaplastes ornatus*	450	2	-	-	-	72.0	2	16.0	149.0[b]	-
	Mecopodinae										
15	*Mecopoda elongata*	3699	2	27.24	2	0.74	0.0	2	0.0	-	-
	Tettigoniinae										
16	*Tettigonia viridissima*	1450	1	78.63	1	5.4	250.0	1	17.2	454.0	1
17	*Tettigonia cantans*	1204	1	52.6	1	4.4	154.4	1	12.8	-	-
18	*Gampsocleis glabra*	885	5	36.32	5	4.1	61.22	5	6.9	216.88	4
19	*Decticus verrucivorus*	1618	3	56.09	3	3.5	123.42	3	7.6	169.56	3
20	*Platycleis affinis*	576	5	13.78	5	2.4	23.05	5	4.0	75.14	5
21	*Platycleis albopunctata*	479	3	12.20	3	2.5	14.37	3	3.0	71.7	2
22	*Platycleis nigrosinata*	409	1	11.56	1	2.8	14.56	1	3.6	-	-
23	*Metrioptera saussuriana*	509	4	13.05	4	2.6	29.33	4	5.8	100.45	4
24	*Metrioptera bicolor*	438	3	19.22	3	4.4	23.78	3	5.4	54.63	3
25	*Metrioptera roeselii*	345	3	15.73	3	4.6	20.23	3	5.9	40.24	3
26	*Sepiana sepium*	529	2	15.98	2	3.0	23.87	2	4.5	39.55	2
27	*Yersinella raymondi*	200	2	2.26	3	1.1	11.0	1	5.5	20.83	2
28	*Anonconotus alpinus*	604	6	4.94	6	0.8	7.71	6	1.3	59.02	5
29	*Antaxius pedestris*	716	1	25.69	1	3.6	89.83	1	12.5	532.5	1
30	*Pholidoptera griseoaptera*	498	2	16.34	2	3.3	37.09	2	7.4	84.6	2
31	*Eupholidoptera spp1*	1233	1	56.40	1	4.6	103.6	1	8.4	197.0	1
32	*Eupholidoptera spp2*	1042	1	34.93	1	3.4	135.82	1	13.0	-	-
	Conocephalinae										
33	*Conocephalus discolor*	150	2	2.38	2	1.6	12.20	2	8.1	-	-
34	*Ruspolia nitidula*	556	3	2.21	3	0.4	1.59	3	0.3	51.13	2
	Meconematinae										
35	*Cyrtaspis scutata*	182	6	6.72	6	3.7	9.86	6	5.4	21.3	2
36	*Meconema meridionale*	97	8	1.7	8	1.8	0.0	8	0.0	17.26	5
37	*Meconema thalassinum*	94	4	0.56	4	0.6	0.0	4	0.0	4.86	5

Table 4.1 (continued).

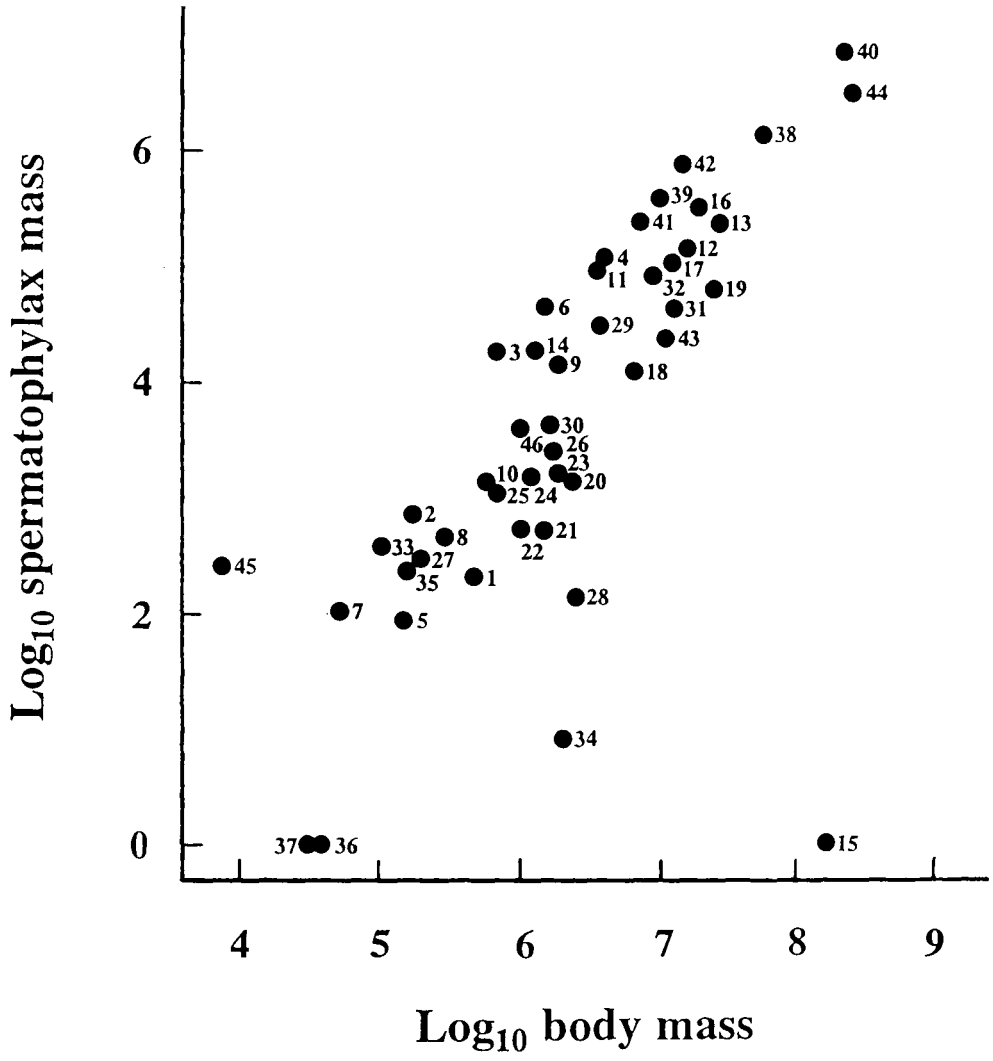
	Sub-families and species	Male weight (mg)	(n)	Ampulla weight (mg)	(n)	% of male weight	Sp'lax weight (mg)	(n)	% of male weight	Sperm number ($x10^4$)	(n
	Ephippigerinae										
38	*Ephippiger ephippiger*	2313	5	148.97	6	6.4	468.76	5	20.3	-	-
39	*Ephippiger terrestris*	1091	1	60.80	1	5.6	270.0	1	24.7	-	-
40	*Ephippigerida taeniata*	4075	17	156.81	17	3.8	947.01	17	23.2	-	-
41	*Ephippigerida saussureiana*	945	1	46.0	1	4.9	219.8	1	23.3	-	-
42	*Steropleurus stali*	1296	1	90.9	1	7.0	362.5	1	28.0	-	-
43	*Uromenus rugiscollis*	1143	9	62.72	9	5.5	79.01	9	6.9	170.25	4
	Pycnogastrinae										
44	*Pycnogaster inermis*	4397	2	308.7	2	7.0	669.85	2	15.2	1020.0	1
	Zaprochilinae										
45	Gen.Nov.22.sp1.	48[c]	-	-	-	-	ca 10[c&d]	-	ca 20[c&d]	21.97[e]	-
	Listeroscelidinae										
46	*Requena verticalis*	400[f]	-	13.07[g]	-	3.3	36.2[g]	-	9.1	93.35[g]	-

a) K.Reinhold, pers comm; b) Helversen & Helversen 1991; c) Simmons & Bailey 1990; d) Gwynne & Baile 1988; e) Simmons & Gwynne 1991; f) Gwynne 1990b; g) Simmons et al 1993.

Fig 4.3 Relation between male body mass and spermatophylax mass for 46 species of tettigoniid. The numbers adjacent to each dot correspond with species listed in table 4.1.

Fig 4.4 Relation between male body mass and ampulla mass for 44 species of tettigoniid. The numbers adjacent to each dot correspond with species listed in table 4.1.

4.3


Log10 spermatophylax mass
Log10 body mass
0
2
4
6
4
5
6
7
8
9
40
44
38
42
39
16
41
13
12
4
17
11
32
19
6
31
29
43
3
14
9
18
30
46
26
23
10
20
25
24
2
21
8
33
22
27
45
35
1
28
7
5
34
37
36
15

4.4

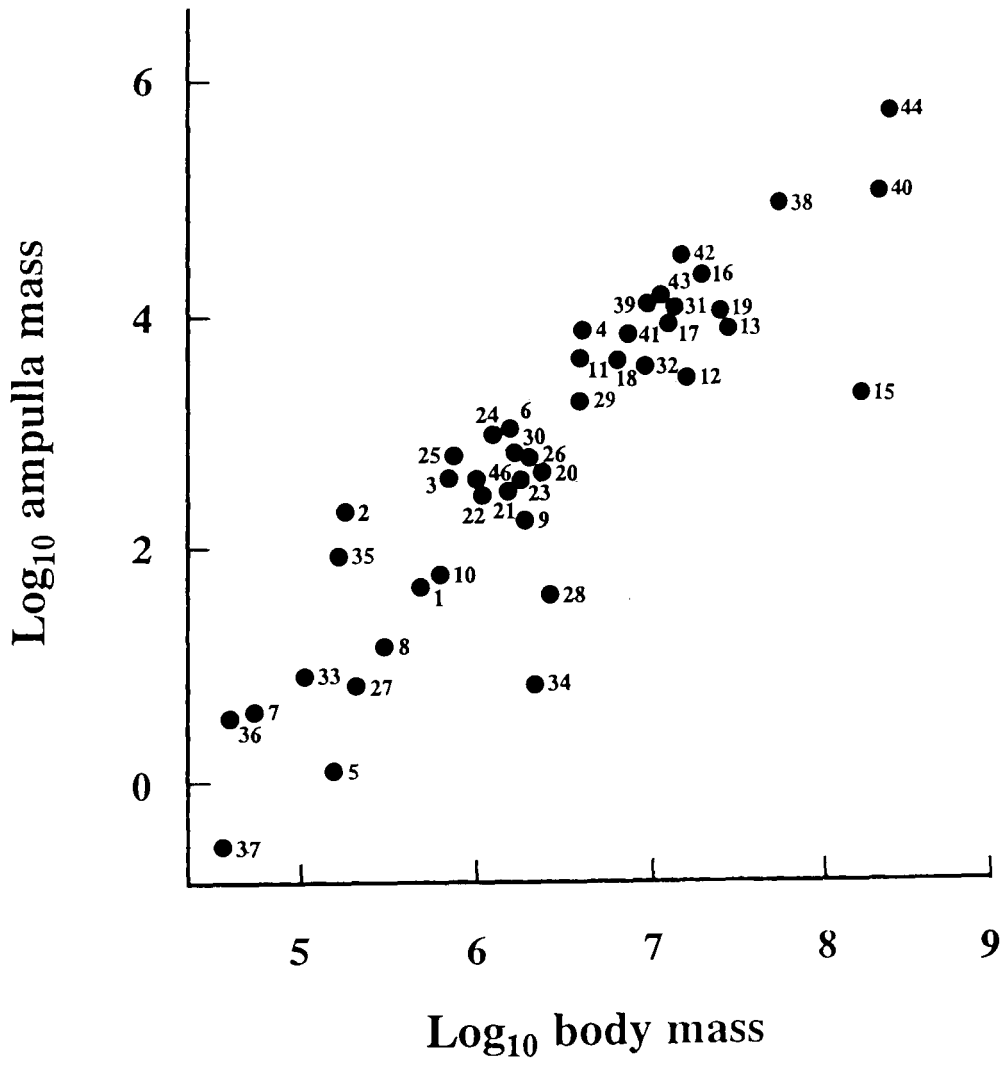

Log10 ampulla mass
Log10 body mass
0
2
4
6
5
6
7
8
9
44
40
38
42
16
43
39
31
19
4
41
17
13
11
18
32
12
15
29
24
6
30
25
26
3
46
20
23
22
21
9
2
35
10
1
28
8
33
27
34
7
36
5
37

Fig 4.5 Relation between male body mass and sperm number for 35 species of tettigoniid. The numbers adjacent to each dot correspond with species listed in table 4.1.

4.5

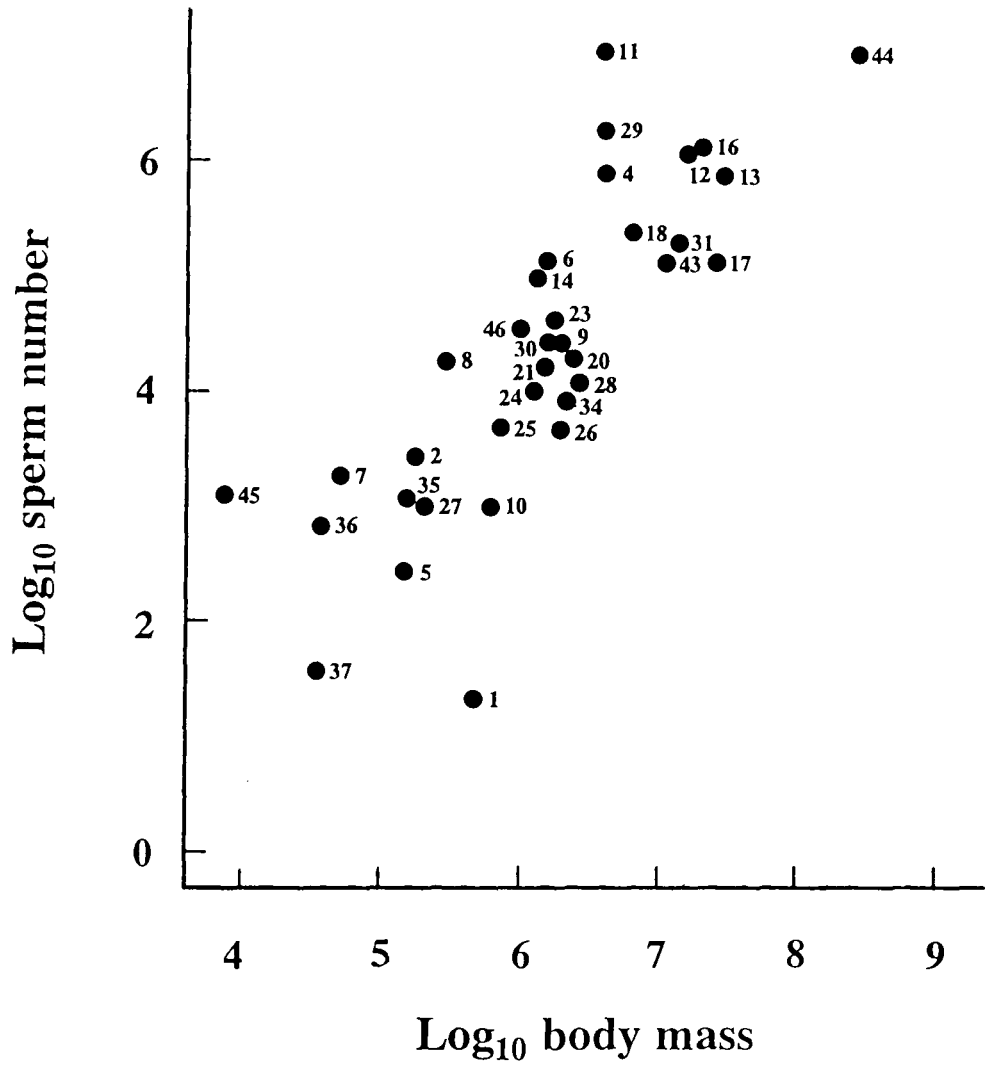
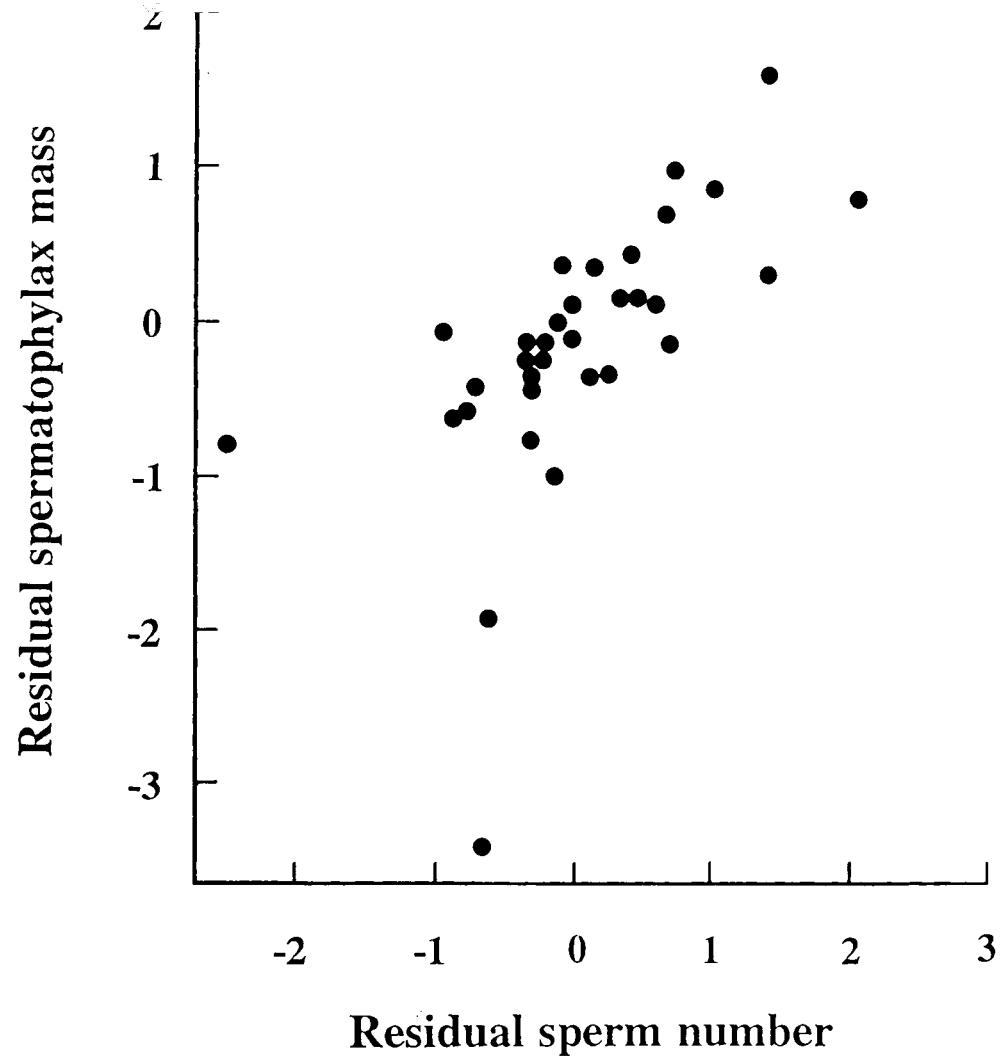

Log10 sperm number
6
4
2
0
4
5
6
7
8
9
Log10 body mass
11
44
29
16
12
13
4
18
31
43
17
6
14
23
46
9
30
20
8
21
28
24
34
25
26
2
7
45
35
27
10
36
5
37
1

Fig 4.6 Relation between residual spermatophylax mass (residuals from the linear regression of spermatophylax mass against male body mass, see fig 4.3) and residual sperm number (residuals from the linear regression of sperm number against male body mass, see fig 4.5) for 33 species of tettigoniid.

Fig 4.7 Relation between residual spermatophylax mass (residuals from the linear regression of spermatophylax mass against male body mass, see fig 4.3) and residual ampulla mass (residuals from the linear regression of ampulla mass against male body mass, see fig 4.4) for 41 species of tettigoniid.

4.6


Residual spermatophylax mass
1
0
-1
-2
-3
-2
-1
0
1
2
3
Residual sperm number

4.7

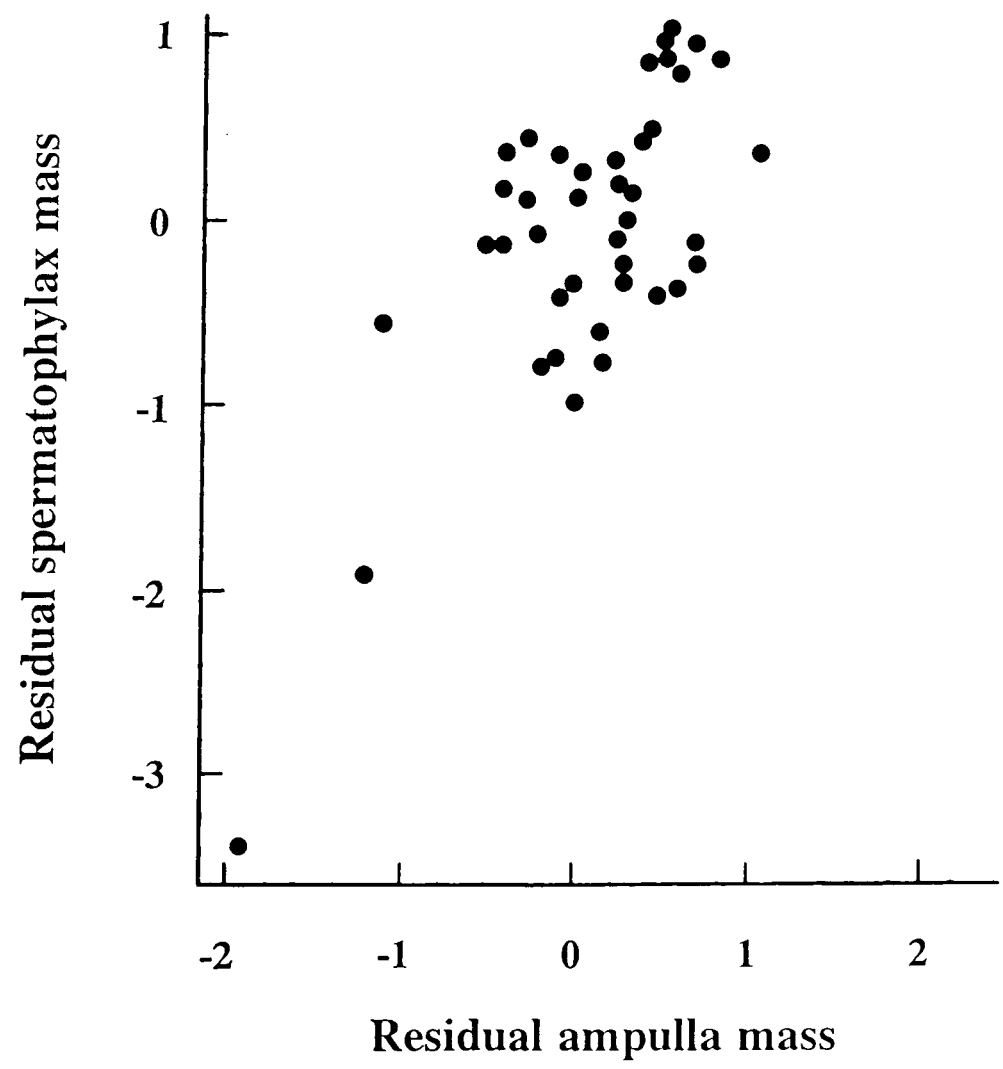
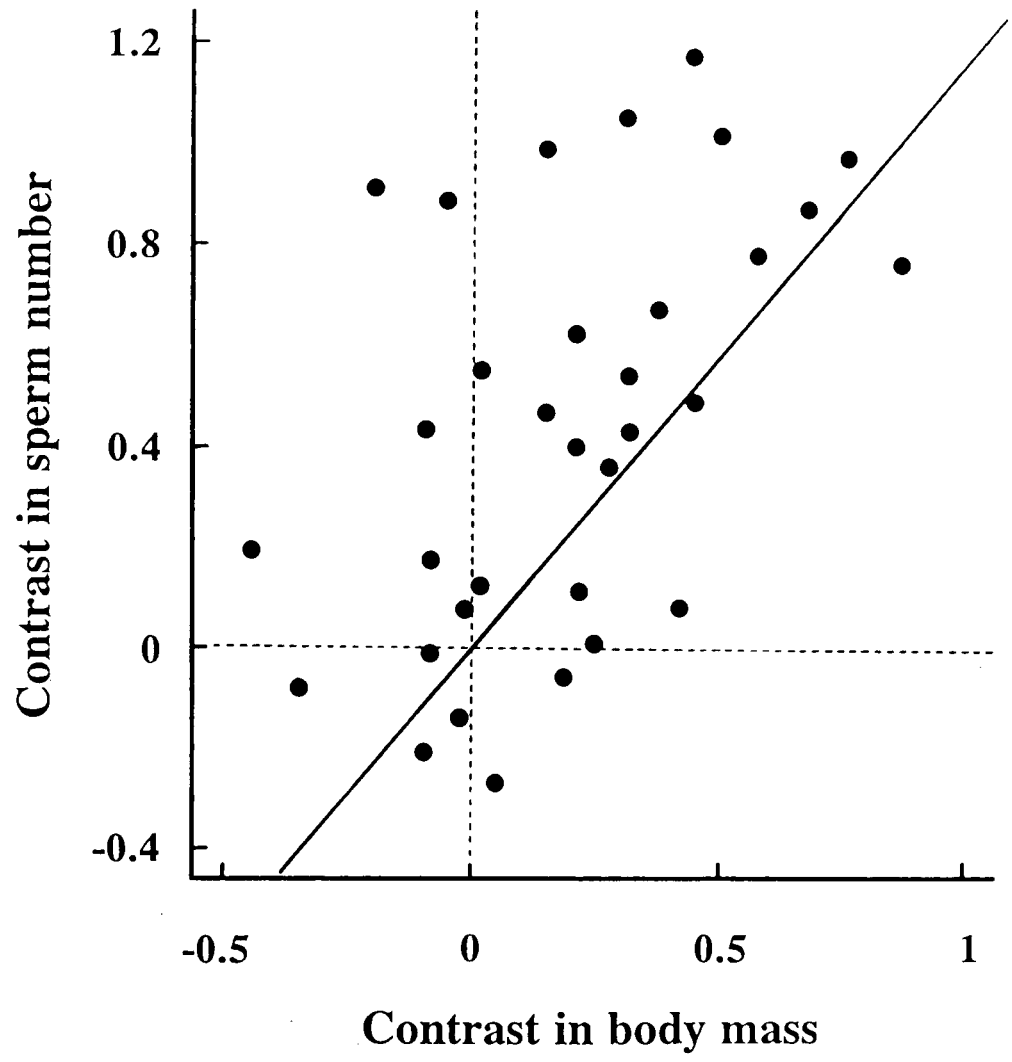

Residual spermatophylax mass
1
0
-1
-2
-3
-2
-1
0
1
2
Residual ampulla mass

Fig 4.8 Contrasts in sperm number against contrasts in body mass for the bushcrickets studied (slope = 1.18 $\pm$ 0.21, t_{31} = 5.52, $p << 0.001$, r^2 = 0.49).

Fig 4.9 Contrasts in spermatophylax mass against contrasts in body mass for the species for which sperm number data were available (slope = 1.30 $\pm$ 0.22, t_{31} = 5.9, $p << 0.001$, r^2 = 0.52).

4.8


1.2
0.8
0.4
0
-0.4
Contrast in sperm number
-0.5
0
0.5
1
Contrast in body mass

4.9

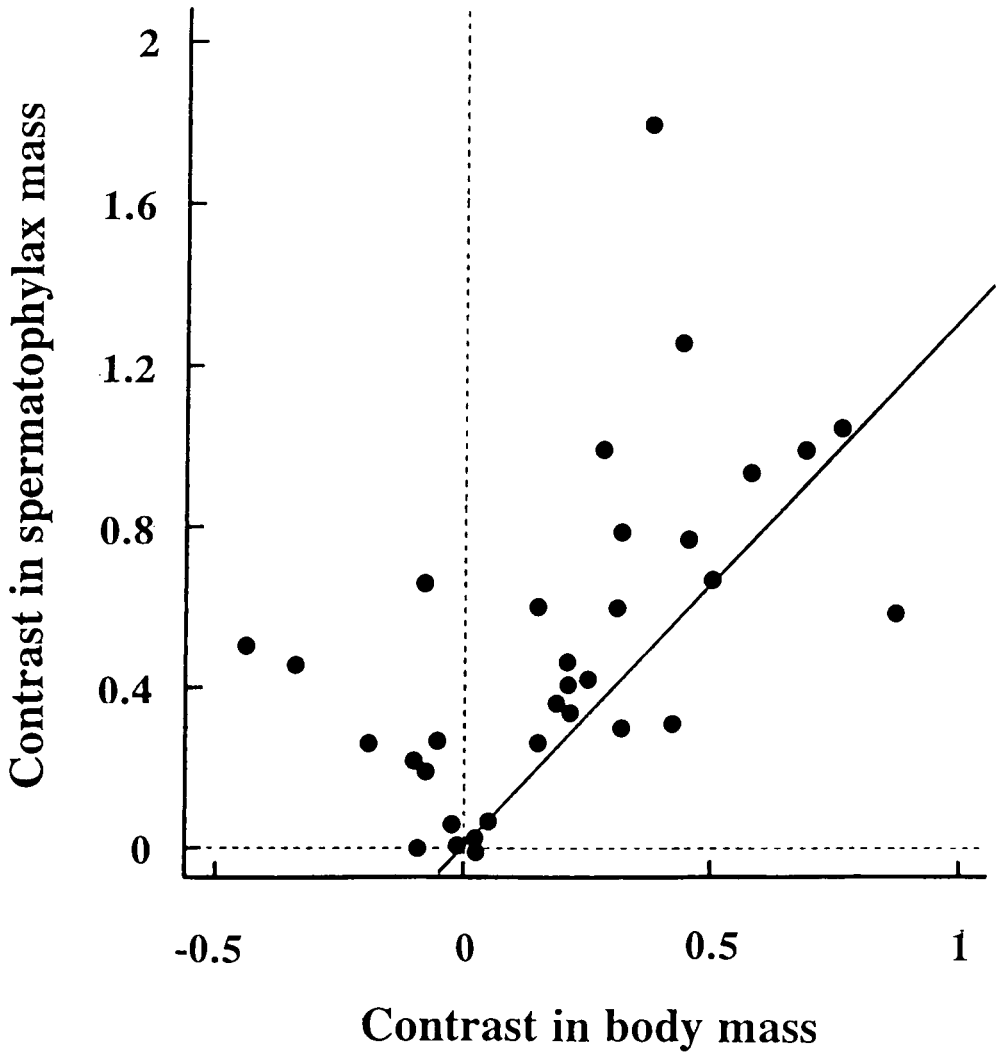

2
1.6
1.2
0.8
0.4
0
Contrast in spermatophylax mass
-0.5
0
0.5
1
Contrast in body mass

Fig 4.10 Residual contrasts in spermatophylax mass (residuals from the linear regression of spermatophylax contrasts against body weight contrasts) against residual contrasts in sperm number for the different bushcrickets studied (slope = 0.64 ± 0.14, $t_{31} = 4.43$, $p < 0.001$, $r^2 = 0.38$).

4.10

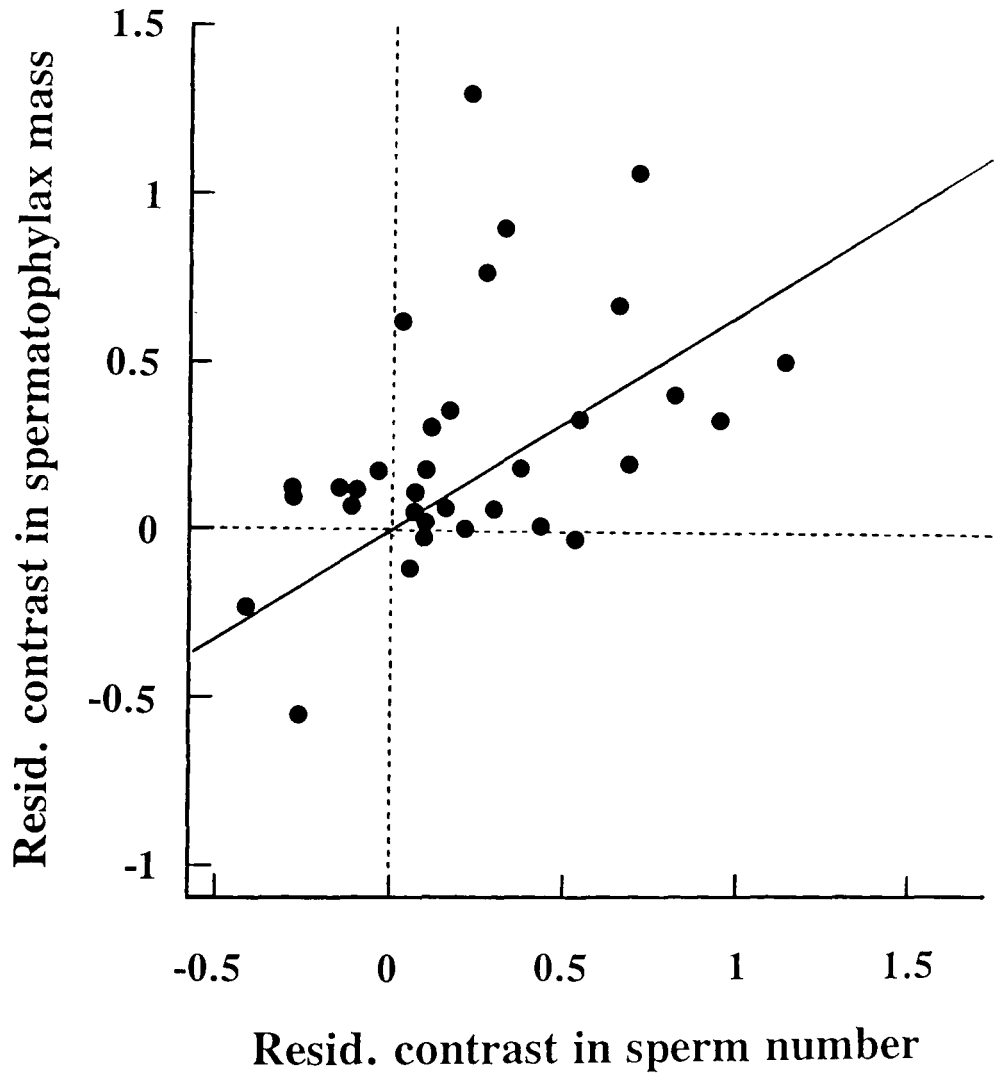
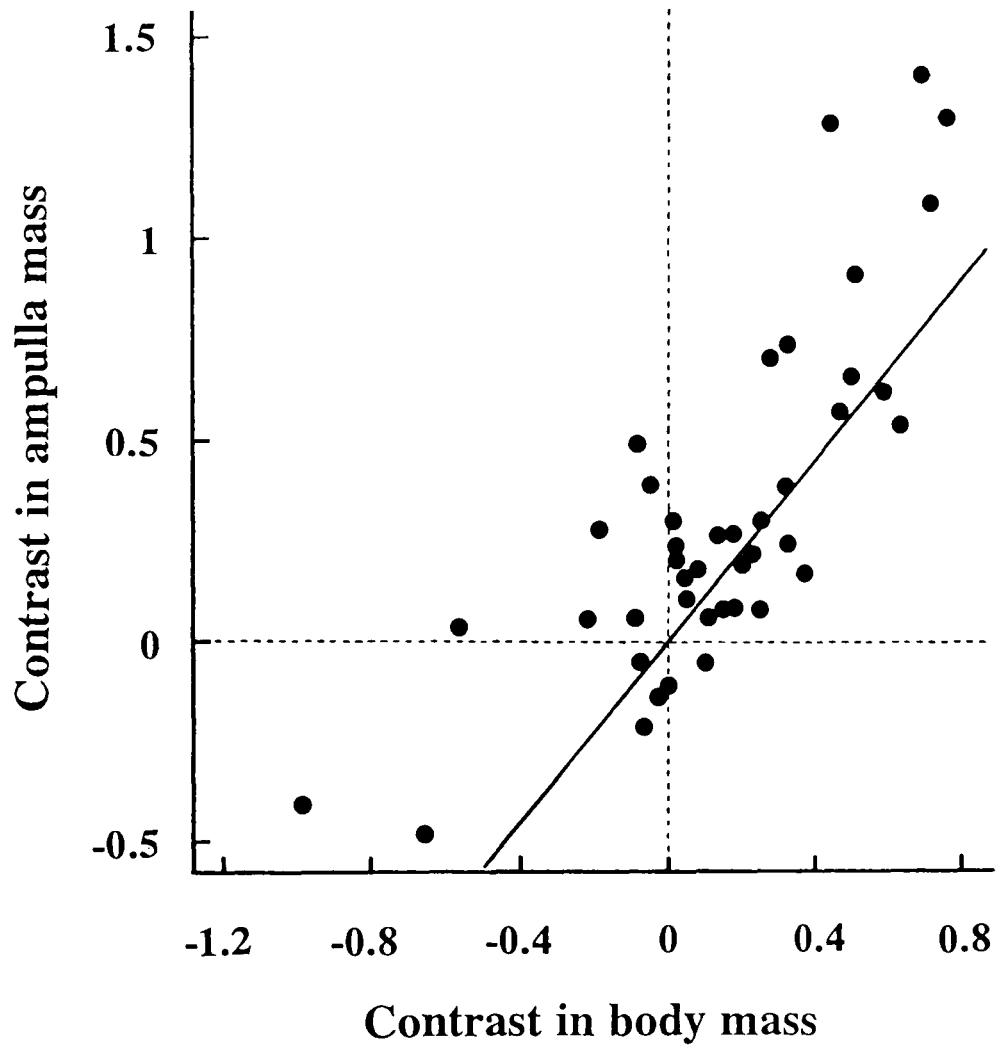

1.5
1
0.5
0
-0.5
-1
-0.5
0
0.5
1
1.5
Resid. contrast in spermatophylax mass
Resid. contrast in sperm number

Fig 4.11 Contrasts in ampulla mass against contrasts in body mass in the bushcrickets studied (slope = 1.12 $\pm$ 0.13, t_{40} = 8.7, p<< 0.001, r^2 = 0.65).

Fig 4.12 Spermatophylax contrasts against body weight contrasts for the species for which ampulla mass data were available (slope = 1.17 $\pm$ 0.20, t_{39} = 5.9, p<< 0.001, r^2 = 0.47). The arrow indicates an outlier (contrast number 37) which was excluded from the calculation of the regression line (with the outlier included, slope = 0.72 $\pm$ 0.24, t_{40} = 2.98, p< 0.01, r^2 = 0.18).

4.11


1.5
1
0.5
0
-0.5
Contrast in ampulla mass
-1.2
-0.8
-0.4
0
0.4
0.8
Contrast in body mass

4.12

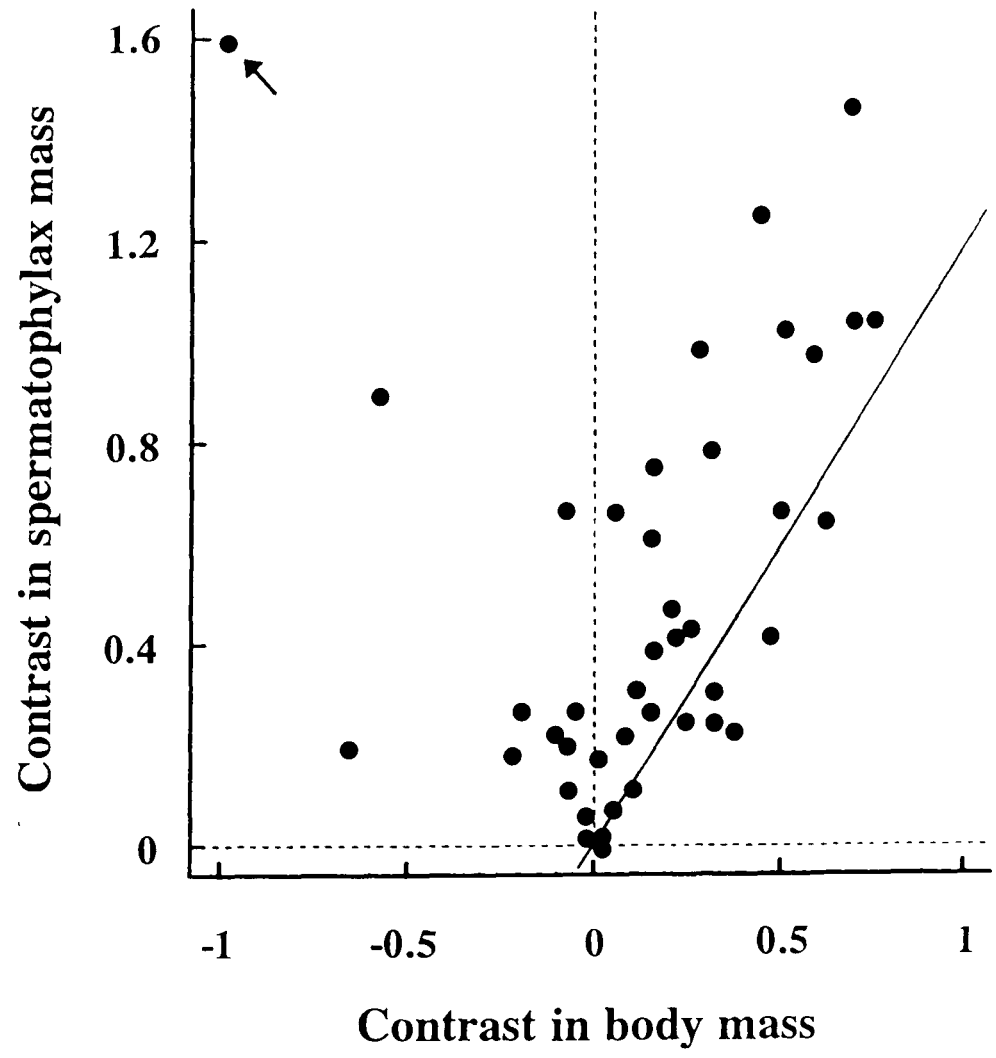
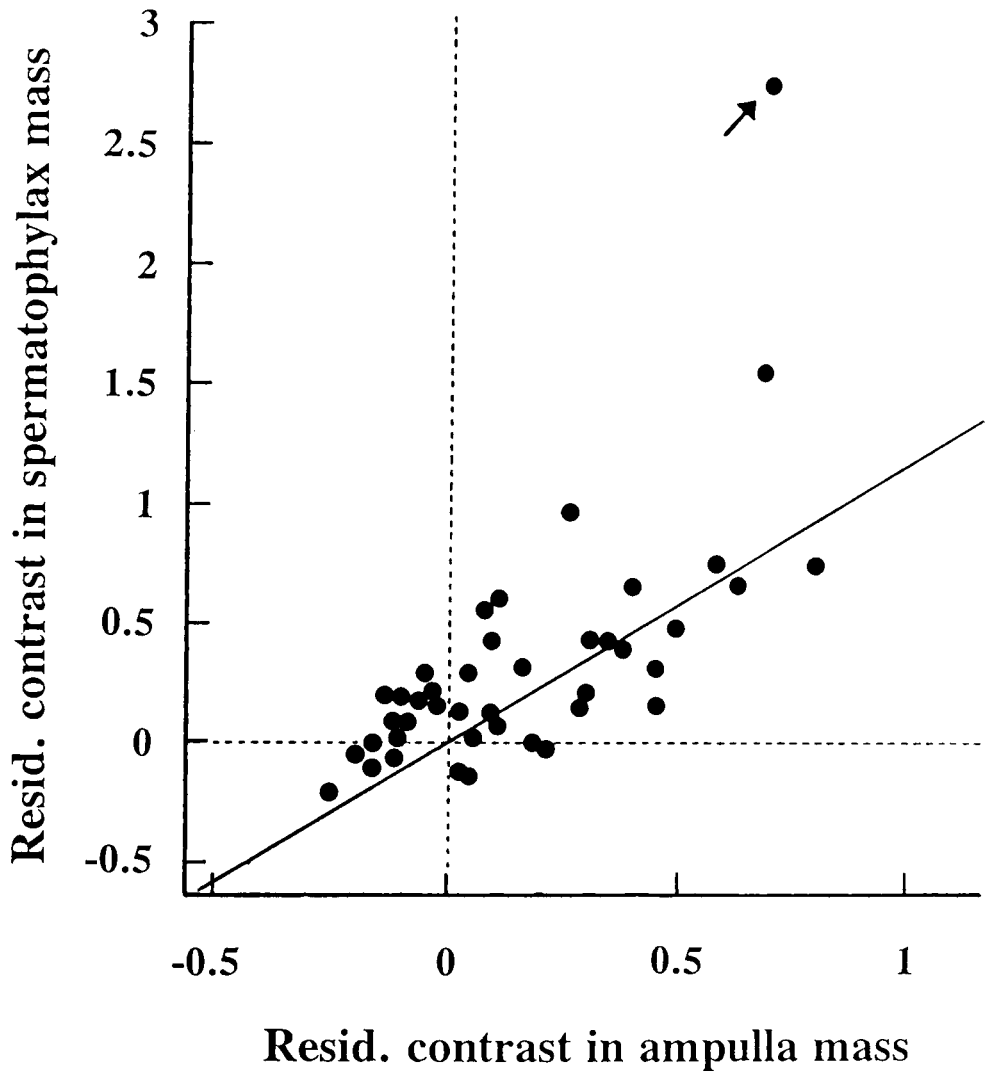

1.6
1.2
0.8
0.4
0
Contrast in spermatophylax mass
-1
-0.5
0
0.5
1
Contrast in body mass

Fig 4.13 Residual contrasts in spermatophylax mass (residuals from the linear regression of spermatophylax contrasts against body weight contrasts) against residual contrasts of ampulla mass for the bushcrickets studied (slope = 1.16 $\pm$ 0.14, $t_{39} = 8.2$, $p << 0.001$, $r^2 = 0.63$). The arrow indicates contrast number 37, which was excluded both from the calculation of the regression line used to generate the spermatophylax residuals, and from the present analysis (with this point included in both regression analyses, slope = 1.49 $\pm$ 0.17, $t_{40} = 8.72$, $p << 0.001$, $r^2 = 0.65$).

4.13


Resid. contrast in spermatophylax mass
3
2.5
2
1.5
1
0.5
0
-0.5
-0.5
0
0.5
1
Resid. contrast in ampulla mass

Again, the slope of this relationship is not significantly different from 1 (t_{31} = 1.36, N.S.). As predicted by the ejaculate-protection hypothesis, a positive relationship was found between residual contrasts of spermatophylax mass and residual contrasts of sperm number (fig. 4.10; slope = 0.64 ± 0.14, t_{31} = 4.43, $p < 0.001$, r^2 = 0.38). The slope of this regression is significantly less than 1 (t_{31} = 2.57, $p < 0.05$). Thus, allowing for male body weight, a ten times evolutionary increase in sperm number would appear to be associated with a 4.4 (anti-log of 0.64) times increase in spermatophylax mass.

For the set of contrasts using species for which ampulla mass data were available, contrasts in ampulla mass were positively related to contrasts in body mass (fig. 4.11; slope = 1.12 ± 0.13, t_{40} = 8.7, $p << 0.001$, r^2 = 0.65), with a slope which is not significantly different from 1 (t_{40} = 0.92, N.S.).

The relationship between contrasts in spermatophylax mass and contrasts in body mass for this data set is presented in fig. 4.12. The arrow in fig 4.12 indicates an apparent outlier (contrast number 37 in fig. 4.2a). This point represents the contrast between mean data values for the subfamily Phaneropterinae and the subfamily Mecopodinae. Data for only a single species of mecopodine were available (*Mecopoda elongata*). This species is comparatively very large and is unusual in that the males do not produce a spermatophylax. By contrast, the phaneropterines for which data were available are relatively small in terms of body size, but produce relatively large spermatophylaxes. Thus the contrast in spermatophylax mass between the two sub-families was strongly positive, while the contrast in body mass was strongly negative. This situation is very different from the apparent trend. Because the anomalous position of this data point appears to have arisen due to the small sample size of mecopodines used, I have performed the analysis both with and without this outlier. For this data set, contrasts in spermatophylax mass were

positively related to contrasts in body mass (fig. 4.12; with outlier excluded: slope = 1.17 ± 0.20, t_{39} = 5.9, p<< 0.001, r^2 = 0.47; with outlier included: slope = 0.72 ± 0.24, t_{40} = 2.98, p<0.01, r^2 = 0.18). Whether or not the outlier is included, the slope of this regression is not significantly different from 1 (outlier excluded: t_{39} = 0.85, N.S.; outlier included: t_{40} = 1.17, N.S.).

As predicted by the ejaculate-protection hypothesis, a positive relationship was found between residual contrasts of spermatophylax mass and residual contrasts of ampulla mass (fig. 4.13; outlier excluded: slope = 1.16 ± 0.14, t_{39} = 8.2, p<< 0.001, r^2 = 0.63; outlier included: slope = 1.49 ± 0.17, t_{40} = 8.72, p<< 0.001, r^2 = 0.65). With the apparent outlier excluded both from the calculation of the regression line used to generate the residuals (see fig. 4.12) and from the regression of residual contrasts of spermatophylax mass against residual contrasts of ampulla mass, the slope of this relationship was not significantly different from 1 (t_{39} = 1.14, N.S.). However, with the apparent outlier included in both analyses, the slope of the relationship between residual contrasts of spermatophylax mass and residual contrasts of ampulla mass is significantly greater than 1 (t_{40} = 2.89, p<0.01).

4.4 *Discussion.*

The results of this comparative study support the ejaculate-protection hypothesis for the evolution of spermatophylax size. As predicted by this hypothesis, a positive relationship was found between changes in spermatophylax size and changes in ampulla size and between changes in spermatophylax size and changes in sperm number, with male body weight controlled for.

In an independent comparative study which used mainly Australian species of

bushcricket (as opposed to the mainly European set of species used here), Wedell (in press) also provides evidence to support the ejaculate-protection hypothesis for the evolution of spermatophylax size. Wedell (in press) analysed generic values to try to avoid the pitfalls of comparing related taxa. She found a significant positive relationship between spermatophylax size and ampulla size across genera, after removing the effect of male body size.

The paternal investment hypothesis does not predict a relationship between spermatophylax size and ampulla size or sperm number, because selection is envisaged as acting on spermatophylax size alone, elaborating it beyond the size necessary for complete sperm transfer (see Gwynne 1986b, 1988b, 1990a). However, it might be argued, *a posteriori*, that the relationship between spermatophylax and ampulla size across taxa could be due to selection for paternal investment acting to enlarge both the ampulla and the spermatophylax as proteinaceous nutrient investments. This would not, however, account for the relationship between changes in spermatophylax size and changes in sperm number demonstrated in this study. It is interesting that all evolutionary changes in this study appear to be in direct proportion (ie. slope = 1) except for the relationship between changes in relative sperm number and changes in relative spermatophylax mass (dependent variable), the slope of which is significantly less than 1. This might be because changes in spermatophylax eating time (= time available for sperm transfer) may not be directly proportional to changes in spermatophylax mass.

It is of course possible that genes for spermatophylax size, ampulla size and sperm number are linked. If this were the case then an increase in spermatophylax size through selection for paternal investment would automatically lead to an increase in ampulla size and sperm number. However, this would only occur if the production of a larger amount of sperm, for example, had a negligible cost. Otherwise,

individuals carrying genes for spermatophylax size and sperm number which were not linked would be at a selective advantage and the linkage might be expected to break down over evolutionary time. The production of large amounts of sperm does appear to have a significant cost (see Dewsbury 1982; chapter 6). Although selection should favour rapid replenishment of sperm, empirical evidence suggests that males may be limited in their capacity to produce sperm (Dewsbury 1982). For example, in the bushcricket *Leptophyes laticauda*, the number of sperm produced is markedly lower both in recently mated males and in recently adult males. The number of sperm produced steadily increases, over a period of at least 30 days, both with time elapsed since the last mating and with male age at mating in this species (chapter 6), suggesting that sperm production is costly.

It should be noted that although comparative evidence to support the ejaculate-protection hypothesis for the evolutionary enlargement of the spermatophylax appears to be strong, this does not rule out the possibility that selection for paternal investment might also have been important in this process. However, Wedell (1993b) provides comparative evidence which fails to support the paternal investment hypothesis. The paternal investment hypothesis predicts that selection should act to increase the nutritional value of the spermatophylax (Wedell 1993b). If larger spermatophylaxes are the product of selection for paternal investment, they might therefore be expected to have a higher nutritional value than smaller spermatophylaxes, ie. have a higher concentration of protein (the spermatophylax consists largely of protein and water, Bowen et al 1984; Reinhold & Heller 1993; Wedell 1993b). Wedell (1993b) found, however, that the converse is true: although larger spermatophylaxes tend to contain a higher total amount of protein, there is a significant negative correlation between the size of the spermatophylax and the percentage protein wet weight, across species. This would seem to suggest a history of selection to increase spermatophylax bulk cheaply (ie. by increasing the

concentration of water) rather than selection to increase the nutritional quality of the spermatophylax.

The benefit to males of producing a larger ejaculate\ more sperm (and hence a larger spermatophylax) may arise from the selective pressures associated with sperm competition (see chapter 1, section 1.1.2; chapter 2, section 2.1.1). In species from a number of different taxa, including a tettigoniid (see Wedell 1991), it has been found that when two males mate with the same female, there is a positive relationship between the probable amount of sperm transferred by a given male, relative to the competing male, and the proportion of eggs fertilised by that male (Martin et al 1974; Dewsbury 1984; Sakaluk 1986b; Dickinson 1986, 1988; Simmons 1987; Muller & Eggert 1989; Ginsberg & Huck 1989; Parker et al 1990; Wedell 1991; Thornhill & Sauer 1991; Simmons & Parker 1992). These studies include species which show sperm mixing (eg. Simmons 1987; Wedell 1991) and those in which some degree of last-male sperm precedence occurs (eg. Dickinson 1988; Simmons & Parker 1992).

The transfer of a greater amount of sperm\ ejaculate, in insects at least, may also benefit a male by inducing a longer non-receptive refractory period in the female (for tettigoniids see Gwynne 1986b, Wedell & Arak 1989; Simmons & Gwynne 1991; for Lepidoptera see Labine 1964; Obara et al 1975; Sugawara 1979; Rutowski 1980; Rutowski et al 1981; Rutowski 1984; Oberhauser 1989, 1992; He & Tsubaki 1991; for Mecoptera see Thornhill 1976b), though there may be sexual conflict over the duration of this period (Simmons & Gwynne 1991). Wedell (in press) provides evidence to support the hypothesis that selection on males to induce longer refractory periods in their mates may have been important in the evolution of larger volumes of ejaculate and larger spermatophylaxes in tettigoniids. In a comparative study, she found a positive relationship between the duration of the female refractory period

and ampulla mass (an estimate of ejaculate volume) and spermatophylax mass, across genera. Another benefit to a male of transferring a greater quantity of sperm\ ejaculate is that this may result in a hastening of the onset and an increase in the rate of oviposition following mating (for tettigoniids, see Wedell & Arak 1989; for insects in general, see reviews of Leopold 1976; Chen 1984), thereby increasing the probability that a female will lay eggs before mating with another male.

Given these benefits of producing a larger ejaculate\more sperm, male bushcrickets harbouring genes to produce both a larger ejaculate\more sperm and a larger spermatophylax to ensure its transfer (larger spermatophylaxes take longer for a female to eat and therefore result in an increase in the time available for ejaculate transfer, Sakaluk 1985; Wedell & Arak 1989; chapter 6) might generally be expected to be at a selective advantage. In the face of sperm competition, selection on sperm number and hence spermatophylax size might proceed as an intraspecific arms race, with males continually being selected to produce larger sperm loads and larger spermatophylaxes than rival males. However, one cost of producing a larger ejaculate and larger spermatophylax would be an increase in the recovery period required between matings (see Dewsbury 1982, Simmons 1990a, Heller & Helversen 1991; Hayashi 1993). Genes for the production of a larger sperm load\ volume of ejaculate and a larger spermatophylax would only be expected to spread, therefore, if the benefit to a male of fertilising a greater proportion of a given female's eggs in the event of sperm competition outweighed the cost of a reduction in the number of females a male could inseminate in his lifetime.

Circumstances which might favour the evolution of a larger ejaculate\ more sperm and hence a larger spermatophylax may include an increase in the intensity of sperm competition. Sperm competition theory predicts that when the probability of sperm competition is high, males will be selected to transfer relatively more sperm per

mating (Short 1979; Parker 1982, 1984, 1990a, 1990b; Moller 1988a, 1988b, 1991a, 1991b). In accordance with this prediction, comparative studies of butterflies (Svard & Wicklund 1989), frogs (Kusano et al 1991), birds (Moller 1991a) and mammals (Kenagy & Trombulak 1986) including cervids (Clutton-Brock et al 1982), equiids (Ginsberg & Rubenstein 1990) and primates (Short 1979; Harcourt et al 1981; Harvey & Harcourt 1984; Moller 1988a) have demonstrated that in species in which there is a greater degree of polyandry, hence a greater risk of sperm competition, males produce relatively larger ejaculates\more sperm or at least have relatively larger testes (see reviews of Moller 1991b; Harvey & May 1989; Gomendio & Roldan 1993). Species with relatively larger testes have, in turn, been found to produce relatively more sperm per ejaculate in birds (Moller 1988b) and mammals (Moller 1989) including primates (Moller 1988a). Even intraspecific studies have demonstrated that males appear to increase the number of sperm ejaculated as the immediate risk of sperm competition increases (in a fruitfly, Gage 1991; in a tenebrionid beetle, Gage & Baker 1991; in a gryllid cricket, Gage 1993; in humans, Baker & Bellis 1989; in rodents, Bellis et al 1990; see also He & Tsubaki 1992, who found that in a noctuid moth, males reared at high population densities, where the subsequent risk of sperm competition is likely to be higher, produce larger spermatophores than males reared in solitary conditions).

In Svard & Wicklund's study of interspecific variation in ejaculate volume (spermatophore mass) in butterflies, an estimate of the degree of polyandry in each species was obtained by calculating the mean number of spermatophores found in the bursa copulatrix of wild-caught females. Although female bushcrickets do not retain empty spermatophores within their genital tracts, a method does exist whereby the number of times a female has mated can be determined, at least for members of the sub-family Tettigoniinae. In this sub-family, a separate ejaculate pouch, or spermatodose, is apparently formed within the spermatheca after each mating

(Boldyrev 1915; Gwynne 1984c). The mean number of spermatodoses in the spermatheca of wild-caught females near the end of the season could be compared for a number of species of tettigoniine differing in relative spermatophylax size\sperm number. It would thus be possible to test the hypothesis that an increase in the degree of polyandry would favour the evolution of larger sperm loads\ volumes of ejaculate accompanied by larger spermatophylaxes. However, the situation is complicated by the fact that a high degree of polyandry is likely to favour males that are able to induce longer refractory periods in their mates. Consequently, a paradoxical situation might arise whereby females of species in which the degree of polyandry has been greater during recent evolutionary history may exhibit longer refractory periods and hence have a lower observed mating frequency.

4.5 *Summary.*

There are two main hypotheses concerning the selective pressures important in the evolutionary enlargement of the spermatophylax in bushcrickets. The paternal investment hypothesis proposes that while the spermatophylax originated as an adaptation to ensure complete sperm\ ejaculate transfer, elaboration of spermatophylax size proceeded through selection for paternal investment. The ejaculate-protection hypothesis, on the other hand, proposes that the evolutionary enlargement of the spermatophylax proceeded through selection to ensure the transfer of larger ejaculates\ more sperm. The ejaculate-protection hypothesis predicts that evolutionary changes in spermatophylax size should be positively correlated with evolutionary changes in ampulla size (ie.ejaculate volume) and sperm number. The paternal investment hypothesis, however, does not predict a relationship between these variables because selection is envisaged as acting on the spermatophylax alone, enlarging it beyond the size necessary for complete sperm\

ejaculate transfer. Here I present the results of a comparative study designed to test the ejaculate-protection hypothesis. Measurements of spermatophylax mass, ampulla mass, sperm number and male body mass were taken for a variety of species of European bushcricket and the data was analysed using the independent comparisons method. This method corrects for similarity between taxa resulting from common ancestry and involves converting the data set into a series of statistically independent contrasts. As predicted by the ejaculate-protection hypothesis, a positive relationship was found, across taxa, between changes in spermatophylax size and changes in ampulla size and sperm number, with male body mass controlled for. The data, therefore, support the hypothesis that the evolutionary enlargement of the spermatophylax has proceeded through selection to ensure the transfer of larger ejaculates\ more sperm.

5. Has the Large Spermatophylax in Bushcrickets Undergone a Change in Function?

5.1 *Introduction.*

Gwynne (1986b, 1988b, 1990a) suggested that the large spermatophylax in tettigoniids may have undergone a change in function. He proposed that while the spermatophylax appears to have originated in the context of intrasexual selection as a protective device to avoid premature removal of the ampulla, elaboration of spermatophylax size may have proceeded through natural selection for male parental investment (see chapter 1).

In addition to evidence that spermatophylax-feeding can result in an increase in egg weight and number (Gwynne 1984a, 1988a; Simmons 1990a; but see also Gwynne et al 1984; Wedell & Arak 1989; Reinhold & Heller 1993, in which no effect has been found), studies of sperm transfer in the bushcricket *Requena verticalis* appear to lend some support to this hypothesis: Gwynne et al (1984) demonstrated that in this species, 75% of sperm appears to leave the ampulla in half the mean time taken by females to finish eating the spermatophylax. Similarly, Gwynne (1986b) found that both a "full complement" of sperm, and substances which induce a typical refractory period in the female, appear to be transferred to the spermatheca in half the mean spermatophylax-eating time. Thus it is widely quoted that the spermatophylax of *R.verticalis* (which contributes to a mean loss of 12% or 19% of male body weight at mating, Bowen et al 1984; Gwynne 1990b) is twice as large as necessary to ensure complete sperm transfer, and that its relatively large size is therefore unlikely

to be maintained by selection on males to ensure complete sperm transfer.

Studies of other species, however, have revealed a closer correspondence between mean spermatophylax-eating duration and the time taken for complete sperm transfer. This has been found both in species which produce small spermatophylaxes (Sakaluk 1984; Wedell & Arak 1989) and those which produce large spermatophylaxes (Simmons & Gwynne 1991; Reinhold & Heller 1993).

The alternative hypothesis, therefore, is that the large spermatophylax fulfils the same primary function as the small spermatophylax (ie. to ensure complete sperm\ejaculate transfer) and has evolved to its large size for the protection of a larger volume of ejaculate (see chapter 4).

In order to test these hypotheses, I examined the amount of sperm remaining in the ampulla as a function of time in relation to the mean time taken by females to consume the spermatophylax and remove the ampulla in *Leptophyes punctatissima* Bosc. and *L.laticauda* Friv. (Tettigoniidae; Phaneropterinae). These two congeneric species appear to be closely related (they are placed in the same species-group by Bei-bienko 1965), yet they differ greatly in spermatophylax size: *L.punctatissima* produces a small spermatophylax contributing to a mean loss of 5.6% (2.3 - 8.5% n = 45) of male body weight, while *L.laticauda* produces a large spermatophylax, contributing to a mean loss of 22.9% (11.3 - 32.7% n = 60) of male body weight (see chapter 7).

If the small spermatophylax of *L.punctatissima* and the large spermatophylax of *L.laticauda* are both maintained by selection to prevent removal of the ampulla before complete sperm transfer, there is no reason to expect that the shape of the sperm-transfer curve relative to mean spermatophylax-eating time will differ

between the two species. If, however, the large spermatophylax of *L.laticauda* has been enlarged beyond the sperm-protection function, sperm depletion should occur proportionately sooner in this species - ie. the rate of sperm transfer relative to mean spermatophylax-eating time should be greater in *L.laticauda* than in *L.punctatissima*.

5.2 *Methods.*

5.2.1 *The species.*

L.punctatissima (the speckled bushcricket) occurs in most of Europe from Spain to southern Scandinavia, including southern England, and eastwards to Yugoslavia and the western U.S.S.R. (Marshall & Haes 1988; Bei-Bienko 1965). *L.laticauda* has a more restricted distribution centred around the southern Alps, and occurs from southeastern France to Yugoslavia (Bei-bienko 1965).

L.punctatissima used in this experiment were derived from mixed European stocks kept in continuous laboratory culture since the mid 1970s, occasionally being supplemented with new individuals. The *L.laticauda* were obtained from a laboratory colony derived from adults collected in the Italian Alps in 1983 and Southeastern France in 1991. Both species were reared under conditions outlined by Hartley & Dean (1974) (see also appendix 1). Early instars were given buttercup (*Ranunculus* spp), dock (*Rumex* spp) and *Buddleia*. Later instars were given *Buddleia* alone, though they would also accept birch (*Betula* spp) and bramble (*Rubus fruticosus*). Sexes were separated before the final moult. On becoming adult, individuals were uniquely marked on the pronotum using "Humbrol" enamel paint. Adult male *L.laticauda* were kept in individual cages (plastic sweet jars with a large hole covered by nylon mesh in the lid for ventilation). This was necessary to prevent

males from mounting one another and biting holes in one another's dorsal tergites - a common occurrence in crowded cages, which appears to have the adverse effect of conditioning males against the usual response to female mounting.

In these experiments only virgin females (n= 62 for *L.punctatissima*, n= 48 for *L.laticauda*) were used. All males used (n= 62 for *L.punctatissima*, n= 48 for *L.laticauda*) had been adult for at least 12 days and had not previously mated for at least 8 days. This was done to ensure that males of both species were likely to have built up sperm-reserves to a reasonable level before being allowed to mate (see chapter six). Males of both species appeared to show an increase in calling activity at two different times of the day: early to mid-morning and mid-afternoon to early evening. Matings were set up during either period. Individual pairs (n= 62 for *L.punctatissima*, n= 48 for *L.laticauda*) were placed in small black-nylon mesh cages (approx. 10 x 10 x 10cm) and were observed until mating had taken place.

5.2.2 *The duration of spermatophylax consumption.*

After mating, females were observed intermittently until they were close to finishing the spermatophylax, at which point they were observed continuously until they had finished the spermatophylax and removed the ampulla. The time from the end of copulation until the female had fully consumed the spermatophylax (ie. finished chewing the last mouthful) was recorded, along with time taken for the female to remove the ampulla after having finished the spermatophylax. Additional data on ampulla attachment duration resulting from spermatophylax consumption for *L.punctatissima* were obtained from experiments presented in chapter six. In total, data on the duration of spermatophylax consumption were obtained for 37 separate females\spermatophores in the case of *L.punctatissima* and ten separate

females\spermatophores in the case of *L.laticauda*.

5.2.3 *Rate of sperm transfer.*

To determine the rate at which sperm leaves the ampulla, the ampulla was removed with watchmaker's forceps at intervals after the end of copulation. The ampulla was then placed in a plastic vial in a known volume (0.2 or 0.4 mls for *L.laticauda* and 0.05 mls for *L.punctatissima*) of physiological locust saline. The ampulla was crushed and its contents were suspended by thorough mixing with watchmaker's forceps for 5 mins. This was found to result in an even suspension of sperm. A portion of each sample was then transferred to a haemocytometer (Neubauer, improved). The number of sperm in the centre grid were counted under a microscope. Two sub-samples were counted from each sample and a mean value was taken. This value was multiplied by the appropriate dilution factor to give the total sperm number in the original sample.

5.2.4. *Analysis.*

In order for the sperm-depletion curves of the two species to be directly comparable, the number of sperm in the ampulla was converted to a percentage of the mean sperm number of full spermatophores for each species. This was plotted against ampulla attachment duration as a percentage of the mean duration of ampulla attachment resulting from the consumption of the spermatophylax for each species. Non-linear regression analyses were performed on the data to generate the sperm-transfer curve for each species (non-linear regression uses a Marquardt search algorithm to determine estimates that minimise the residual sum of squares). These analyses were performed using STATGRAPHICS v.2.6 (Manugistics Inc., U.S.A.),

and the equation fitted in each case was:

$$\% \text{ sperm} = \exp(a + b.\ \% \text{ time})$$

Where a and b are the fitted constants.

The sperm-transfer curves of the two species were compared using a maximum likelihood method. This is a test of whether 2 regression lines (one for each species) account for more of the combined data set than one line - ie. whether the lines are significantly different for each species. The residual sum of squares (SS) from the non-linear regressions on the data for each species were recorded (the combined $SS = SS_2$). One line was then fitted to the combined data set and the residual sum of squares accounted for by the model recorded ($= SS_1$). The log-likelihood ratio is then:

$$S = -2 \log (SS_2/SS_1)$$

S is distributed as Chi^2 with 1 degree of freedom.

The sperm transfer curve relative to mean spermatophylax eating time for *L.punctatissima* (small spermatophylax) was also compared with that of *R.verticalis* using the method outlined above. Re-constructed data from Gwynne et al (1984) for *R.verticalis* were used and converted as above. The mean number of sperm in full spermatophores for *R.verticalis* was taken, from the graph in Gwynne et al (1984), as the mean number of sperm contained in ampullae attached for less than five minutes ($= 1520 \times 10^3$ sperm, $n = 6$). The mean duration of ampulla attachment resulting from the consumption of the spermatophylax in *R.verticalis* ($= 316$ min, $n = 13$) was also taken from Gwynne et al (1984).

Mean values are cited ± standard error.

5.3 *Results.*

The mean time taken for females to consume fully the spermatophylax was found to be 42.6 ± 1.81 min (range: 22 - 74 min, n=37) for *L.punctatissima* and 338.3 ± 20.28 min. (range: 225 - 462 min., n=10) for *L.laticauda*. Females in all cases started to eat the ampulla directly after having finished the spermatophylax.

The mean number of sperm in full spermatophores was found to be 115.4 x 10^3 ± 12.6 x 10^3 sperm (range: 47.25 x 10^3 - 248 x10^3 sperm, n=16) in *L.punctatissima* and 1687.6 X 10^3 ± 128.8 x 10^3 sperm (range: 894 x 10^3 - 3104 x 10^3 sperm, n=17) in *L.laticauda*.

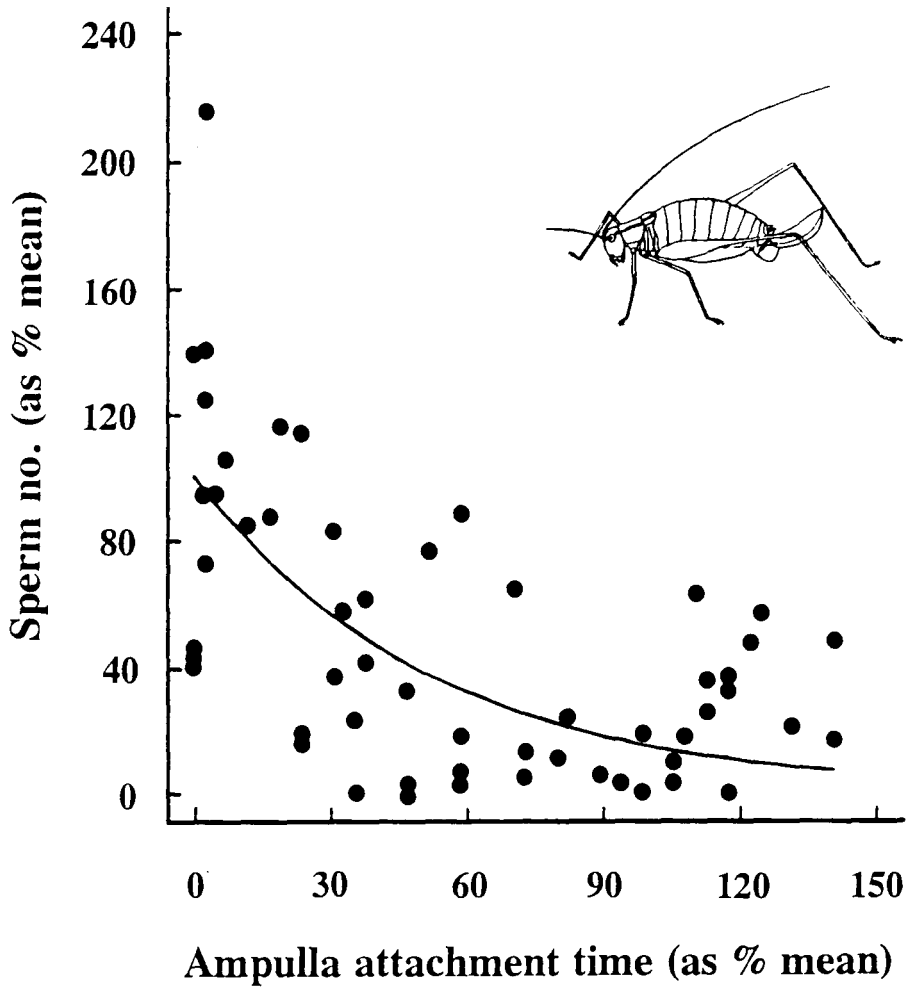
The lines fitted by the non-linear regression of sperm remaining in the ampulla (as % of mean sperm number in full spermatophores) against ampulla attachment time (as % of mean spermatophylax eating time) for *L.punctatissima* and *L.laticauda* are shown in figs 5.1 and 5.2. The line fitted by non-linear regression on the combined data sets for *L.punctatissima* and *L.laticauda* is shown in fig. 5.3. No significant difference in the shape of the sperm-transfer curves relative to mean spermatophylax eating time was found between *L.punctatissima* (small spermatophylax) and *L.laticauda* (large spermatophylax) (S = 0.054, N.S.).

The line fitted by the non-linear regression of sperm remaining in the ampulla (as % mean sperm number of full spermatophores) against time (as % mean

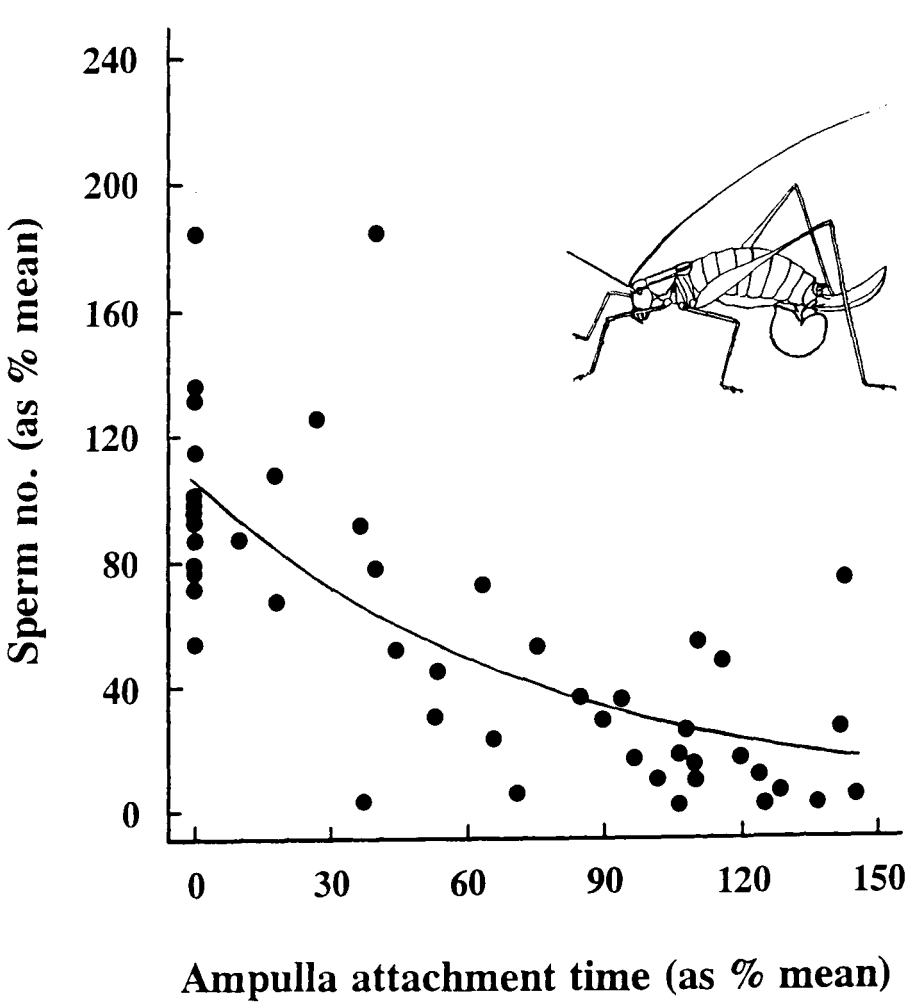
Fig 5.1 Line fitted by the non-linear regression of sperm remaining in the ampulla (expressed as a percentage of the mean number of sperm in full ampullae) as a function of time (expressed as a percentage of the mean duration of ampulla attachment resulting from the consumption of the spermatophylax = mean spermatophylax-eating time) in *Leptophyes punctatissima*. Each point represents the number of sperm remaining in a separate spermatophore, transferred to a separate female.

Fig 5.2 As above, for *L. laticauda*.

5.1


240
200
160
120
80
40
0
Sperm no. (as % mean)
0
30
60
90
120
150
Ampulla attachment time (as % mean)

5.2


240
200
160
120
80
40
0
Sperm no. (as % mean)
0
30
60
90
120
150
Ampulla attachment time (as % mean)

5.3 Line fitted by the non-linear regression of sperm remaining in the ampulla (as % mean sperm number) as a function of time (as % mean spermatophylax-eating time) for the combined data sets of *L. laticauda* (dots) and *L. punctatissima* (stars).

5.3

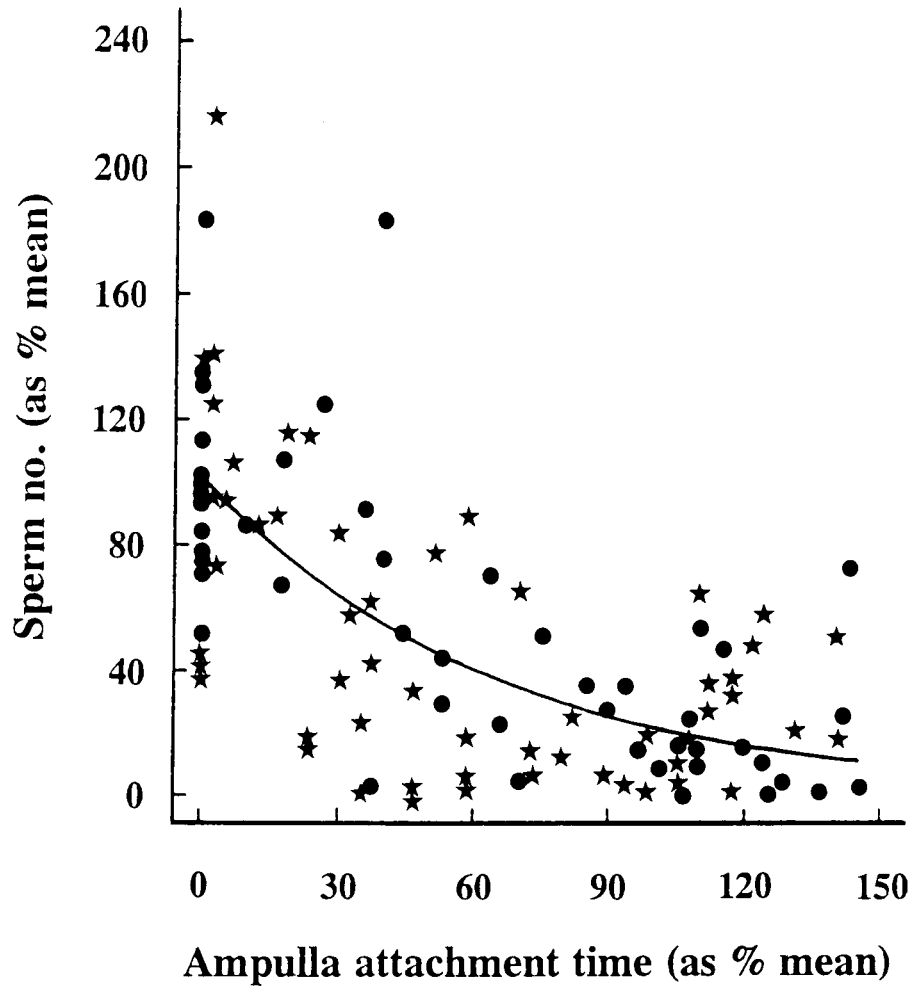
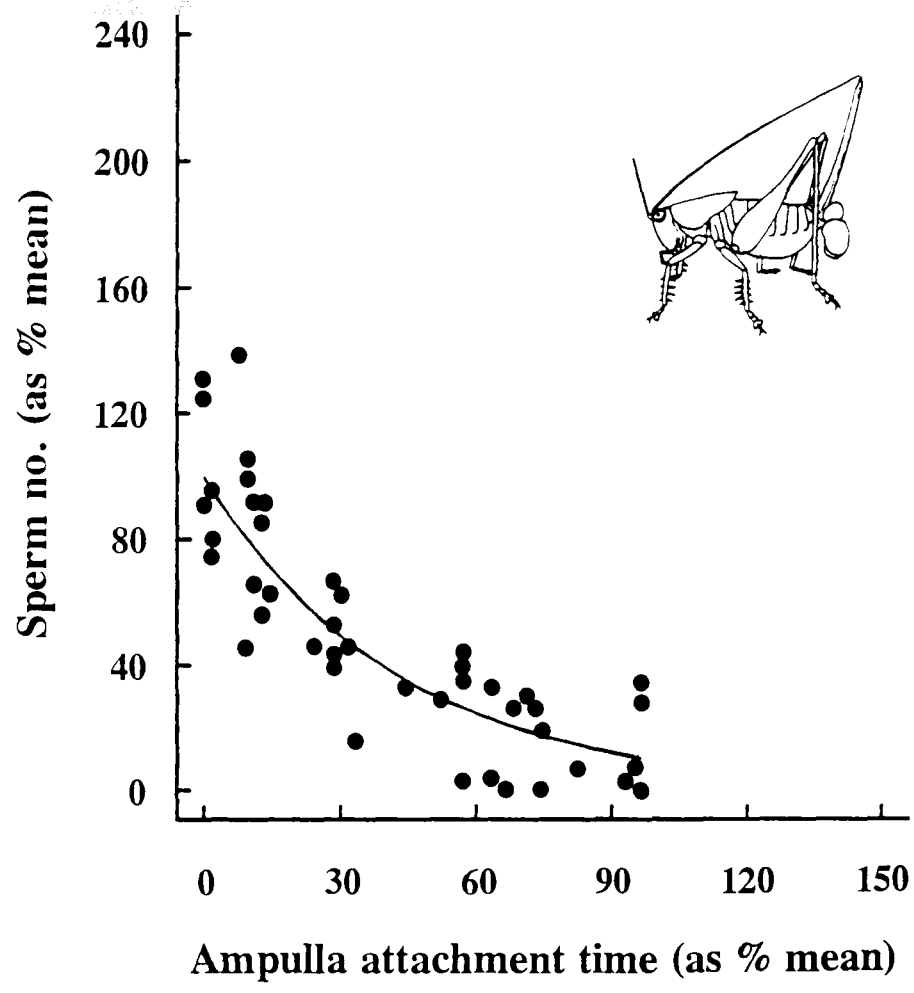

240
200
160
120
80
40
0
Sperm no. (as % mean)
0
30
60
90
120
150
Ampulla attachment time (as % mean)

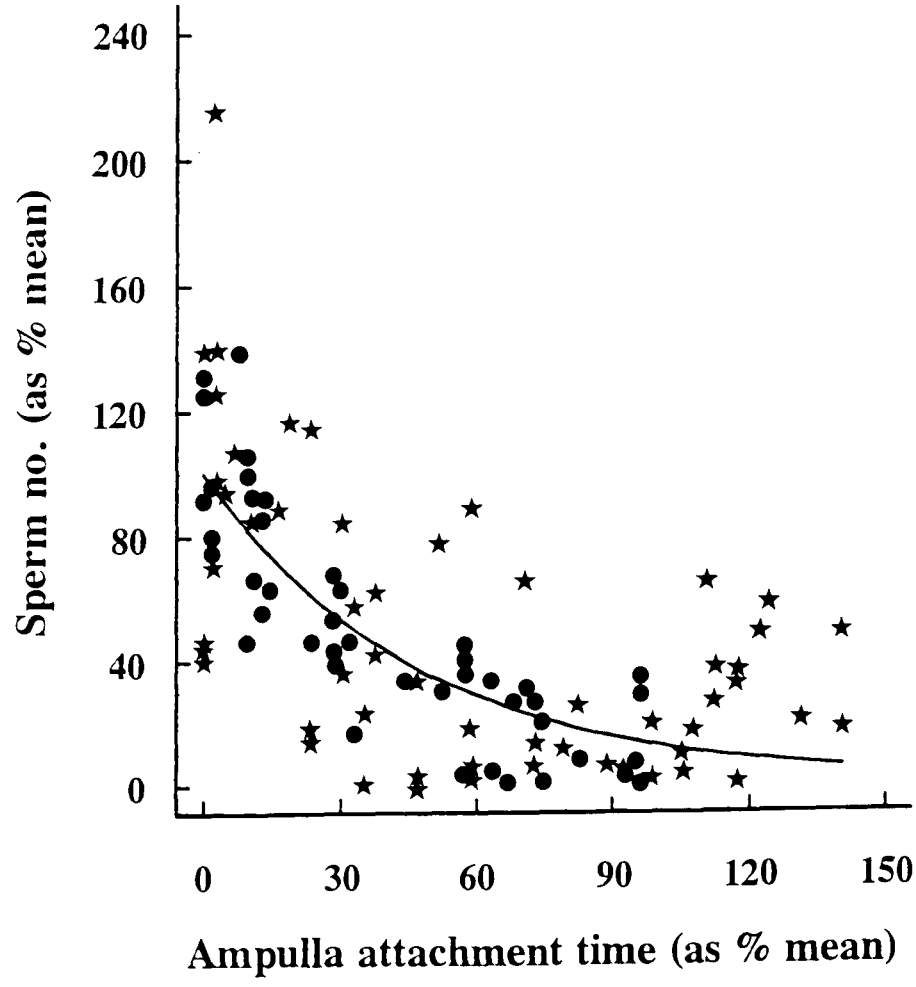
Fig 5.4 Line fitted by the non-linear regression of sperm remaining in the ampulla (as % mean sperm number) as a function of time (as % mean spermatophylax-eating time) in *Requena verticalis* (data re-constructed from Gwynne et al 1984; the drawing of *R. verticalis* and its spermatophore was based on a photograph in Gwynne 1983b).

Fig 5.5 Line fitted by the non-linear regression of sperm remaining in the ampulla (as % mean sperm number) as a function of time (as % mean spermatophylax-eating time) for the combined data sets of *R. verticalis* (dots) and *L. punctatissima* (stars).

5.4


240
200
160
120
80
40
0
Sperm no. (as % mean)
0
30
60
90
120
150
Ampulla attachment time (as % mean)

5.5


240
200
160
120
80
40
0
Sperm no. (as % mean)
0
30
60
90
120
150
Ampulla attachment time (as % mean)

spermatophylax-eating time) for *R.verticalis* is given in fig. 5.4. The line fitted by the non-linear regression on the combined data sets for *L.punctatissima* and *R.verticalis* is given in fig. 5.5. There was no significant difference in the shape of the sperm-transfer curves in relation to mean spermatophylax-eating time between *L.punctatissima* (small spermatophylax) and *R.verticalis* (medium-large spermatophylax) (S = 0.02, N.S.).

5.4 *Discussion.*

No significant difference in the shape of the sperm-transfer curve in relation to the mean time taken for females to eat the spermatophylax was found between *L.punctatissima* (small spermatophylax) and *L.laticauda* (large spermatophylax) or between *L.punctatissima* (small spermatophylax) and *R.verticalis* (medium-large spermatophylax). Therefore, the hypothesis that the large spermatophylax in tettigoniids has undergone a change in function, ie. has been enlarged beyond the size necessary to ensure complete sperm transfer, is rejected. Conversely, the results support the hypothesis that the large spermatophylax fulfils the same primary function as the small spermatophylax (ie. to ensure complete sperm transfer).

Within all three species, there is considerable variation in the number of sperm remaining in the ampulla at any given moment following spermatophore transfer: some ampullae seem to have transferred almost all their contents in about half the mean spermatophylax eating time, while other ampullae still contain a fair proportion of sperm beyond the mean spermatophylax eating time. It should be noted that this variation would make it difficult to detect a significant difference in the shape of the sperm-transfer curves between *L.punctatissima* and the other species.

The cause of this variation is unclear: in addition to experimental error, the variation could either be due to variation in the amount of sperm in the ampulla at zero minutes or variation in the rate of sperm transfer. It is difficult to distinguish between spermatophores which initially contained a large amount of sperm and have transferred a large proportion of their contents, and those which initially contained a small amount of sperm and have transferred little of their contents.

Ideally, both the number of sperm remaining in the ampulla at any given moment and the number of sperm transferred to the spermatheca for the same spermatophore should be measured in order to calculate the exact percentage of sperm transferred with time. Reinhold & Heller (1993) have done just this using the bushcricket *Poecilimon veluchianus*. This species produces a very large spermatophylax contributing to a loss of 26% of male body weight at mating (Heller & Helversen 1991). They found that the time necessary for sperm transfer was highly variable: some spermatophores had discharged the majority of their contents in about 1/3 of the mean spermatophylax eating time, while others still contained over 50% of their initial sperm number at about 1.3 x the mean spermatophylax eating time. By about 1.75 x the mean spermatophylax eating time, however, all spermatophores had transferred more than 70% of their sperm. In addition, Reinhold & Heller (1993) compared the proportion of spermatophores that had transferred a substantial proportion of their sperm to the spermatheca with the proportion of spermatophores not completely consumed as a function of spermatophore attachment duration. They estimated that about 19% of spermatophores are consumed before having transferred a substantial part of their sperm in this species. Reinhold & Heller (1993) conclude that the large spermatophylax of *P. veluchianus* seems to fulfil a sperm-protection function.

Gwynne's (1986b) conclusion that the spermatophylax of *R. verticalis* is twice as large as necessary to ensure complete sperm transfer was based on his finding no significant difference between the mean number of sperm transferred to the spermatheca at 1/2 the mean spermatophylax eating time and the mean number transferred at the mean spermatophylax eating time in this species. However, given that the amount of sperm available for transfer and the rate of sperm transfer (see above) may be subject to considerable variation within species, it is perhaps not surprising that no significant difference was found. From the plot of the number of sperm remaining in the ampulla with time for *R. verticalis* (see fig 5.4, data from fig.2 in Gwynne et al 1984), it appears that the time required for complete sperm transfer actually corresponds fairly well with the mean spermatophylax eating time in this species. Furthermore, inspection of the data reveals that 2 out of the 5 spermatophores (ie. 40%) which were attached for the mean spermatophylax eating time still contain approximately 30-35% of the mean sperm number present at 0 min. The data from Gwynne et al (1984) do not therefore support the contention that the spermatophylax of *R. verticalis* is larger than necessary to ensure *complete* sperm transfer.

The benefits to a male bushcricket of transferring all of the sperm or ejaculate contained in the spermatophore probably include an increase in the chance of fertilising a greater proportion of the eggs of a given female in the event of sperm competition (see Wedell 1991; see also chapter 1, section 1.1.2; chapter 2, section 2.1.1), the induction of a longer non-receptive refractory period in the female (see Wedell & Arak 1989; Simmons & Gwynne 1991; see also chapter 1, section 1.1.2.b), a hastening of the onset of oviposition following mating, and an increase in the rate of oviposition (see Wedell & Arak 1989; see also chapter 1, section 1.1.2.c). The latter three factors together increase the chance that the female will lay eggs before mating with another male.

It is worth noting that the difference in the mean sperm number for *L.punctatissima* and *L.laticauda* is consistent with the hypothesis that the evolutionary enlargement of the spermatophylax in tettigoniids has proceeded through selection to ensure the transfer of larger amounts of sperm\ejaculate. Mean spermatophylax weight in proportion to male body weight is approximately 4 x greater in *L.laticauda* than in *L.punctatissima* and, accordingly, mean sperm number in proportion to male body weight is 5.6 x greater in *L.laticauda.* Comparative studies of a number of tettigoniid species have also lent support to this hypothesis (see Wedell, in press; chapter 4). These studies have demonstrated that when male body size is controlled for, there is a positive relationship, across species, between spermatophylax size and ampulla size (ie.ejaculate volume) (Wedell, in press, Wedell 1993b; chapter 4) and between spermatophylax size and sperm number (chapter 4), as predicted.

Though data suggest that the large spermatophylax performs the same primary function as the small spermatophylax, ie. to ensure complete sperm\ejaculate transfer, this does not preclude the possibility of the spermatophylax additionally functioning as paternal investment at no extra cost to the male (Reinhold & Heller 1993). In *R.verticalis* and a species of zaprochiline, the potential for the spermatophylax to function as paternal investment has been demonstrated (Gwynne 1988a, 1988b; Simmons 1990a). However, in at least six other species of bushcricket (*Poecilimon veluchianus*, *P.affinis*, *Metaplastes ornatus*, *Decticus verrucivorus*, *Leptophyes punctatissima* and *Steropleurus stali*), three of which (*P.veluchianus*, *M.ornatus* and *S.stali*) produce very large spermatophylaxes (>20 % of male body weight), a paternal investment function seems improbable as males appear to be unlikely to fertilise a significant proportion of the eggs which stand to benefit from their spermatophylax nutrients (Helversen & Helversen 1991; Heller &

Helversen 1991; Achmann et al 1992; Reinhold & Heller 1993; Wedell 1993a; chapter 7).

5.5 *Summary.*

There are two hypotheses concerning the evolutionary enlargement of the spermatophylax in tettigoniids. The first proposes that the large spermatophylax has undergone a change in function: while the spermatophylax may have originated as an adaptation to ensure complete sperm\ejaculate transfer, elaboration of spermatophylax size may have proceeded through selection for paternal investment. The alternative hypothesis proposes that the large spermatophylax may retain the same primary function as the small spermatophylax and the evolutionary enlargement of the spermatophylax may have proceeded through selection to ensure the transfer of larger volumes of ejaculate\ numbers of sperm. The former hypothesis predicts that in species with proportionately larger spermatophylaxes, the rate of sperm transfer relative to the time taken for females to fully consume the spermatophylax should be greater than in species with proportionately smaller spermatophylaxes. The latter hypothesis, on the other hand, predicts that the shape of the sperm transfer curve in relation to mean spermatophylax consumption time should not differ between species with large or small spermatophylaxes. I tested these hypotheses by examining the mean spermatophylax consumption duration and number of sperm remaining in the ampulla as a function of time in the tettigoniids *Leptophyes punctatissima* and *L.laticauda*. The former species produces a small spermatophylax contributing to a loss of 5.6% of male body weight at mating, while the latter produces a much larger spermatophylax contributing to a loss of 23% of male body weight. I compared the shapes of the curves generated by non-linear regression of sperm remaining in the ampulla (as % mean sperm number) against

time (as % mean spermatophylax consumption duration) for *L.punctatissima* and *L.laticauda* and for *L.punctatissima* and *Requena verticalis* (with re-constructed data from Gwynne et al 1984) using a maximum likelihood method. No significant difference in the shape of the sperm transfer curves relative to mean spermatophylax eating time were found between *L.punctatissima* (small spermatophylax) and *L.laticauda* (large spermatophylax) or between *L.punctatissima* and *R.verticalis* (medium-large spermatophylax). Therefore, the hypothesis that the large spermatophylax has undergone a change in primary function is rejected.

6. Intraspecific Variation in Spermatophylax Size.

6.1 *Introduction.*

In the tettigoniid *Meconema meridionale*, which does not produce a spermatophylax, prolonged copulation following spermatophore transfer appears to function to prevent the female from eating the ampulla before complete ejaculate transfer (see chapter 3). In concordance with this hypothesis, males of this species appear to adjust the duration of copulation in relation to the size of ampulla (and probably, therefore, volume of ejaculate) they have produced (see chapter 3).

The hypothesis that the spermatophylax similarly functions to ensure complete ejaculate transfer (ejaculate-protection hypothesis) predicts that males should adjust spermatophylax size in relation to the amount of sperm or volume of ejaculate they can produce (or adjust the amount of sperm \ volume of ejaculate in relation to the size of spermatophylax they are able to produce); ie. spermatophylax size should be correlated with ampulla size and sperm number. Although both the spermatophylax and ampulla are component parts of the spermatophore, they are secreted by different sets of accessory glands (Ander 1939). Therefore, their sizes need not be physiologically linked.

An increase in spermatophore size with male age at mating has been found in two species of Lepidoptera (Oberhauser 1988, He & Tsubaki 1992) and the cockroach (order Dictyoptera) *Diploptera punctata* (Woodhead 1986). Sperm number was also found to increase with male age at mating in the latter species (Woodhead 1986). A decrease in spermatophore size in recently mated males and\or an increase in

spermatophore size with time since last mating has been found in the Trichoptera (Khalifa 1949b) and at least 18 species of Lepidoptera (Khalifa 1950b; Srivastava & Srivastava 1957; Outram 1971; Sims 1979; Rutowski 1979; Boggs 1981; Rutowski 1984; Svard 1985; Rutowski & Gilchrist 1986; Svard & Wicklund 1986; Oberhauser 1988; Svard & Wicklund 1989; Marshall & McNeil 1989; Lederhouse et al 1990; He & Tsubaki 1992; Royer & McNeil 1993). A similar phenomenon has also been found in the spermatophylax-producing megalopteran *Protohermes* (Hayashi 1993) and the spermatophylax-producing tettigoniids *Orchelimum* (Gwynne 1983; Faever 1983), *Poecilimon* (Heller & Helversen 1991) and *Requena* (Simmons et al 1992, see also Davies & Dadour, 1989, who found an increase in the weight of the accessory glands which secrete the spermatophylax with time since last mating in *Requena*). These studies measured the size of the spermatophylax and ampulla as a unit.

Here I examine the effect of male age at first mating and time since last mating on ampulla size, sperm number and spermatophylax size in *Leptophyes laticauda* - a species of tettigoniid which produces a large spermatophylax (male weight loss at mating = 11 - 33 % of male body weight, see chapter 7). I test the prediction that spermatophylax size should be correlated with ejaculate volume and sperm number. I also present data examining an assumption of the ejaculate-protection hypothesis, namely that the production of a larger spermatophylax should result in a longer duration of ampulla attachment.

6.2 *Methods.*

6.2.1 *Experiment 1: variation in spermatophylax size, ampulla size and sperm number in* L.laticauda.

For details on the origin of *L.laticauda* stocks used in this experiment and rearing methods, see chapter 5. Sexes were separated prior to the final moult. On becoming adult, males were individually marked with "Humbrol" enamel paint and the date of the final moult for each male was recorded. Two data sets were collected: 1) to examine the effect of male age at first mating on spermatophylax size, ampulla size, and sperm number produced at the first mating; 2) to examine the effect of time since last mating on spermatophylax size, ampulla size and sperm number. To produce the first data set, virgin males (n= 18 males) of known age were selected and placed in black nylon mesh observation cages (measuring approx. 10 x 10 x 10cm) with receptive (ie.acoustically responding) females. Only one pair were placed in each cage. To produce the second data set, males which had previously mated at least once at a known date and had produced a large spermatophore at the last mating (ie.20 - 30 % male pre-mating body weight) were used (n= 29 males). Male age was not held constant in this latter case, though all males used had been adult for at least 12 days. Virgin females approximately 10-20 days old were used in this experiment.

Cages were observed until mating had taken place, after which the entire spermatophore was removed using watchmaker's forceps. The spermatophore (spermatophylax + ampulla) was weighed, to the nearest 0.01 mg, on a Cahn 25 electrobalance. The ampulla was then separated from the spermatophylax and

weighed separately. Rather than weighing the spermatophylax separately, the mass of the ampulla was subtracted from the mass of the entire spermatophore to calculate spermatophylax mass. This reduced handling of the spermatophylax which, being mucoid in consistency, is somewhat prone to desiccation. The ampulla was then placed in a known volume of physiological locust saline (generally 0.4 ml). Sperm counts were conducted using the method outlined in chapter 5. Males were weighed after mating on an electrobalance accurate to 1mg.

The effect of male age at mating on the number of sperm produced by male *L.punctatissima* was also examined using the methods outlined above, though the ampulla, being much smaller than that of *L.laticauda*, was placed in a smaller volume of locust saline (0.05 ml). The origin of the *L.punctatissima* stocks used and rearing methods are detailed in chapter 5. Twenty *L.punctatissima* males were used in this experiment.

6.2.2 *Experiment 2: Spermatophore mass and the duration of ampulla attachment.*

The effect of spermatophylax mass on the duration of ampulla attachment was examined in the tettigoniids *L.punctatissima* (mean spermatophore mass as % of male pre-mating body mass = 5.6 % , range 2.3 - 8.5 %, n = 45, see Chapter 7), *Poecilimon schmidti* (spermatophore = 14.3 % male body mass, range 7.2 - 18.95 %, n = 60, see Chapter 2, table 2.1) and *Steropleurus stali* (spermatophore = 27% male body mass, range 16 - 37%, n = 51, see chapter 3). All animals used were derived from laboratory colonies. Rearing methods and origin of laboratory stocks are outlined in appendix 1. On becoming adult, bushcrickets were individually marked on the pronotum with "Humbrol" enamel paint. Stridulating males and

sexually receptive females (ie. acoustically responding) of each species were selected at random. Males were weighed on an electrobalance accurate to 1mg, and single pairs (n= 26 for *L.punctatissima*, n= 47 for *P.schmidti*, n= 20 for *S.stali*) were placed in black nylon mesh observation cages (10 x 10 x 10cm). These were observed until mating had taken place. The time at which copulations ended were noted and the mated males were re-weighed within 30 min. of the end of copulation. The post-mating weight of individual males was subtracted from the pre-mating weight to estimate the weight of the spermatophore. As the spermatophylax forms the greatest percentage of the spermatophore in these species (about 85 %, calculated from data presented in chapter 4), variation in spermatophore mass within species is likely to reflect variation in the mass of the spermatophylax. The time taken for females to consume fully the spermatophylax and begin to eat the ampulla was noted in each case. Means are cited $\pm$ standard error.

6.3 *Results.*

6.3.1 *Experiment 1: variation in spermatophylax size, ampulla size and sperm number in* L.laticauda.

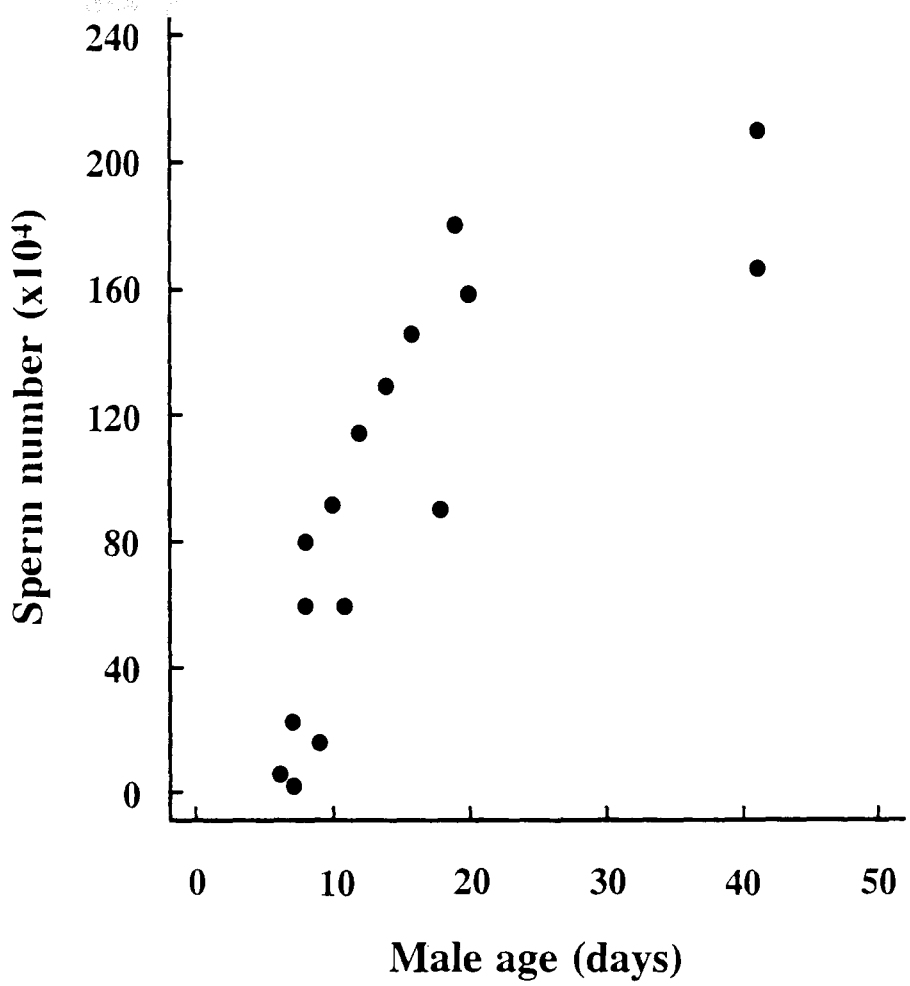
6.3.1.a *Male age at first mating.*

Spearman's rank correlation revealed positive relationships between the age of virgin male *L.laticauda* at first mating and the number of sperm produced at the first mating (r_s=0.91, n=16, p<0.001, fig. 6.1), male age at first mating and ampulla mass (r_s=0.92, n=18, p<0.01, fig. 6.2) and male age at first mating and spermatophylax mass (r_s=0.79, n=18, p<0.01, fig. 6.3). A positive relationship

Fig 6.1 Number of sperm produced as a function of male age at first mating in *L.laticauda* ($r_s = 0.91$, $n = 16$, $p < 0.01$).

Fig 6.2 Ampulla mass as a function of male age at first mating in *L.laticauda* ($r_s = 0.92$, $n = 18$, $p < 0.01$).

6.1


Sperm number (x10⁴)
240
200
160
120
80
40
0
0
10
20
30
40
50
Male age (days)

6.2

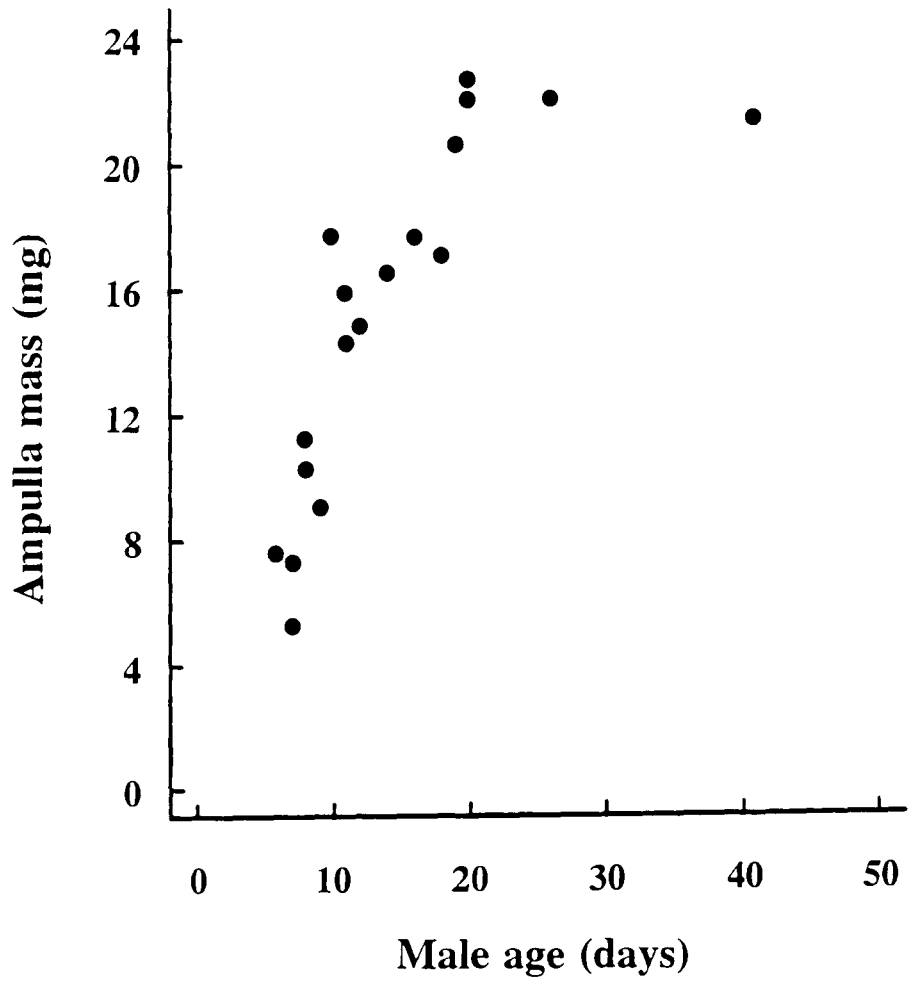
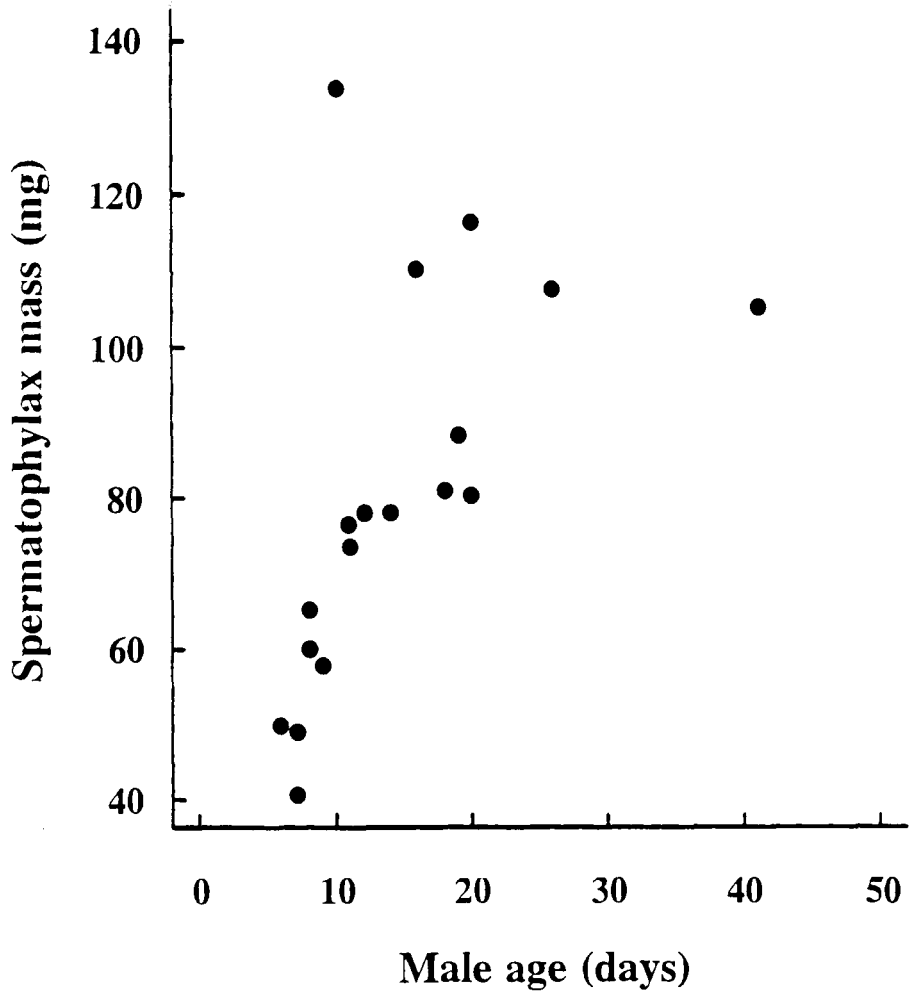

Ampulla mass (mg)
24
20
16
12
8
4
0
0
10
20
30
40
50
Male age (days)

Fig 6.3 Spermatophylax mass as a function of male age at first mating in *L.laticauda* ($r_s = 0.79$, $n=18$, $p<0.01$).

Fig 6.4 Number of sperm produced as a function of male age at first mating in *L.punctatissima* ($r_s = 0.94$, $n= 20$, $p<<0.001$).

6.3


Spermatophylax mass (mg)
140
120
100
80
60
40
0
10
20
30
40
50
Male age (days)

6.4

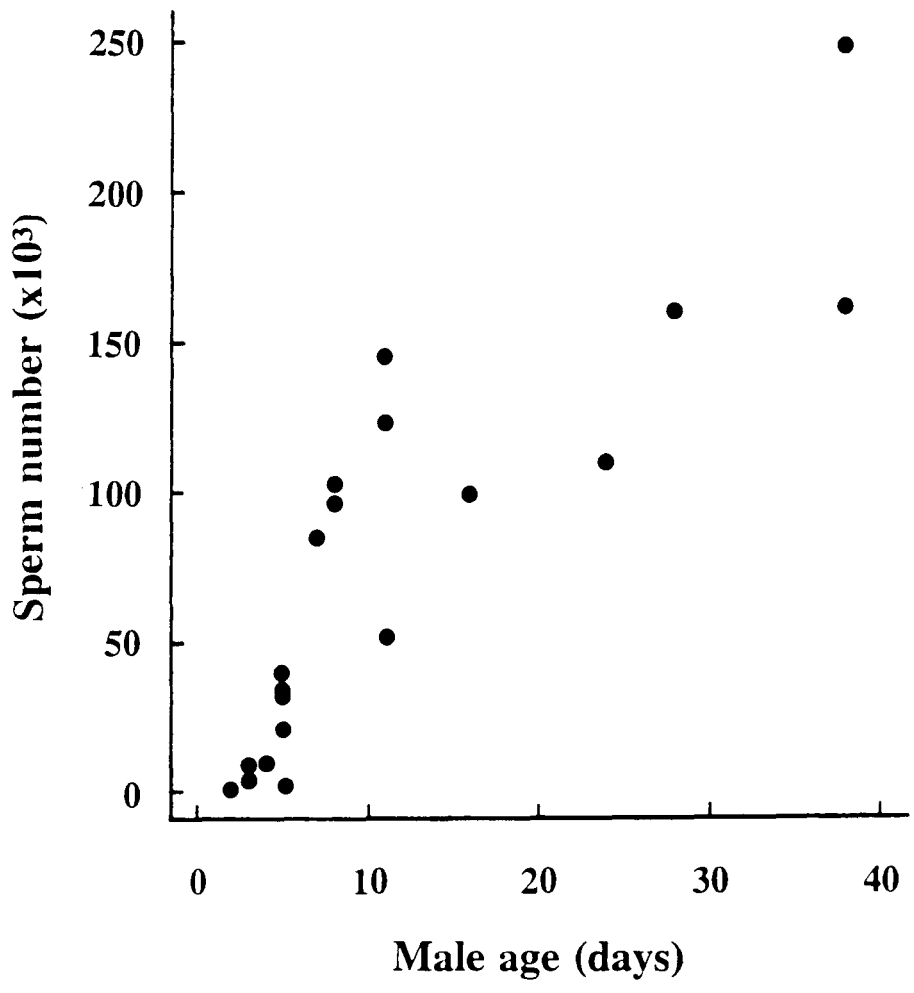
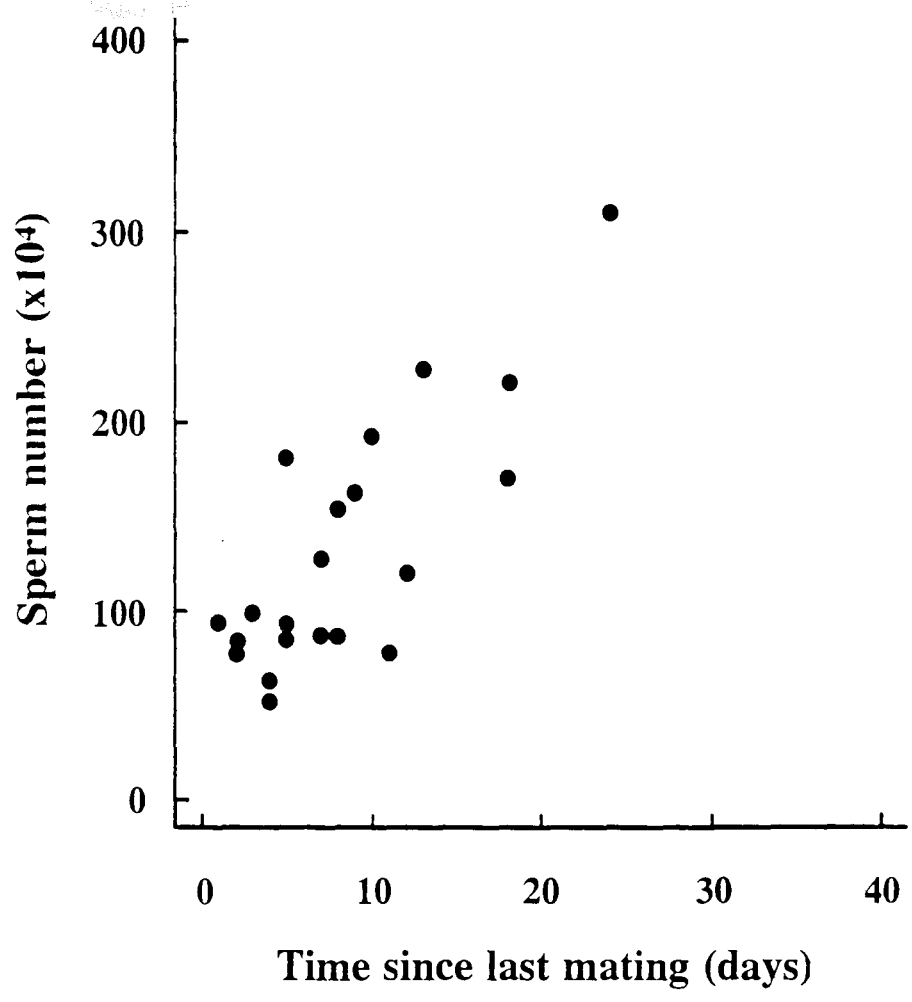

Sperm number (x10³)
250
200
150
100
50
0
0
10
20
30
40
Male age (days)

Fig 6.5 Number of sperm produced as a function of time since the previous mating in *L.laticauda* (r_s= 0.66, n=21, p<0.01).

Fig 6.6 Ampulla mass as a function of time since the previous mating in *L.laticauda* (r_s= 0.83, n=29, p< <0.001).

6.5


Sperm number (x10⁴)
400
300
200
100
0
0
10
20
30
40
Time since last mating (days)

6.6

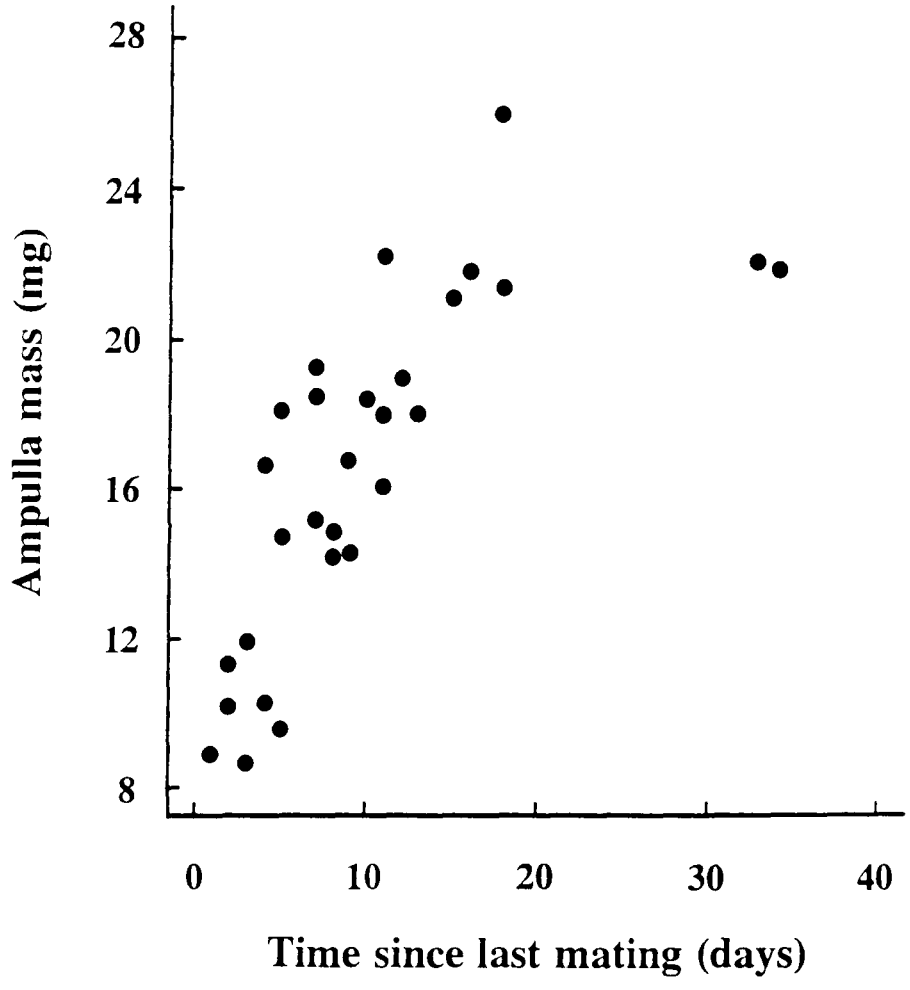
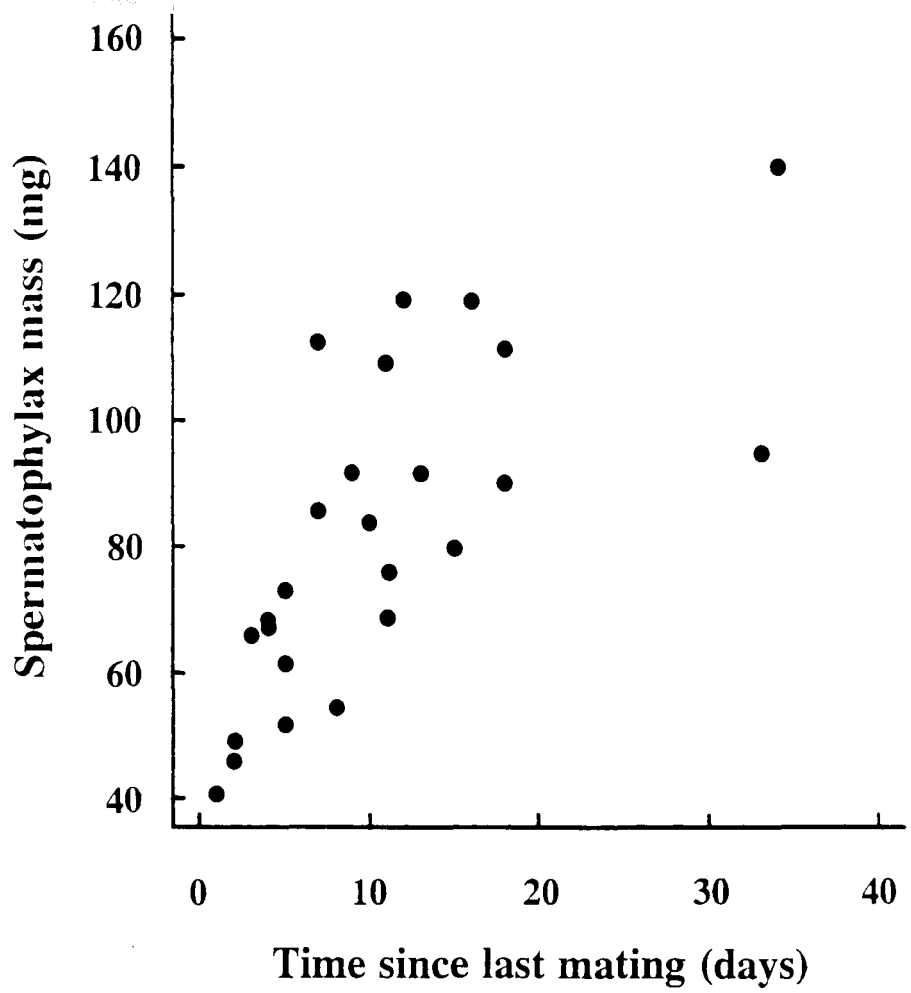

Ampulla mass (mg)
28
24
20
16
12
8
0
10
20
30
40
Time since last mating (days)

Fig 6.7 Spermatophylax mass as a function of time since the previous mating in *L.laticauda* (r_s= 0.81, n=25, p< < 0.001).

Fig 6.8 Correlation between spermatophylax mass and sperm number in *L.laticauda* (r= 0.56, 32 d.f., p< 0.01).

6.7


Spermatophylax mass (mg)
160
140
120
100
80
60
40
0
10
20
30
40
Time since last mating (days)

6.8

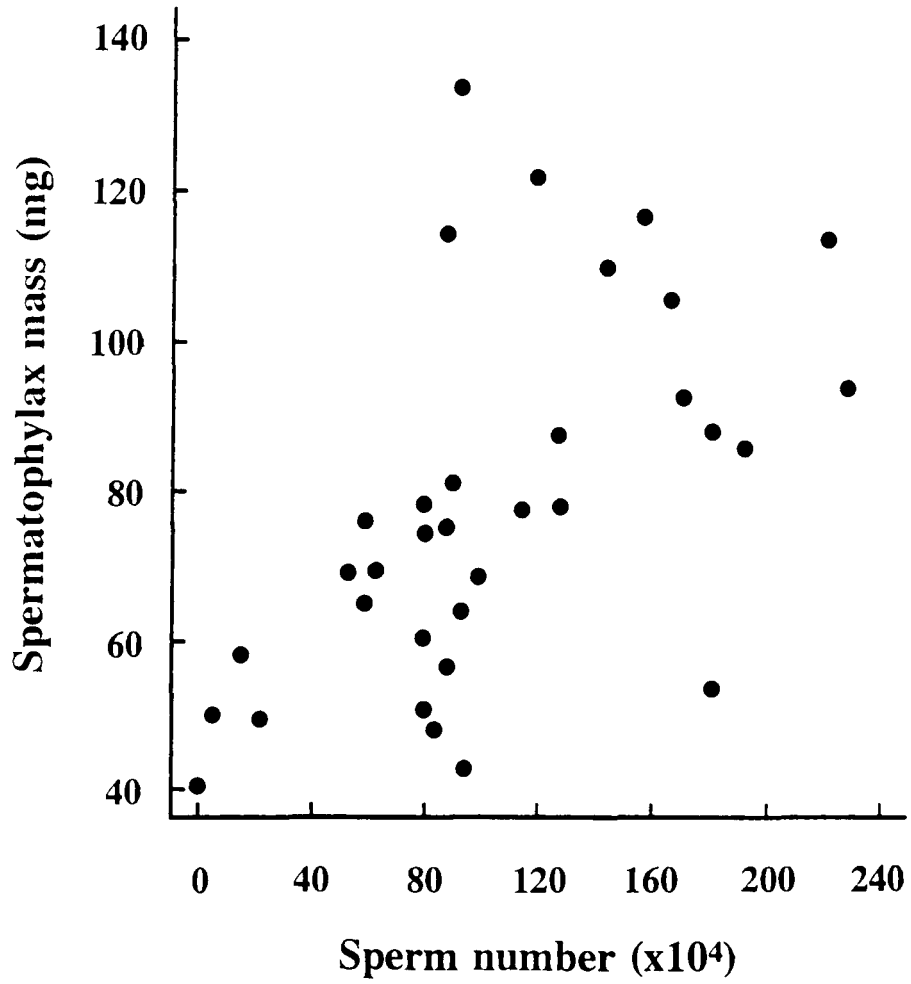
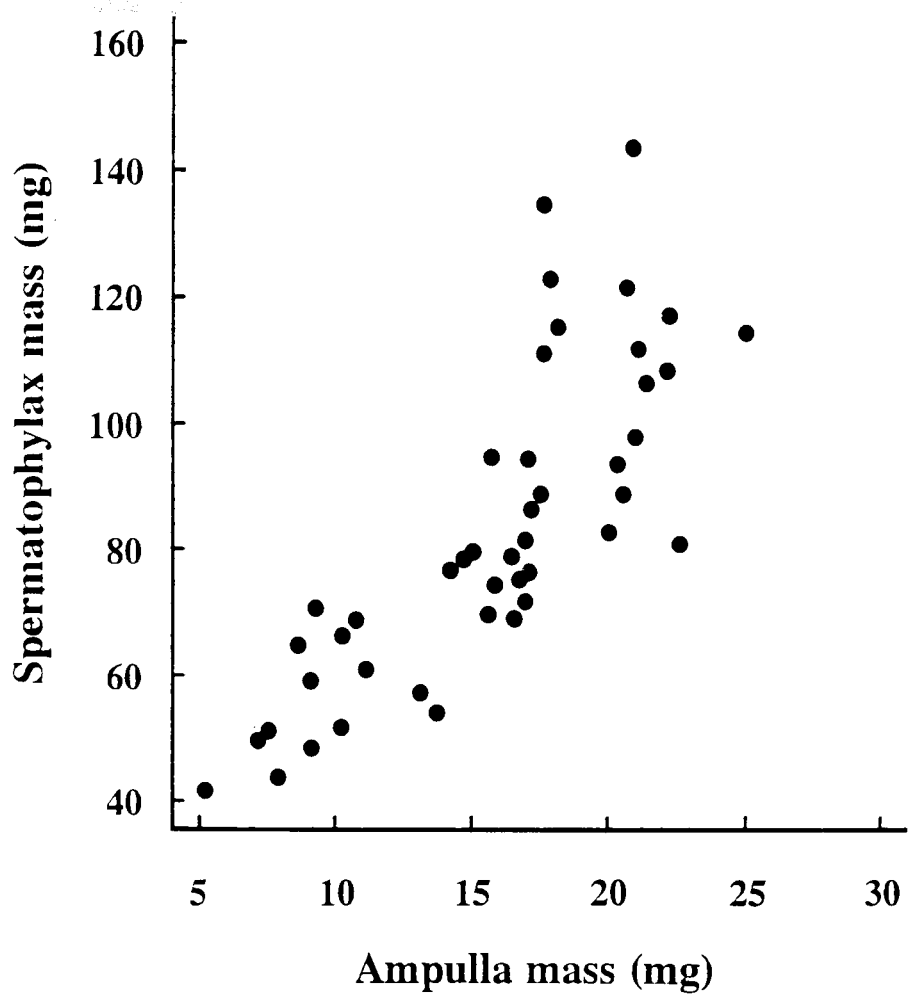

Spermatophylax mass (mg)
140
120
100
80
60
40
0
40
80
120
160
200
240
Sperm number (x10⁴)

Fig 6.9 Correlation between spermatophylax mass and ampulla mass in *L.laticauda* ($r = 0.80$, 43 d.f., $p << 0.001$).

Fig 6.10 Correlation between ampulla mass and sperm number in *L.laticauda* ($r = 0.75$, 32 d.f., $p << 0.001$).

6.9


Spermatophylax mass (mg)
160
140
120
100
80
60
40
5
10
15
20
25
30
Ampulla mass (mg)

6.10

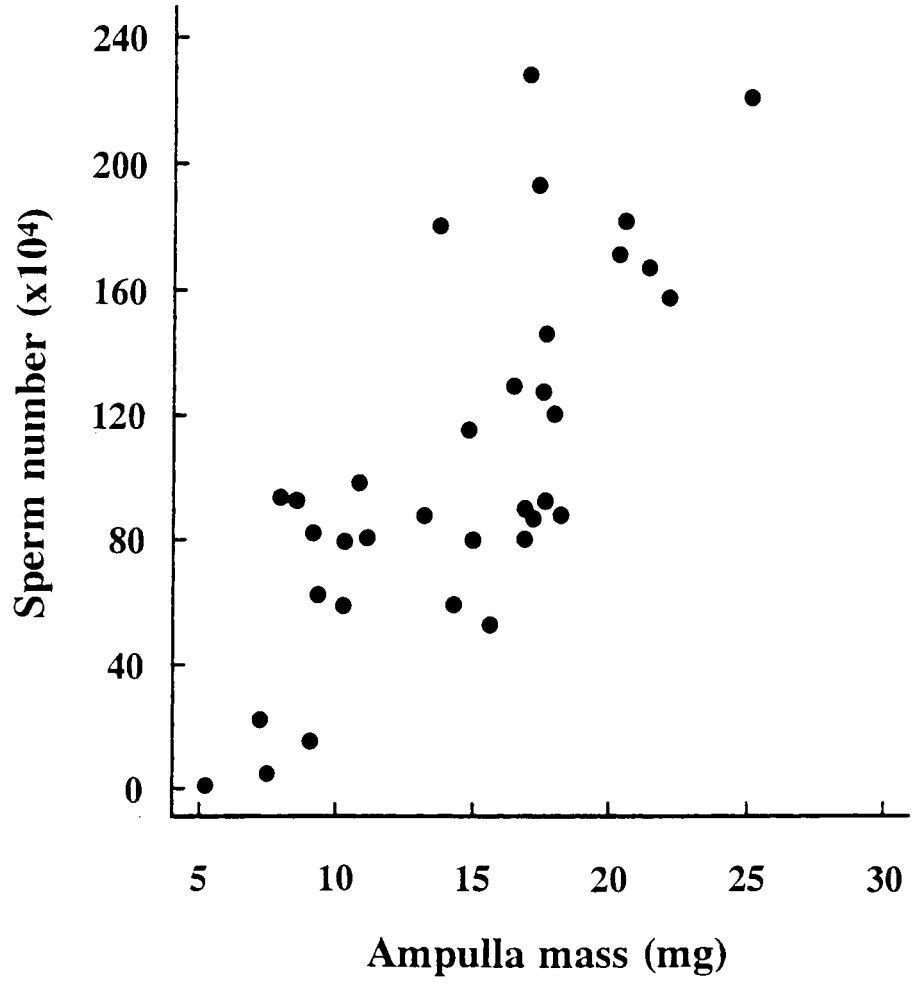
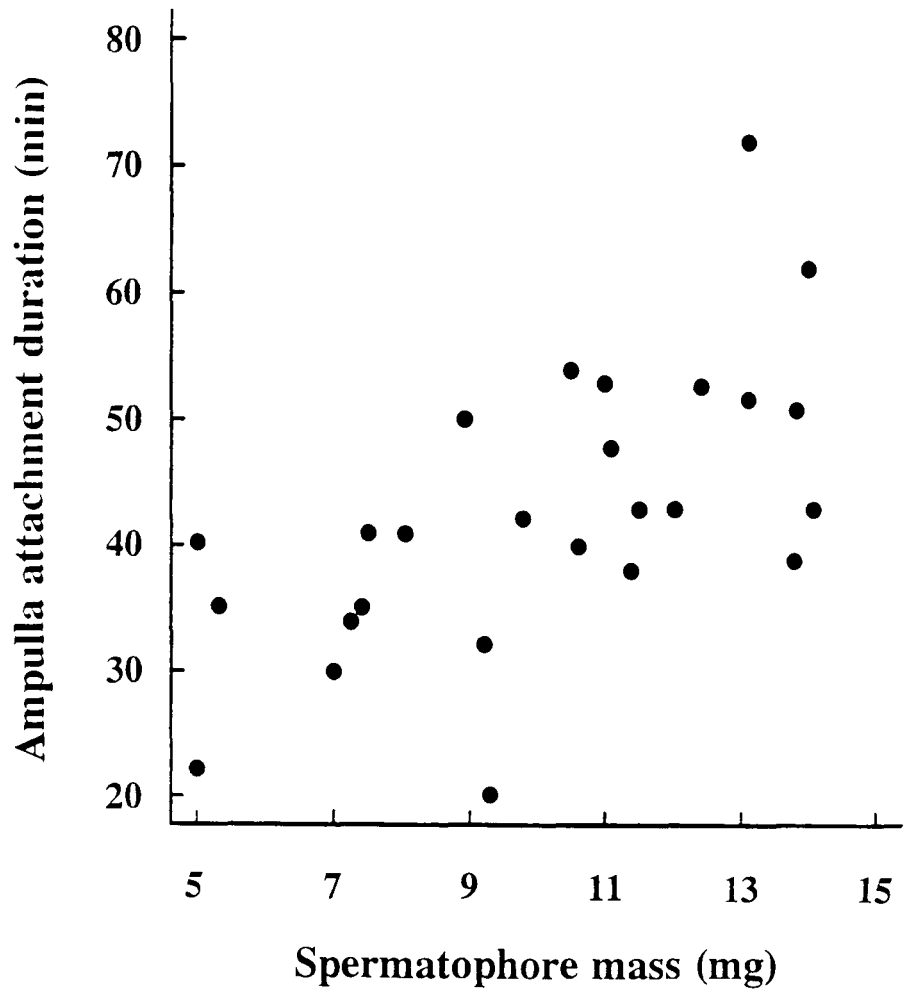

Sperm number (x10⁴)
240
200
160
120
80
40
0
5
10
15
20
25
30
Ampulla mass (mg)

Fig 6.11 The relationship between spermatophore mass and the duration of ampulla attachment in *L.punctatissima* ($r = 0.63$, 24 d.f., $p < 0.01$).

Fig 6.12 The relationship between spermatophore mass and the duration of ampulla attachment in *Poecilimon schmidti* ($r = 0.65$, 45 d.f., $p << 0.001$).

6.11


Ampulla attachment duration (min)
80
70
60
50
40
30
20
5
7
9
11
13
15
Spermatophore mass (mg)

6.12

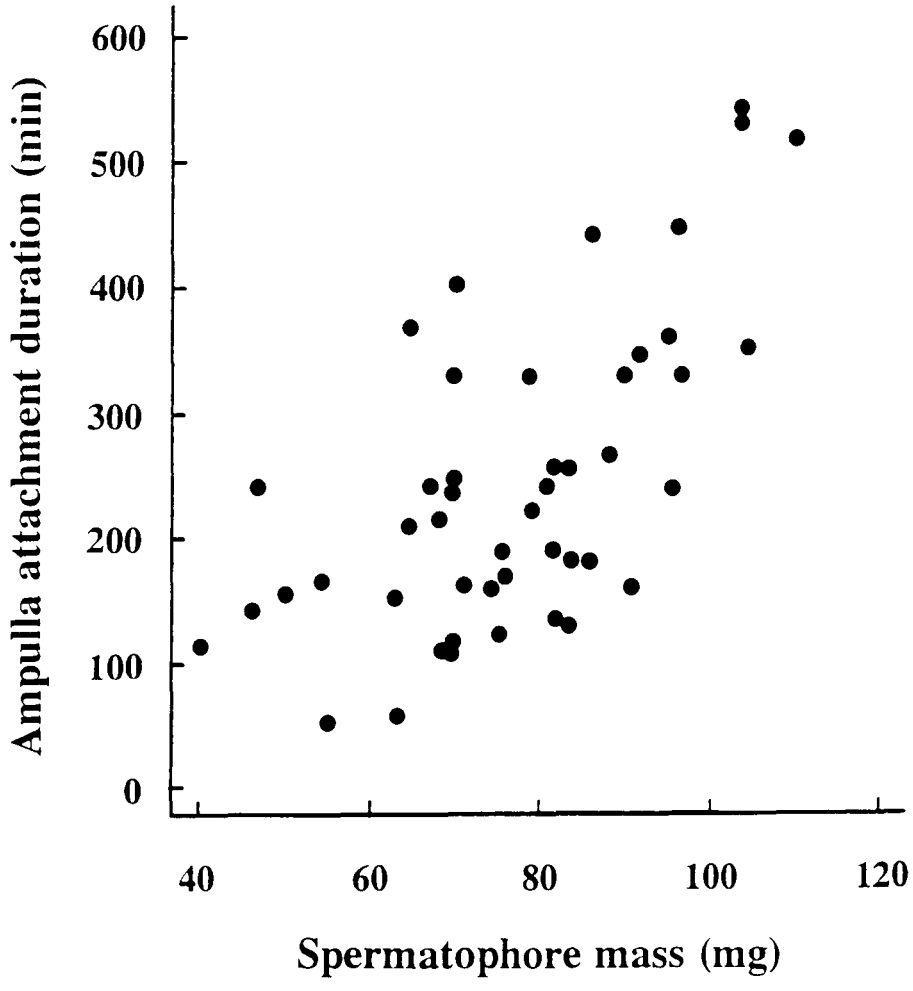
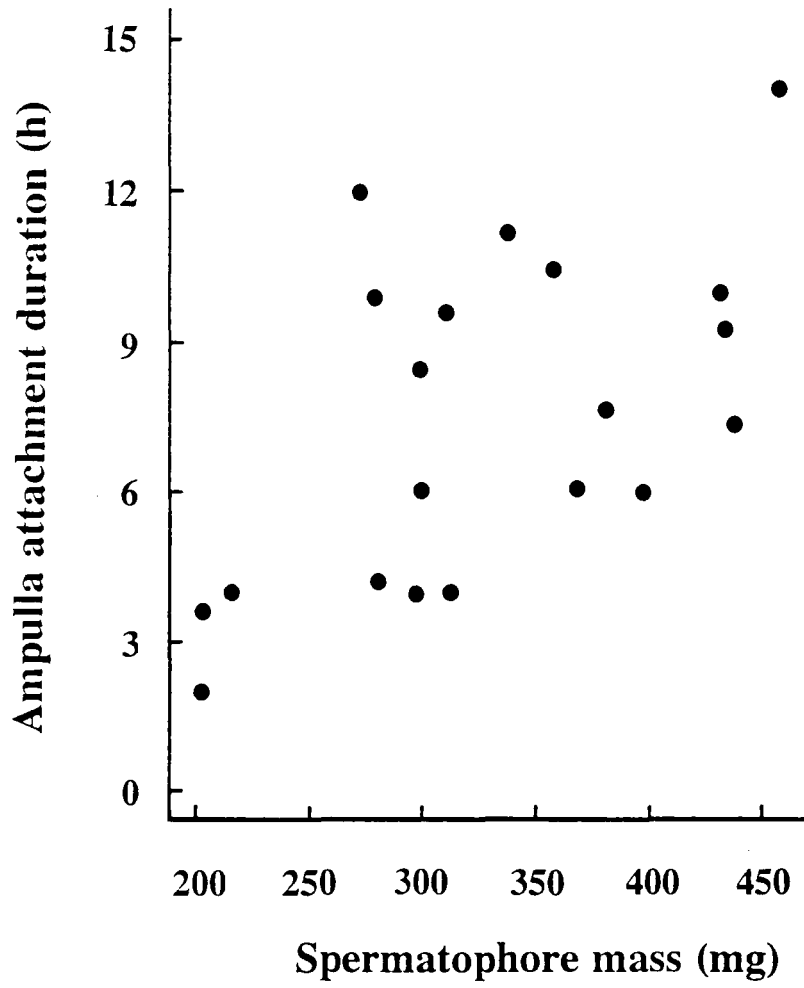

Ampulla attachment duration (min)
600
500
400
300
200
100
0
40
60
80
100
120
Spermatophore mass (mg)

Fig 6.13 The relationship between spermatophore mass and the duration of ampulla attachment in *Steropleurus stali* ($r = 0.56$, 18 d.f., $p < 0.05$).

6.13


Ampulla attachment duration (h)
15
12
9
6
3
0
200
250
300
350
400
450
500
Spermatophore mass (mg)

between male age at first mating and number of sperm produced was also found for *L.punctatissima* (r_s=0.94, n=20, p< <0.001, fig. 6.4).

In order to disentangle the effects of the different variables on spermatophylax size, multiple regression analysis was performed on $\log_{10}$ transformed data, with spermatophylax mass as the dependent variable and male age at first mating, male body mass, number of sperm produced and ampulla mass at first mating as independent variables. Of these independent variables, ampulla mass was the only one that contributed significantly to spermatophylax size ($\log_{10}$ spermatophylax mass = 1.08 ($\pm$ 0.09 S.E.) + 0.71 ($\pm$ 0.08) $\log_{10}$ ampulla mass; r^2 = 0.85, $F_{1,13}$ = 78.2, p< < 0.001). Once ampulla mass was allowed for, the other variables contributed nothing further to spermatophylax mass.

6.3.1.b *Time since last mating.*

Spearman's rank correlation revealed positive relationships between time elapsed since the last mating for male *L.laticauda* and number of sperm produced (r_s=0.66, n=21, p<0.01, fig. 6.5), time since last mating and ampulla mass (r_s=0.83, n=29, p< <0.001, fig 6.6) and time since last mating and spermatophylax mass (r_s=0.81, n=25, p< < 0.001, fig. 6.7).

Multiple regression analysis was performed on $\log_{10}$ transformed data, with spermatophylax mass as the dependent variable and male body mass, sperm number, ampulla mass, time since last mating and male age as independent variables. Complete sets of these variables were only obtained for 14 of the 29 males used for this data set. Of the independent variables, ampulla mass and male age were found to be the best predictors of spermatophylax mass ($\log_{10}$ spermatophylax mass = 1.18 ($\pm$ 0.12) + 1.07 ($\pm$ 0.10) $\log_{10}$ ampulla mass - 0.39 ($\pm$ 0.08) $\log_{10}$ male age; r^2 =

0.89, $F_{2,11} = 52.7$, $p << 0.001$). Ampulla mass was found to have a significant positive effect on spermatophylax mass, while male age, after controlling for the effect of ampulla mass, had a significant negative effect on spermatophylax mass.

6.3.1.c *Spermatophylax size and ejaculate volume\sperm number in* L.laticauda.

As predicted by the sperm-protection hypothesis, a positive correlation (using the combined data set) was found between spermatophylax mass and sperm number ($r=0.56$, 32 d.f., $p<0.001$, fig. 6.8) and between spermatophylax mass and ampulla mass ($r=0.80$, 43 d.f., $p << 0.001$, fig. 6.9). As might be expected, a positive correlation was also found between ampulla mass and sperm number ($r=0.75$, 32 d.f., $p << 0.001$, fig. 6.10). No significant relationship was found between male size (mass after mating) and ampulla mass ($r=0.2$, 32 d.f., $p=0.17$ NS), spermatophylax mass ($r=0.17$, 32 d.f., $p=0.33$ NS) or sperm number ($r=0.2$, 32 d.f., $p=0.26$ NS). Consequently, controlling for male size in correlations between these variables was not necessary.

Multiple regression analysis, using $\log_{10}$ transformed data, was used to examine the effects of sperm number, ampulla mass and male body mass on spermatophylax mass for this data set. Ampulla mass was found to be the best predictor of spermatophylax mass ($\log_{10}$ spermatophylax mass $= 1.06 (\pm 0.08) + 0.72 (\pm 0.07)$ $\log_{10}$ ampulla mass; $r^2 = 0.74$, $F_{1,32} = 96.7$, $p << 0.001$). The slope of this regression is significantly less than 1 ($t_{33} = 4$, $p < 0.001$). Thus, a ten times increase in the mass of the ampulla produced would appear to be associated with a 5.2 (anti-log of 0.72) increase in the mass of the spermatophylax produced.

6.3.2 *Experiment 2: spermatophore mass and the duration of ampulla attachment.*

Females of all three species tended to begin to eat the ampulla directly after having finished the spermatophylax. As might be expected, a positive correlation between spermatophore mass and the duration of ampulla attachment (= time taken for the female to consume fully the spermatophylax) was found in *Leptophyes punctatissima* ($r=0.63$, 26 d.f., $p<0.001$, fig. 6.11), *Poecilimon schmidti* ($r=0.65$, 47 d.f., $p<<0.001$, fig. 6.12) and *Steropleurus stali* ($r=0.56$, 20 d.f., $p<0.05$, fig. 6.13). In the case of *S.stali*, 7 out of the 27 females observed ate only about half of the spermatophylax on the day of mating. Five of these resumed feeding the next day and finished the ampulla 20.5 - 24 hours following its transfer, while two left the remainder of the spermatophylax and ampulla uneaten (this eventually fell off about 72 hours following spermatophore transfer in both cases). These 7 females were excluded from the analysis.

6.4 *Discussion.*

6.4.1 *Spermatophylax size and sperm number \ ejaculate volume.*

As predicted by the ejaculate-protection hypothesis, spermatophylax mass in *L.laticauda* was found to co-vary with ampulla mass and sperm number: all three variables increased with male age at mating and time since last mating. Consequently, a positive correlation was found between spermatophylax mass and sperm number and spermatophylax mass and ampulla mass. A positive relationship between spermatophylax mass and ampulla mass has also been found in the

tettigoniids *Decticus verrucivorus* (Decticinae, spermatophore about 9% male body weight) (Wedell & Arak 1989, Wedell 1993a), *Metrioptera roeselii* (Decticinae, spermatophore about 10% male body weight) (Wedell 1993b), *Ephippiger ephippiger* (Ephippigerinae, spermatophore about 28% male body weight) (Wedell 1993b), *Ephippigerida taeniata* (Ephippigerinae, spermatophore about 28% male body weight) (Nixon 1992) and in the gryllid *Gryllodes supplicans* (spermatophore about 3% of male body weight) (Gage 1993; but see Sakaluk & Smith 1988, in which no significant relationship between ampulla and spermatophylax mass was found in *G.supplicans*). In the latter two species, a positive correlation between spermatophylax size and sperm number was also found. In *Requena verticalis* (Listroscelidinae, spermatophore about 12% or 19% of male body weight), however, no relationship between spermatophylax mass and ampulla mass, spermatophylax mass and sperm number or ampulla mass and sperm number has been found (Simmons et al 1993b).

While the positive relationship between spermatophylax size and ampulla size, and spermatophylax size and sperm number in *L.laticauda* could suggest, as predicted, that males are adjusting the size of the spermatophylax in relation to the volume of ejaculate \ number of sperm they are able to produce (or adjusting the number of sperm \ volume of ejaculate in relation to the size of spermatophylax they are able to produce), it could equally be interpreted to indicate that young or recently mated males are physiologically limited in their ability to produce both spermatophylax and ampulla material and sperm. If this is the case, the positive relationship between spermatophylax mass and ampulla mass \ sperm number may not be adaptive. However, the results of multiple regression analyses support the hypothesis that males are adjusting spermatophylax size in relation to the volume of ejaculate produced: ampulla mass was found to be a better indicator of spermatophylax mass than either the age of virgin males at their first mating or time elapsed since the last

mating (in males that had mated previously). In fact, having allowed for ampulla mass, male age at mating (in males that had mated previously) was found to have a negative effect on the mass of the spermatophylax produced.

6.4.2 *Spermatophylax size and ampulla attachment duration.*

It was found that larger spermatophores (ie. larger spermatophylaxes) result in a longer duration of ampulla attachment in the tettigoniids *L.punctatissima*, *Poecilimon schmidti* and *Steropleurus stali*. This study confirms, therefore, one of the assumptions of the ejaculate-protection hypothesis for spermatophylax function. A positive correlation between spermatophylax size and ampulla attachment duration has also been found in the tettigoniid *D.verrucivorus* (Wedell & Arak 1989) and the gryllid *Gryllodes supplicans* (Sakaluk 1984, 1985). A similar relationship between the size of the nuptial gift offered by a male and the time subsequently available for sperm transfer has been found in the mecopterans *Hylobittacus apicalis* (Thornhill 1976b) and *Panorpa* (Thornhill 1979, Thornhill & Sauer 1991) and the dipteran *Empis borealis* (Svensson et al 1990).

6.4.3 *Spermatophore size and male mating strategies.*

It is interesting that male *L.laticauda* will mate the day after a previous mating, or when recently adult, and produce a small spermatophylax and a small ampulla containing less sperm, rather than waiting until able to produce a larger spermatophylax and more sperm. Dewsbury (1982) suggested that the selective pressures associated with sperm competition and costly ejaculates should lead males not to inseminate as many females as possible, but to ensure that the amount of ejaculate delivered to each female provides effective paternity. By delaying mating

until able to produce a large spermatophylax and more sperm, a male bushcricket could increase his chances of fertilising a greater proportion a female's eggs (see Wedell & Arak 1989; Wedell 1991). Alternatively, a male could maximise mating frequency and adopt a strategy of mating whenever the opportunity arises, even if unable to produce a full-size spermatophore.

In the Lepidoptera, a group in which spermatophore size also increases with time since last mating (see section 6.1), both strategies appear to occur: Oberhauser (1988) found that recently mated male monarch butterflies (*Danaus plexippus*) were as likely to copulate as virgins, and therefore seem to adopt a strategy of copulating whenever possible even though this may result in the transfer of a smaller spermatophore. In *Pieris protodice*, however, recently mated males show reduced courtship persistence (Rutowski 1979), suggesting that they adopt a strategy of delaying re-mating until able to produce a larger spermatophore.

While male *L.laticauda* will mate when unable to produce a full-size spermatophore, the probability of males mating or the intensity of the male's sexual signalling activity with time since last mating or male age (and, therefore, spermatophore size) was not measured. Therefore it is uncertain which of the above strategies male *L.laticauda* adopt as a rule. In the related bushcricket *Poecilimon veluchianus*, the probability of a male re-mating increases over a period of 3 days following mating, as does spermatophore size (Heller & Helversen 1991; see also Hayashi 1993 who found a similar phenomenon in the spermatophylax-producing megalopteran *Protohermes grandis*). In *Requena verticalis*, males allowed constant access to females will re-mate, on average, 2.6 days after their first mating (Gwynne 1990b). However, male calling activity increases over the first four days following an initial mating (Simmons et al 1992; see also Sakaluk et al 1987 who found that mating results in a short-term reduction in male signalling activity in the

spermatophylax-producing haglid *Cyphoderris*). Over this period the accessory glands which produce the spermatophylax steadily increase in mass (Davies & Dadour 1989). In other species of bushcricket, the resumption of male calling activity appears to be influenced by the recovery of the male's accessory glands (in a zaprochiline, Simmons 1990 and in *Ephippiger ephippiger*, Busnel et al 1956, Busnel & Dumortier 1955).

It appears therefore that while male bushcrickets may be able to mate before their accessory glands \ sperm supplies have been fully replenished, male sexual signalling activity may be suppressed until males are able to produce larger spermatophores. This fits with the ideas of Dewsbury (1982) ie. that males should delay mating to ensure that the amount of ejaculate transferred to each female provides effective paternity in the face of sperm competition. Another benefit of delaying re-mating until able to produce a larger spermatophore could result from possible effects of spermatophylax nutrients on offspring fitness (see Gwynne 1988a; Simmons 1990 who found that spermatophylax feeding results in an increase in egg weight and number in bushcrickets, but see also Gwynne et al 1984; Wedell & Arak 1989; Reinhold & Heller 1993 who found no such effect). The duration of the male refractory period following mating in different species (at least in the Orthoptera and Megaloptera) appears to depend upon the relative size of spermatophore characteristically produced: species with smaller spermatophores appear to require less time to replenish their resources and consequently have shorter re-mating intervals (Hayashi 1993).

It would be of interest to measure the intensity of calling in male *L.laticauda* in relation to male age and time since last mating. However, the situation in *L.laticauda* is complicated by the fact that the male call appears to function not only in mate attraction but also in male-male interactions: when solitary, males produce

isolated chirps but when in the presence of other calling males they produce a rapid series of chirps or "aggressive song" (Hartley 1991). Males will produce aggressive song almost immediately after the end of copulation if in the vicinity of other calling males (pers.obs.). Measurements of calling activity with male age or time since last mating in *L.laticauda* would therefore have to be conducted upon acoustically isolated males.

6.5 *Summary.*

The hypothesis that the spermatophylax functions to allow complete ejaculate transfer predicts that a male should adjust spermatophylax size in relation to the volume of ejaculate or number of sperm he is able to produce: ie. spermatophylax size and sperm number \ ejaculate volume should covary. In this chapter, I examine the variation in spermatophylax size, ampulla size and sperm number in relation to male age and time since last mating in the tettigoniid *Leptophyes laticauda* - a species which produces a large spermatophylax. As predicted, it was found that spermatophylax size covaries with sperm number and ampulla size (ie. ejaculate volume): all three variables were found to increase with male age at first mating (in previously virgin males) and time since last mating (in non-virgin males). Consequently, a positive correlation between spermatophylax size and sperm number and between spermatophylax size and ampulla size was found. While such a relationship is predicted by the ejaculate-protection hypothesis, it could be that males are physiologically limited in their capacity to produce spermatophylax and ampulla material and sperm when recently mated or of a young age. If this is the case, the positive relationship between spermatophylax size and sperm number \ ejaculate volume may not be adaptive. The results of multiple regression analyses, however, support the hypothesis that males adjust the size of the spermatophylax in relation to

the volume of ejaculate produced: ampulla mass was found to be a better predictor of spermatophylax mass than either male age at first mating (in previously virgin males) or time elapsed since the last mating (in non-virgin males). In this chapter, I also examine an assumption of the ejaculate- protection hypothesis, that the production of a larger spermatophylax should result in a longer duration of ampulla attachment. As expected, a positive correlation was found, within species, between estimated mass of the spermatophylax transferred and ampulla attachment duration in the bushcrickets *L.punctatissima*, *Poecilimon schmidti* and *Steropleurus Stali*.

7. Patterns of Sperm Precedence in Bushcrickets.

7.1 *Introduction.*

Sperm competition occurs when the ejaculates of two or more males overlap in time in the reproductive tract of the same female and consequently compete for the fertilisation of the female's eggs (Parker 1970). This phenomenon is prevalent in insects, where females may store sperm in a viable condition for long periods of time in specialised sperm storage organs (spermathecae) and frequently mate with more than one male (Parker 1970; Ridley 1988, 1990). From the male's perspective, sperm competition leads to two opposing selective forces: selection to avoid or reduce subsequent competition from the sperm of another male and selection to displace previously stored sperm from the female's sperm stores. The eventual outcome in terms of the proportion of a female's eggs fertilised by her first or last mate (ie. the pattern of sperm precedence) may, at least in part, reflect the extent to which adaptation in the one direction outdoes adaptation in the other (Parker 1970; Boorman & Parker 1976; for reviews of the mechanisms of sperm precedence in insects, and other groups, see Thornhill & Alcock 1983; Birkhead & Hunter 1990; Parker et al 1990; Lessels & Birkhead 1990). It should be noted, however, that the pattern of sperm precedence may also reflect the female's best interests (see Walker 1980; Eberhard 1990; Birkhead & Moller 1993).

Studies of sperm competition in insects have revealed considerable variation between species in the mean proportion eggs fertilised by the second (or last) male. This measure is referred to as the P_2 value (Boorman & Parker 1976). The most common pattern of sperm use in insects seems to be last-male precedence (see Birkhead &

Hunter 1990). Ridley (1989) reviewed sperm competition data for 57 insect species. Of these 44 (77%) showed some degree of last-male sperm precedence ($P_2 > 0.5$) while only 11 (19%) showed first-male precedence.

The pattern of sperm precedence is currently known for only four species of tettigoniid. Even within this small sample, a great deal of variation between species has been found: in *Requena verticalis*, virtually complete first-male sperm precedence occurs (Gwynne 1988b); in *Decticus verrucivorus*, there is sperm mixing (mean $P_2 = 0.5$) (Wedell 1991); while in *Poecilimon veluchianus* and *Metaplastes ornatus* there is pronounced last-male sperm precedence (mean P_2 for *P.veluchianus* = 0.9) (Achmann et al 1992; Helversen & Helversen 1991). In *M.ornatus*, males insert their specialised sub-genital plate into the female's genital chamber prior to spermatophore transfer and stimulate the release of stored sperm, apparently by simulating the passage of eggs past the female's spermathecal opening (Helversen & Helversen 1991).

A knowledge of the pattern of sperm precedence in tettigoniids is important because of its bearing on the potential of the spermatophylax to function as a form of paternal investment. In order for nuptial gifts to function as paternal investment, they must have a positive effect on offspring fitness and\or number and the donating male must stand a chance of fertilising a significant proportion of the eggs which benefit from his gift nutrients (see Wickler 1985, 1986; Gwynne 1986b; Sakaluk 1986b; Simmons & Parker 1989; chapter 1, section 1.1.3). Whether the latter can be the case will depend, in part, upon the pattern of sperm precedence, the female refractory period and the time taken for the female to lay eggs containing male-donated nutrients. Where the rate of incorporation of male donated nutrients into eggs is rapid and the female refractory period is long, however, the pattern of sperm precedence may be relatively unimportant in this context (see Simmons 1990a).

In *R.verticalis*, the pattern of first-male sperm precedence means that a male mating with a virgin will fertilise a large proportion of the eggs in which his spermatophylax nutrients are incorporated (Gwynne 1988b). Furthermore, these eggs appear to benefit from the spermatophylax nutrients (Gwynne 1988a). Under these circumstances, the spermatophylax has the potential to function as paternal investment. However, there is the problem that a male mating with a non-virgin will stand little chance of fertilising eggs which may benefit from his nutrients (see chapter 1, section 1.1.3).

Where sperm mixing or, in particular, last-male sperm precedence occurs, the time taken for the female to lay eggs containing male-donated nutrients, and the duration of the female's refractory period become important in determining the potential of the spermatophylax to function as paternal investment (see Wickler 1985, 1986; Gwynne 1986b; Sakaluk 1986b; Simmons & Parker 1989; Chapter 1, section 1.1.3). In *D.verrucivorus*, *P.veluchianus* and probably *M.ornatus*, females are likely to have re-mated before laying eggs containing nutrients donated by the previous male (Helversen & Helversen 1991; Heller & Helversen 1991; Achmann et al 1992; Reinhold & Heller 1993; Wedell 1993a). Furthermore, in *D.verrucivorus* at least, the female is likely to re-mate many times over the period in which the first male's nutrients are incorporated into her eggs (Wedell 1993a). This will result in one male's spermatophylax nutrients being donated to eggs which are predominantly fertilised by other males. The spermatophylax is unlikely, therefore, to function as paternal investment in these species (Helversen & Helversen 1991; Heller & Helversen 1991; Achmann et al 1992; Reinhold & Heller 1993; Wedell 1993a).

Here, I examine the pattern of sperm precedence and female refractory periods in

the tettigoniids *Leptophyes punctatissima*, *L.laticauda* (Phaneropterinae) and *Steropleurus stali* (Ephippigerinae). I also describe the mating behaviour and spermathecal morphology of these species - information which may help in the generation of hypotheses to explain the mechanism of sperm precedence (see Walker 1980, Birkhead & Hunter 1990).

7.2 *Methods.*

7.2.1 *The species.*

Details of the origin and maintenance in captivity of the *Leptophyes laticauda* and *L.punctatissima* stocks used in these experiments are given in chapter five and appendix 1.

The *Steropleurus stali* used in these experiments were obtained from a laboratory colony derived from adults collected in the Sierra de Guadarrama, Spain, in 1988. Rearing conditions for this species were based on those outlined by Hartley & Dean (1974) for another species of ephippigerine (see appendix 1 for details). Sexes were separated prior to the final moult. On becoming adult, all individuals were uniquely marked on the pronotum with different colour combinations of "Humbrol" enamel paint.

7.2.2 *Sperm precedence studies.*

Studies of sperm precedence in the three species were conducted using the "irradiated male" technique (see Parker 1970, Boorman & Parker 1976). This

technique involves mating a virgin female with two different males, one of which has been irradiated. The irradiation induces a high level of dominant lethal mutations in the chromosomes of the sperm. Although the sperm remain active and are capable of fertilisation, most of the resulting zygotes die in early embryonic development (Boorman & Parker 1976). Consequently eggs that hatch or develop whole embryos are likely to have been fertilised by the normal (N) male, while those that do not are likely to have been fertilised by the irradiated (R) male. In order to control for the possibility that sperm from the R and N-males differ in their competitive ability (see, for example Zimmering & Fowler 1966; Parker & Smith 1975), both N,R (ie. female mated first to an N-male then to an R-male) and R,N mating patterns are performed and an average of the obtained values of sperm precedence is taken. In order to correct for eggs that are fertilised by the N male but fail to hatch or to develop whole embryos, and for eggs fertilised by the R male that do hatch\develop whole embryos, the expected proportion of these egg types are generated by mating virgin females singly to individual N or R males. The proportion of offspring fertilised by irradiated sperm in either R,N or N,R matings (P_R) will then be given by:

$$P_R = (1 - x/p) + \{ z/p \times \frac{1-(x/p)}{1-(z/p)} \} \qquad (7.1)$$

where x = observed proportion of eggs hatching\with whole embryos, p = mean proportion hatching\with whole embryos in control N matings, z = mean proportion hatching\with whole embryos in control R matings (equation from Boorman & Parker 1976). The first part on the right-hand side corresponds to the depression in fertility caused by the R sperm, while the second part is the expected proportion of hatching eggs\whole embryos which are products of R sperm (Boorman & Parker 1976). Where irradiation induces 100% early embryonic mortality (ie. z = 0), the latter will disappear. Where the R male mates first (ie. R,N matings), P_2 (the

proportion of eggs fertilised by the last male) = $1\text{-}P_R$. Where the R male mates second (ie. N,R matings), $P_2 = P_R$.

Males of all three species used in this experiment had been adult for at least a week. This was to ensure that males were sexually active and able to produce spermatophores. For each species, groups of five males at a time were sterilised by irradiation with a caesium-137 Gammacell source at a dose of 7.4 Krads (8 mins at 923.3 rads/min). Females of all three species were mated about ten days after the final moult. Individual pairs were placed in black nylon-mesh cages (approx. 10cm x 10cm x 10cm) and were observed until mating had taken place. For *S.stali* all matings were set up in the morning as this corresponds with the period of greatest male singing activity. For *L.punctatissima* and *L.laticauda*, males appeared to be most active over two separate periods: the early to mid morning and mid afternoon to early evening. Matings were set up in either period. For each species, four mating combinations were arranged: 1) **R** matings (females mated once to an irradiated male; n= 4 for *L.laticauda*; n= 3 for *L.punctatissima*; n= 4 for *S.stali*), 2) **N** matings (female mated once to a normal male; n= 3 for *L.laticauda*; n= 3 for *L.punctatissima*; n= 7 for *S.stali*), 3) **N,R** matings (female mated first to an N-male and then to an R-male; n= 3 for *L.laticauda*; n= 7 for *L.punctatissima*; n= 4 for *S.stali*), 4) **R,N** matings (female mated first to an R-male and then to an N-male; n= 5 for *L.laticauda*; n= 5 for *L.punctatissima*; n= 4 for *S.stali*). In the double-mating categories, females were generally re-mated upon the termination of their refractory periods following the first mating (see 7.2.4).

After mating, females were individually housed in mesh-topped plastic canisters and provided with a sprig of *Buddleia* leaves in a water-filled vial for food. *L.punctatissima* were also given bramble (*Rubus fruticosus*) leaves, while the *S.stali* were given dock (*Rumex* spp) leaves and a pinch of wheat germ. Food was replaced

at weekly intervals. Females were also given a suitable medium for oviposition. For *L.laticauda* this consisted of blocks of polyurethane foam ("wet foam" for flower arrangements) approximately 5cm x 5cm x 5cm. Female *L.punctatissima* were given 5 discs of polythene sheet under their water container. Eggs are readily laid between these discs (Deura & Hartley 1982). Female *S.stali* were provided with sand (about 5cm deep) in the base of their canisters in which to lay eggs.

Eggs were collected from each female at weekly intervals from the last mating over a period of four weeks. Eggs from each week from each female were placed on filter paper on moist cotton-wool in individual petri-dishes. These eggs were incubated at 30°c for one month followed by 25°c for two months. After this treatment, initial diapause is at a minimal level and most fertile eggs should contain whole embryos ready to enter embryonic diapause (Hartley & Warne 1972; Deura & Hartley 1982; Hartley 1990; Hartley, pers. comm). The number of successfully developed eggs for each female was determined by immersing the eggs in a petri-dish of water, illuminating them with obliquely transmitted light and observing them through a low-power binocular microscope. The water renders the eggshells sufficiently transparent to allow easy assessment of development. The proportions of eggs containing whole embryos in the different experimental groups were used to calculate P_2 values (proportion of eggs fertilised by the second male to mate) using formula 7.1 above (from Boorman & Parker 1976).

7.2.3 *Mating behaviour.*

Observations of mating behaviour for each species were made concurrently with the sperm precedence experiment above and with experiments presented in chapters five and six. Observations were based on 45 separate pairs of *L.punctatissima*, 60 separate pairs of *L.laticauda* and 51 separate pairs of *S.stali*. Sexually active

(ie.stridulating) males were placed with receptive (ie.acoustically responding) females, one pair per observation cage (measuring 10cm x 10cm x 10cm). Males were weighed prior to and shortly after mating on an electrobalance accurate to 1mg in order to estimate the mass of the spermatophore. Cages were observed until the female mounted the male at which point all behaviours were carefully recorded and timed. The time spent by the female on the male before copulation was recorded along with the time from the start of copulation to the secretion of the spermatophore and time from spermatophore secretion to the end of copulation. The behaviour of males and females following copulation were also noted.

7.2.4 *Female refractory periods.*

Experiments to examine the duration of the female refractory period following mating were conducted concurrently with the sperm precedence experiments above. For all three species, on each day following an initial mating, females (which had been allocated at random to the double-mating categories) were placed next to a cage of stridulating males of the same species and observed for 30 min before being placed with sexually active males (of the appropriate type, ie N or R, see, 7.2.2) one pair per observation cage, and observed intermittently for at least three hours. Females of all three species produce a response-song to male calls when sexually receptive (Hartley et al 1974; Hartley & Robinson 1976; Hartley 1991). The termination of the female refractory period, therefore, is relatively easy to detect. However, in order to be certain that females had regained receptivity, female refractory periods were measured as the intervals between actual matings. For *L.punctatissima*, preliminary observations suggested that females might regain receptivity on the same day as an initial mating. Therefore, for a group of females (n = 7), newly mated males were removed from the observation cages after the initial mating and replaced by stridulating males. Pairs were then observed intermittently (approx. every 15

min) until the females re-mated. The time from end of a female's first mating to the beginning of her second was recorded. The number of eggs laid by females during the refractory period was also noted for each species. In all, data on the duration of the refractory period were obtained for seven different *L.punctatissima* females, fifteen different *L.laticauda* females and ten different *S.stali* females.

7.2.5 *Spermathecal morphology.*

Spermathecae were dissected out of females which had died of age. These were placed in a petri-dish of water under a binocular microscope and were drawn with the aid of a drawing-tube attachment. For *S.stali*, the spermatheca of a single female which had mated once, approximately 4 weeks before death, but had been prevented from ovipositing was drawn. For *L.laticauda*, the spermathecae of three such females were drawn, along with the spermatheca of a virgin female, for comparison. The number of sperm in the three spermathecae from the non-virgin *L.laticauda* were counted using the method outlined in chapter five. For *L.punctatissima*, the spermatheca of a single virgin female was drawn.

All means are quoted $\pm$ standard error.

7.3 *Results.*

7.3.1 *Sperm-precedence studies.*

The mean number of eggs laid by females over a four-week period in each experimental group for each species are given in table 7.1. In *L.punctatissima*,

Table 7.1: Mean ± standard error number of eggs laid over 4 weeks in each treatment for each species (sample size in brackets; N = mated to normal male; R = mated to irradiated male; NR = mated first to a normal male, then to an irradiated male; RN = mated first to an irradiated male, then to a normal male)

Treatment	Species		
	L.laticauda	*L.punctatissima*	*S.stali*
N	146.3 ± 22.2 (3)	35.0 ± 4.6 (3)	119.0 ± 12.4 (7)
R	79.8 ± 7.5 (4)	35.0 ± 7.8 (3)	140.0 ± 35.5 (4)
NR	108.0 ± 17.0 (3)	92.1 ± 11.1 (7)	125.3 ± 32.2 (4)
RN	86.4 ± 16.4 (5)	104.0 ± 10.2 (5)	208.0 ± 25.0 (4)

Table 7.2: P_2 values (proportion of eggs fertilised by the last male to mate) for individual females of each species (RN = mated first to an irradiated male, then to a normal male; NR = mated first to a normal male, then to an irradiated male).

Treatment	Species		
	L.laticauda	*L.punctatissima*	*S.stali*
RN	0.28 0.20 0.45 0.01 0.0	0.31 0.06 0.98 0.37 0.94	0.85 0.63 0.08 0.92
NR	-0.19 (ie.0) -0.04 (ie.0) -0.2 (ie.0)	0.54 0.09 0.63 0.94 0.91 0.88 0.93	1.0 0.98 1.0 0.97
Mean ± S.E.	0.12 ± 0.06	0.63 ± 0.1	0.8 ± 0.11

females in the doubly-mated categories laid significantly more eggs than females in the singly-mated categories over the four-week period (non-parametric ANOVA testing an *a priori* directional prediction (number of eggs laid by doubly-mated females > number of eggs laid by singly-mated females), $z=3.37$, $p<0.001$). However, no such difference was found in *L.laticauda* ($z=-0.23$, NS) or in *S.stali* ($z=1.57$, NS), though the result for the latter species is close to significance. The difference in fecundity between singly and doubly mated *L.punctatissima* is analysed further in chapter 8.

For *L.punctatissima*, the mean egg viability for N females was high (v= proportion viable= 0.81 ± 0.04, n=3), while eggs produced by R females showed almost complete early embryonic mortality (v= 0.007 ± 0.007, n=3). A very similar pattern occurred in *L.laticauda* (v for N females= 0.75 ± 0.1, n=3; v for R females= 0.005 ± 0.003, n=4). In *S.stali*, the mean egg viability for N females was lower (v= 0.65 ± 0.06) and eggs produced by R females showed complete early embryonic mortality (v= 0, n=4).

For *L.punctatissima*, the mean proportion of eggs fertilised by the second male to mate (P_2) in R,N matings was 0.53 ± 0.18 (n=5). The mean P_2 value for N,R matings was 0.70 ± 0.12 (n=7). The overall mean P_2 value was 0.63 ± 0.1 (range: 0.06 - 0.98, n=12) (Table 7.2) indicating random sperm mixing with, perhaps, a slight second-male advantage.

For *L.laticauda*, the mean P_2 for R,N matings was 0.19 ± 0.08 (n=5) while the mean P_2 for N,R matings was -0.14 ± 0.05 (n=3). A negative P_2 value occurred in this case because the mean proportion of viable eggs from N,R matings was higher than the mean proportion of viable eggs in the N group. Considering the small sample sizes, this was probably due to random sampling error. Being a proportion,

the negative P_2 value should be taken as zero. The overall mean P_2 value for *L.laticauda* was 0.12± 0.06 (range 0 - 0.45, n=8) (table 7.2) indicating pronounced first-male sperm precedence.

For *S.stali*, the mean P_2 value for N,R matings was 0.99± 0.008 (n=4) while the mean P_2 for R,N matings was 0.62± 0.19 (n=4). The overall mean P_2 value for *S.stali* was 0.8± 0.11 (range 0.08 - 1, n=8) indicating pronounced last-male sperm precedence. Out of the 8 females, 7 showed last-male sperm precedence while only one showed first-male precedence (see table 7.2). Although this could reflect a failed second mating, the small sample size does not warrant the exclusion of this data point.

Non-parametric analysis of variance (Meddis 1984) revealed a significant difference between the P_2 values of the three species (H_2 = 13.65, $p<0.01$).

7.3.2 *Mating behaviour.*

7.3.2.a *L.punctatissima.*

The typical sequence of events in a successful mating encounter for *L.punctatissima* was as follows: after the pair had made antennal contact, the male would turn his back to the female, arching it downwards. The female would then walk forwards, palpating the dorsal surface of the male's back. When the female was fully mounted upon the male and palpating the dorsal tergites adjacent to the male's reduced tegmina, the male would reach the tip of his abdomen upwards and backwards and clasp the base of the ovipositor with his cerci. The inwardly curved tips of the cerci link with grooves either side of the base of the ovipositor. The mean time taken from

the female mounting to the male linking his cerci (ie. the start of copulation) was 0.56± 0.11 min (0.13 - 1.32 min, n=11). The copulatory position consisted of the female mounted above the male, both facing in the same direction, though with the male's front end slightly bent towards the substrate. Deposition of the spermatophore began 1.18± 0.07 min (0.93 - 1.43 min, n=7) after the start of copulation. Copulation ended 2.1± 0.27 min (0.73 - 3.12 min, n=7) later. The total copulation duration was 4.15± 0.23 min (1.66 - 8 min, n=27). As a result of spermatophore production, males lost an average of 5.6± 0.26 % (2.28 - 8.49 %, n=45) of their body weight. Following copulation, the female would generally walk away, while the male would remain stationary. The male appeared to show no further attention to the female after copulation. The male would groom his genital region 30 sec - 3 min after the end of copulation and would shudder violently 7± 1.2 min (4 - 11 min, n=6) after the end of copulation. Males were observed to resume stridulation 69.7± 4.1 min (60 - 91 min, n=7) after copulation had ended. One male was observed to transfer a spermatophore to a different female 217 min after a previous copulation, though this was smaller than the first. The female would bend double, detach most of the spermatophylax and begin to eat it 11.48± 0.69 min (1.67 -17min, n=32) following the termination of coupling. The female tended to eat the ampulla directly after having finished the spermatophylax, 42.57± 1.81 min (22 - 74 min, n=37) after the end of copulation (chapter 5).

7.3.2.b *L.laticauda*

Mating in *L.laticauda* was similar to that in *L.punctatissima*, though when the female was mounted upon the male and palpating the dorsal tergites adjacent to the male's reduced tegmina, the male would reach the end of his abdomen backwards and upwards, grasp the base of the ovipositor with his cerci then, unlike *L.punctatissima*, would release it almost instantaneously before repeating the action.

This behaviour continued for an average of 3.32± 0.42 min (1.5 - 7 min, n=14) before copulation began. The ampulla was secreted 0.88± 0.04 min (0.67 - 1.22 min, n=16) from the start of copulation. The large spermatophylax followed and copulation ended 2.83± 0.19 min (1.83 - 4.85 min, n=18) later. The total duration of copulation was 3.87± 0.17 min (2.81 - 5.85 min, n=20). Males lost an average of 22.86± 0.66 % (11.33 - 32.7 %, n= 60) of their body weight at mating as a result of spermatophore production. As in *L.punctatissima*, after copulation, the female would generally walk away while the male would remain stationary and appeared to pay no further attention to the female. The male would groom his genitalia before shuddering violently about 6 min after the end of copulation. In the presence of other singing males, male *L.laticauda* would begin aggressive song (see Hartley 1991; chapter 6) as little as 5 min after the end of copulation. Males have been observed to mate again on the day following a previous mating, though this results in the transfer of a smaller spermatophore (see chapter 6). The female would bend double and begin to consume the large spermatophylax 2.58± 0.75 min (0.08 - 9 min, n=12) after the end of copulation. Unlike *L.punctatissima*, females did not detach the spermatophylax but would take small bites, straighten up, chew, then bend again to take further bites. Females began to eat the ampulla directly after having finished the spermatophylax, 338.3± 20.38 min (225 - 462 min, n=10) after the end of copulation (chapter 5).

7.3.2.c *S.stali*.

In *S.stali*, as in the previous two species, pairs would make antennal contact then the female would mount the male, palpating his dorsal tergites. When the female's head was above the male's pronotum, the male would reach the tip of his abdomen backwards and upwards with his cerci raised and would probe in the region of the

female's subgenital plate. Copulation began when the strongly sclerotised spurs on the inner tip of each cercus of the male engaged with the sockets situated either side of the eighth sternite (sub-genital plate) of the female (see Hartley & Warne, 1984 for illustrations of the male cercal spurs and female sternal sockets of two species of ephippigerine; see also Rentz 1972 for illustrations of the same in a species of decticine). At about this point, the male's genitalia were partly everted and the titillators were inserted between the base of the ovipositor and the posterior flap of the subgenital plate, into the female's genital chamber (See Hartley & Warne, 1974 for an illustration of the titillators of a species of ephippigerine). About 1 - 3 min after the start of copulation, the male would bend underneath the female into a c-shape and would grasp the ovipositor with his first and second pairs of legs. The fleshy, everted genitalia of the male would pulsate regularly, about once every 2 seconds, and the titillators would move rhythmically in the female's genital chamber until the bilobed ampulla began to be secreted, 13.9$\pm$ 0.98 min (10 - 20 min, n=10) after the start of copulation. The large spermatophylax followed and when it was fully secreted, 1.74$\pm$ 0.18 min (0.5 - 3.15 min, n = 16) later, the female would begin to walk and the male would disengages his cerci and drop away. The total duration of copulation was 16.16$\pm$ 0.94 min (10 - 24 min, n=19). The spermatophore represented an average of 27$\pm$ 0.74 % (15.8 - 36.7 %, n=51) of male body weight. After copulation, the male tended to remain stationary and, as in the other two species, would groom his genital region and appeared to pay the female no further attention. *S.stali* males did not resume singing until 3$\pm$ 0.14 days (2 - 4 days, n=13) following mating and would re-mate after this time. At an average of 2.6 $\pm$ 0.5 min (1-5min, n=10) after the end of copulation, females would bend double and begin to consume the spermatophylax. As in *L.laticauda*, the spermatophylax was not removed but was eaten one mouthful at a time. The ampulla was eaten directly after the female had finished the spermatophylax. Out of 27 females observed, 20 ate the whole of the spermatophylax on the day of mating,

taking an average of 7.49± 0.74 hours (2 - 14 hours) to do so before eating the ampulla (data from chapter 6), while 7 females ate only about half of the spermatophylax then stopped eating it. Five of these resumed feeding the next day and finished the ampulla 20.5 - 24 hours from the start of copulation, while the other 2 left the remainder of the spermatophore uneaten. This eventually dropped to the ground 72 hours after the end of copulation (chapter 6).

7.3.3 *Female refractory periods.*

The mean female refractory period for *L.punctatissima* was found to be 185.43± 38.26 min (75 - 384 min, n=7) when females were given the opportunity to re-mate on the same day as a previous mating. Females would almost invariably re-mate on the day following a previous mating. For *L.laticauda*, the mean female refractory period was found to be 6.87± 1.02 days (1 - 15 days, n=15). The mean female refractory period for *S.stali* was found to be 4.8± 0.36 days (3 - 7 days, n=10).

None of the female *L.punctatissima* which re-mated on the same day as a previous mating showed any sign of oviposition behaviour (ie. probing at the crevices between the wooden supports of the observation cage with the ovipositor) between matings. This is not surprising because the *L.punctatissima* in this experiment generally mated for the second time before nightfall and females of this species do not generally oviposit until this time of day (Duncan 1960; Deura & Hartley 1990; pers. obs.). Female *L.punctatissima* which re-mated on the day following an initial mating did, however, oviposit overnight. Detailed counts of the number of eggs laid overnight were not made, though observations suggest that females tended to lay about 4 eggs in this period. This figure agrees with the mean weekly egg production of about 25 eggs for this species under similar environmental conditions (Deura & Hartley 1990; chapter 8) and roughly corresponds with Wedell's (in press) estimate

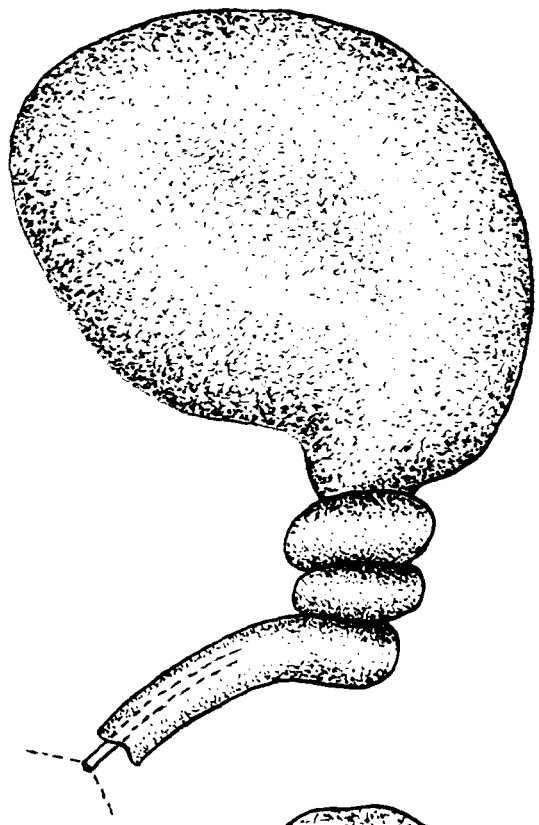
of the mean number of eggs laid over period of 1.6 days for this species (5.7 eggs). During the mean refractory period of about 7 days, female *L.laticauda* lay about 25 eggs (chapter 8). The mean number of eggs laid by female *S.stali* during their first refractory period was found to be 18.2$\pm$ 5.25 (0 - 67 eggs, n=20).

7.3.4 *Spermathecal morphology.*

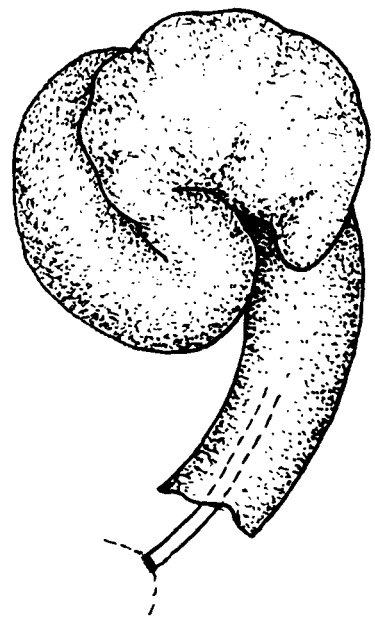
The spermatheca of *S.stali* consists of a spheroid, sac-like bladder with a narrow, convoluted spermathecal duct which opens into the genital chamber. The spermatheca of a singly-mated female *S.stali* is illustrated in fig. 7.1a. The spermathecae of *L.punctatissima* and *L.laticauda* are very similar in shape to one another and differ from that of *S.stali* in that the spermathecal bladder is more elongated and is distinctly pointed. The spermathecae of virgin *L.punctatissima* and *L.laticauda* are illustrated in figs. 7.1b and 7.1c, respectively. As the spermatheca of *L.laticauda* receives more sperm, the spermathecal bladder appears to become more elongated, up to a point. The spermathecae of three singly-mated *L.laticauda* containing progressively larger amounts of sperm are illustrated in Figs. 7.2a, 7.2b and 7.2c. The number of sperm contained in these spermathecae were 297 x 10^3, 634 x 10^3 and 1303 x 10^3 sperm, respectively.

Fig. 7.1 a) Spermatheca (sperm-storage organ) of a singly-mated female *Steropleurus stali*; **b)** spermatheca of a virgin female *Leptophyes punctatissima*; **c)** spermatheca of a virgin female *L. laticauda*. Scale bars represent 1mm in each case.

a



b



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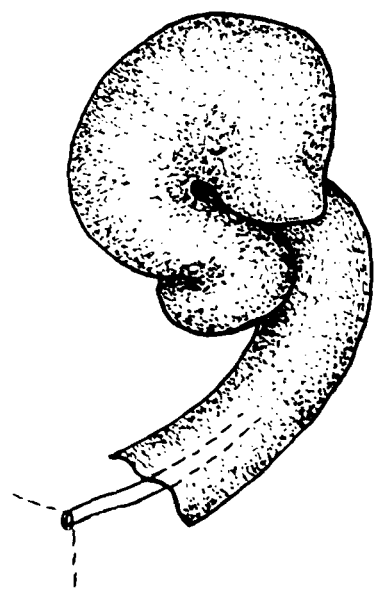
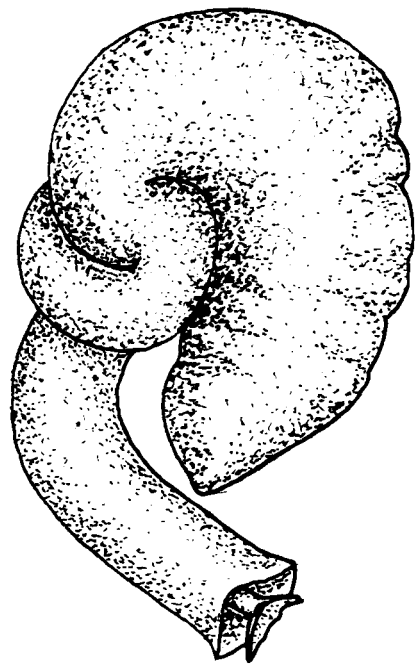
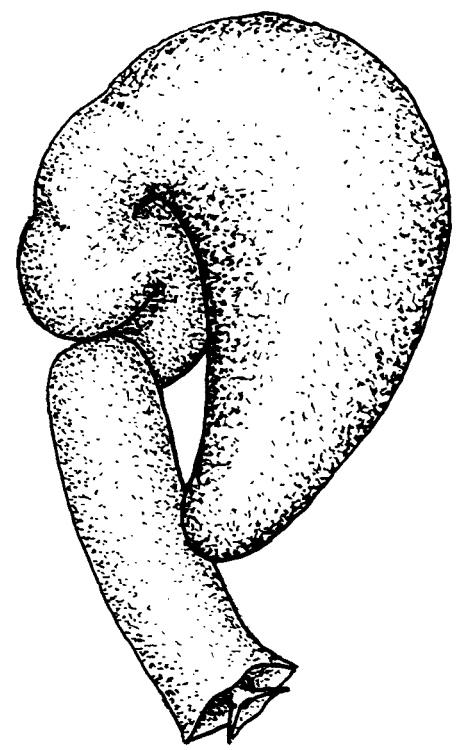


Fig 7.2 a), b) & c) Spermathecae of three singly-mated *L.laticauda*, containing progressively larger amounts of sperm. Scale bars represent 1mm in each case.

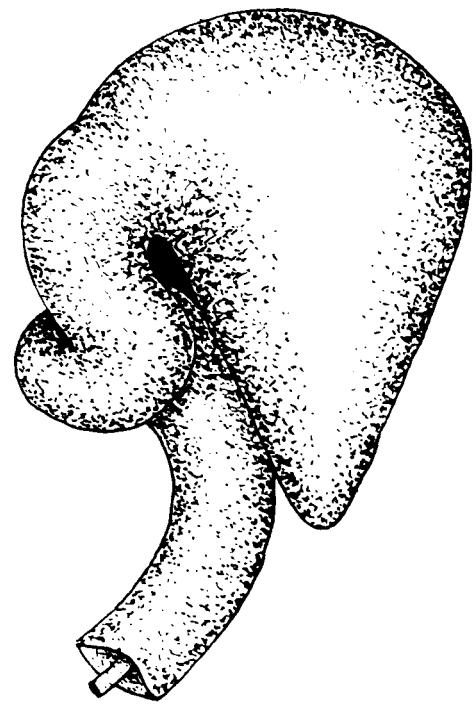
a



b



C



7.4 *Discussion.*

7.4.1 *The potential of the spermatophylax to function as paternal investment in* L.laticauda, L.punctatissima *and* S.stali.

In *L.laticauda*, as in *Requena verticalis* (see Gwynne 1988b), the pattern of pronounced first-male sperm precedence means that when a male mates with a virgin female, he will have a high probability of fertilising eggs in which nutrients from his spermatophylax might be incorporated. This will occur regardless of the duration of the female's post-mating refractory period or the time taken to lay eggs containing these nutrients. The spermatophylax, therefore, has the potential to function as paternal investment in *L.laticauda* when males mate with virgins, if nutrients from the spermatophylax result in an increase in offspring fitness or number (however, no significant effect of spermatophylax-feeding on egg weight or number has been found in this species, see chapter 8). On the other hand, when a male mates with a non-virgin at the end of her first refractory period, the pattern of first-male sperm precedence means that nutrients from the spermatophylax would be donated to eggs which will be predominantly fertilised by sperm from another male. The spermatophylax would be unlikely to function as paternal investment in this case (Wickler 1985). It could be argued, however, that since males that have mated with virgins are the only ones that are likely to leave a significant number of progeny, the fact that the spermatophylax is unlikely to function as paternal investment when a male mates with a non-virgin is irrelevant to the argument that the spermatophylax is maintained by selection for paternal investment.

With pronounced first-male sperm precedence, males that are able to determine female mating status and preferentially mate with virgins will be at a selective advantage. In the spider *Frontinella pyramitela* (Linypiidae) and the fly *Culicoides melleus* (ceratopogonidae), species in which there is first-male sperm precedence (Linley 1975; Austad 1982), there is evidence that males are able to assess female mating status during courtship and copulation attempts (Linley & Hinds 1975; Suter 1990). In *R.verticalis*, no evidence of male discrimination in favour of virgin females per. se. has been found in the lab. or in the field (Lynam et al 1992; Simmons et al, in press). However, males can apparently assess female age as they show a preference for younger females (Simmons et al, in press) and produce smaller spermatophylaxes when mating with older females (Simmons et al 1993). The latter phenomenon also occurs in *D.verrucivorus* (Wedell 1992), a species which shows sperm mixing (Wedell 1991). In the field, older females are probably more likely to be non-virgins. Simmons (1993) proposed that the production of smaller spermatophylaxes by *R.verticalis* males when mating with older females fits with the paternal investment hypothesis for spermatophylax function: due to first-male sperm precedence, male *R.verticalis* will have a low probability of fertilising eggs when mating with non-virgin females and, therefore, they reduce the degree of paternal investment accordingly. An alternative hypothesis to account for this phenomenon might be that older females differ in their rate of spermatophylax consumption or tendency to eat the spermatophore. Observations of *L.laticauda* and *S.stali* suggest that older females frequently eat only half of the spermatophylax and leave the remainder of the spermatophore attached. In this case, production of a large spermatophylax would be un-necessary to prevent the female from removing the ampulla prematurely.

In *L.laticauda*, laboratory observations do not suggest male discrimination against non-virgins; the sperm precedence study would not have been possible if such

discrimination were pronounced. Nevertheless, the observation that the mean time taken from the female mounting the male to the start of copulation is about six times greater in *L.laticauda* (mean: 3.32 $\pm$ 0.42 min, range: 1.5 - 7 min, see section 7.3.2.b) than in *L.punctatissima* (mean 0.56 $\pm$ 0.11 min, range 0.13 - 1.32 min, see section 7.3.2.a) could reflect the presence in *L.laticauda* of an adaptation to help discriminate against non-virgins. During this pre-copulatory period, male *L.laticauda* repeatedly engage their cerci with the base of the female's ovipositor (ie. begin copulation) before pulling away again. A prolonged pre-copulatory period might facilitate male assessment of female virginity (see Suter 1990). The male may assess the degree of female receptivity (see Linley & Hinds 1975) and may require female "courtship persistence" in this period. However, similar pre-copulatory behaviour also occurs in *Poecilimon veluchianus* (pers. obs.). This species shows pronounced last-male sperm precedence (Achmann et al 1992), therefore there is no advantage to males, in this context, in discriminating in favour of virgins. The significance of this pre-copulatory behaviour, which also occurs in several members of the sub-family Tettigoniinae (pers. obs), remains to be investigated.

In *L.punctatissima*, with its sperm mixing, and, to a greater extent, *S.stali*, with its last-male sperm precedence, the duration of the female refractory period in relation to the time taken for females to lay eggs containing nutrients from the spermatophylax becomes important in determining the potential of the spermatophylax to function as paternal investment. In both these species, as in most other bushcrickets observed, females tend to store mature eggs in their oviducts, both before becoming sexually receptive and throughout their reproductive lives (pers.obs.). Such eggs will have undergone vitellogenesis and will be unable to incorporate further nutrients. These stored eggs will be oviposited before any subsequently-developed eggs. Because fertilisation occurs as eggs are laid, this will result in a time delay between mating and the male's sperm being able to fertilise the

eggs which might contain nutrients from his spermatophylax.

In *L.punctatissima*, females will re-mate as little as 185 min. after a previous mating, in which time they are unlikely to lay any eggs at all (see section 7.3.3). If females re-mate the following day, they may lay about 4 eggs overnight. From first becoming adult to reaching receptivity (a period of about a week, pers.obs.), female *L.punctatissima* become quite rotund with stored eggs. On average, female body weight increases by 60 % during this period (Mean female weight on becoming adult = 0.2± 0.008g, range: 0.1533 - 0.23g, n=10; mean female body weight when first sexually receptive = 0.3265± 0.0136g, range: 0.2513 - 0.3960g, n=11). Females may contain up to 60 mature eggs when first sexually receptive (pers.obs.). Even after females have begun to oviposit, they still contain a large number of stored eggs: a random sample of females dissected after they had oviposited for 1 - 4 weeks contained an average of 45± 8.0 mature eggs (range: 13 - 67 eggs, n=7). Therefore, the 4 eggs which females may lay between matings, if they re-mate the next day, are highly unlikely to contain spermatophylax nutrients from the female's most recent mate. From the mean number of stored eggs and the rate of egg-laying, it would take females about ten days before they could begin to lay eggs developed directly after a given mating, which could contain the male's spermatophylax-nutrients. In this period, females are likely to re-mate several times, assuming that they tend to re-mate at the end of their refractory periods. Taking into account the pattern of sperm precedence, nutrients from one male's spermatophylax are therefore likely to be donated to eggs which will predominantly be fertilised by other males, as in *D.verrucivorus* (Wedell 1993). It should be noted, however, that this assumes that behaviour in the lab. (ie. female refractory periods and the number of mature eggs stored) is comparable to that in the field. If this is the case, the small spermatophylax of *L.punctatissima* is unlikely to function as paternal investment.

In *S.stali*, the mean female refractory period was found to be about 5 days, during which time females laid an average of 18 eggs (range 0 - 67 eggs) (see section 7.3.3). As in *L.punctatissima*, females tend to be rotund with stored eggs (containing up to 100 mature eggs, pers. obs.) when first sexually receptive, at an average of 9.7$\pm$ 0.72 days (range: 6 - 13 days, n=10) after becoming adult (pers.obs.). Furthermore, they continue to have a large number of mature eggs in storage throughout their lives: a random group of females dissected after having oviposited for 1 - 2 weeks contained an average of 62$\pm$ 9.6 mature eggs (range: 26 - 100, n=6). Because the mature eggs in storage will be laid first, the average of 18 eggs laid in a female's refractory period are unlikely to be able to contain spermatophylax nutrients from her most recent mate. If a female re-mates at the end of her refractory period, therefore, eggs which stand to benefit from the spermatophylax nutrients of a given male are likely to be predominantly fertilised by the sperm of subsequently-mating males, taking into account the last-male sperm precedence in this species. Assuming that females tend to re-mate at the end of their refractory periods in the field (this species often occurs at high densities in the field, P.Bateman, pers. comm., so females are unlikely to have trouble in finding a mate), it seems, therefore, that the large spermatophylax of *S.stali* (contributing to a loss of up to 37% of male body weight at mating) is unlikely to be maintained by selection for paternal investment.

7.4.2 *Possible mechanisms of sperm precedence in* L.punctatissima, L.laticauda *and* S.stali.

The mean P_2 value of 0.63 together with the large degree of variation in P_2 values in *L.punctatissima* (range: 0.06 - 0.98, table 7.2) suggest a pattern of random mixing of sperm from both males within the spermatheca. Similar intraspecific variation in

P_2 values around a mean value approximating 0.5 has been found in the tettigoniid *Decticus verrucivorus* (Wedell 1991) and the Gryllids *Gryllodes supplicans* (Sakaluk 1985) and *Gryllus bimaculatus* (Simmons 1987), species in which sperm-mixing is presumed to occur (see also Parker et al 1990 who found that the pattern of sperm precedence in *G.bimaculatus* fitted their model of random sperm mixing). In these three species, it appears that the intraspecific variation in P_2 values can, at least in part, be explained by the relative number of sperm transferred by either the first or last male to mate: the male which has transferred the most sperm fertilises a greater proportion of the female's eggs (Sakaluk 1985; Simmons 1987; Wedell 1991).

In *L.laticauda*, a pattern of first-male sperm precedence was found. This is relatively unusual amongst insects (see Ridley 1989). Two possible mechanisms of first-male sperm precedence have been suggested for insects. These are:

1) **Mating plugs**. The first male to mate with the female deposits an obstruction (sperm-plug or mating-plug) in the female's reproductive tract which prevents the entry of sperm from subsequently mating males (Reviewed by Parker 1970; Boorman & Parker 1976, see also Parker & Smith 1975; Matsumoto & Suzuki 1992);

2) **Spermathecal filling.** The first male fills the female's sperm-storage organ to capacity, creating a back-pressure that prevents further sperm from entering (Retnakaran 1974; Walker 1980; see also Lessels & Birkhead 1990 who modelled this as a theoretically possible mechanism of first-male sperm precedence, with reference to birds).

In Lynyphiid spiders, first-male sperm precedence may result from the morphology of the spermatheca: there are separate tubes for the entry and departure of sperm on

opposite sides of the spermatheca (Austad 1982; Austad 1984; Watson 1991). Consequently, sperm from the first-male may be positioned closer to the opening leading to the oviduct, resulting in a "first in, first out" bias in sperm precedence favouring the first male (Watson 1991).

In *L.laticauda*, the spermatheca has only a single tube through which sperm both enter and leave (see figs. 7.1 and 7.2). Consequently, the latter mechanism of first-male sperm precedence may be ruled out. Dissection of females mated 1 - 6 weeks previously (n=10), together with microscopic examination of the reproductive tract has failed to reveal the presence of any obvious obstructions to further sperm transfer, such as a broken spermatophore tube (cf Parker & Smith 1975) or a mucoid plug. However, the sperm-plug hypothesis cannot be ruled out on this basis because a barrier to further inseminations might take a subtle form and might be damaged by dissection or overlooked. Furthermore, such a barrier might only be temporary and might have been dissolved by the female prior to dissection.

The hypothesis that the first male to mate in *L.laticauda* may fill the spermatheca to capacity, thus preventing further sperm from entering, is an interesting possibility because, if this were the case, the difference in sperm precedence between *L.laticauda* and *L.punctatissima* might be accounted for by the difference in mean sperm number between the two species. *L.laticauda* males produce, on average, about 15 times more sperm per spermatophore than *L.punctatissima* males, or 5.6 times more sperm if the difference in male body weight between the two species is taken into account (chapter 5). The larger sperm load of *L.laticauda* might fill the spermatheca to capacity, resulting in first male sperm precedence, while the smaller sperm load of *L.punctatissima* might be insufficient to fill the spermatheca, allowing the mixing of ejaculates from different males to occur. Preliminary data suggest that the spermatheca of *L.laticauda* may have a limited capacity: the spermatheca in once

mated females was found to contain an average of $744.7 \times 10^3 \pm 295.6 \times 10^3$ sperm (range: 297×10^3 - 1303×10^3 sperm, n=3) which is less than half the mean number of sperm contained in the spermatophore of mature males ($=1687.6 \times 10^3 \pm 128.8 \times 10^3$ sperm, range: 894×10^3 - 3104×10^3 sperm, n=17, chapter 5). However, the sample size was small and more data are needed. Furthermore, the three once-mated females had mated about 4 weeks previously (though they were prevented from laying eggs in this period). Sperm might have died in storage during this time or could have been digested by the female.

The hypothesis that first-male sperm precedence results from spermathecal filling by the first male could be tested in two ways. Firstly, the number of sperm in the spermathecae of singly and doubly-mated females could be compared. The spermathecal filling hypothesis predicts that the spermathecae of singly and doubly-mated females should not differ in the number of sperm they contain. However, the sperm-plug hypothesis would also predict this. In order to distinguish between the sperm-plug and spermathecal-filling hypotheses, the amount of sperm transferred by the first male (ie. the duration of spermatophore attachment) could be manipulated and its effect on the degree of first-male sperm precedence examined (eg. using the sterile-male technique, Boorman & Parker 1976). The spermathecal-filling hypothesis predicts that if the first male fails to introduce a sufficient amount of sperm to fill the spermatheca, first-male sperm precedence should break down and sperm-mixing should occur. The sperm-plug hypothesis, on the other hand, predicts that no sperm from the second mating should enter the spermatheca, regardless of the amount of sperm transferred by the first male.

In *S.stali*, data suggest a pattern of last-male sperm precedence (mean $P_2 = 0.8$), though more replicates would be desirable to confirm this pattern since one of the eight females in this experiment showed first-male precedence. Three principal

mechanisms of last-male sperm precedence have been proposed for insects (see Birkhead & Hunter 1990; Parker et al 1990; Lessels & Birkhead 1990). These are:

1) **Stratification**. This occurs when sperm from later inseminations lie in a more favourable position for fertilisation (ie. nearest to the exit of the spermatheca) than sperm from earlier inseminations leading to a "last in, first out" second male bias. Walker (1980) proposed that this is more likely to occur in species with elongate or tubular spermathecae (but see Ridley, 1989, who, in a comparative study, failed to find a significant relationship between spermathecal shape and the pattern of sperm precedence in insects);

2) **Volumetric displacement** (= sperm flushing). Sperm from the last male enter the spermatheca (presumably under pressure) and force out sperm which are already there (eg. Etman & Hooper 1979; Ono et al 1989; Parker et al 1990; Parker & Simmons 1991);

3) **Direct removal \ stimulating the female to release stored sperm.** Males may physically scoop sperm from previous inseminations out of the female's reproductive tract using specially modified parts of the intromittent organs (as, for example, in the Odonata: Waage 1979, 1984; Siva-Jothy 1987; Siva-jothy & Tsubaki 1989) or may stimulate the female to release previously stored sperm prior to transferring their own (eg. Helversen & Helversen 1991).

Alternatively, last-male sperm precedence might result from the mortality of sperm in the females reproductive tract (Lessels & Birkhead 1990), the killing or immobilisation of earlier sperm by later sperm (Silberglied et al 1984) or the selective manipulation of stored ejaculate by females (Birkhead & Moller 1993).

In *S.stali*, the spermatheca is a membranous, spheroid, sac-like structure (fig 7.1a). According to Walker (1980), therefore, stratification would be unlikely to occur, though it would probably be unwise to exclude the possibility of stratification on this basis alone. Volumetric displacement of sperm also seems unlikely in *S.stali*. Unlike the tree cricket *Truljalia* and the yellow dung-fly *Scatophaga*, species in which volumetric displacement is the proposed mechanism of sperm precedence (Ono et al 1989; Parker et al 1990; Parker & Simmons 1991), sperm in *S.stali* is transferred via a spermatophore. This, together with the long, narrow and convoluted spermathecal duct in this species (see fig 7.1a) suggests that sperm are unlikely to be delivered either in the correct position or under sufficient pressure to displace sperm already stored in the spermatheca (the spermatophore tube does not reach into the spermathecal duct, pers. obs.).

Sperm removal\ stimulation of the female to release previously stored sperm would seem to be more feasible as the mechanism of last-male sperm precedence in *S.stali*. Prior to spermatophore transfer, *S.stali* males insert a pair of barbed titillators into the female's genital chamber and appear to move them rhythmically as the everted genitalia of the male pulsate. This behaviour continues for an average duration of 14 min (10 - 20 min, see section 7.3.2.c) before the spermatophore is transferred. Although the titillators are unlikely to be able to reach up into the spermathecal duct (they are too short, pers.obs.), it is possible that their movement might stimulate the release of previously stored sperm by the female, as do movements of the male's specialised sub-genital plate within the female's genital chamber in the bushcricket *Metaplastes ornatus* (Helversen & Helversen 1991). Alternatively, the prolonged copulation prior to spermatophore transfer might be a mate-assessment period (Wedell 1992) or may be necessary for the formation of the large spermatophore (see chapter 3, part 1 for further discussion of this subject). The latter explanation

would seem unlikely, however, because certain other *Steropleurus* species, which produce equally large spermatophores, require only a matter of minutes for spermatophore formation following the start of copulation (0.87 - 2 min in *S.martorelli*, *S.brunneri* and *S.perezi*, chapter 3), as in *L.laticauda*. It would be interesting to compare the pattern of sperm precedence in one of these *Steropleurus* species with that of *S.stali*.

The hypothesis that male *S.stali* stimulate the female to release stored sperm prior to spermatophore transfer could be tested by comparing the number of sperm in the spermatheca of singly-mated females with that of doubly-mated females in which the second male is allowed a full copulation but the spermatophore is removed before sperm transfer. If the release of stored sperm occurs, females in the latter category should have significantly fewer sperm in their spermathecae than females in the former category (see Helversen & Helversen 1991).

That last-male sperm precedence in *S.stali* may be a passive consequence of the mortality of sperm from the first male seems unlikely because second matings occurred soon after first matings (about 5 days) in the sperm precedence experiment. Furthermore, singly-mated females had a reasonably high hatching success over the 4 week period and the spermatheca of a singly-mated female dissected after 4 weeks contained large numbers of active sperm. Insufficient data are available to comment on the possible occurrence in *S.stali* of the other two potential mechanisms of last-male sperm precedence mentioned above.

7.5 *Summary.*

In order for the spermatophylax to function as paternal investment, the donating male must stand a chance of fertilising a significant proportion of the eggs which stand to benefit from his spermatophylax nutrients. Whether this can be the case will depend, in part, upon the pattern of sperm use by multiply-mated females (= the pattern of sperm precedence), the female refractory period and the time taken for the female to lay eggs containing male-donated nutrients. I examined the pattern of sperm precedence in the bushcrickets *Leptophyes laticauda*, *L.punctatissima* and *S.stali*, using the sterile-male technique. The duration of the female refractory periods and the number of eggs laid in the refractory period were also noted. In addition, I examined the mating behaviour and spermathecal morphology of these species - information which can be useful in the generation of hypotheses to explain the mechanism of sperm precedence. In *L.laticauda*, a pattern of first-male sperm precedence was found (mean proportion of a doubly-mated female's eggs fertilised by the last male = P_2 = 0.12); therefore, a male mating with a virgin will fertilise a large proportion of the eggs in which his spermatophylax nutrients may be incorporated. The large spermatophylax of this species will, therefore, have the potential to function as paternal investment, if nutrients from the spermatophylax result in an increase in offspring fitness and\or number. However, there is the problem that a male mating with a non-virgin will stand little chance of fertilising eggs which might benefit from his spermatophylax nutrients. In *L.punctatissima*, sperm mixing with, perhaps, a slight last-male bias, was found (mean P_2 = 0.63), while data for *S.stali* indicated pronounced last-male sperm precedence (mean P_2 = 0.8). As a result of the large number of mature eggs typically stored by females of these species, together with the duration of the female refractory periods and the

mean number of eggs laid in these periods, it appears that females are likely to have re-mated before laying eggs which could contain nutrients donated by a given male. Taking into account of the patterns of sperm precedence in these two species, this means that one male's spermatophylax nutrients are likely to be incorporated into eggs which will be predominantly fertilised by the sperm of subsequently-mating males. The small spermatophylax of *L.punctatissima* and the large spermatophylax of *S.stali* are unlikely, therefore, to be maintained by selection for paternal investment. In this chapter, I also discuss possible mechanisms of sperm precedence in the three species.

8. The Effect of Spermatophylax Consumption on Female Reproductive Output in Bushcrickets.

8.1 *Introduction.*

The hypothesis that the spermatophylax in bushcrickets is maintained by selection for paternal investment proposes that a male benefits from spermatophylax production through the spermatophylax nutrients consumed by his mate being used to increase the fitness and\or number of the male's own offspring (see chapter 1, section 1.1.3). In support of this hypothesis, spermatophylax proteins have been found to be incorporated into developing eggs in the bushcrickets *Requena verticalis* (Bowen et al 1984), *Decticus verrucivorus* (Wedell 1993a) and a species of zaprochiline (Simmons & Gwynne 1993). Furthermore, in *R.verticalis* and the zaprochiline, females receiving a spermatophylax at mating have been found subsequently to produce more and\or heavier eggs than females experimentally deprived of the spermatophylax (Gwynne 1984a, 1988a; Simmons 1990a; Simmons & Bailey 1990; but see Gwynne et al 1984 who found no effect of spermatophylax feeding on egg weight or number in *R.verticalis*). In *R.verticalis*, Gwynne (1988a) found a significant negative relationship between the mean weight of eggs produced by females and the proportion of progeny which died before emergence. This suggests that the production of heavier eggs may amount to the production of fitter offspring.

In the bushcrickets *D.verrucivorus* and *Poecilimon veluchianus*, however, no difference in the number or weight of eggs produced has been found between females deprived of the spermatophylax and those allowed to consume the

spermatophylax (Wedell & Arak 1989; Reinhold & Heller 1993). This casts doubt, therefore, as to whether an increase in egg weight and\or number through spermatophylax feeding is a general phenomenon in bushcrickets.

In this chapter, I examine the effect of spermatophylax consumption by females on the number and weight of eggs subsequently produced in the bushcrickets *Leptophyes laticauda* and *L.punctatissima* and present preliminary data for *Steropleurus stali* and *S.asturiensis*. Because the effects of male-derived nutrients on female fecundity are expected to be more pronounced when the level of nutrients available to females are below the amount necessary for maximum fecundity (Gwynne et al 1984, Gwynne 1984a; Boggs 1990), the effect of spermatophylax consumption on female reproductive output is examined here using females maintained on both normal and impoverished diets in *L.laticauda* and on impoverished diets only in *S.stali* and *S.asturiensis*. In this chapter, I also further analyse the difference in fecundity between singly and doubly-mated female *L.punctatissima* (see chapter 7, section 7.3.1).

8.2 *Methods.*

8.2.1 *Experiment 1: the effect of spermatophylax consumption on female fecundity in* L.laticauda *on both normal and restricted diets.*

Male *L.laticauda* produce a large spermatophylax which contributes to a mean loss of 23% (range: 11 - 33%) of male body weight at mating (see chapter 7). The origin and maintenance in captivity of the *L.laticauda* stocks used in this experiment are outlined in chapter 5. Newly adult females were housed individually in plastic sweet

jars with nylon mesh set into the lid for ventilation. These females were assigned at random to one of two experimental groups: in the "normal-diet" group, females were fed *ad libitum* with *Buddleia* leaves (n= 20 females), while in the "restricted-diet" group, females were provided with only one *Buddleia* leaf (approximately 8cm long) per week (n= 11 females - the sample size was initially larger though, unfortunately, six females in this category died prematurely). In both categories, the *Buddleia* leaves were placed in a vial of water to maintain freshness. A sample of eight females from each dietary group were weighed when newly adult and again when first showing signs of sexual receptivity (ie. showing response stridulation or attempting to mount a male). Pronotum length was measured (to the nearest 0.1 mm) for all females, using a pair of vernier callipers. On each day following the final moult, each female was placed in a mesh observation cage (measuring approximately 10cm x 10cm x 10cm) with a stridulating male and observed for 1 hour. If females mated, they were assigned to one of two further experimental groups: in the "plus-spermatophylax" group, females were allowed to consume fully the spermatophylax after mating (n= 15 females, including 10 "normal-diet" females and 5 "restricted-diet" females); in the "minus-spermatophylax" group, females were deterred from eating the spermatophylax by sprinkling it with sand (n= 16 females, including 10 "normal-diet" females and 6 "restricted-diet" females). This technique was taken from Reinhold & Heller (1993). Females with spermatophylaxes treated in this way invariably left them uneaten. At about 6 hours after spermatophore deposition, the entire spermatophore of females in the "minus-spermatophylax" category was removed with forceps. This time corresponds to the mean time taken for females to consume the spermatophylax and eat the ampulla (see chapter 5). Counts of the number of sperm remaining in the spermatophore after this time indicated that the "minus-spermatophylax" treatment did not interfere with sperm transfer. While, therefore, females in the "minus-spermatophylax" category received no spermatophore nutrients from the male, they presumably received the same

amount of ejaculate as females in the "plus spermatophylax" category.

Following mating, females were returned to their individual cages and were provided with a block of polyurethane foam each (measuring approx. 5cm x 5cm x 5cm) in which to lay eggs. Females were maintained on the same diet (normal or restricted diet) after mating as before mating. On each week following mating over a period of 4 weeks, the block of polyurethane foam from each female was removed and replaced with another. For each female, the number of eggs produced in each week following mating were recorded for a period of four weeks and a random sample of 12 eggs from each week were weighed to the nearest 0.01mg on a Cahn 25 electrobalance.

The effects of spermatophylax feeding and diet on the number of eggs laid over the four-week period and on mean egg weight were determined by 2-way analysis of variance, with spermatophylax feeding (plus or minus-spermatophylax) and diet (normal or restricted diets) as the main factors and female pronotum length as a co-variate.

8.2.2 *Experiment 2: the effect of spermatophylax consumption and double-mating on female fecundity in* L.punctatissima.

In the experiments presented in chapter seven, it was found that doubly-mated female *L.punctatissima* laid significantly more eggs over a 4 week period than singly-mated females. This difference could be due either to the effects of the extra spermatophylax material consumed by doubly-mated females or to the effects of the extra ejaculate received by these females (substances in the ejaculate which stimulate oviposition have been documented in a number of insects, see reviews of Leopold

1976; Chen 1984; chapter 1, section 1.1.2.c). Here, I attempt to examine separately the effects of the receipt of an extra ejaculate and the effects of the consumption of extra spermatophylax material on egg number and egg weight in *L.punctatissima*. The design of this experiment is a two-way analysis of variance, with spermatophylax feeding (plus or minus-spermatophylax) and number of matings (single or double matings) as the main factors.

The origin and maintenance in captivity of the *L.punctatissima* stocks used in this experiment are outlined in chapter 5. All females were mated at about ten days following the final moult. Individual females were placed in black nylon mesh observation cages (measuring approx. 10cm X 10cm X10cm), each containing a stridulating male, and were observed until mating had taken place. Females were then assigned at random to one of four experimental categories:

1) **Single mating, plus spermatophylax**: females were allowed a single mating and were left to consume the spermatophylax (n= 12 females).

2) **Single mating, minus spermatophylax**: females were prevented from consuming the spermatophylax following mating by placing them on a short stick (approx 15cm long) and either blowing on them or encouraging them to move by changing the orientation of the stick each time they attempted to bend double to take a bite out of the spermatophylax (n= 12 females). At about 43 mins after spermatophore deposition, the entire spermatophore was removed from females in this category, using watchmaker's forceps. This period of time corresponds to the mean spermatophore attachment time which results from spermatophylax consumption (see chapter 5). Counts of the number of sperm remaining in the spermatophore after this time indicated that the "minus-spermatophylax" treatment did not interfere with the process of sperm transfer.

3) **Double-mating, plus spermatophylax**: females were re-mated upon the termination of the first refractory period (generally less than 1 day) and were allowed to consume the spermatophylax in both matings (n= 12 females).

4) **Double-mating, minus spermatophylax**: females were re-mated upon the termination of the first refractory period and were prevented from consuming the spermatophylax (as in (2), above) in both matings (n= 8 females).

After mating, females were individually housed in mesh-topped plastic canisters and were each provided with a sprig of *Buddleia* leaves and a bramble (*Rubus* sp) leaf in a water-filled vial for food. This was replaced each week. Each female was also provided with five disks of polythene sheet positioned under the water container, as a medium for oviposition. Eggs were collected from each female at weekly intervals from the last mating over a period of four weeks. For each female, the number of eggs laid in the first week and the total number of eggs laid over the four-week period were recorded and a sample of 12 randomly-selected eggs were weighed to the nearest 0.01 mg on a Cahn 25 electrobalance.

The effects of spermatophylax feeding and the effects of double-mating on the dependent variables (mean egg weight, number of eggs laid in the first week, number of eggs laid in four weeks) were determined by two-way analysis of variance with spermatophylax consumption (plus or minus- spermatophylax) and number of matings (single or double- mating) as the main factors.

It should be noted that the "single-mating, plus-spermatophylax" and "double-mating, plus-spermatophylax" categories in this experiment incorporate females from

the sperm competition experiment presented in chapter 7, with the addition of a few additional replicates; these two experiments are not independent. The "single-mating, minus- spermatophylax" and the "double-mating, minus- spermatophylax" treatments were performed at the same time as the above-mentioned treatments and used individuals from the same experimental stock and lab. generation.

8.2.3 *Experiment 3: the effect of spermatophylax consumption on female reproductive output in* S.stali *and* S.asturiensis - *a preliminary experiment.*

S.stali and *S.asturiensis* both produce large spermatophylaxes, contributing to a mean loss of 27% of male body weight at mating (chapter 3, part 1). Individuals of both species used in these experiments were obtained from laboratory colonies derived from adults collected in Spain in 1985 and 1988 by Dr. J.C.Hartley. Rearing conditions for both species were based on those outlined by Hartley & Dean (1974) for another species of ephippigerine. Shortly after becoming adult, females of both species were maintained on a diet of *Buddleia* alone (as opposed to the usual lab. diet for these species which includes wheat-germ and various herbs). This probably represents a restricted diet for these omnivorous species. The *S.stali* females were maintained on the restricted diet after mating had occurred (ie. from about ten days after the final moult) while the *S.asturiensis* were maintained on this diet from directly after the final moult.

When sexually receptive, at about ten days following the final moult, individual females of each species were placed in mesh observation cages (measuring approx. 10cm x 10cm x 10cm) with individual stridulating males of the appropriate species and were observed until mating occurred. After mating, females were allocated to

one of two experimental categories: in one the female was allowed to eat the spermatophylax (n= 6 for *S.asturiensis*; n= 4 for *S.stali*); in the other, the spermatophylax was carefully cut from the sperm-ampulla directly after mating (n= 6 for *S.asturiensis*; n= 4 for *S.stali*). Because females in which the spermatophylax was removed had a tendency to eat the sperm-ampulla immediately, these females were placed in tubes for about 6 hours which were not wide enough to allow them to bend double and remove the ampulla. This time roughly corresponds to the mean ampulla attachment time which results from spermatophylax-feeding in these species (see chapter 7). After this treatment, females were uniquely marked with "Humbrol" paint on the pronotum and were individually housed in mesh-topped plastic canisters which were floored with sand (approx. 5cm deep) as a medium for oviposition. Sprigs of six small *Buddleia* leaves were placed in a vial of water in each cage as a food source. These were changed each week. Sand from each container was sieved on a daily basis up until the death of each female and any eggs present were counted and individually weighed to the nearest 0.01 mg on a Cahn 25 electrobalance.

One-way analysis of variance was used to examine the effect of spermatophylax consumption on egg weight, lifetime fecundity, the time interval between mating and oviposition, the rate of oviposition and female lifespan. The rate of oviposition was measured as the time taken to lay 33 eggs. This figure, although somewhat arbitrary, was chosen because it corresponds to half the mean number of ovarioles in both species. Means are cited $\pm$ standard error.

8.3 *Results.*

8.3.1 *Experiment 1: the effect of spermatophylax consumption and diet on fecundity in* L.laticauda.

There was no significant difference in the mean number of days taken from the final moult to the onset of sexual receptivity between normal and restricted-diet females (mean for normal- diet females = 10.3 $\pm$ 0.6 days, range 7-15 days, n= 16; mean for restricted-diet females = 10.3 $\pm$ 0.4 days, range 9-12 days, n= 7; 1-way ANOVA $F_{1,21}$ = 0.001, $p>0.05$). While there was no difference in mean body weight when first adult between normal and restricted-diet females (mean body weight for normal-diet females = 0.33 $\pm$ 0.01g, n= 8; mean body weight for restricted-diet females = 0.33 $\pm$ 0.17g, n= 7; 1-way ANOVA $F_{1,13}$= 0.0, $p>0.05$), females in the restricted diet category were significantly lighter than females in the normal diet category at the onset of sexual receptivity (mean body weight for restricted-diet females= 0.47 $\pm$ 0.03g, n=7; mean body weight for normal-diet females= 0.60 $\pm$ 0.03g, n= 8; 1-way ANOVA $F_{1,13}$= 8.9, p= 0.01).

Neither diet nor spermatophylax feeding were found to have a significant effect on egg weight (table 8.1). Diet was, however, found to have a significant positive effect on egg number, while spermatophylax feeding had no significant effect. There were no significant interactions (table 8.1). The co-variate female pronotum length was found to have no significant effect on either egg weight or egg number and was excluded from the final analysis.

Table 8.1 A) Analysis of the number of eggs produced, over a 4 week period, and the weight of eggs (mean weight of up to 48 eggs per female) with treatments (plus or minus spermatophylax; restricted or normal diets) in experiment 1.

Analysis of variance

Source of variation	Number of eggs			Egg weight		
	F	df	p	F	df	p
Spx. feeding	0.0	1	NS	0.2	1	NS
Diet	43.9	1	<0.001	0.3	1	NS
Interaction	0.08	1	NS	0.73	1	NS
Error		27			27	

Table 8.1 B) Mean number and weight of eggs (mean weight of up to 48 eggs per female, in mg) laid by females in each treatment (means are cited ± S.E.; numbers in brackets are the number of females in each treatment; spx=spermatophylax).

Diet	Mean no. eggs		Mean egg weight	
	+spx	-spx	+spx	-spx
Normal	99.9±6.3(10)	97.8±6.3(10)	2.17±0.05(10)	2.25±0.05(10)
Restricted	48.4±8.9(5)	50.5±8.1(6)	2.19±0.07(5)	2.17±0.06(6)

8.3.2 *Experiment 2: the effect of spermatophylax consumption and double-mating on fecundity in* L.punctatissima.

Two-way analysis of variance revealed that number of matings (single versus double-mating) had a significant positive effect on the number of eggs laid by females in the first week following mating and on the number of eggs laid over the four-week period, while spermatophylax feeding (minus versus plus-spermatophylax) had no significant effect on these variables (table 8.2). Neither spermatophylax feeding nor number of matings had a significant effect on mean egg weight. There were no significant interactions (table 8.2).

8.3.3 *Experiment 3: the effect of spermatophylax consumption on female reproductive output in* S.stali *and* S.asturiensis.

One-way analysis of variance revealed no significant difference between plus or minus-spermatophylax treatments in total egg production, egg weight, days from mating to oviposition or time taken to lay 33 eggs either in *S.stali* or in *S.asturiensis* (table 8.3). There was, however, a positive effect of spermatophylax consumption on female lifespan from mating in *S.stali*, but not in *S.asturiensis* (table 8.3).

Table 8.2: A) Analysis of the number of eggs produced in the first week following mating, number of eggs in 4 weeks and the weight of eggs (mean weight of 12 eggs per female) with treatments (plus or minus spermatophylax; single or double mating) in experiment 2.

Analysis of variance

Source of variation	Eggs in first week			Eggs in 4 weeks			Egg weight		
	F	df	p	F	df	p	F	df	p
Spermatophylax feeding	1.3	1	NS	0.4	1	NS	0.02	1	NS
Mating	21.9	1	<0.001	66.1	1	<0.001	0.04	1	NS
Interaction	2.5	1	NS	0.01	1	NS	0.12	1	NS
Error		39			29			27	

Table 8.2: B) Mean number of eggs laid in the first week, mean number of eggs laid in 4 weeks and mean egg weight (mean weight of 12 eggs per female, in mg) for females in each treatment (means are cited ± S.E.; numbers in brackets are the number of females in each treatment; spx = spermatophylax).

No. matings	Mean eggs in first week		Mean eggs in 4 weeks		Mean egg weight	
	+spx	-spx	+spx	-spx	+spx	-spx
Single	23.3±2.4(12)	22.2±2.5(11)	40.1±6.9(8)	36.4±7.4(7)	1.62±0.03(7)	1.61±0.03(6)
Double	31.2±2.4(12)	38.0±2.9(8)	98.0±5.7(12)	93.2±8(6)	1.61±0.02(12)	1.62±0.03(6)

Table 8.3. Means and analysis (analysis of variance) of the number of eggs produced, egg weight, time to lay 33 eggs and female lifespan from mating in experiment 3 with treatments (females prevented from eating the spermatophylax or not) in A) *S.asturiensis* and B) *S.stali* (means are cited ± S.E.; numbers in brackets are the number of females in each treatment; spx=spermatophylax).

	Treatment means ±S.E. (n)				
Variables	+spx	-spx	**F**	**df**	**p**
A) *S.asturiensis*					
Total egg number	71.5±11.7(6)	50.5±5.35(6)	2.68	1,10	NS
Egg weight (mg)	3.28±0.09(6)	3.22±0.15(6)	0.12	1,10	NS
Time from mating to oviposition (days)	9.66±2.84(5)	8.67±1.74(6)	0.09	1,9	NS
Time to lay 33 eggs (days)	21.2±6.11(5)	30.83±2.87(6)	2.29	1,9	NS
Female lifespan from mating (days)	37.33±4.27(6)	38.67±1.48(6)	0.09	1,10	NS
B) *S.stali*					
Total egg number	95.25±15.16(4)	93.0±15.13(4)	0.01	1,6	NS
Egg weight (mg)	3.90±0.18(4)	3.73±0.15(4)	0.53	1,6	NS
Time from mating to oviposition (days)	4.0±1.08(4)	5.75±2.5(4)	0.41	1,6	NS
Time to lay 33 eggs (days)	7.00±2.27(4)	11.25±5.92(4)	0.45	1,6	NS
Female lifespan from mating (days)	45±5.49(4)	30.5±1.9(4)	6.23	1,6	<0.05

8.4 *Discussion.*

8.4.1 *The effect of spermatophylax consumption on female reproductive output.*

No significant difference in either the number of eggs produced or the weight of these eggs was found between females allowed to consume the spermatophylax and females prevented from doing so either in *L.punctatissima* or in *L.laticauda*, even when, in the case of the latter species, females were maintained on a restricted diet. Preliminary experiments using *S.stali* and *S.asturiensis*, both using low-quality diets, also failed to reveal a significant effect of spermatophylax consumption on these variables. It should be noted, however, that the sample sizes used in experiments involving the latter two species were not particularly large. It is possible that with larger sample sizes a significant positive effect of spermatophylax consumption on the dependent variables might have been found in these species.

The spermatophylax produced by male *L.punctatissima* is relatively small (contributing to a mean loss of 5.6% of male body weight at mating, see chapter 7), therefore the paternal investment hypothesis would not necessarily predict a positive effect of spermatophylax-feeding on female reproductive output in this species (see Gwynne 1990a). The spermatophylaxes produced by male *L.laticauda*, *S.stali* and *S.asturiensis*, however, are all very large - often amounting to over thirty percent of male body weight in each species (pers. obs., see also chapter 7; chapter 2, table 2.1 and chapter 3). The paternal investment hypothesis would therefore predict a positive effect of spermatophylax consumption on female reproductive output in

these species (see Gwynne 1990a).

Preliminary studies of the bushcrickets *Poecilimon schmidtii* and *Ephippiger ephippiger* (spermatophores representing approx. 14% and 28% of male body weight, respectively, see chapter 2, table 2.1) have also failed to find a significant effect of spermatophylax-feeding on the number or weight of eggs produced (Derby 1990, unpublished honours project), as have studies of *P.veluchianus* (spermatophore 28% of male body weight; Reinhold & Heller 1993) and *Decticus verrucivorus* (spermatophore approx. 9% of male body weight; Wedell & Arak 1989) even when, in the latter species, females were maintained on low-quality diets.

Studies of the bushcricket *Requena verticalis*, which produces a spermatophylax amounting to approx. 12.5% (Gwynne 1990b) to 19% (Gwynne 1986b) of male body weight, have yielded conflicting results: Gwynne et al (1984) found no effect of spermatophylax feeding on egg weight or number in this species when females were maintained on "normal" diets, while Gwynne (1988a) found a positive effect of spermatophylax consumption on these variables in both restricted and normal-diet categories. A positive effect of spermatophylax consumption on egg weight and number has also been found in a zaprochiline bushcricket (spermatophore about 20% of male body weight; Simmons 1990a; Simmons & Bailey 1990) when females were maintained on a relatively poor food source. Overall, however, the generality of the phenomenon of an increase in egg weight and\or number as a result of spermatophylax-feeding in bushcrickets would seem to be in doubt.

There could, of course, be more subtle effects of spermatophylax-feeding on offspring fitness than an increase in egg weight. While, therefore, the failure to find a positive effect of spermatophylax-feeding on egg weight or number does not

provide support for the paternal investment hypothesis, it does not refute it. Furthermore, it is possible that the selective pressures responsible for the maintenance of the spermatophylax might differ in different species. In *R.verticalis* and the zaprochiline, for example, it appears the spermatophylax has the potential to function as paternal investment, in light of the positive effects of spermatophylax consumption on female reproductive output which have been demonstrated (Gwynne 1984a, 1988a; Simmons 1990a; Simmons & Bailey 1990) and the fact that the spermatophylax-donating male appears to be likely to fertilise a significant proportion of the eggs which benefit from his nutrients in these species (Gwynne 1988b; Simmons 1990a; Simmons & Gwynne 1993). It should be noted, however, that the spermatophylax also appears to function as a means of ensuring complete sperm\ejaculate transfer in these species (see Simmons & Gwynne 1991; Gwynne et al 1984; chapter 5; but see also Gwynne 1986b, who argues that the spermatophylax is larger than necessary to ensure complete sperm transfer in *R.verticalis*). The effects of spermatophylax feeding on egg weight and\or number might, therefore, be seen as incidental benefits to the male of the production of a spermatophylax which are achieved at no extra cost to the male.

In other species of bushcricket including *D.verrucivorus* (wedell 1993a), *Poecilimon veluchianus* and *P.affinis* (Heller & Helversen 1991; Achmann et al 1992; Reinhold & Heller 1993), *Metaplastes ornatus* (Helversen & Helversen 1991) and probably *Steropleurus stali* and *Leptophyes punctatissima* (chapter 7) evidence suggests that a male is unlikely to be able to fertilise a significant proportion of the eggs which might benefit from his spermatophylax nutrients in light of the female re-mating interval, pattern of oviposition and the pattern of sperm precedence. The spermatophylax in these species (three of which- *P.veluchianus*, *M.ornatus* and *S.stali*- produce very large spermatophylaxes, representing over 20% of male body weight, Heller & Helversen 1991; Helversen & Helversen 1991; Chapter 7) is

unlikely, therefore, to be maintained by selection for paternal investment.

It should be noted that here, I am primarily concerned with the possible benefits, in terms of an increase in egg weight or number, resulting from spermatophylax feeding from the perspective of the male which produced the spermatophylax. Experiments examining the female's perspective (ie. the benefits to females of multiple mating in terms of receiving male-donated nutrients) might offer females a wider range of spermatophylaxes than the experiments in this chapter (as in Gwynne 1984a and Gwynne 1988a) and might concentrate more on the possible benefits to a female from spermatophylax consumption in terms of reduced foraging activity (see Boggs 1990) and increased lifespan. Here, I did examine the effect of spermatophylax consumption on female lifespan in *S.asturiensis* and *S.stali*, when females were maintained on low-quality diets, and found a significant positive effect in *S.stali*. Recent studies of the crickets *Gryllodes sigillatus* and *Gryllus veletis* (Burpee & Sakaluk 1993), the butterfly *Pieris napi* (Wiklund et al 1993) and the beetle *Callosobruchus maculatus* (Fox 1993) have found that females given unlimited mating opportunities lived significantly longer than females given restricted mating opportunities (though only when females were maintained on a starvation diet in the case of *C.maculatus*). These studies propose that this difference in lifespan is due to the benefits accrued through the digestion of spermatophore nutrients obtained through multiple-mating. While an increase in female longevity through spermatophore consumption is clearly a benefit from the female's perspective, it is unlikely to constitute a benefit of spermatophore production for individual spermatophore-donating males in these polyandrous species.

8.4.2 *The effect of diet on female reproductive output.*

A negative effect of a restricted diet on the number of eggs laid, but not egg weight, was found in *L.laticauda*: females maintained on a restricted diet laid, on average, approximately half as many eggs over a period of four weeks as females receiving a normal diet. A similar decrease in the number of eggs produced with a decrease in diet quality has been found in other bushcrickets (Gwynne 1988a; Wedell & Arak 1989; Simmons & Gwynne 1993) and is well documented in insects in general (see reviews of Johansson 1964 and Engelmann 1970). In the zaprochiline bushcricket studied by Simmons & Gwynne (1993), the difference in fecundity between females maintained on high and low-quality diets appeared to be due to the fact that females on the low-quality diet allocated a greater proportion of the nutrients obtained from both the general diet and spermatophylax consumption to somatic maintenance rather than to reproduction (Simmons & Gwynne 1993).

8.4.3 *The effect of double-mating on female reproductive output.*

In *Leptophyes punctatissima*, doubly-mated females laid, on average, more than twice as many eggs over a four-week period than females allowed only a single mating. This difference appeared to be due to the extra ejaculate received by doubly-mated females (or to mechanical stimulation through mating itself) rather than the extra spermatophore material consumed by these females. An increase in fecundity as a result of multiple-mating is widespread in insects (Ridley 1988) and appears to occur equally in species in which males produce spermatophores or other "nuptial gifts" at mating and those in which males do not. This strongly suggests that some factor other than the use nutrients from the spermatophore or from other nuptial gifts is

responsible for the increase in fecundity in multiply mated females (although the use of nutrients in the ejaculate itself to increase fecundity cannot be ruled out on this basis).

The proximate (ie. causal) explanation for an increase in fecundity by doubly-mated female insects may be that it results from the receipt of greater quantities of oviposition stimulants in the ejaculate. The occurrence of such substances is well documented in crickets (see chapter 1, section 1.1.2.c) and other insects (see reviews of Leopold 1976 and Chen 1984). Why it should be in the female's interests to respond to these substances by an increase in the number of eggs laid is another question. Recent studies of adders suggest that females may benefit from receiving more than one ejaculate through an increase in offspring viability resulting from enhanced sperm-competition in the female's reproductive tract (Masden et al 1992). Alternatively, females of certain species may hold back eggs until they have received extra ejaculates simply because they receive insufficient viable sperm per ejaculate to fertilise a full complement of eggs (see Masden et al 1992; Fox 1993 for reviews of the benefits to females of multiple-mating and the receipt of extra ejaculates). In this context, it may be significant to note that while an increase in fecundity as a result of double-mating was found in *L.punctatissima*, no such difference was found in *L.laticauda* (see chapter 7). While the maximum number of eggs produced by females of both species are similar (see chapter 7), *L.laticauda* males transfer about 6 times more sperm, relative to male body weight, than *L.punctatissima* males (see chapter 5). Perhaps, then, *L.punctatissima* females are sperm-limited, while *L.laticauda* females are not.

The increase in the number of eggs laid as a result of double-matings per se (as opposed to extra spermatophore nutrients obtained from double-mating) demonstrated in this study and in the comparative study of Ridley (1988) highlights

the importance of controlling for the amount of ejaculate transferred in studies which aim to examine the effect of an increase in spermatophore (or other "nuptial gift") nutrients on female fecundity. A number of such studies have not controlled for the amount of ejaculate received (or, more precisely, for the potential amount of oviposition-stimulants received) and have nevertheless reported positive effects of male nuptial-gift nutrients on female fecundity. These include studies of the coleopteran *Melolontha melolontha* (Landa 1960), the orthopterans *Melanoplus* (Riegert 1965), *Chorthippus brunneus* (Butlin et al 1986), *Gryllodes sigillatus* and *Gryllus veletis* (Burpee & Sakaluk 1993), the dipterans *Drosophila pseudoobscura* (Turner & Anderson 1983) and *D.mojavensis* (Markow et al 1990) and the lepidopterans *Colias eurytheme* (Rutowski et al 1987), *Papilio xuthus* (Watanaabe 1988), *Danaus plexippus* (Oberhauser 1989) and *Pieris napi* (Wiklund et al 1993).

8.5 *Summary.*

The paternal investment hypothesis for the maintenance of the spermatophylax in bushcrickets proposes that a male benefits from the production of a spermatophylax through his mate using nutrients from the spermatophylax to increase the fitness and\or number of the male's own offspring. An increase in the weight and number of eggs laid as a result of spermatophylax feeding has been demonstrated in two species of bushcricket but has not been found in others. Here, I examine the difference in egg weight and number between females allowed to consume the spermatophylax and those prevented from doing so, with the degree of insemination held constant, in the bushcrickets *L.laticauda*, *L.punctatissima*, *S.stali* and *S.asturiensis*. Because the effects of male-derived nutrients on female fecundity are expected to be more pronounced when females are food-limited, experiments using *L.laticauda* were conducted on females maintained on both restricted and normal

diets while experiments on *S.stali* and *S.asturiensis* were conducted on females maintained on restricted diets only. I also further examine the difference in fecundity between singly and doubly mated female *L.punctatissima*; I attempt to examine separately the effects of the consumption of extra spermatophore material and the receipt of extra ejaculate from double matings on the number of eggs laid by comparing the mean fecundities of singly and doubly-mated females allowed to consume the spermatophore and singly and doubly-mated females prevented from consuming the spermatophore but receiving a full ejaculate in each case. No effect of spermatophylax consumption on egg weight or number was found in *S.stali*, *S.asturiensis*, *L.punctatissima* or *L.laticauda*, even, in the latter case, when females were maintained on a restricted diet. The restricted diet did, however, have a significant negative effect on the number of eggs laid in *L.laticauda*, but no effect on egg weight. A positive effect of spermatophylax consumption on female lifespan from mating was found in *S.stali*. The failure to find a significant positive effect of spermatophylax consumption on female reproductive output in the above species casts doubt as to the generality of the phenomenon of an increase in egg weight and number as a result of spermatophylax consumption in bushcrickets. While the results do not support the paternal investment hypothesis, they do not refute it because spermatophylax feeding might have more subtle effects on offspring fitness than an increase in egg weight. In *L.punctatissima*, doubly-mated females were found to lay over twice as many eggs over a four week period as singly-mated females. This difference appeared to be due to the receipt of extra ejaculate from the double matings as opposed to the consumption of extra spermatophore material: mating (single v double-mating) was found to have a significant positive effect on the number of eggs laid, while spermatophore feeding (plus v minus-spermatophore) was not. This highlights the importance of controlling for the amount of ejaculate received in studies which aim to examine the effects of spermatophore nutrients on female fecundity.

Conclusions.

Data suggest that the spermatophylax originated as an adaptation to protect the ejaculate, functioning to prevent the female from eating the ampulla before complete ejaculate transfer (chapter 2 & 3; Boldyrev 1915; Gerhardt 1913, 1914; Gwynne 1990a): the spermatophylax appears to be analogous to a range of adaptations found in males of the sub-order Ensifera, which may be interpreted as functioning to counteract the tendency of females to eat the ampulla prematurely. These adaptations include prolonged copulation following spermatophore transfer, feeding the female with glandular secretions following spermatophore transfer, post-copulatory mate-guarding and multiple copulations with the same female (chapter 2; Boldyrev 1915; Alexander & Otte 1967a). The occurrence of prolonged copulation following spermatophore transfer appears to be associated with the total loss of the spermatophylax in the meconematine bushcricket *Meconema* and with the considerable reduction in spermatophylax size in the ephippigerine bushcricket *Uromenus rugiscollis* (chapter 3). This supports the hypothesis that prolonged copulation and the spermatophylax are analogous in function.

The subsequent evolutionary enlargement of the spermatophylax appears to have accompanied the evolutionary enlargement of ejaculate volume and sperm number, i.e. appears to have proceeded to facilitate the transfer of larger ejaculates (chapter 4; Wedell, in press). A comparative study of 43 species of bushcricket revealed a positive relationship, across taxa, between evolutionary changes in spermatophylax size and changes in ampulla size (i.e. ejaculate volume) and sperm number, with male body weight controlled for (chapter 4; see also Wedell, in press, who also found a positive relationship between spermatophylax mass and ampulla mass across taxa in an independent comparative study of bushcrickets).

The current function of the large spermatophylax appears to be the same as that of the small spermatophylax, i.e. to ensure complete sperm \ ejaculate transfer. No significant difference in the shape of the sperm transfer curve relative to the mean duration of spermatophylax consumption was found between *Leptophyes punctatissima* (small spermatophylax) and *L.laticauda* (large spermatophylax) or between *L.punctatissima* and *Requena verticalis* (medium-large spermatophylax) (chapter 5). Furthermore, in *L.laticauda*, males appear to adjust the size of the spermatophylax in relation to the amount of sperm or volume of ejaculate they are able to produce: a positive relationship was found between spermatophylax mass and sperm number and between spermatophylax mass and ampulla mass (i.e. ejaculate volume) (chapter 6). Other studies of crickets and bushcrickets have also suggested that the spermatophylax functions to ensure complete sperm transfer. These include studies of species with small spermatophylaxes (*Gryllodes supplicans*: Sakaluk 1984; *Decticus verrucivorus*: Wedell & Arak 1989; Wedell 1991) and those with relatively large spermatophylaxes (*Poecilimon veluchianus*: Reinhold & Heller 1993; a zaprochiline bushcricket: Simmons & Gwynne 1991; but see Gwynne et al 1984 and Gwynne 1986b, who argued that the spermatophylax of *R.verticalis* is larger than is necessary to ensure complete sperm and\or ejaculate transfer).

The possibility that the spermatophylax additionally functions as paternal investment cannot, however, be ruled out on this basis. In order for male-donated nutrients to function as paternal investment they must 1) have a positive effect on offspring fitness and\or number and 2) the nutrient-donating male must stand to fertilise most or all of the offspring which benefit from his nutrients (see Simmons & Parker 1989). A positive effect of spermatophylax consumption on egg weight and\or number has previously been documented in some species of bushcricket (in a zaprochiline bushcricket: Simmons 1990a; in *R.verticalis*: Gwynne 1984a, 1988a,

but see Gwynne et al 1984, who found no effect in this species) though has not been found in others (*D.verrucivorus*: Wedell & Arak 1989; *Poecilimon veluchianus*: Reinhold & Heller 1993). In this study, no effect of spermatophylax consumption on female reproductive output was found in *L.punctatissima*, *L.laticauda*, or *Steropleurus*, even when, in the latter two cases, females were maintained on a restricted diet.

In the bushcrickets *R.verticalis* and a zaprochiline, the possibility appears to exist that the spermatophylax functions secondarily as a form of paternal investment. This is because the male appears to stand a good chance of fertilising a significant proportion of the eggs which benefit from his spermatophylax nutrients (Gwynne 1988b; Simmons 1990a; Simmons & Gwynne 1993). However, in *L.punctatissima* and *Steropleurus stali* (though not in *L.laticauda*) it appears that the spermatophylax-donating male is unlikely to fertilise eggs in which his nutrients might be incorporated, in light of the short female re-mating interval, the pattern of last-male sperm precedence and the pattern of oviposition. The enormous spermatophylax of *S.stali* is therefore unlikely to function as paternal investment. Recent studies suggest that in a number of other bushcricket species (*D.verrucivorus*: Wedell 1993a; *Poecilimon veluchianus* and *P.affinis*: Heller & Helversen 1991; Achmann et al 1992; Reinhold & Heller 1993; *Metaplastes ornatus*: Helversen & Helversen 1991), including some with very large spermatophylaxes (*P.veluchianus* and *M.ornatus*), the spermatophylax is also unlikely to function as paternal investment, for the above reasons. In conclusion, while the paternal investment hypothesis lacks generality, the ejaculate-protection hypothesis seems to be more widely applicable and appears to successfully account for the origin, evolutionary enlargement and current function of the spermatophylax in bushcrickets.

References

Achmann R, Heller K G, Epplen J T (1992) Last-male sperm precedence in the bushcricket *Poecilimon veluchianus* (Orthoptera, Tettigonioidea) demonstrated by DNA fingerprinting. Mol Ecol **1**: 47-54.

Ander K (1939) Comparative anatomical and phylogenetic studies on the Ensifera (Saltatoria). Opusc Entomol Suppl 2.

Alcock J (1981a) Notes on the reproductive behaviour of some Australian thynnine wasps (Hymenoptera: Tiphiidae). J Kansas Entomol Soc **54**: 681-693.

Alcock J (1981b) Seduction on the wing. Nat Hist **90**: 36-41.

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

Alexander R D, Borgia G (1979) On the origin and basis of the male-female phenomenon. In Blum M S, Blum N A (eds),**Sexual Selection and Reproductive Competition in the Insects**. Academic press, New York, pp 417-440.

Alexander R D, Brown W L (1963) Mating behaviour and the origin of insect wings. Misc Pub Mus Zool Univ Michigan **628**: 1-19.

Alexander R D, Otte D (1967a) The evolution of genitalia and mating behaviour in crickets (Gryllidae) and other orthoptera. Misc Pub Mus Zool Univ Michigan **133**: 1-62.

Alexander R D, Otte D (1967b) Cannibalism during copulation in the brown bush cricket, *Hapithus agitator* (Gryllidae). Fla Ent **50** (2): 79-87.

Austad S N (1982) First male sperm priority in the bowl and doily spider, *Frontinella pyramitela* (Walckenaar). Evolution **36**: 777-785.

Austad S N (1984) Evolution of sperm priority patterns in spiders. In: Smith R L (ed), **Sperm Competition and the Evolution of Animal Mating Systems**. Academic Press, Orlando pp 223-249.

Backus V L, Cade W H (1986) Sperm competition in the field cricket *Gryllus integer* (Orthoptera: Gryllidae). Fla Ent **69** (4):722-727.

Baker R R, Bellis M A (1989) Number of sperm in human ejaculates varies in accordance with sperm competition theory. Anim Behav **37**: 867-869.

Barrett P (1991) **Keeping Wetas in Captivity**. Wellington Zoological Gardens, New Zealand.

Baumann H (1974) Biological effects of paragonial substances PS_1 and PS_2 in female *Drosophila funebris*. J Insect Physiol **20**: 2347-2363.

Baumgartner (1910) Observations on the Gryllidae IV: copulation. Kansas Univ Sci Bull **5**: 323-345.

Bei-Bienko G Ya (1965) **Fauna of the U.S.S.R.: Orthoptera vol.2 no.2; Tettigonioidea; Phaneropterinae**. Israel programme for scientific translations, Jerusalem.

Bell P D (1979) Mate choice and mating behaviour in the black horned tree cricket, *Oecanthus nigricornis* (Walker). (M.Sc. thesis). University of Toronto, Toronto.

Bell P D (1980) Multimodal communication in the black-horned tree cricket. *Oecanthus nigricornis* (Walker) (Orthoptera: Gryllidae). Can J Zool **58**: 1861-1868.

Bellis M A, Baker R R, Gage M J G (1990) Variation in rat ejaculates is consistent with the kamikaze sperm hypothesis. J Mamm **71**: 479-480.

Bellman H (1988) **A Field Guide to the Grasshoppers and Crickets of Britain and Northern Europe**. Collins, London.

Bentur J S, Dakshayani K, Mathad S B (1977) Mating induced oviposition and egg production in the crickets *Gryllus bimaculatus* De Geer and *Plebiogryllus guttiventris* Walker. Z Angew Entomol **84**: 129-135.

Berenguier P (1907) Biologie de l'*Isophya pyrenaea* Serville, variete nemausensis (nov). Bull Soc Etude Sc Nat 1907: 1-13.

Bidochka M J, Snedden W A (1985) Effect of nuptial feeding on the mating behaviour of female ground crickets. Can J Zool **63**: 207-208.

Beier M (1955) Ordnung Saltatoptera (Saltatoria, Latreille 1817). Bronn's Kl Ordn Tierriechs. **6**: 34-304.

Birkhead T R, Hunter F M (1990) Mechanisms of sperm competition. Trends Ecol Evol **5**(2): 48-51.

Birkhead T R, Moller A P (1993) Female control of paternity. Trends Ecol Evol **8** (3): 100-104.

Bleton A (1942) Notes sur la biologie, au Maroc, d'*Eugaster spinulosus* Joh (Orthoptere, Tettigoniidae). Extr Bull Soc Nat Maroc **22**: 89-95.

Boak C R B (1984) Natural history and acoustic behaviour of a gregarious cricket. Behaviour **89**: 241-250.

Boggs C L (1981) Selection pressures affecting male nutrient investment at mating in heliconiine butterflies. Evolution **35** (5): 931-940.

Boggs C L (1990) A general model of the role of male-donated nutrients in female insects' reproduction. Am Nat **136**: 598-617.

Boggs C L, Gilbert L E (1979) Male contribution to egg production in butterflies: first evidence for transfer of nutrients at mating. Science **206**: 83-84.

Boggs C L, Watt W B (1981) Population structure of Pierid butterflies IV: genetic and physiological investment in offspring by male *Colias*. Oecologia **50**: 320-324.

Boldyrev B Th (1912) Begattung und spermatophoren bei *Tachycines asynamorus* Adel. Rev Russe d'Entomol **12**: 552-570.

Boldyrev B Th (1913) Die begattung und der spermatoporenbau bei der maulwarfsgrille (*gryllotalpa gryllotalpa* L). Zool Anz **42**: 592-605.

Boldyrev B Th (1915) Contributions a l'etude de la structure des spermatophores et des particularites de la copulation chez Locustidae et Gryllodea. Horae Soc Entom Ross **41**(6):1-245.

Boldyrev B Th (1927) Copulation and spermatophores of *Gryllomorpha dalmatina* (Ocsk) (Orthoptera: Gryllidae). Eos **3**: 279-288.

Boldyrev B Th (1928a) Einige episoden aus dem geschlechtsleben von *Discoptila fragosoi* Bol. Rev Russe Entomol (Moscow) **22**: 137-147.

Boldyrev B Th (1928b) Biological studies on *Bradyporus multituberculatus* F.W.(Orth. Tettig.). Eos **4**: 13-56.

Boorman E B, Parker G A (1976) Sperm (ejaculate) competition in *Drosophila melanogaster* and the reproductive value of females to males in relation to female age and mating status. Ecol Ent **1**: 145-155.

Bornemissza G F (1964) Sex attractant of male scorpion flies. Nature **203**: 786-7.

Boucher L, Huignard J (1987) Transfer of secretions from the spermatophore to the female insect in *Caryedon serratus*: analysis of the possible trophic role of these secretions. J Insect Physiol **33**: 949-957.

Boudou-Saltet P, Capolongo D (1975) Le spermatophore chez quelques especes de *Dolichopoda* (Orthoptera: Rhaphidophoridae) de Grece et d'Italie. Biologia Gallo- Hellenica **6** (1): 3-8.

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

Bownes M, Partridge L (1987) Transfer of molecules from ejaculate to females in *Drosophila melanogaster* and *D.pseudoobscura*. J Insect Physiol **33**: 941-947.

Breed M D (1983) Cockroach mating systems. In: Gwynne D T, Morris G K (eds), **Orthopteran Mating Systems: sexual competition in a diverse group of insects**. Westview Press, Colorado, pp 268-284.

Brown K S (1984) Adult obtained pyrrolizidine alkaloids defend ithomiine butterflies against a spider predator. Nature **309**: 707-709.

Brower J H (1975) Sperm precedence in the Indian meal moth *Plodia interpunctella*. Ann Ent Soc Amer **68**: 78-80.

Burpee D M, Sakaluk S K (1993) Repeated matings offset costs of reproduction in female crickets. Evol Ecol **7**: 240-250.

Burr M, Campbell B P, Uvarov B P (1923) A contribution to our knowledge of the orthoptera of Macedonia. Trans Ent Soc Lond 1923: 110-169.

Buskirk R E, Frohlich C, Ross K G (1984) The natural selection of sexual cannibalism. Am Nat **123**: 612-625.

Busnel R-G, Dumortier B (1955) Etude du cycle genital du male d'ephippiger et son rapport avec le comportement acoustique. Bull Soc Zool Paris **80**: 23-26.

Busnel R-G, Dumortier B, Busnel M-C (1956) Recherches sur le comportement acoustique des ephippigeres (Orthopteres, Tettigoniidae). Bull Biol Fr Belg **90**: 219-286.

Butlin R K, Woodhatch C W, Hewitt G M (1987) Male spermatophore investment increases female fecundity in a grasshopper. Evolution **41**: 221-225.

Campan M, Demai F (1983) Le comportement sexuel de *Nemobius sylvestris* (Orthoptera: Gryllidae). Biol Behav **3**: 185-204.

Carayon J (1964) Un cas d'offrande nuptiale chez les Heteropteres. C R Acad Sci Paris **259**: 4815-4818.

Clutton-Brock T H, Guiness F E, Albon S D (1982) **Red Deer: behaviour and ecology of two sexes**. Chicago University Press, Chicago.

Chao W Y (1981) The distribution, translocation and function of semen in the female reproductive system of the army moth (*Leucania separata*). Acta Entomol Sin **24**: 135-141.

Chen P S (1984) The functional morphology and biochemistry of insect male accessory glands and their secretions. Ann Rev Entomol **29**: 233-255.

Chopard L (1951) **Faune de France 56: Orthopteroides**. Lechevalier, Paris.

Daly M (1978) The cost of mating. Am Nat **112**: 771-774.

Dambach M, Lichtenstein L (1978) Zur ethologie der Africanischen grille *Phaeophilacris spectrum* Saussure. Z Tierpsychol **46**: 14-29.

Davey K G (1965) **Reproduction in the Insects**. Oliver & Boyd, Edinburgh & London.

David K (1936) Beitrage zur anatomie und lebensgeschichte von *Osmylus chrysops* L. Z Morph Okol Tier **31**: 151-206.

Davies P M, Dadour I R (1989) A cost of mating by male *Requena verticalis* (Orthoptera: Tettigoniidae). Ecol Ent **14**: 467-469.

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

Deura K, Hartley J C (1990) Egg laying behaviour of the bushcricket *Leptophyes punctatissima*. The Entomologist. **109** (2): 100-105.

Derby E (1990) The spermatophore, its importance in mating and female fecundity in the bushcrickets *Poecilimon schmidti* and *Ephippiger ephippiger*. Unpublished honours project, Department of life sciences, university of Nottingham, U.K.

Destephano D B, Brady V E (1977) Prostaglandin and prostaglandin synthetase in the cricket *Acheta domesticus*. J Insect Physiol **23**: 905-911.

Destephano D B, Brady V E, Lovins R E (1974) Synthesis of prostaglandin by reproductive tissue of the house cricket *Acheta domesticus*. Prostaglandins **6**: 71-79.

Destephano D B, Brady V E, Woodall L B (1976) Partial characterisation of prostaglandin synthetase in the reproductive tract of the male house cricket *Acheta domesticus*. Prostaglandins **11**: 261-273.

Dewsbury D A (1982) Ejaculate cost and male choice. Am Nat **119**: 601-610.

Dewsbury D A (1984) Sperm competition in muroid rodents. In: Smith R L (ed) **Sperm Competition and the Evolution of Animal Mating Systems.** Academic press, Orlando, pp 547-571.

Dickinson J L (1986) Prolonged mating in the milkweed leaf beetle *Labidomera clivicollis clivicollis* (coleoptera: Chrysomelidae): a test of the "sperm-loading" hypothesis. Behav Ecol Sociobiol **18**: 331-338.

Dickinson J L (1988) Determinants of paternity in the milkweed leaf beetle. Behav Ecol Sociobiol **23**: 9-19.

Dodson G N, Morris G K, Gwynne D T (1983) Mating behaviour of the primitive orthopteran genus *Cyphoderris* (Haglidae). In Gwynne D T, Morris G K (eds), **Orthopteran Mating Systems: sexual competition in a diverse group of insects**. Westview press, Boulder, colorado, pp 305-318.

Downes J A (1970) The feeding and mating behaviour of the specialised Empidinae (Diptera): observations on four species of *Rhanphomyia* in the high arctic and a general discussion. Can Ent **102**: 769-791.

Downes J A (1978) Feeding and mating in the insectivorous Ceratopogoninae (Diptera). Mem Ent Soc Can no. **104**

Drummond B A II (1984) Multiple mating and sperm competition in the Lepidoptera. In Smith R L (ed), **Sperm Competition and the Evolution of Animal Mating Systems**. Academic Press, Orlando.

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

Dussourd D E, Harvis C A, Meinwald J, Eisner T (1991). Pheromonal advertisement of a nuptial gift by a male moth (*Utetheisa ornatrix*). Proc Natl Acad Sci USA **88**: 9224-9227.

Eades D C (1964) General biology and geographic variation of *Ceuthophilus guttulosus* Walker (Orthoptera: Gryllacrididae: Rhaphidophorinae). Trans Amer Ent Soc **90**: 73-110.

Eberhard W G (1990) Inadvertant machismo? Trends Ecol Evol **5** (8): 263.

Edmunds M (1975) Courtship, mating and possible sex pheromones in 3 species of Mantodea. Ent Mon Mag **111**: 53-57.

Edwards F W (1920) Some records of predacious Ceratopogoninae (Diptera). Ent Mon Mag **56**: 203.

Eisner T, Meinwald J (1987) Alkaloid-derived pheromones and sexual selection in Lepidoptera. In: Prestwich G D, Blomquist G J (eds), **Pheromone Biochemistry**. Acad Press, London, pp 251-269.

Eluwa M C (1979) Observations on the spermatophore and post-copulatory behaviour of 3 west-African bush-crickets (Orthoptera; Tettigoniidae). Ent Mon Mag **114**: 1-7.

Engebreston J A, Mason W H (1980) Transfer of ^{65}Zn at mating in *Heliothis viriscens*. Environ Entomol **9**: 119-121.

Engelhardt V (1915) On the structure of the alluring gland in *Isophya acuminata* Br-W. Bull Soc Entomol Moscow **1**: 58-63.

Engelmann F (1970) **The Physiology of Insect Reproduction**. Pergamon Press, Oxford.

Erlich A H, Erlich P R (1978) Reproductive strategies in butterflies I: mating frequency, plugging and egg number.J Kansas Entomol Soc **51**: 666-697.

Etman A A M, Hooper G H S (1979) Sperm precedence of the last mating in *Spodoptera litura*. Ann Entomol Soc Am **72**: 119-120.

Evans A R (1988) Mating systems and reproductive strategies in three Australian gryllid crickets: *Bobilla victoriae* (Otte), *Balamara gidya* (Otte) and *Teleogryllus commodus* (Walker) (Orthoptera: Gryllidae: Nemobiinae; Trigonidiinae; Gryllinae.). Ethology **78**: 21-52.

Fabré J H (1899) **Souvenirs Entomologiques**, vol 6. DeLagrave, Paris. (Translated into English in: **The Life of the Grasshopper**. The Works of J.H.Fabré. Hodder & Stoughton, London 1917).

Faever M (1983) Pair formation in the katydid *Orchelimum nigripes* (Orthoptera: Tettigoniidae). In: Gwynne D T & Morris G K (eds) **Orthopteran Mating Systems: sexual competition in a diverse group of insects**. Westview press, Boulder, Colorado, pp 205-239.

Felsenstein J (1985) Phylogenies and the comparative method. Am Nat **125**: 1-15.

Field L H, Sandlant G (1983) Aggression and mating behaviour in the Stenopelmatidae (Orthoptera: Ensifera) with reference to New Zealand Wetas. In: Gwynne D T, Morris G K (eds) **Orthopteran Mating Systems: sexual competition in a diverse group of insects**. Westview press, Boulder, Colorado, pp 120-146.

Fitzpatrick S M, McNeil J N (1989) Lifetime mating potential and reproductive success in males of the true armyworm *Pseudaletia unipuncta* (Haw) (Lepidoptera: Noctuidae). Funct Ecol **3**: 37-44.

Fosberg J, Wiklund C (1989) Mating in the afternoon: time-saving in courtship and remating by females of the polyandrous butterfly *Pieris napi* L. Behav Ecol Sociobiol **25**: 349-356.

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

Friedel T, Gillot C (1976) Male accessory gland substance of *Melanoplus sanguinipes*: an oviposition stimulant under the control of the corpus allatum. J Insect Physiol **22**: 489-495.

Friedel T, Gillot C (1977) Contribution of male-produced proteins to vitellogenesis in *Melanoplus sanguinipes*. J Insect Physiol **23**: 145-151.

Fulton B B (1915) The tree crickets of New York: life history and bionomics. N Y Agric Exp Sta Tech Bull **42**: 3-47.

Fulton B B (1931) A study of the genus *Nemobius* (Orthoptera: Gryllidae). Ann Entomol Soc Am **24**: 205-237.

Funk D H (1989) The mating of tree crickets. Sci Amer August: 42-49.

Gabutt P D (1954) Notes on the mating behaviour of *Nemobius sylvestris*. Anim Behav **2**: 84-88.

Gage A (1993) Unpublished Honours thesis, Dept Life Science, University of Nottingham, U.K.

Gage M J G (1991) Risk of sperm competition directly affects ejaculate size in the mediterranean fruit fly. Anim Behav **42**: 1036-1037.

Gage M J G, Baker R R (1991) Ejaculate size varies with socio-sexual situation in an insect. Ecol Ent **16**: 331-337.

Galliart P L, Shaw K C (1991) Role of weight and acoustic parameters, including nature of chorusing, in the mating success of males in the katydid *Amblycorypha parvipennis* (Orthoptera: Tettigoniidae). Fla Ent **74**: 453-464.

Gangwere S K (1961) A monograph on food selection in Orthoptera. Trans Amer Ent Soc **87**: 67-225.

Gangwere S K, Morales Agacino E (1973) Food selection and feeding behaviour in Iberian Orthopteroidea. An INIA, Ser Prot Veg **3** (1973): 251-343.

Gelin H (1908) Sur la rusticite du *Cyrtaspis scutata* Charp. (orth). Bull Soc Ent Fr, 1908 pp 293-294.

Gerhardt U (1913) Copulation und spermatophoren von Grylliden und Locustiden. Zool Jb Syst **35**: 416-532.

Gerhardt U (1914) Copulation und spermatophoren von Grylliden und Locustiden 2. Zool Jb Syst **37**: 1-64.

Gerhardt U (1921) Neue studien uber copulation und spermatophoren von Grylliden und Locustiden. Acta Zool **11**: 293-327.

Ginsberg J R, Huck U W (1989) Sperm competition in Mammals. Trends Ecol Evol **4** (3): 74-79.

Ginsberg J R, Rubenstein D I (1990) Sperm competition and variation in zebra mating behaviour. Behav Ecol Sociobiol **26**: 427-434.

Given B (1954) Evolutionary trends in the Thynninae (Hymenoptera: Tiphiidae) with special reference to feeding habits of Australian species. Trans R Ent Soc Lond **105**: 1-10.

Goetghebuer M (1914) Notes a propos de l'accouplement de *Johannsenomyia nitida* Mc Q. Ann Soc Ent Belg **58**: 202.

Gomendio M, Roldan E R S (1993) Mechanisms of sperm competition: linking physiology and behavioural ecology. Trends Ecol Evol **8** (3): 95-100.

Gorochov A V (1988) (in Russian) The classification and phylogeny of grasshoppers (Gryllida = Orthoptera, Tettigonioidea). In Pomerenko A (ed) **The Cretaceous Biocoenotic Crisis and the Evolution of Insects**. " Hayka", Moscow, pp 145-190.

Greenfield M D (1982) The question of paternal investment in Lepidoptera: male contributed proteins in *Plodia interpunctella*. J Invert Reprod **5**: 323-330.

Grzeschik K H (1969) On the systematics, biology and ethology of *Eugaster* Serv. Form et Functio **1**: 46-110.

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

Gwynne D T (1982) Mate selection by female katydids (Orthoptera: Tettigoniidae; *Conocephalus nigropleurum*). Anim Behav **30**: 734-738.

Gwynne D T (1983a) Male nutritional investment and the evolution of sexual differences in the Tettigoniidae and other Orthoptera. In: Gwynne D T, Morris G K (eds) **Orthopteran Mating Systems: sexual competition in a diverse group of insects**. Westview press, Boulder Colorado, pp 337-366.

Gwynne D T (1983b) Coy conquistadors of the sagebrush. Nat Hist **92**: 70-75.

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

Gwynne D T (1984b) Male mating effort, confidence of paternity and insect sperm competition. In: Smith R L (ed) **Sperm Competition and the Evolution of Animal Mating Systems**. Academic press, Orlando, pp 117-149.

Gwynne D T (1984c) Sexual selection and sexual differences in mormon crickets (Orthoptera: Tettigoniidae, *Anabrus simplex*). Evolution **38**: 1011-1022.

Gwynne D T (1985) Role reversal in katydids: habitat influences reproductive behaviour (Orthoptera: Tettigoniidae, *Metaballus* sp). Behav Ecol Sociobiol **16**: 355-361.

Gwynne D T (1986a) Reply to stepfathers in insects and their pseudo-parental investment. Ethology **71**: 74-77.

Gwynne D T (1986b) Courtship feeding in katydids: investment in offspring or in obtaining fertilisations? Am Nat **128**: 342-352.

Gwynne D T (1987) Mating behaviour of *Neoconocephalus ensiger* (Orthoptera: Tettigoniidae) with notes on the calling song. Can Ent **109**: 237-242.

Gwynne D T (1988a) Courtship feeding and the fitness of female katydids. Evolution **42**: 545-555.

Gwynne D T (1988b) Courtship feeding in katydids benefits the mating male's offspring. Behav Ecol Sociobiol **23**: 373- 377.

Gwynne D T (1989) Does copulation increase the risk of predation? Trends Ecol Evol **4**: 54-56.

Gwynne D T (1990a) The katydid spermatophore: evolution of a parental investment. In: Bailey W J, Rentz D F C (eds) **The Tettigoniidae: biology, systematics and evolution**. Crawford House Press, Bathurst, pp 27-40.

Gwynne D T (1990b) Testing parental investment and the control of sexual selection in Katydids: the operational sex ratio. Am Nat **136**: 474-484.

Gwynne D T (1991) Sexual competition among females: what causes courtship role reversal? Trends Ecol Evol **4**: 118-121.

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). Aust J Zool **32**: 15-22.

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

Halliday T R, Arnold S J (1987) Multiple mating by females: a perspective from quantitative genetics. Anim Behav **35**: 939-941.

Hancock J L (1905) The habits of the striped meadow cricket (*Oecanthus fasciatus* Fitch). Am Nat **39**: 1-11.

Harcourt A H, Harvey P H, Larson S G, Short R V (1981) Testis weight, body weight and breeding system in primates. Nature **293**: 55-57.

Hartley J C (1990) Egg biology of the Tettigoniidae. In: Bailey W J and Rentz D C F (eds.) **The Tettigoniidae: biology, systematics and evolution**. Crawford House Press, Bathurst, pp 41-70.

Hartley J C (1991) The use of sound by *Leptophyes Laticauda*. Metaleptia **13** (1): 4-6.

Hartley J C, Dean R L (1974) *Ephippiger cruciger* as a laboratory insect (Orthoptera: Tettigoniidae). J Nat Hist **8**: 349-354.

Hartley J C, Robinson D J (1976) Acoustic behaviour in both sexes of the speckled bush-cricket *Leptophyes punctatissima*. Physiol Ent **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). Anim Behav **22**: 382-389.

Hartley J C, Warne A C (1972) The developmental biology of the egg stage of the Western European Tettigoniidae (Orthoptera). J Zool, Lond **168**: 267-298.

Hartley J C, Warne A C (1984) Taxonomy of the *Ephippiger ephippiger* complex (*ephippiger*, *cruciger* and *cunii*) with special reference to the mechanics of copulation. Eos **60**: 43-54.

Harvey P H, Harcourt A H (1984) Sperm competition, testes size and breeding system in primates. In: Smith R L (ed) **Sperm Competition and the Evolution of Animal Mating Systems**. Academic Press, Orlando, pp 589-600.

Harvey P H, May R M (1989) Out for the sperm count. Nature **337**: 508-509.

Harvey P H, Pagel M D (1991) **The Comparative Method in Evolutionary Biology**. Oxford University Press, Oxford.

Harvey P H, Purvis A (1991) Comparative methods for explaining adaptations. Nature **351**: 619-624.

Harz K (1969) **Die Orthopteren Europas**, Vol 1. W.Junk B V Publ, The Hague.

Hayashi F (1992) Large spermatophore production and consumption in dobsonflies *Protohermes* (Megaloptera: Corydalidae). Jpn J Ent **60**: 59-66.

Hayashi F (1993) Male mating costs in two insect species (*Protohermes*, Megalpotera) that produce large spermatophores. Anim Behav **45**: 343-349.

He Y (1992) Spermatophore size and its effects on male and female reproduction in the armyworm *Pseudaletia separata*. Proc XIX Int Cong Entomol, Abstracts.

He Y, Tsubaki Y (1991) Effects of spermatophore size on female re-mating in the armyworm, *Pseudaletia separata*, with reference to larval crowding. J Ethol **9**: 47-50.

He Y, Tsubaki Y (1992) Variation in spermatophore size in the armyworm, *Pseudaletia separata* (Lepidoptera: Noctuidae) in relation to rearing density. Appl Entomol Zool **27** (1): 39-45.

Heller K G (1984) Zur bioakustik und phylogenie der gattung *Poecilimon* (Orthoptera, Tettigoniidae, Phaneropterinae). Zool Jb Syst **111**: 69-117.

Heller K G (1988) **Bioakustik der Europaischen laubheuschrecken**. Verlag Josef Magraf, Weihersheim.

Heller K G (1990) Evolution of song pattern in East-Mediterranean Phaneropterinae: constraints by the communication system. In Bailey W J, Rentz D C F (eds) **The Tettigoniidae: biology, systematics and evolution.** Crawford House Press, Bathurst, pp 130-151.

Heller K G, von Helversen D (1991) Operational sex ratio and individual mating frequencies in two bushcricket species (Orthoptera, Tettigonioidea, *Poecilimon*). Ethology **89**: 211-228.

von Helversen D, von Helversen O (1991) Pre-mating sperm removal in the bushcricket *Metaplastes ornatus* Ramme 1931 (Orthoptera: Tettigonioidea, Phaneropteridae). Behav Ecol Sociobiol **28**: 391-396.

Hennig W (1981) **Insect Phylogeny**. J Wiley & Sons, Chichester.

Hihara F (1981) Effects of the male accessory gland secretions on oviposition and remating in females of *Drosophila melanogaster*. Zool Mag **90**: 307-316.

Hohorst W (1937) Die begattungsbiologie der grylle *Oecanthus pellucens* Scolpi. Z Morphol Okol Tiere **32**: 227-275.

Houghton C O (1909) Observations on the mating habits of *Oecanthus*. Ent News **20**: 274-279.

Hubbel T H (1936) A monographic revision of the genus *Ceuthophilus*. University of Florida Pub Biol Science Ser 2 (1).

Hubbel T H, Norton R M (1978) The systematics and biology of the cave-crickets of the North American tribe Hadenoecini (Orthoptera: Saltatoria: Ensifera: Rhaphidophoridae: Dolichopodinae). Misc Pub Mus Zool Univ Michigan no. 156.

Huber F (1955) Sitz und bedeutung nervoser zentren fur instinkthandlungen beim mannchen von *Gryllus campestris* L. Z Tierpsychol **12**: 12-48.

Huignard J (1969) Action stimulatrice du spermatophore sur l'ovigenese chez *Acanthoscelides obtectus*. C R Hebd Seanc Acad Sci Paris **268**: 2938-2940.

Huignard J (1983) Transfer and fate of male secretions deposited in the spermatophore of females of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). J Insect Physiol **29**: 55-63.

Huignard J, Quesneau-Thierry A, Barbier M (1977) Isolement, action biologique et evolution des substances paragoniales contenues dans le spermatophore d'*Acanthoscelides obtectus* Say. J Insect Physiol **23**: 351-357.

Hungerford H B (1936) The Mantispidae of the Douglas lake, Michigan, region with some biological observations (Neuroptera). Ent News **47**: 85-88.

Isley F B (1941) Researches concerning the Texas Tettigoniidae. Ecol Monogr **11**: 459-474.

Johansson A S (1964) Feeding and nutrition in reproductive processes in insects. R Ent Soc Lond Symp **2**: 43-55.

Jones K N, Odendaal F J, Ehrlich P R (1986) Evidence against the spermatophore as paternal investment in the checkerspot butterflies (*Euphydryas*: Nymphalidae). Am Midl Nat **116**: 1-6.

Kaltenbach A (1970) Unterlagen fur eine monographie der Saginae II beitrage zur autokologie der gattung *Saga* Charpentier (Saltatoria: Tettigoniidae). Zool Beitr (n.f.) **16**: 155-245.

Kaltenbach A (1990) The predatory Saginae. In: Bailey W J, Rentz, D C F (eds) **The Tettigoniidae: biology, systematics and evolution**. Crawford House Press, Bathurst, pp 280-302.

Kenagy G J, Trombulak S C (1986) Size and function of mammalian testes in relation to body size. J Mammal **67**: 1-22.

Kessel E L (1955) The mating activities of balloon flies. Syst Zool **4**: 97-104.

Kevan D K Mc E (1982) Orthoptera. In: Parker S P (ed) **Synopsis and Classification of Living Organisms**, Vol 2. Mc Graw Hill, New York, pp 252-379.

Key K H L (1970) Orthoptera (Grasshoppers, Locusts, Crickets). In: **The Insects of Australia**. CSIRO (ed), Melbourne University Press, Melbourne.

Khalifa A (1949a) The mechanism of insemination and mode of action of the spermatophore in *Gryllus domesticus*. Quart J Micr Sci **90**: 281-92.

Khalifa A (1949b) Spermatophore production in Trichoptera and some other insects. Trans R Ent Soc Lond **100**: 449-471.

Khalifa A (1950a) Sexual behaviour in *Gryllus domesticus* L. Behaviour **2**: 264-274.

Khalifa A (1950b) Spermatophore production in *Galleria mellonella* L. (Lepidoptera). Proc Roy Entomol Soc **25**a: 33-40.

Knowlton N, Greenwell S R (1984) Sperm competition: the influence of female interests. In: Smith R L (ed) **Sperm Competition and the Evolution of Animal Mating Systems**. Academic Press, Orlando, pp 61-84.

Kusano T, Toda M, Fukuyama K (1991) Testes size and breeding systems in Japanese anurans with special reference to large testes in the treefrog *Rhacophorus arboreus* (Amphibia: Rhacophoridae). Behav Ecol Sociobiol **29**: 27-31.

Labine P A (1964) Population biology of the butterfly *Euphydryas editha*. 1. Barriers to multiple inseminations. Evolution **18**: 335-336.

Landa V (1960) Origin, development and function of the spermatophore in the cockchafer (*Melolontha melolontha* L.) Acta Soc Entomol Czech **57**: 297-316.

Lederhouse R C, Ayres M P, Scriber J M (1990) Adult nutrition affects male virility in *Papilio glaucus* L. Funct Ecol **4**: 743-751.

Leinhardt R (1921) Le mechanisme de la stridulation chez *Cyrtaspis scutata* Charp. (Orth. Meconematinae) Ann Soc Entomol Fr **90**: 156-160.

Leopold R A (1976) The role of male accessory glands in insect reproduction. Ann Rev Entomol **21**: 199-221.

Leroy Y (1969) Quelques aspects de la reproduction des Tettigonioides de Trinidad (Orthoptera). Ann Soc Ent Fr **5**: 775-798.

Lessels C M, Birkhead T R (1990) Mechanisms of sperm competition in birds: mathematical models. Behav Ecol Sociobiol **27**: 325-337.

Linley J R (1975) Sperm supply and its utilisation in doubly-inseminated flies, *Culicoides melleus*. J Insect Physiol **21**: 1785-1788.

Linley J R, Hinds M J (1975) Quantity of the male ejaculate influenced by female unreceptivity in the fly *Culicoides melleus*. J Insect Physiol **21**: 281-285.

Liske E, Davies W J (1984) Sexual behaviour of the chinese praying mantis. Anim Behav **32**: 916-917.

Liske E, Davies W J (1987) Courtship and mating behaviour of the chinese praying mantis *Tenodera aridifolia sinesis*. Anim Behav **35**: 1524-1537.

Loher W (1979) The influence of Prostaglandin E2 on oviposition in *Teleogryllus commodus*. Ent Exp & Appl **25**: 107-119.

Loher W (1981) The effect of mating on female sexual behaviour of *Teleogryllus commodus* Walker. Behav Ecol Sociobiol 9: 219-225.

Loher W (1989) Temporal organisation of reproductive behaviour. In: Huber F, Moore T E (eds) **Cricket Behaviour and Neurobiology**. Cornell Univ Press, Ithica, New York.

Loher W, Dambach M (1989) Reproductive behaviour. In: Huber F & Moore T E (eds) **Cricket Behaviour and Neurobiology**. Cornell University Press, Ithaca, New York.

Loher W, Edson K (1973) The effect of mating on egg production and release in the cricket *Teleogryllus commodus*. Ent Exp & Appl **16**: 483-490.

Loher W, Rence B (1978) The mating behaviour of *Teleogryllus commodus* (Walker) and its central and peripheral control. Z Tierpsychol **46**: 225-259.

Low B S (1978) Environmental uncertainty and the parental strategies of marsupials and placentals. Am Nat **112**: 197-213.

Lynam A J, Morris S, Gwynne D T (1992) Differential mating success of virgin female katydids *Requena verticalis* (Orthoptera: Tettigoniidae). J insect Behav **5**: 51-59.

Magnhagen C (1991) Predation risk as a cost of reproduction. Trends Ecol Evol **6**: 183-186.

Mann T (1984) **Spermatophores: development, structure, biochemical attributes and role in the transfer of spermatozoa**. Springer Verlag, Berlin.

Markow T A (1988) *Drosophila* males provide a material contribution to offspring sired by other males. Func Ecol **2**: 77-79.

Markow T A, Ankney P F (1984) *Drosophila* males contribute to oogenesis in a multiple-mating species. Science **224**: 302-303.

Markow T A, Gallagher P D, Krebs R A (1990) Ejaculate-derived nutritional contribution and female reproductive success in *Drosophila mojavensis* (Patterson & Crow). Func Ecol **4**: 67-73.

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

Marshall L D (1982) Male nutrient investment in the Lepidoptera: what nutrients should males invest? Am Nat **112**: 197-213.

Marshall L D, Mc Neil J N (1989) Spermatophore mass as an estimate of male nutrient investment: a closer look in *Pseudaletia unipuncta* (Haworth) (Lepidoptera: noctuidae). Funct Ecol **3**: 605-612.

Martin P A, Reimers T J, Lodge J R, Dzuik P J (1974) The effects of ratios and numbers of spermatozoa mixed from two males on the proportions of offspring. J Reprod Fert **39**: 251-258.

Masden T, Shine R, Loman J, Hakansson T (1992) Why do female adders copulate so frequently? Nature (lond) **355**: 440-441.

Matsumoto K, Suzuki N (1992) Effectiveness of the mating plug in *Atrophaeneura alcinous* (Lepidoptera: Papilionidae). Behav Ecol Sociobiol **30**: 157-163.

Mays D L (1971) Mating behaviour of nemobiine crickets *Hygronemobius*, *Nemobius* and *Pteronemobius* (Orthoptera: Gryllidae). Fla Ent **54**: 113-126.

Mbata K J (1992a) The biology and host-plant specificity of *Acanthoplus speiseri* Brancsik (Orthoptera: Tettigoniidae: Hetrodinae) a pest of grain crops. J Ent Soc Sth Afr **55**: 99-106.

Mbata K J (1992b) Some observations on the reproductive behaviour of *Acanthoplus speiseri* Brancsik (Orthoptera: Tettigoniidae: Hetrodinae). Insect Sci Applic **13**: 19-26.

Meddis R (1984) **Statistics Using Ranks**. Blackwell, Oxford.

Meixner A J, Shaw K C (1986) Acoustic and associated behaviour of the coneheaded katydid *Neoconocephalus nebrascensis* (Orthoptera: Tettigoniidae). Ann Ent Soc Amer **79**: 554-565.

Mercier L (1914) Caractere sexuel secondaire chez les panorpes. Le role des glandes salivaires des males. Arch Zool Exp Gen **55**: 1-5.

Moller A P (1988a) Ejaculate quality, testes size and sperm competition in primates. J Human Evol **17**: 487-488.

Moller A P (1988b) Testes size, ejaculate quality and sperm competition in birds. Biol J Linn Soc **33**: 273-283.

Moller A P (1989) Ejaculate quality, testes size and sperm production in mammals. Func Ecol **3**: 91-96.

Moller A P (1991a) Sperm competition, sperm depletion, paternal care and relative testis size in birds. Am Nat **137**: 882-906.

Moller A P (1991b) Sperm competition and sperm counts: an evaluation of the empirical evidence. In Baccetti B (ed) Comparative spermatology 20 years after; papers presented at the 6th international congress of spermatology, Sienna, Italy, August 30- september 5, 1990. Sereno Symposia Publications from Raven Press, pp 775-777.

Morris G K (1971) Aggression in male conocephaline grasshoppers (Tettigoniidae). Anim Behav **19**: 132-137.

Morris G K (1971) Mating systems, paternal investment and aggressive behaviour of acoustic orthoptera. Fla ent **62**: 9-17.

Morris G K (1980) Calling display and mating behaviour of *Copiphora rhinoceros* Pictet (Orthoptera: Tettigoniidae). Anim Behav **28**: 42-51.

Muller J K, Eggert A K (1989) Paternity assurance by "helpful" males: adaptations to sperm competition in burying beetles. Behav Ecol Sociobiol **24**: 245-249.

Mullins D E, Keil C B (1980) Paternal investment of urates in cockroaches. Nature **283**: 567-569.

Mullins D E, Keil C B, White R H (1992) Maternal and paternal nitrogen investment in *Blatella germanica* (L) (Dictyoptera; Blattellidae). J Exp Biol **162**: 55-72.

Nagao T, Schimozawa T (1987) A fixed time interval between 2 behavioural elements in the mating behaviour of male crickets, *Gryllus bimaculatus*. Anim Behav **35**: 122-130.

Nixon K (1992) A study of mate choice and the spermatophore in a species of bushcricket (Orthoptera: Tettigoniidae). Unpublished Joint-honours project, Dept. Life Science, University of Nottingham, U.K.

Obara Y, Tateda H, Kuwabara M (1975) Mating behaviour of the cabbage white butterfly *Pieris rapae crucivora*. 5. Copulatory stimuli inducing changes of female response patterns. Zool Mag **84**: 71-76.

Oberhauser K S (1988) Male monarch butterfly spermatophore mass and mating strategies. Anim Behav **36**: 1384-1388.

Oberhauser K S (1989) Effects of spermatophores on male and female monarch butterfly reproductive success. Behav Ecol Sociobiol **25**: 237-246.

Oberhauser K S (1992) Rate of ejaculate breakdown and intermating intervals in monarch butterflies. Behav Ecol Sociobiol **31**: 367-373.

Ono T, Siva-Jothy M T, Kato A (1989) Removal and subseqent ingestion of rival's semen during copulation in a tree cricket. Physiol Entomol **14** (2): 195-202.

Otte D, Cade W (1983) African crickets (Gryllidae) 3. On the African species of *Valarifictorus* Randell (Gryllinae, Modicogryllini). Proc Acad Nat Sci Philadelphia **135**: 241-253.

Outram I (1971) Aspects of mating in the spruce budworm *Choristoneura fumiferana* (Lepidoptera, Tortricidae). Can Entomol **103**: 1121-1128.

Oschmann M (1991) Verbreitung und Lebensweise der Eichenschrecke *Meconema thalassinum* (De Geer) (Saltatoria, Tettigoniidae). Ent Nach Ber **35** (2): 105-107.

Pagel M D, Harvey P H (1989) Comparative methods for examining adaptation depend on evolutionary models. Folia primatologica **53**: 203-220.

Parker G A (1970) Sperm competition and its evolutionary consequences in the insects. Biol Rev **45**: 525-567.

Parker G A (1982) Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. J Theor Biol **96**: 281-294.

Parker G A (1984) Sperm competition and the evolution of animal mating strategies. In: Smith R L (ed) **Sperm Competition and the Evolution of Animal Mating Systems**. Academic Press, Orlando, pp 1-60.

Parker G A (1990a) Sperm competition games: raffles and roles. Proc R Soc Lond Ser B **242** (1304): 120-126.

Parker G A (1990b) Sperm competition games: sneaks and extra-pair copulations. Proc R Soc Lond ser B **242**: 127-133.

Parker G A, Simmons L W (1989) Nuptial feeding in insects: theoretical models of male and female interests. Ethology **82**: 3-26.

Parker G A, Simmons L W (1991) A model of constant random sperm displacement during mating: evidence from *Scatophaga*. Proc R Soc Lond B **246**: 107-115.

Parker G A, Simmons L W, Kirk H (1990) Analysing sperm competition data: simple models for predicting mechanisms. Behav Ecol Sociobiol **27**: 55-65.

Parker G A, Smith J L (1975) Sperm competition and the evolution of the pre-copulatory passive phase behaviour in *Locusta migratoria migratoriodes*. J Entomol **49**: 155-171.

Petersson E (1991) Effects of re-mating on the fecundity and fertility of female caddis flies *Mystacides azurea*. Anim Behav **41**: 813-818.

Pickford P, Gillot C (1971) Insemination in the migratory grasshopper *Melanoplus sanguinipes* (Fabr). Can J Zool **49**: 1583-1588.

Pickford P, Gillot C (1976) Effect of varied copulatory periods of *Melanoplus sanguinipes* (Orthoptera: Acrididae) females on egg hatchability and hatchling sex ratios. Can Ent **108**: 331-335.

Piersol W H (1907) The curious mating habits of the fly *Rivellia boscii*. Am Nat **41**: 465.

Pitnick S, Markow T A, Riedy M F (1991) Transfer of ejaculate and incorporation of male-derived substances by females in the Nannoptera species group (Diptera: Drosophilidae) Evolution **45**: 774-780.

Pivnick K A, McNeil J N (1987) Puddling in butterflies: sodium affects reproductive success in *Thymelicus lineola*. Physiol Entomol **12**: 461-472.

Power J H (1958) On the biology of *Acanthoplus bechuanus* Per. (Orthoptera: Tettigoniidae). J Ent Soc Sth Africa **21**: 376-381.

Preston-Mafham K (1990) **Grasshoppers and Mantids of the World**. Blamford, London.

Principi M M (1985) Lo spermatophoro nei Neurotteri Crisopidi. Frustula Entomol **21**: 143-159.

Pritchard G (1967) Laboratory observations on the mating behaviour of the island fruit fly *Rioxa pornia* (Diptera: Tephritidae). J Aust Entomol Soc **6**: 127-132.

Quinn J S, Sakaluk S K (1986) Pre-zygotic male reproductive effort in insects: why do males provide more than sperm? Fla Ent **69**: 84-94.

Ragge D (1955) **The Wing Venation of the Orthoptera Saltatoria**. Brit Mus (Nat Hist), London.

Reigert P W (1965) Effects of grouping, pairing and mating on the bionomics of *Melanoplus bilituratus* (Walker) (Orthoptera: Acrididae) Can Entomol **97**: 1046-1051.

Reinhold K, Heller K G (1993) The ultimate function of nuptial feeding in the bushcricket *Poecilimon veluchianus* (Orthoptera: Tettigoniidae: Phaneropterinae). Behav Ecol Sociobiol **32**: 55-60.

Rentz D C F (1963) Biological observations on the genus *Decticita* (Orthoptera: Tettigoniidae). Wasm J Biol **21**: 91-94.

Rentz D C F (1972) The lock and key as an isolating mechanism in katydids. Amer sci **60**: 750-755.

Rentz D C F, Coless D H (1990) A classification of the shield-backed katydids (Tettigoniinae) of the world. In: Bailey W J, Rentz D C F (eds) **The Tettigoniidae: biology, systematics and evolution**. Crawford House Press, Bathurst, pp 352-377.

Retnakaran A R (1974) The mechanism of sperm precedence in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can Ent **106**: 1189-1194.

Richards A M (1961) The life history of some species of Rhaphidophoridae (Orthoptera). Tr R Soc N Z **88**: 121-137.

Richards A M (1973) A comparative study of the biology of the giant wetas *Deinacrida heteracantha* and *D.fallai* (Orthoptera: Henicidae) from New Zealand. J Zool Lond **169**: 195-236.

Ridley M (1988) Mating frequency and fecundity in insects. Biol Rev **63**: 509-549.

Ridley M (1989) The incidence of sperm displacement in insects: four conjectures, one corroboration. Biol J Linn Soc Lond **38**: 349-367.

Ridley M (1990) The control and frequency of mating in insects. Func Ecol **4**: 75-84.

Riegert P W (1965) Effects of grouping, pairing and mating on the bionomics of *Melanoplus bilituratus* (Walker). Can Ent **97**: 1046-1051.

Rigalleau L (1936) Observations sur les Ephippigeres. Rev Zool Agr **35**: 113-182.

Ritchie M G, Butlin R K, Hewitt G M (1989). Assortative mating across a hybrid zone in *Chorthippus parallelus* (Orthoptera: Acrididae). J Evol Biol **2**: 339-352.

Roeder K D (1935) An experimental analysis of the sexual behaviour of the praying mantis (*Mantis religiosa*). Biol Bull **69**: 203-220.

Roeder K D (1976) **Nerve Cells and Insect Behaviour.** Harvard University Press, Cambridge, Massachusets.

Rost R, Honegger H W (1987) The timing of premating and mating behaviour in a field population of the cricket *Gryllus Campestris* L. Behav Ecol Sociobiol **21**: 279-289.

Roth L M (1967) Uricose glands in the accessory sex gland complex of male Blatteria. Ann Ent Soc Amer **60**: 1203-1211.

Roth L M (1969) The evolution of tergal glands in the Blatteria. Ann Ent Soc Amer **62**: 176-208.

Roth L M, Dateo G B Jr (1964) Uric acid in the reproductive system of the cockroach *Blattela germanica*. Science **146**: 782-784.

Roth L M, Dateo G B Jr (1965) Uric acid storage and excretion by accessory glands of male cockroaches. J Insect Physiol **11**: 1023-1029.

Royer L, McNeil R (1993) Male investment in the European corn-borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae): impact on female longevity and reproductive performance. Func Ecol **7**: 209-215.

Rubenstein D I (1989) Sperm competition in the water strider *Gerris regimis*. Anim Behav **38**: 631-636.

Rutowski R L (1979) The butterfly as an honest salesman. Anim Behav **27** (4): 1269-1270.

Rutowski R L (1980) Courtship solicitation by females of the checkered white butterfly, *Pieris protodice*. Behav Ecol Sociobiol **7**: 113-117.

Rutowski R L (1984) Production and use of secretions passed by males at copulation in *Pieris protodice* (Lepidoptera, Pieridae). Psyche **91**: 141-152.

Rutowski R L, Gilchrist G W (1986) Copulation in *Colias eurytheme* (Lepidoptera: Pieridae): patterns and frequency. J Zool **207**: 115-124.

Rutowski R L, Gilchrist G W, Terkanian B (1987) Female butterflies mated with recently mated males show reduced reproductive output. Behav Ecol Sociobiol **20**: 319-322.

Rutowski R L, Long C E, Marshall L D and Vetter R S (1981) Courtship solicitation by *Colias* females (Lepidoptera: Pieridae). Am Midl Nat **105**: 334-340.

Rutowski R L, Newton M, Schaefer J (1983) Interspecific variation in the size of the nutrient investment made by male butterflies during copulation. Evolution **37**: 708-713.

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

Sakaluk S K (1985) Spermatophore size and its role in the reproductive behavior of the cricket *Gryllodes supplicans* (Orthoptera: Gryllidae). Can J Zool **63**: 1652-1656.

Sakaluk S K (1986a) Is courtship feeding by male insects paternal investment? Ethology **73**: 161-166.

Sakaluk S K (1986b) Sperm competition and the evolution of nuptial feeding behavior in the cricket, *Gryllodes supplicans* (Walker). Evolution **40**: 584-593.

Sakaluk S K (1987) Reproductive behaviour of the decorated cricket *Gryllodes supplicans* (Orthoptera: Gryllidae): calling schedules, spatial distribution and mating. Behaviour **100**: 202-225.

Sakaluk S K (1991) Post-copulatory mate guarding in decorated crickets. Anim Behav **41**: 207-216.

Sakaluk S K, Cade W H (1980) Female mating frequency and progeny production in singly and doubly mated house and field crickets. Can J Zool **58**: 404-411.

Sakaluk S K, Cade W H (1983) The adaptive significance of female multiple matings in house and field crickets. In: Gwynne D T, Morris G K (eds) **Orthopteran Mating Systems: sexual competition in a diverse group of insects**. Westview Press, Boulder Colorado, pp 319-336.

Sakaluk S K, Morris G K, Snedden W A (1987) Mating and its effect on acoustic signalling behavior in a primitive orthopteran *Cyphoderris strepitans* (Haglidae): the cost of feeding females. Behav Ecol Sociobiol **21**: 173-178.

Sakaluk S K, Smith R L (1983) Inheritance of male parental investment in an insect. Am Nat **132**: 594-601.

Saltet P (1969) Le spermatophore chez un orthoptere Rhaphidoporinae (*Dolichopoda linderi* Duf). Annales de Speleologie **24**: 533-539.

Sanger K (1987) The role of temporary immobility in the mating behaviour of *Saga natoliae* Serville and *Saga ephippigera* Fischer de Waldheim (Ensifera: Tettigoniidae). Zool Anz **219**: 66-69.

Schal G, Bell W J (1982) Ecological correlates of paternal investment of urates in a tropical cockroach. Science **218**: 170-172.

Schmidt G H (1990) A new species of *Gymnoproctus* Karsch 1887 (Grylloptera: Tettigoniidae: Heteroderinae) from Kenya with some notes on its biology. Trop Zool **3** (2): 121-137.

Sharov A G (1967) Phylogeny of the Orthopteroidea. Reprint, Israel Program for Scientific Translations, Jerusalem, 1971.

Shaw K C, Galliart P (1987) Acoustic and mating behaviour of a Mexican katydid, *Pterophylla beltrani* (Orthoptera: Tettigoniidae). Fla Entomol **70**: 354-368.

Short R V (1979) Sexual selection and its component parts, somatic and genital selection, as illustrated by man and the great apes. Adv Study Behav **9**: 131-158.

Silberglied R E, Shepherd J G, Dickinson J L (1984) Eunuchs: the role of apyrene sperm in Lepidoptera? Am Nat **123**: 255-265.

Simmons L W (1986) Female choice in the field cricket *Gryllus bimaculatus* (De Geer). Anim Behav **34**: 1463-1470.

Simmons L W (1987) Sperm competition as a mechanism of female choice in the field cricket *Gryllus bimaculatus*. Behav Ecol Sociobiol **21**: 197-202.

Simmons L W (1988a) The contribution of Multiple mating and spermatophore consumption to the lifetime reproductive success of female field crickets (*Gryllus bimaculatus*). Ecol Ent **13**: 57-69.

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

Simmons L W (1990a) Nuptial feeding in tettigoniids: male costs and rates of fecundity increase. Behav Ecol Sociobiol **27**: 43-47.

Simmons L W (1990b) Post-copulatory guarding, female choice and the levels of gregarine infection in the field cricket, *Gryllus bimaculatus*. Behav Ecol Sociobiol **26**: 403-407.

Simmons L W (1991) On the post-copulatory guarding behaviour of male field crickets. Anim Behav **42**: 504-505.

Simmons L W (1992) Quantification of role reversal in relative parental investment in a bush cricket. Nature **358**: 61-63.

Simmons L W (1993) Some constraints on reproduction for male bushcrickets, *Requena verticalis* (Orthoptera: Tettigoniidae) diet, size and parasite load. Behav Ecol Sociobiol **32**: 135-139.

Simmons L W, Bailey W J (1990) Resource influenced sex roles of zaprochiline tettigoniids (Orthoptera: tettigoniidae). Evolution **44** (7): 1853-1868.

Simmons L W, Craig M, Llorens T, Schinzig M, Hosken D (1993) Bushcricket spermatophores vary in accord with sperm competition and parental investment theory. Proc R Soc Lond B **251**: 183-186.

Simmons L W, Gwynne D T (1991) The refractory period of female katydids (Orthoptera: Tettigoniidae): sexual conflict over the re-mating interval? Behav Ecol **2** (4): 276-282.

Simmons L W, Gwynne D T (1993) Reproductive investment in bushcrickets: the allocation of male and female nutrients to offspring. Proc R Soc Lond B **252**: 1-5.

Simmons L W, Llorens T, Schinzig M, Hosken D, Craig M (in press) Sperm competition selects for male mate choice and protandry in the bushcricket *Requena verticalis* (Orthoptera: Tettigoniidae). Anim Behav.

Simmons L W, Parker G A (1989) Nuptial feeding in insects: mating effort versus paternal investment. Ethology **81**: 332- 343.

Simmons L W, Parker G A (1992) Individual variation in sperm competition success in yellow dung flies, *Scatophaga stercoraria*. Evolution **46**: 366-375.

Simmons L W, Teal R J, Maier M, Standish R J, Bailey W J, Withers P C (1992) Some costs of reproduction for male bushcrickets, *Requena verticalis* (Orthoptera: Tettigoniidae), allocating resources to mate attraction and nuptial feeding. Behav Ecol Sociobiol **31**: 57-62.

Sims S R (1979) Aspects of mating frequency and reproductive maturity in *Papilio zelacaon*. Amer Midl Natur. **102**: 36-50.

Sismondo E (1980) Physical characteristics of the drumming of *Meconema thalassinum*. J Insect Physiol **26**: 209-212.

Siva-jothy M T (1987) Variation in copulation duration and the resultant degree of sperm removal in *Orthetrum cancellatum* (L) (Libellulidae: Odonata). Behav Ecol Sociobiol **20**: 147-151.

Siva-jothy M T, Tsubaki Y (1989) Variation in copulation duration in *Mnais pruinosa pruinosa* Selys (Odonata: Calopterygidae) 1. Alternative mate-searching tactics and sperm precedence. Behav Ecol Sociobiol **24**: 39-45.

Solymar B D, Cade W H (1990) Heritable variation for female mating frequency in field crickets *Gryllus integer*. Behav Ecol Sociobiol **26**: 73-76.

Srivastava U S, Srivastava B P (1957) Notes on spermatophore formation and transfer of sperm in the female reproductive organs of *Leucinodes orbonalis* Guen. (Lepidoptera, Pyraustidae). Zool Anz **156**: 258-266.

Stanley-Samuelson D W, Peloquin J J, Loher W (1986) Egg laying in response to prostaglandin injections in the Australian field cricket *Teleogryllus commodus*. Physiol Entomol **11**: 213-219.

Steele R H (1986a) Courtship feeding in *Drosophila subobscura* I. The nutritional significance of courtship feeding. Anim Behav **34**: 1087-1098.

Steele R H (1986b) Courtship feeding in *Drosophila subobscura* II. Courtship feeding by males influences female mate choice. Anim Behav **34**: 1099-1108.

Steiner P (1930) Studien an *Panorpa communis* L I. Zur biologie. Z Morphol Oekol Tier **17**: 1-25.

Sugawara P (1979) Stretch reception in the bursa copulatrix of the butterfly *Pieris rapae crucivora* and its role in behaviour. J Comp Physiol A **130**: 191-199.

Suter R B (1990) Courtship and the assessment of virginity by male bowl and doily spiders. Anim Behav **39**: 307-313.

Svärd L (1985) Paternal investment in a monandrous butterfly, *Pararge aegeria*. Oikos **45**: 66-70.

Svärd L, Wicklund C (1986) Different ejaculate delivery strategies in first versus subsequent matings in the swallowtail butterfly *Papilio machaon* L. Behav Ecol Sociobiol **18**: 325-330.

Svärd L, Wicklund C (1988) Fecundity, egg weight and longevity in relation to multiple mating in females of the monarch butterfly. Behav Ecol Sociobiol **23**: 39-43.

Svärd L, Wicklund C (1989) Mass and production rate of ejaculates in relation to monandry-polyandry in butterflies. Behav Ecol Sociobiol **24**: 395-402.

Svärd L, Wicklund C (1991) The effect of ejaculate mass on female reproductive output in the European swallowtail butterfly, *Papilio machaon* (L) (Lepidoptera: Papilionidae). J Insect Behav **4** (1): 33-41.

Svensson B O G, Petersson E, Frisk M (1990) Nuptial gift size prolongs copulation duration in the dance fly *Empis borealis*. Ecol Ent **15**: 225-229.

Tempere G (1923) Note sur *Cyrtaspis scutata* Charpentier (Orthoptere - Locustaire). Proc Verb Soc Linn Bordeaux **75**: 76-81.

Thornhill R (1976a) Sexual selection and paternal investment in insects. Am Nat **110**: 153-163.

Thornhill R (1976b) Sexual selection and nuptial feeding behaviour in *Bittacus apicalis* (Insecta: Mecoptera). Am Nat **110**: 529-548.

Thornhill R (1979) Male and female sexual selection and the evolution of mating strategies in insects. In: Blum M S & Blum N A (eds) **Sexual Selection and Reproductive Competition in Insects**. Academic Press, New York, pp 81-121.

Thornhill R (1980) Rape in *Panorpa* scorpionflies and a general rape hypothesis. Anim Behav **28**: 52-59.

Thornhill R (1981) *Panorpa* (Mecoptera: Panorpidae) scorpionflies: systems for understanding resource defence polygyny and alternative male reproductive efforts. Ann Rev Ecol Syst **12**: 355-386.

Thornhill R (1984) Alternative hypotheses for traits beleived to have evolved by sperm competition. In: Smith R L (ed) **Sperm Competition and the Evolution of Animal Mating Systems**. Academic Press, Orlando, pp 151-178.

Thornhill R, Alcock J (1983) **The Evolution of Insect Mating Systems**. Cambridge, Massachusettes: Harvard University Press.

Thornhill R, Gwynne D T (1986) The evolution of sexual differences in insects. Amer Sci **74**: 382-389.

Thornhill R, Sauer R K (1991) The notal organ of the scorpionfly (*Panorpa vulgaris*): an adaptation to coerce mating duration. Behav Ecol **2**: 156-164.

Tinkham E R, Rentz, D C (1969) Notes on the bionomics and distribution of the genus *Stenopelmatus* in central california with the description of a new species (Orthoptera: Gryllacrididae). Pan Pac Entomol **45**: 4-14.

Toschi C A (1965) The taxonomy, life histories and mating behaviour of the green lacewings of Strawberry canyon (Neuroptera: Chrysopidae). Hilgardia **36**: 391-431.

Trevitt S, Partridge L (1991) A cost of receiving sperm in the female fruitfly *Drosophila melanogaster*. J Insect Physiol **37** (6): 471-475.

Trivers R L (1972) Parental investment and sexual selection. In: Campbell B (ed), **Sexual Selection and the Descent of Man, 1871-1971**. Aldine Publishing Company, Chicago, pp 136-179.

Turner M E, Anderson W W (1983) Multiple mating and female fitness in *Drosophila*. Evolution **37**: 714-723.

Waage J K (1979) Dual function of the damselfly penis: sperm removal and sperm transfer. Science **203**: 916-918.

Waage J K (1984) Sperm competition and the evolution of odonate mating systems. In: Smith R L (ed), **Sperm Competition and the Evolution of Animal Mating Systems**. Academic Press, Orlando, pp 251-290.

Walker T J (1978) Post-copulatory behavior of the two-spotted tree cricket, *Neoxabea bipunctata*. Fla Ent **61**: 39-40.

Walker T J (1980) Reproductive behaviour and mating success of male short-tailed crickets: differences within and between demes. Evol Biol **13**: 219-260.

Walker T J (1983) Mating modes and female choice in short-tailed crickets (*Anurogryllus arboreus*). In: Gwynne D T, Morris G K (eds) **Orthopteran Mating Systems: sexual competition in a diverse group of insects.** Westview press, Boulder, Colorado, pp 240-267.

Walker T J (ed) (1984) Mole Crickets in Florida. Fla Agric Stn Bull **846**: 1-54.

Walker T J, Gurney B A (1967) The metanotal glands as a taxonomic character in *Oecanthus* of the United States. Proc Ent Soc Wash **69**: 157-161.

Walker T J, Masaki S (1989) Natural history. In: Huber F, Moore T E (eds) **Cricket Behaviour and Neurobiology**, Cornell Uni Press, Ithaca & London.

Walker W F (1980) Sperm utilisation strategies in the nonsocial insects. Am Nat **115**: 780-779.

Warne A C (1970) The Biology of the Eggs of the Tettigoniidae. Ph.D. thesis, University of Nottingham, U.K.

Watanaabe M (1988) Multiple matings increase the fecundity of the yellow swallowtail butterfly, *Papilio Xuthus*, in summer generations. J Insect Behav **1**: 17-30.

Watson P J (1991) Multiple paternity and first-male sperm precedence in the sierra dome spider, *Linyphia litigiosa* Keyserling (Linyphiidae). Anim Behav **41**: 135-148.

Wedell N (1991) Sperm competition selects for nuptial feeding in a bushcricket. Evolution **45** (8): 1975-1978.

Wedell N (1992) Protandry and mate assessment in the wartbiter *Decticus verrucivorus* (Orthoptera: Tettigoniidae). Behav Ecol Sociobiol **31**: 301-308.

Wedell N (1993a) Mating effort or paternal investment? Incorporation rate and cost of male donations in the wartbiter. Behav Ecol Sociobiol **32**: 239-246.

Wedell N (1993b) Variation in nuptial gift quality in bushcrickets. In Wedell N, **Evolution of Nuptial Gifts in Bushcrickets.** Doctoral dissertation, Department of Zoology, University of Stockholm, Stockholm 1993.

Wedell N (in press) Spermatophore size in bushcrickets: comparative evidence for nuptial gifts as sperm protection devices. Evolution.

Wedell N, Arak A (1989) The Wartbiter spermatophore and its effect on female reproductive output (Orthoptera: Tettigoniidae, *Decticus verrucivorus*). Behav Ecol Sociobiol **24**: 117-125.

Wheeler W M (1924) The courtship of the Calobates. J Hered **15**: 485.

Wickler W (1985) Stepfathers in insects and their pseudo- parental investment. Z Tierpsychol **69**: 72-78.

Wickler W (1986) Mating costs versus parental investment: a reply to Gwynne. Ethology **71**: 78-79.

Wiklund C, Kaitala A, Lindfors V, Abenius J (1993) Polyandry and its effect on female reproduction in the green-veined white butterfly (*Pieris napi* L.). Behav Ecol Sociobiol **33**: 25-33.

Withycombe C L (1922) Notes on the biology of some British Neuroptera (Planipennia). Trans Entomol Soc Lond **71**: 501-594.

Woodhead A P (1985) Sperm mixing in the cockroach *Diploptera punctata*. Evolution **39**: 159-164.

Woodhead A P (1986) Male age: effect on mating behaviour and success in the cockroach *Diploptera punctata*. Anim Behav **34**: 1874-1879.

Zeh D W, Smith R L (1985) Paternal invesment by terrestrial arthropods. Am Zool **25**: 785-805.

Zimmering S, Fowler G N (1966) X-irradiation of the *Drosophila* male and its effects on the number of sperm transferred to the female. Z Vererbungsl **98**: 150-151.

Zuk M (1987) The effects of gregarine parasites, body size and time of day on spermatophore production and sexual selection in field crickets. Behav Ecol Sociobiol **21**: 65-72.

Appendix 1: Collecting & Rearing Methods.

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Collecting Methods.

A variety of species of bushcricket were collected by Dr. J.C. Hartley, Mrs D. Hartley and myself from Spain in August 1990 and from France in August 1990 and August 1991. I collected further specimens from N. Greece in July 1991 and S.W. England in September 1990 and 1991. Collecting localities for each species are given in appendix table 1.

The most effective collecting method in the field was simply to stalk singing males (which could be detected by ear or with the aid of a bat-detector) and to catch them with the aid of a glass vial or a plastic specimen jar. This was placed in front of the insect, which was then encouraged to jump into the jar. Females were located by careful searching in the vicinity of singing males. Another method used to locate bushcrickets was careful examination of vegetation, especially patches of vegetation caught by early-morning sunlight, in which bushcrickets are prone to bask. Species living in grassy habitats could often be "flushed" out of the vegetation simply by walking through it. Species living in trees and bushes, such as the oak bushcricket *Meconema thalassinum* were collected by "beating"- ie. by hitting the vegetation with a large stick in order to dislodge the insects and cause them to fall onto a white beating-tray held below.

Bushcrickets were generally collected as adults. These were housed in specially constructed card boxes during the collecting trips. The boxes were designed to be stored flat when empty. The lids were fitted with black nylon mesh panels to allow

Appendix table 1. Collecting localities, laboratory diets and oviposition sites for the different species of bushcricket studied. R=living rose leaves, J= *Juncus* stems, S= sand, Pd= between polythene disks, P= polyurethane foam, Cw= cotton wool, Hg= hollow grass stems, G= amongst tightly-packed grass, Pl= pithy plant stems. Most species were collected by Dr J.C.Hartley and myself. Those species collected by J.C.H. alone are indicated.

Species	Diet in captivity	Ovipos. site	Collecting locality
Phaneropterinae			
Phaneroptera nana	*Ranunculus* & *Buddleia*	R	Seranon, Bases Alpes, SW France & Nr Couisa, French Pyrenees.
P.falcata	"	"	Various localities in S & W France.
Tylopsis lilifolia	"	J	Nr Col de Vil Rouge, French Pyrenees & Provence.
Barbitistes serricauda	"	S	Seranon, Bases Alpes, France.
Leptophyes punctatissima	"	P.d.& C.w.	Various localities in England & France
L.laticauda	"	P	Seranon, Basses Alpes, France & Italian Alps.
L.albovittata	"	-	Nr Psarades, Verno, N Greece.
L.bosci	"	Pl	Cesara, N W Italy.
Poecilimon schmidti	"	S	Yugoslavia (by J.C.H).
P.jonicus	"	"	Psarades, Verno, N E Greece.
P.veluchianus	"	"	Greece, eggs sent by K.Reinhold.
P.affinis	"	"	"
Metaplastes ornatus	"	"	Nr Litohoro, Mt Olympus, Greece
Polysarcus scutatus	", plus *Rumex* & *Plantago*	"	Col de Allios (alt. 2250m), French Alps

Mecopodinae			
Mecopoda elongata	*Buddleia*	S	Malaysia (from a dealer).
Tettigoniinae			
Tettigonia viridissima	*Taraxacum*, *Rumex*, *Aglais*, wheatgerm, grasshoppers & other live insects.	S	Nr El Serrat, Andorra, Pyrenees.
T.cantans	"	"	Cesara, NW Italy.
Gampsocleis glabra	wheat-seedlings, wheatgerm, grass-hoppers.	"	Nr Vielle Fort, SE Sevennes, S France.
Decticus verrucivorus	"	"	French Alps.
D.albifrons	"	"	Nr Col de Vil Rouge, French Pyrenees & Provence.
Platycleis affinis	Wheat-seedlings, wheatgerm, flowering grasses.	P & Cw	Nr Les Sables d' l'Onne, Vendee, W France.
P.albopunctata	"	"	Buddleigh Salterton, S.Devon.
P.nigrosignata	"	"	Vourvourou, Sithonia Greece.
P.tesselata	"	Hg & Cw	Nr Tremp, Spanish Pyrenees.
Metrioptera saussureiana	"	P & Cw	East of Mont Dore, Massif central, France.
M.bicolor	"	"	Seranon, Basses Alpes, SE France.
M.brachyptera	"	"	Aylesbere common, Devon.
M.roeselii	"	Hg	Borehamwood, Herts & Badney Forest, Lincs (by D.Fox).
Sepiana sepium	"	Cw	Seranon, Basses Alpes, SE France.

Yersinella raymondi	*Taraxacum*, *Rumex*, *Buddleia*, flowers of eg. *Taraxacum* & *Ranunculus*, wheat-germ.	P & Cw	Seranon, Basses Alpes, SE France.
Anonconotus alpinus	"	S	Col de Allios (alt. 2250m), French Alps.
Antaxius pedestris	"	"	Seranon, Basses Alpes, SE France.
A.hispanicus	"	"	Nr Viu de Hevata, Spanish Pyrenees.
Pholidoptera griseoaptera	"	P & Cw	Silverton, Nr Exeter, Devon.
Eupholidoptera spp1	"	S	Yugoslavia (by J.C.H)
Eupholidoptera spp2	"	"	Psarades, NE Greece.
Pachytrachis	"	"	Yugoslavia (by J.C.H)
Conocephalinae			
Conocephalus discolor	Wheat-seedlings & wheatgerm.	P & G	Seranon, Basses Alpes, SE France & various localities in Sothern France.
C.dorsalis	"	J	Buddleigh Salterton, S Devon.
Ruspolia nitidula	"	G	Various localities in S France.
Meconematinae			
Cyrtaspis scutata	Aphids, *Drosophila*	Cw	Landevielle, Vendee, W.France.
Meconema meridionale	"	"	Mercus, Ariege, French Pyrenees.
M.thalassinum	"	"	Wollaton Park, Nottingham.
Ephippigerinae			
Ephippiger ephippiger	*Rumex*, *Buddleia* wheatgerm, flowers of, eg., *Taraxacum*.	S	Col de Front Froid, French Pyrenees.

E.terrestris	"	S	Seranon, Basses Alpes, SE France.
E.perforatus	"	"	Spain (by J.C.H.)
Ephippigerida taeniata	"	"	Nr Zahra de los Atunes, S Spain.
E.saussureiana	"	"	Spanish Pyrenees (by J.C.H.).
E.zapateri	"	"	Cuenca, Spain.
Baetica ustulata	"	"	Sierra Nevada (alt. 2750m), S Spain.
Callicrania monticola	"	"	French Pyrenees (by J.C.H)
Steropleurus stali	"	"	Sierra de Guadarrama (by J.C.H).
S.asturiensis	"	"	Spain (by J.C.H.)
S.andalusius	"	"	Sierras Nr Malaga, S Spain.
S.martorelli	"	"	Sierra Nevada (alt. 1580m), S Spain.
S.catalaunicus	"	"	Viu de Hevata, Spanish Pyrenees.
S.brunneri	"	"	Cuenca, Spain.
S. perezi	"	"	Cuenca, Spain.
Uromenus rugiscollis	" & *Plantago* & *Taraxacum*.	P	Nr Chantonnay & Landevielle, Vendee, W.France.
Pycnogastrinae			
Pycnogaster inermis	" & *Ranunculus*	S	Sierra Nevada (alt. 2750m), S Spain.

viewing and ventilation. A sleeve was fitted in the back of each box to allow easy access. The boxes measured approx. 15cm x 23cm x 8cm when constructed. Different species were generally kept in separate boxes and sexes were also separated when space allowed. Fresh vegetation and\or live food were placed in the cages each day during the collecting trips. All individuals collected were brought back to the laboratory at Nottingham, where they were kept in a heated greenhouse in cages containing a suitable medium for oviposition.

Rearing Methods.

Treatment of eggs.

Oviposition sites used by different species of bushcricket are fairly diverse. Some typically lay eggs in soil, some in dead vegetation of various kinds, while others have more specialised requirements and insert their eggs in the edges of living leaves.

Species which lay eggs in soil were provided with a layer of sand (about 2" deep) in which to oviposit. Eggs could be easily sieved from this medium. Species which typically lay eggs in fibrous plant tissue or decaying wood would often lay eggs in polyurethane foam ("wet" foam for flower-arrangements) or in damp cotton wool. Eggs could easily be extracted from the polyurethane foam by crumbling it over a sieve or by crumbling it in water (the eggs sink and the foam floats). Eggs laid in cotton wool, on the other hand, were difficult to extract and had to be teased out individually. Certain species which lay eggs in leaves (ie *Phaneroptera*) would not accept alternative egg-laying sites and eggs had to be painstakingly extracted from the vegetation. Some species which lay eggs in plant stems (*Tylopsis lilifolia* and

Conocephalus dorsalis) were found to lay eggs readily in dry *juncus* stems. The collection of eggs from the pithy interior of such stems was not too difficult. Other stem-laying species such as *Metrioptera roeselii* and *Platycleis tesselata* would only oviposit in thin, hollow grass stems, which made extraction of the eggs difficult. Details of the oviposition media accepted in captivity by each species are given in appendix table 1.

After collection, eggs were washed, dried and placed on a layer of filter paper, on damp cotton wool, in petri-dishes. These were then transferred to incubators held at the appropriate temperature. It was essential to keep the eggs damp because prolonged drying causes them to collapse and the embryos to die.

Many European bushcrickets are biennial and spend about 20 months in the egg stage in the field (Deura & Hartley 1982; Hartley & Warne 1972; Hartley 1990). A number of species have both an initial diapause, which proceeds any significant amount of embryogenesis, and an embryonic diapause, normally at the whole-embryo stage (at the 3/4 embryo stage in *Antaxius*) (Hartley & Warne 1972).

The initial diapause is often variable and in many cases can be eliminated to some extent by immediate high incubation temperatures (see Deura & Hartley 1982; Hartley & Warne 1972; Hartley 1990). The embryonic diapause appears to be less easy to eliminate and is terminated only after prolonged cooling. Consequently, in the majority of species (especially those in the Phaneropterinae, Tettigoniinae and Ephippigerinae), eggs were initially incubated at 30°c for one month, followed by 25°c for 2 months, before being moved to 8°c for at least 3 months (eggs could be stored at this temperature for at least 8 months) in order to complete the embryonic diapause. Hatching usually occurred within about 1 month of the eggs being raised

to 16°c, following the period of cooling. Hatching is often inhibited by higher temperatures (Hartley & Warne 1972). There were a number of notable exceptions to this pattern. For example: eggs of *Meconema* (Meconematinae) required incubation at 16°c for 1 month, followed by 10°c for 3 months, before being raised to 15°c, after which hatching occurred within 2 months; eggs of *Ruspolia* and *Conocephalus* (Conocephalinae) required 1-2 months at 20°c (after which time some occasionally hatched), followed by 1 month at 10°c (at the 1/2 embryo stage), before being raised to 20°c again, after which hatching occurred within 1-2 months; eggs of *Mecopoda* (Mecopodinae) did not require cooling at any stage and hatched after 8 weeks at 25°c. More complete details of the incubation requirements of tettigoniid eggs are given in Hartley & Warne (1972) and Hartley (1990).

Housing.

Upon hatching, nymphs were transferred, via an aspirator, to plastic canisters (measuring approx 10cm x 10cm x 15cm) with black nylon mesh inserted into the lids for ventilation. About 20 small nymphs were generally kept per cage, though in carnivorous species, a maximum of about 4 were kept per cage. For both carnivorous and vegetarian species, fresh vegetation was provided in small water-filled bottles or vials. This was changed about once a week (details of the feeding requirements of various species are given in section 2.3 below and in appendix table 1). The mouths of the bottles were always plugged with cotton wool to prevent the nymphs from drowning. The vegetation provided food, a moulting platform and moisture for the nymphs.

Older nymphs and adults were kept in larger cages consisting of a wooden frame (1ft x 1ft x 2ft), covered with black nylon gauze. These cages were designed to fit over 2ft x 1ft seed trays. A sleeve at the front of each cage allowed easy access, without

the risk of nymphs escaping. Once again, fresh vegetation in pots of water were placed in each cage. As an alternative, the seed tray could be planted with a mixture of vegetation (Warne 1970; Hartley, pers.comm), particularly *poa*, dock (*Rumex* spp) ribwort plantain (*Plantago lanceolata*), groundsel (*Senecio vulgaris*) and buttercup (*Ranunculus*). Small nymphs and even adults could be maintained in this growing environment, which could last for a number of weeks if watered regularly. This method was valuable in the winter when fresh food-plants were often hard to find.

Cages were kept in a heated greenhouse, at a daytime temperature of 20-25°c and a night-time temperature of not less than 15°c. Cages were positioned directly underneath electric strip-lights to provide a source of radiant heat and were exposed to a 14 hour photoperiod. One problem with keeping the bushcrickets in a greenhouse was that on particularly sunny days in late spring and summer, the greenhouse tended to overheat unless the doors and windows were left open. On more than one occasion, this sadly resulted in the death of a large amount of experimental and breeding stock.

Feeding requirements.

The dietary requirements of European bushcrickets are quite varied, although most species are omnivorous to some extent. Some bushcrickets, such as *Tettigonia*, *Gampsocleis* (Tettigoniinae), *Meconema* and *Cyrtaspis* (Meconematinae) are almost entirely predaceous. In the laboratory, these species were reared on aphids, *Drosophila* and, for the larger species such as *Tettigonia viridissima*, grasshoppers, surplus *Leptophyes* and caterpillars. A large number of species in the Ephippigerinae and Tettigoniinae are more widely omnivorous and would feed on the leaves of

various broad-leaved herbs (eg *Rumex* spp, *Ranunculus* spp, *Plantago lanceolata*) and flowers of a number of species such as dandelion (*Taraxacum*) and buttercup (*Ranunculus*). These bushcricket species were also given wheat-germ and would occasionally feed on both live and dead insects when given the opportunity. Other species, including the tettigoniines *Metrioptera* and *Platycleis* and the conocephalines *Conocephalus* and *Ruspolia*, tended towards gramnivory and were reared largely on wheat-seedlings, grass seed heads and flowers, and wheat-germ. The phaneropterines and the mecopodine *Mecopoda*, on the other hand, appeared to be entirely vegetarian and could be reared well on a mixture of buttercup (*Ranunculus* spp), dock (*Rumex* spp) and *Buddleia*. Adults could be maintained on *Buddleia* alone. In fact, *Buddleia* proved to be useful fodder for a number of omnivorous and vegetarian species. Details of the food accepted by various species in captivity are given in appendix table 1. The dietary preferences of a large number of European bushcrickets, representing several subfamilies, has been described in detail by Ganwere & Morales Agacino (1973). Gangwere (1961) gives a more general review of food-selection in different bushcricket families.

Under the conditions outlined above, newly-hatched nymphs of most species could be raised to maturity in about 6 weeks (apart from *Mecopoda* which required at least 4 months to reach adulthood).

Appendix 2: table 2.1. The mating and post-mating behaviour of male ensiferans (from chapter 2).

Table 2.1 The mating and post-mating behaviour of male ensiferans. **PC** = prolonged copulation following spermatophore transfer; **SPX** = spermatophylax production; **MG** = post-copulatory mate-guarding; **MM** = multiple matings with the same female; **GL** = feeding the female with glandular secretions; **OTH** = any other behaviour wl could be interpreted as a means of countering female tendancy to eat spermatophores before complete sperm transfer; ref = source of reference. 0 = behaviour absent; + = behaviour present; ? = insufficient information. Numbers in brackets refer to references listed at the end of the table. P.o.= personal observation. See ch.2 for discus

Super-families, familes, sub-families and species.	Post-mating "behaviour"							Notes
	PC	SPX	MG	MM	GL	OTH	ref	
GRYLLOIDEA								
GRYLLIDAE								
Gryllinae								
Gryllus campestris	0	0	+	+	0	0	1,3,4	Mating behaviour is quite similar in all studied members of this genus (1).
G.bimaculatus	0	0	+	+	0	0	1,2,7	Copulation lasts for 30s to 1 min (1,2,3,4). The spermatophore represents about
G.pennsylvanicus	0	0	+	+	0	0	1	0.2% of male body weight (5). Immediately upon separating from the female, the
G.veletis	0	0	+	+	0	0	1	male turns about, antennates the female's dorsum briefly and enters into a
G.firmus	0	0	+	+	0	0	1	distinctive post-copulatory behaviour (mate-guarding) (6). The male stands
G.bermudiensis	0	0	+	+	0	0	1	immobile with his antennae directed forwards, or laterally, across the female's
G.assimilis	0	0	+	+	0	0	1	back (6). If the female starts to move or attempts to eat the spermatophore,
G.rubens	0	0	+	+	0	0	1	the male jerks forwards and antennates her until she again becomes motionless
G.integer	0	0	+	+	0	0	1	(6,1,7). Guarding behaviour lasts for 40 to 60 min in *G.bimaculatus* (7) and 75
G.vernalis	0	0	+	+	0	0	1	min in *G.campestris* (3). In *G.bimaculatus*, females who choose to remain
G.fultoni	0	0	+	+	0	0	1	with their mates remove and eat their spermatophore only when their mate
G.personatus	0	0	+	+	0	0	1	regains sexual receptivity and resumes courtship (5). Females who leave their
G.vocalis	0	0	+	+	0	0	1	mates tend to remove the spermatophores of smaller males sooner, possibly
G.armatus	0	0	+	+	0	0	1	reflecting post-mating mate choice (5). Males re-mate, on average, 48 min after a previous copulation (2). A male may mate 2 to 3 times with the same female in the space of 3 hours (5,8).
Acheta domesticus	0	0	+	+	0	0	1,9,10	Copulation lasts for 30s (1,9). Following copulation, the male is aggressive to other male crickets and maintains contact with the female, re-gaining her with a rapid searching behaviour if lost (1,9). During guarding behaviour, the male lays his antennae across the female's dorsum (9). The male antennates the female and jerks his body backwards and forwards whenever the female attempts to detach the spermatophore (9). During guarding, the female remains immobile most of the time, especially when antennated by the male (9). The duration of guarding behaviour is about 60 min (1,9,10). The male forms another spermatophore 15 to 65 min after copulation and resumes courtship. Sometime after this, the female rubs or chews off the old spermatophore (1,9).

Gryllodes sigillatus	0	+	+	0	0	0	1,10, 11,12, 13	Copulation lasts 2 min (11). The spermatophore bears a spermatophylax an represents an average of 3.1% (0.1 - 6%) of male body weight (12). The female detaches the spermatophylax within 1 to 5 secs of dismounting (1,12 The female takes an average of 40 min to finish the spermatophylax and usu removes the ampulla 1-7 min later (13). Post-copulatory mate guarding also in this species, though this behaviour is "less intense" than in *Gryllus* or *Acheta* (1). During guarding, the male faces away from the female, directin his cerci towards her (10). In response to female movement, the male either turns to antennate the female or remains in the guarding posture and perfor a "push-up", briefly raising his body by extending his legs (10). Females te to become "restless" shortly before or after finishing the spermatophylax (1 The male follows his mate and makes random searching movements in the immediate area should the female wander out of range of his antennae (10). Males have been observed to "head-butt" females in apparent response to female movements toward spermatophore eating (10). Guarding behaviour lasts for 32 min (10). Following copulation, males do not mate again for an average of 251 min (10).
Teleogryllus commodus	0	0	+	+	0	0	1,4,14, 15,16	Copulation lasts for 3 min (4). The spermatophore represents 0.7 % of male body weight (14). After copulation, the male remains close to the female and lays his antennae across the female's back. The male responds to female movements with antennal flagellation, body rocking and sometimes an aggressive chirp (4). Males have also been observed to "head-butt" females attempting to remove the spermatophore (15). Should the female wander, the the male persues her and re-gains antennal contact, at which point the female tends to quiet down (4). The mean duration of guarding is 53 min (14) to 83 min (4). The female eats the spermatophore within 13 min of the end of guarding (4). Males will re-mate 35 - 40 min following the termination of the guarding period (4). In a confined space, the same pair may re-mate 3-4 times in a 12 hour period (16). Multiple-matings between the same pair have also been observed in habitat simulations (14).
Modicogryllus conspersus	0	0	+	?	0	0	1	Copulation lasts for 3 min (1). Male post-copulatory behaviour is similar to that of *Acheta* (1). The female removes the spermatophore 30 min after copulation and eats it 6 min later (1). The male forms another spermatophore 24 min after copulation (1). Copulation was not repeated in the space of the 40 min observation period in the one case observed (1).
Modicogryllus frontalis	0	0	?	?	0	0	33	

Teleogryllus spp	0	+	?	+	0	0	1	Copulation lasts for 4 to 4.5 min (1). Following copulation, the female rem and eats the spermatophylax. One pair of copulations by the same male and female occurred 15 min apart (1).
Valarifictorus micado	0	0	?	?	0	0	1	Copulation lasts 3 min (1). The male produces a new spermatophore 40 to 5 min after copulation. It appears that the pair remain close together in an end-end position for a while after copulation, though the post-copulatory behaviour of the male has not been observed carefully (1).
Valarifictorus shimba	?	+	?	?	?	?	18	
Miogryllus verticalis	0	0	+	+	0	0	1	Copulation lasts for 4 min (1). The male begins to stridulate after copulatio and the pair remain in an end-end position with their cerci touching. This position is resumed following disturbance (1). In one case, copulation was repeated after mate-guarding behaviour lasting for 1 hour (1).
Discoptila fragosoi	+	0	0	0	+	0	19	Copulation lasts for 15 - 90 min (19). The male retains the spermatophore, holding it in place during the prolonged copulatory act. The female chews at a secretion, produced by the male's metanotal glands, from the underside of the male's raised tegmina just before and during copulation (19). The male withdraws the empty spermatophore and eats it upon the termination of copulation, at which point he takes no futher notice of the female (19).
Gryllomorpha dalmatina	0	+	0	0	0	0	20	Copulation lasts for 5 to 12 min (20). The female removes the spermatophylax 1 min after the end of copulation and eats it. It takes the female up to 2 hours to finish the spermatophylax, at which point she removes and eats the ampulla (20).
Gryllopsis sp	0	0	?	?	0	0	1	Copulation lasts for less than 3 min (1).
Nemobiinae								
Nemobius sylvestris	0	0	+	+	+	0	1,21, 22,23, 24	Copulation lasts for only about 1 sec (21). The male transfers two spermatophores to the female in two separate copulations. Following each copulation, the male exhibits mate-guarding behaviour, maintaining contact with the female and directing his antennae across the female's body (21). In some cases, the female repeatedly mounts the male and palpates the surface of his tegmina following copulation (22). The first spermatophore is retained for 30-60 min prior to being removed and eaten by the female. The second spermatophore is 3 times the diameter of the first and is transferred 60-70 min after the first mating (1,21,22,23,24).

Neonemobius cubensis	+	0	+	+	+	0	24	In *Neonemobius*, *Allonemobius* and *Eunemobius*, the female mounts the mal
N.mormonius	+	0	+	+	+	0	24	briefly (for 0.5-2 sec in *Neonemobius* and *Allonemobius*, for 14-20 sec in
Allonemobius ambitiosus	+	0	+	+	+	0	24	*Eunemobius*), without the transfer of a spermatophore, at the beginning of e
A.sparsalsus	+	0	+	+	+	0	24	mating sequence (24). The male resumes courtship and forms a spermatoph
A.fasciatus	+	0	+	+	+	0	24	to 10 min later. After 20-40 min of male courtship, the female mounts for a
Eunemobius carolinus	+	0	+	+	+	0	24	second time and receives a spermatophore. The pair remain coupled for 20-
E.melodius	+	0	+	+	+	0	25	min (24) (up to 45 min in *A. fasciatus* (25)), while the female feeds on a sec
								from specialised spurs situated on the male's hind tibiae. From 5-10 min after the end of copulation, the female removes the spermatophore by wipin against the substrate. She then eats it (though in some cases the male eats th spermatophore). The male resumes courtship and the mating sequence is repeated (24,25). In *A.fasciatus*, males have been observed to actively persu mates after the end of copulation, suggesting the presence of mate guarding
Pteronemobius heydeni	0	0	?	?	0	0	26	Copulation lasts for about 1 min (26). Following copulation, the female rests for a while before removing the spermatophore with her hind legs. Glandular feeding has not been observed in this species (26).
Hygronemobius alleni	0	0	+	+	0	0	24	Copulation lasts for less than 1 sec (24). The female retains the spermatophore for an average of 8 min (3.2-15 min) before wiping it off and eating it. The female takes 3.5-15 min to finish eating the spermatophore. During this time, the male re-locates the female, antennates her, then resumes courtship (24). One pair were observed to mate 3 times at intervals of 24 and 76 min (24).
Bobilla victorae	0	0	+	+	0	0	14	Copulation lasts for less than 1 sec (14). The spermatophore represents 0.85% of male body weight (14). The female eats the spermatophore 1-3 min after the end of copulation. The male resumes stridulation a few seconds later. He produces another spermatophore 4-9 min after copulation and monopolises the female for repeated mating. The same pair may mate up to 7 times, with an average of 24 min (18-37 min) between copulations. Males can produce up to 15 spermatophores (mean= 11.5) in a period of 8 hours (14). Males guard females only if they attempt to leave after copulation (14).
Brachytrupinae								
Anurogryllus arboreus	+	0	0	0	0	0	1,27, 28	Copulation lasts for 10-16 min (27,28) or 47 min (1). The male retains the spermatophore during and after copulation (1,27,28). The male calls to attract other females during copulation and may mate up to 3 times in a single evening (27).

Eneopterinae								
Hapithus agitator	+	0	+	+	0	+*	29	Copulation lasts for 7-13 min (29).* The female feeds on the male's tegmin during copulation. The spermatophore is rubbed off, or simply falls off, sh after copulation and is then eaten by either the male or female (29). One ma was seen to produce another spermatophore 12 min after copulation (29). Tl pair remain together after mating and the male is aggressive toward other individuals (29).
Orocharis sp	0	0	0	+	0	0	30,31	Immediately after recieving her first spermatophore, the female rubs it off a
O.saltator	0	0	0	+	0	0		begins to eat it. She then re-mounts the male, who transfers a second spermatophore while the female is eating the first. When she has finished eating, the female dismounts once again and removes the second spermatophore which she also begins to eat. Meanwhile, the male, who has produced a third spermatophore, initiates yet another coupling. The entire sequence is repeated many times (30,31). *O.saltator* pairs may remain together for as long as 3.5 hours, during which time the male produces a continuous flow of spermatophores (producing up to 20). Each spermatophore contains relatively few sperm (about 0.1x as many as are contained in a typical *Hapithus* spermatophore) (31).
Trafalisca lurida	?	0	?	?	?	?	30	The female palpates the male's metanotum, behind his raised tegmina, prior-to, and possibly during, copulation. While in copulation, the female reaches round and eats the spermatophore (30). This sequence was observed 3 times (30).
Oecanthinae								
Oecanthus	0	0	0	?	+	0	37	Copulation lasts from a few seconds (31) to 1 min (32). Following copulation, the
O.pellucens	0	0	0	?	+	0	32,33	female remains astride the male and feeds on secretions from his metanotal glands.
O.fultoni	0	0	0	?	+	0	34	Should the female dismount, the male resumes active courtship (stridulation,
O.argentinus	0	0	0	?	+	0	1	tremulation and sometimes antennation) and solicits re-mounting by backing
O.californicus	0	0	0	?	+	0	1	toward her. The female often responds by re-mounting and resuming feeding.
O.quadripunctatis	0	0	0	?	+	0	1	After a total feeding duration of approximately 30 min (7.5-30 min or 12-18
O.pini	0	0	0	?	+	0	35	min in *O.pellucens* (32,33); 5-20 min in *O.latipennis* (31), 30 min in *O.fultoni* (34)
O.nigricornis	0	0	0	?	+	0	38	and up to 65 min in *O.pini* (35)), the female dismounts, removes the spermatophore
O.latipennis	0	0	0	?	+	0	31	within 1 min and eats it (1,31,32,33,34,35,36,37,38). In *O.argentinus*, a second spermatophore may be transferred within 70 min of the first (35). *O.nigricornis* males require 30-60 min between copulations in order to produce another spermatophore (38).

Neoxabea bipunctata	0	0	0	?	+	+*	31,37	Mating behaviour resembles that of *Oecanthus* (37). As in *Oecanthus*, the f feeds from the male's metanotal glands after spermatophore transfer. Unlike *Oecanthus*, however, the male hangs upside down during mating, and after the spermatophore has been transferred, *the male drops his hind legs and r them back and forth along the body of the mounted female. This behaviour continue for as long as 45 min and apparently prevents the female from dismounting (31,37). Eventually, the male ceases this activity and the femal dismounts, whereupon she removes the spermatophore and eats it. The unus post-copulatory behaviour of male *N.bipunctata* probably decreases the probability that the female will end metanotal feeding before sperm transfer complete (31,37).
Phalangopsinae								
Amphiacusta spp	+	0	+	+	0	0	1	Copulation lasts for an average of 7 min (1). The male retains the spermatophore throughout and removes and eats it after copulation has ended. The male then resumes stridulation and re-establishes contact with the female A new spermatophore is formed within 5 mins and the pair mate again 24 min after the first copulation (1). All insemination occurs during copulation. The spermatophore tube has a large diameter and is shorter than that of other gryllids studied, perhaps facilitating the rapid evacuation of sperm (1).
Phaeophilacris spectrum	+	0	0	0	0	0	15,39	Copulation lasts for 30-75 min (15,39). The male retains the spermatophore during and after copulation (15,39).
Trigonidiinae								
"Undescribed spp"	0	0	?	+	0	0	40	Three to six spermatophores are transferred in a single bout of mating. The last spermatophore to be transferred is 45x the volume of the others, though all contain sperm (40). The female is known to eat the spermatophores at some point after their transfer (40).
Balamara gidya	0	0	+	0	0	0	14	Copulation lasts for an average of 3 sec (14), during which a large spermatophore (representing 4% of male body weight) is transferred (14). Males behave aggressively toward their mates should they attempt to leave or to remove spermatophores shortly after copulation. Females generally attempt to remove spermatophores only after the male has ceased guarding (14). The long spermatophore tube appears to make it difficult for the female to remove the spermatophore (14). The mean duration of guarding is 11 min (8.5-16 min), while the mean duration of spermatophore attachment is 54 min (12-124 min). Males are able to copulate an average of 3 times in an eight hour period and and require, on average, 8 hours between successive copulations (14).

Cyrtoxipha columbiana	0	0	?	?	0	?	41	Copulation lasts for 1 sec (41).
Mogoplistinae								
Cycloptilum antillarum	0	0	+	?	0	0	42	The male exhibits vigorous mate-guarding behaviour. The female eventually kicks off the spermatophore and eats it (42).
Arachnocephalus vestitus	0	0	?	+	0	0	33	The female eats the spermatophore 4 sec-1.5 min after the end of copulation. Pairs engage in very frequent matings (33).
MYRMECOPHILIDAE								
Myrmecophila americana	?	0	?	?	?	?	43	
GRYLLOTALPIDAE								
Gryllotalpinae								
Gryllotalpa gryllotalpa	0	0	?	?	0	0	23,44, 45	Copulation lasts for 2-3 min (44). The female does not eat the spermatophore immediately, but waits for a period of time before doing so. The empty case of the spermatophore is finally eaten (33).
Neocurtilla hexadactyla	+	0	?	?	0	0	1,47	The spermatophore is transferred 1 min from the start of copulation, but the pair remain with their abdomens touching for over 10 mins. Following the disturbance of one pair, the male was observed to follow the female and resume the end-end position for a further 10 min, without another spermatophore being being transferred (47). After the first disturbance, the female of this pair was seen to chew at part of the spermatophore, though she ceased to do so when disturbed by movements of the male (47).
Scapteriscinae								
Scapteriscus spp	0	0	?	+	0	0	48	The pair copulate briefly (for about 60 sec) (48,15). After the female has dismounted, the male remains stationary for 8-10 min, then resumes courtship. When the male begins the next courtship sequence, the female removes the spermatophore and may eat it. The female may mount and copulate with the same male several times, but once the female becomes unresponsive to the male's courtship attempts, the pair fight until one of them leaves the burrow system (48).

GRYLLACRIDOIDEA

RHAPHIDOPHORIDAE

Rhaphidophorinae

Tachycines asynamorus	0	+	0	0	0	0	49,33	Copulation lasts for 3-4 min (49). The female takes 1 to 1.5 hours to finish the spermatophylax, after which she eats the ampulla (49).

Troglophilinae

Troglophilus cavicola	?	+	?	?	?	?	50	

Ceuthophilinae

Ceuthophilus gracilipes	+?	0	0	0	0	?	51	"Observations indicate that copulation may be lengthy" (51).
C.latens	+?	0	0	0	0	?	51	
C.pallidipes	+?	0	0	0	0	?	51	
C.mescalero	+?	0	0	0	0	?	51	

Dolichopodinae

Dolichopoda euxina	+	0	0	0	0	0	33	Copulation in *Dolichopoda* continues for 1-4 hours (56 min - 2 hours, 12 min in *D.euxina*) following spermatophore transfer (33,53). After copulation, the female consumes secretions which accompany the spermatophore (53).
D.linderi	+	0	0	0	0	0	52	
D.annae	+	0	0	0	0	0	53	
D.matsakisi	+	0	0	0	0	0	53	
D.petrochilosi	+	0	0	0	0	0	53	
D.thasosensis	+	0	0	0	0	0	53	
D.geniculata	+	0	0	0	0	0	53	
Hadenoecus subterraneus	+	0	0	0	0	0	54	Copulation lasts for several hours (54). There is no spermatophylax, but males do secrete a syrupy fluid which presumably serves to help hold the spermatophore in place (54). The male has paired dorsolateral exsertile organs which act as claspers, enclosing the end of the female's abdomen. These are extremely important in holding the partners together (54).
H.cumberlandicus	+	0	0	0	0	0	54	

Macropathinae								
Pachyramma waitomoensis	0	0	0	0	0	0	55	Copulation lasts for 2-3 min (55). After copulation, the insects sometimes separate, but more often, the female returns to the preliminary mating attitu (sitting between the hind-legs of the male, with the fore- and middle-legs of both insects interwoven). The female has never been observed to eat the spermatophore (55). She leaves it in place to dry and fall off (55).
Gymnoplectron longipes	+	0	0	0	0	0	56	Copulation lasts for about 7 hours (56). Spermatophores have not been seen the female (56).
Dahiniinae								
Udeopsylla robusta	0	0	0	+	0	0	1	Males completely insert a miniature spermatophore in brief, rapidly-repeated copulations (1).
GRYLLACRIDIDAE								
Gryllacris sp	?	0	?	?	?	?	57	
STENOPELMATIDAE								
Stenopelmatinae								
Stenopelmatus intermedius	0	+	0	0	0	0	58	Copulation lasts for 5 min (58,59). A lobed spermatophore is attached to the female. A good portion of the male's abdomen is emptied in this process. After copulation, the female begins to eat the externally visible portions of the spermatophore (ie. the spermatophylax) (58,59). Females have been observed to attack and devour the male after mating (58,59).
S.nigrocapitatus	0	+	0	0	0	0	58	
S.fuscus	0	+	0	0	0	0	59	
Henicinae								
Hemideina femortata	0	0	+	0	0	?	60	Copulation last for 6 min in *H.femorata* (60) and for about 2 min in *H.crassidens*, *H.crassicruris* and *H.Thoracica* (56). Male *Hemideina* aggressively defend harems in galleries (holes in trees) against intruding males. Copulation in *H.femorata* occurs most often at the gallery entrance and females tend to remain in the gallery with males afterwards (60). A male will mate throughout the summer with females of his harem (56). After copulation the male may be "aggressive" toward the female (56). In *H.femorata*, the female has been observed to eat the spermatophore only twice in 35 copulations (60). In one case, the male failed to defend the gallery from an intruding male. The female immediately consumed the spermatophore before mating with the new male occupant (60).
H.crassidens	0	0	+	0	0	+	56	
H.crassicruris	0	0	+	0	0	?	56	
H.thoracica	0	0	+	0	0	?	56	

Zealandrosandrus gracilis	+	0	0	0	0	0	61	Copulation lasts for over 2 hours (61).
Deinacridinae								
Deinacrida heteracantha	+	0	+	+	0	0	62	The number of copulations between the same pair varies, but each lasts for hour, during which a single spermatophore is transferred (62). The spermatophore is then dislodged by the male's further copulation attempts. Throughout the whole period of continuous mating, the male and female do normally separate. The male appears to be the dominant partner; the female appears to be passive (62). If a mating pair is disturbed, the female tends to wander, but the male immediately searches for her and they re-mate. The longest period of continuous mating observed in *D.heteracantha* lasted for 9.5 hours, during which 6 spermatophores were transferred (62). The mean number of spermatophores transferred per mating encounter for this species is 3 (range 1-6) (62). The mean number of spermatophores transferred per mating encounter in *D.fallai* is 5 (range 3-8). The spermatophores are apparently never eaten, but, after being dislodged by the male's copulation attempts, they simply dry and fall off (62).
D.fallai	+	0	+	+	0	0	62	
D.connectens	+	0	?	?	0	0	63	Copulation in this species lasts for at least 35 min (63).
Deinacrida spp	+	0	?	?	0	0	56	Mating normally occurs in the early hours of the morning and can last for hours into the following day (56). Small spermatophores are deposited on the subgenital plate of the female. These can be seen a few hours after copulation, but soon dissappear. Females have not been seen to eat the spermatophore (56).
TETTIGONIOIDEA								
HAGLIDAE								
Cyphoderris sp	0	+	0	0	0	0	1	Copulation in *Cyphoderris* lasts for 2-7 min (an average of 3 min in *C.strepitans*) (1,64,65). The female feeds on the male's hind-wings both before and during spermatophore transfer (1). Males loose about 10% of thier body weight at mating, largely as a result of spermatophylax production (64). The female appears to eat the entire spermatophore at some point following copulation (64).
C.strepitans	0	+	0	0	0	0	64	
C.buckelli	0	+	0	0	0	0	65	

TETTIGONIIDAE								*Note: values cited as mean ± standard error throughout.
Tettigoniinae								
Tettigonia viridissima	0	+	0	0	0	0	P.o., 36,33	The spermatophore is transferred 34 ± 11* min (range 21-56 min, n=3) fr start of copulation (p.o.). Copulation then continues for a further 5.7 ± 1.5 (range 3-8 min) (p.o). The spermatophylax contributes to a mean loss of 18 3% (range 13.4-23%, n=3) of male body weight at mating (p.o.). In two c observed, the female began to eat the spermatophylax about 50 min after the end of copulation and took 16 hours to finish eating it before eating the ampulla (p.o.).
T. cantans	0	+	0	0	0	0	P.o., 33	In the one case observed, copulation lasted about 30 min. The spermatophor was transferred near the end of copulation and represented 17% of male body weight (p.o.).
T. caudata	0	+	0	0	0	0	36	The spermatophore is transferred 51 min (41-60 min) from the start of copulation (36). Copulation ends 13 min (11-17 min) later (36).
Gampsocleis glabra	0	+	0	0	0	0	P.o.	The spermatophore is transferred 12 ± 1 min (range 10-12 min, n=3) from the start of copulation (p.o.). Copulation ends 1.6 ± 0.1 min (range 1.3-1.7 min) later (p.o.). The spermatophylax contributes to a loss of 11 ± 0.7% (range 9.1-12.7%, n=5) of male body weight (p.o.). In the one case observed, the female began to eat the spermatophylax 4 min after the end of copulation and took 3 hours, 20 min to finish the spermatophylax before removing and eating the ampulla (p.o).
Metaballus litus	0	+	0	0	0	0	66	Copulation lasts for an average of 16 or 31 min (66). The spermatophore is transferred a few minutes before the end of copulation and represents 24% of male body weight (66).
Decticus albifrons	0	+	0	0	0	0	P.o., 33,70	In the one case observed, the spermatophore was transferred 15 min from the start of copulation (p.o). The spermatophore represented 13.3% of male body weight (p.o.). The female detached the entire spermatophylax after the end of copulation and took 2 hours, 39 min to finish eating it before she ate the ampulla (p.o.). The male was seen to stridulate the following day.

D. verrucivorus	0	+	0	0	0	0	P.o., 67,69, 33,36	The spermatophore is transferred 6.6 ± 1.2 min (range 5-8.9 min, n=3) fr[illegible] the start of copulation (p.o.). Copulation ends 0.8 ± 0.2 min (range 0.4-1.[illegible] min, n=3) later (p.o). The spermatophore represents 11 ± 0.7% (range 9.6[illegible] 11.9%, n=3) (p.o) or 9.5% (5.5-14.8%) (67) or 10.5% (5.7-13%, n=7) ([illegible] male body weight. The female takes an average of 180 min to finish eating [illegible] spermatophylax, after which she eats the ampulla (67). Males will mate agai[illegible] the day after a previous copulation (69).
Platycleis affinis	0	+	0	0	0	0	P.o., 33	Colpulation lasts for 8.3 ± 0.9 min (range 5.8-10 min, n=4) (p.o.). The spermatophore is transferred 7.2 ± 1 min (range 5-8.7 min, n=3) from the start of copulation (p.o.). Copulation ends 0.5 ± 0.2 min (range 0.1-0.8 min, n=3) later (p.o.). The spermatophore represents 6.4 ± 0.4% (range 5.2-7.7%, n=5) of male body weight (p.o.).
P. albopunctata	0	+	0	0	0	0	P.o., 45	The spermatophore is transferred 7 ± 2.3 min (range 4.7-9.2 min, n=2) from the start of copulation (p.o.). Copulation ends 1.2 ± 0.7 min (range 0.5-1.8 min, n=2) later (p.o.). The spermatophore represents 6.3 ± 0.5% (range 5-8%, n=5) of male body weight (p.o.). The female detaches the spermatophylax about 3 min after the end of copulation and takes 40-50 min (n=2) to finish eating it before consuming the ampulla (p.o.). Males resume stridulation within 1 hour of the end of copulation. One male was observed to transfer a spermatophore to another female 1 hour, 41 min from the end of a previous mating (p.o.).
P. tesselata	0	+	0	0	0	0	P.o.	In the single case observed, the spermatophore represented 5% of male body weight (p.o.). The female detached the spermatophylax after the end of copulation and took 1 hour to finish eating it, after which she ate the ampulla (p.o.). The male began to stridulate 20 min after the end of copulation (p.o.).
P. nigrosignata	0	+	0	0	0	0	P.o.	In the single case observed, the spermatophore was transferred 20 min from the start of copulation and the pair separated 1 min later (p.o.). The spermatophore represented 6.4% of male body weight (p.o.).
P. vittata	0	+	0	0	0	0	33	
Metrioptera saussuriana	0	+	0	0	0	0	P.o.	The total duration of copulation is 8 ± 0.8 min (range 6.2-10 min, n=4) (p.o). The spermatophore is transferred 6.3 ± 0.7 min (range 5-7 min, n=3) from the start of copulation. Copulation ends 1 ± 0.1 min (range 0.8-1.2 min, n=3) later (p.o.). The spermatophore represents 8.5 ± 1% (range 6-10%, n=4) of male body weight (p.o.).

M.bicolor	0	+	0	0	0	0	P.o., 23	The mean duration of copulation is 10.5 ± 2.5 min (range 8-13 min, n=2) (p.o.). The spermatophore is transferred toward the end of copulation and represents 10 ± 1% (range 8.8-11.9 %, n=3) of male body weight (p.o.).
M.brachyptera	0	+	0	0	0	0	P.o., 33	The spermatophore is transferred 15.5 ± 2.5 min (range 13-18 min, n=2) from the start of copulation (p.o.). The pair separate 4.6 ± 0.1 min (range 4.7 min, n=2) later (p.o.). The spermatophore represents 11.3 ± 0.7% (ra 10.6-12 %, n=2) of male body weight (p.o.). The female begins to eat the spermatophylax 1-4 min after the end of copulation and takes 138 ± 7 min (range 130-145 min, n=2) to finish it before eating the ampulla (p.o.).
M.roeselii	0	+	0	0	0	0	P.o., 33,45	The spermatophore is transferred 33.5 ± 5.5 min (28-39 min, n=2) from t start of copulation (p.o.). The pair separate 1.2 ± 0.2 min (range 1-1.3 min, n=2) later (p.o.). The spermatophore represents 10.5 ± 1% (range 9.3-12.7%, n=3) of male body weight (p.o.).
Sepiana sepium	0	+	0	0	0	0	P.o., 33	Copulation lasts for 11 ± 2 min (range 9-13 min, n=2) (p.o.). The spermatophore is transferred toward the end of copulation and represents 7.5 ± 0.6% (range 7-8.1%, n=2) of male body weight (p.o.).
Rhacocleis germanica	0	+	0	0	0	0	36	
Pachytrachis spp	0	+	0	0	0	0	P.o.	In the single case observed, the spermatophore was transferred 12 min from the start of copulation and represented 9% of male body weight (p.o.). The pair separated 48 sec after spermatophore transfer (p.o.). The female began to eat the spermatophylax 7 min later and took 2 hours to consume it before eating the ampulla (p.o.).
Yersinella raymondi	0	+	0	0	0	0	P.o.	The spermatophore is transferred 4.1 ± 0.7 min (range 3-6 min, n=4) from the start of copulation and represents 7.3 ± 1% (range 4.8-9%, n=5) of male body weight (p.o.). Copulation ends 1.9 ± 0.2 min (range 1.5-2.5 min, n=4) later (p.o.). The female takes 91 ± 6.4 min (range 80-102 min, n=3) to finish eating the spermatophylax and eats the ampulla 1 min later (P.o.).

Anonconotus alpinus	0	+	0	0	0	0	P.o., 70	This species differs from most other tettigoniids in the manner of coupling. The female does not mount the male, but instead, the male leaps onto the female, grasps her ovipositor or a leg with his jaws then manoeuvres his abdomen and tightly grips the underside of the female's abdomen with his c (70;p.o.). The male stridulates throughout this activity (p.o.). Copulation la for 7.7 ± 1 min (range 4.4-12 min, n=6). The spermatophore is transferre 4.6 ± 0.9 min (range 2.4-3.4 min, n=4) from the start of copulation and th pair separate 2 ± 1 min (range 0.5-5 min, n=4) later (p.o.). The spermatop represents 2 ± 0.2% (range 1.4-2.6%, n=8) of male body weight (p.o.). T female begins to eat the spermatophylax 1-7 min after the end of copulation takes 6-36 min (n=2) to fully consume it before eating the ampulla (p.o.). On two occasions, males were observed to transfer a spermatophore to a different female about 1 hour after the end of a previous mating (p.o.).
Antaxius pedestris	0	+	0	0	0	0	P.o.	In the single case observed, the spermatophore was transferred 24 min from the start of copulation and the pair separated 1.5 min later (P.o). The spermatophore represented 16.0 % of male body weight (p.o.).
A.hispanicus	0	+	0	0	0	0	P.o.	The spermatophore is transferred 34 ± 11 min (range 18-55 min, n=3) from the start of copulation and represents 15.4 ± 0.8% (range 14-17 %, n=3) of male body weight (p.o.). The pair separate 2.3 ± 0.5 min (range 1-3 min, n=4) later (p.o.). In the single case observed, the female took 5 hours to finish eating the spermatophylax and ate the ampulla directly afterwards (p.o.).
Pholidoptera griseoaptera	0	+	0	0	0	0	P.o., 36	Copulation lasts for 8.7 ± 2.5 min (range 4.4-13 min, n=3) (P.o.). The spermatophore is transferred 8.8 ± 2 min (range 6.5-11 min, n=2) (P.o) or 2-19 min, according to (36), from the start of copulation. Copulation ends 2.1 ± 0.1 min (range 2-2.2 min, n=2) or 3-4 min, according to (36), later. The spermatophore represents 11.3 ± 0.4% (range 10.4-12%, n=4) of male body weight (p.o.). In the one case observed, the female took 105 min to finish eating the spermatophylax, at which point she began to eat the ampulla, consuming the external "supplementary reservoirs" (see 33) first, before everting her genital chamber and removing and eating the remainder of the ampulla (p.o).
P.indistincta	0	+	0	0	0	0	33	
Eupholidoptera spp 1	0	+	0	0	0	0	P.o.	In the single case observed, the spermatophore represented 13% of male body weight and copulation ended 2.5 mins after spermatophore transfer (p.o.).

Eupholidoptera spp 2	0	+	0	0	0	0	P.o.	In the single case observed, copulation prior to spermatophore lasted for 46 min (p.o.). Copulation ended 1.7 min after spermatophore transfer and the spermatophore represented 16.4% of male body weight (p.o.).
Idiostatus spp	0	+	0	0	0	0	71	The spermatophore is transferred 15-45 min from the start of copulation and the pair separates shortly afterwards (71). The female eats the medium-size spermatophylax after the end of copulation.
Pediodectes haldemanii	0	+	0	0	0	0	72	Copulation lasts for 15-20 min (72). The female is released following
P.nigromarginatus	0	+	0	0	0	0	72	the transfer of the spermatophore, with its medium-large spermatophylax (7
Anabrus simplex	0	+	0	0	0	0	73,74	Copulation lasts for about 7 min (74). The spermatophore is transferred towards the end of copulation and represents up to 27% (average= 20%) of male body weight (73).
Decticita brevicauda	+?	0	0	0	0	0	75	Copulation lasts for 2 hours (75). Males of this genus do not produce a "seminal mass" (ie. spermatophylax) (75).
Saginae								
Saga ephippigera	0	0*	0	0	0	0	33,76, 77	Copulation lasts for 4-6 min (76). *The spermatophylax is minute and vestigial (33). The male apparently leaves the female immediately after the end of copulation, though (33) observed 1 pair mating 3 times in 2 days. The female does not eat the spermatophore immediately, but apparently leaves it in place for 3-17 hours before eating it (33,76).
S.natoliae	0	+	+?	0	0	0	76,77, 78	Copulation lasts for 8-15 min (76). A small-medium size spermatophylax is produced (76). After copulation, the pair stay together for 60-90 min (76,78). The female apparently eats the spermatophore 20-24 hours after the end of copulation (76).
S.rhodiensis	0	+	0	0	0	0	76,77	Copulation lasts for 3-4 min (76). A small-medium size spermatophylax is is produced and the male moves away from the female shortly after spermatophore transfer (76).

S.campbelli	0	+	0	0	0	0	76,77, 79	Copulation lasts for 2.5-3 min (76). The male leaves the female shortly after transfer of a small-medium size spermatophylax (76). Females of this specie have been observed to eat the male after mating (79).
Hemisaga denticauda	?	+	?	?	?	?	80	
Pachysagella	?	+	?	?	?	?	80	
Conocephalinae								
Conocephalus discolor	0	+	0	0	0	0	P.o., 33,36	Copulation lasts for 19.6 ± 1.8 min (range 10.6-27 min, n=12) (p.o.). The spermatophore is transferred 9.4 ± 1.7 min (range 6.6-14 min, n=4) from the start of copulation and the pair separate 10.2 ± 2 min (range 4.7-14 min, n=4) later (p.o.). The spermatophore represents 12.6 ± 0.5% (range 8-17%, n=21) of male body weight (p.o.). The spermatophylax is translucent and divided into two separate lobes which rest either side of the female's abdomen (33). The female begins to eat the spermatophylax 6 ± 3 min (range 1-16 min, n=5) after the end of copulation (p.o.). She detaches and eats each lobe in turn and takes 91 ± 11 min (range 60-140 min, n=7) to finish the spermatophylax, after which she eats the ampulla (p.o.). Males resume stridulation 26-50 min (n=2) after the end of copulation (p.o.). One male was observed to transfer a spermatophore to another female 3.5 hours after the end of a previous copulation (p.o). The female refractory period was found to be 4 ± 1.2 days (range 2-7 days, n=5).
C.dorsalis	0	+	0	0	0	0	P.o.	Copulation lasts an average of 12 ± 2 min (range 9.8-16 min, n=3) (p.o.). spermatophore is transferred 3.7-14 min (n=2) from the start of copulation and the pair separate 2-6 min (n=2) later (p.o.). The spermatophore represents 8.8 ± 0.7% (range 7.3-9.7 %, n=3) of male body weight (p.o.). In the one case observed, the female took about 80 min to finish eating both lobes of the spermatophylax, at which point she ate the ampulla (p.o.).
C.nigropleurum	0	+	0	0	0	0	81,82	The spermatophore represents an average of 10.8% of male body weight (81,82).
Orchelimum nigripes	0	+	0	0	0	0	83	The spermatophore represents about 10% of male body weight (83).
O.delicatum	0	+	0	0	0	0	80	
Atlanticus testaceus	0	+	0	0	0	0	83	The spermatophore repesents up to 17% of male body weight (average = 10%) (83).

Copiphora rhinoceros	0	+	0	0	0	0	84	Copulation lasts for 3.75 hours (84). The couple separate less than 1 min after spermatophore transfer (84). The female begins to eat the large spermatophylax 12 min from the end of copulation (84).
Vestria viridis	?*	+	0	0	0	0	85	*Copulation lasts for 3 hours (85), though it is unclear at which point during this period the spermatophore is transferred.
Ruspolia nitidula	0	0*	0	0	0	0	P.o., 33,36	The mean duration of copulation is 27 ± 3 min (range 16.3-46 min, n=10) (p.o.) . The spermatophore appears to be transferred about 6 min from the start of copulation (36;p.o.), although it is difficult to ascertain the precise moment of spermatophore transfer, due to its small size. Copulation continues for about 25 min according to (36) and has been observed to last as long as 2 hours (36). The spermatophore represents an average of 1.3 ± 0.3% (range 0.6-2.8%, n=7) of male body weight (p.o.). *The spermatophylax is minute and appears to be vestigial (33). Unlike most other bushcrickets, the ampulla is completely internal in this species (33). At 2.3 ± 0.4 min (range 1-3.5 min, n=5) from the end of copulation, the female begins grooming activity (p.o.). She grooms the length of her ovipositor, her sub-genital plate and the underside of her abdomen (p.o.). During these grooming motions, part of the tiny spermatophylax is usually eaten. However, females leave the ampulla in place for an average of 15.8 ± 1.17 hours (range 1-29 hours, n=31) (p.o.). After this time the ampulla and dried remains of the spermatophylax either fall away or are removed by the female (33;p.o.). Males resume stridulation and will attempt to mate with nearby females an average of 2.4 ± 0.2 min (range 2-3 min, n=7) after the end of copulation (p.o.). One male was observed to transfer a spermatophore to a another female 80 min after a previous copulation (p.o.). The mean female refractory period is 3.9 ± 0.6 days (range 2-7 days, n=10).
Neoconocephalus ensiger	0	0*	0	0	0	0	86	Copulation lasts for 40 min (27-68 min) (86). Females do not groom their genital region following copulation (86). Males resume calling 10 sec to 8.5 min after the end of copulation. *The spermatophylax is minute (86).
N. retusus	0	0*	0	0	0	0	87	Copulation lasts 40 min and *the spermatophylax is minute (87).
N. nebrascensis	0	0*	0	0	0	0	88	Copulation lasts 9-23 min (88) and *a minute spermatophylax is produced (88). The male begins to stridulate 5-10 min after the end of copulation (88). In one case, a male was observed to copulate with another female only 5 mins after the end of the previous mating (88).
Belocephalus subapterous	?	0*	?	?	?	?	80	*The spermatophylax is minute (80).

Microtettigoniinae								
Microtettigonia spp	?	+	?	?	?	?	80	A large spermatophylax is produced (80).
Listeroscelidinae								
Requena verticalis	0	+	0	0	0	0	89,90, 91	The spermatophore represents an average of 12.5% (91) to 19% (89,90) of weight. The female detaches the spermatophylax shortly after the end of copulation and takes about 5 hours to finish eating it before eating the ampu The male refractory period lasts an average of 2.6 days (91).
Phlugis spp	0	+	0	0	0	0	85	Copulation lasts for 1 min (85).
Meconematinae								
Meconema thalassinum	+	0	0	0	0	0	Ch.3, 36	The spermatophore is transferred about 1 min from the start of copulation, after which the pair remain in copulation for 17 ± 1.7 min (range 13-24 min, n=6) (ch 3 The spermatophore lacks a spermatophylax completely and represents an average of only 0.59 ± 0.07% (range 0.47-0.79%, n=4) of male body weight (P.o.). During copulation, the unusually long cerci of the male encompass the end of the female's abdomen and meet on the other side (ch 3). The female eats the spermatophore within 1 min of the end of copulation (ch 3).
M.meridionale	+	0	0	0	0	0	Ch.3	The spermatophore is transferred 53 ± 2.8 sec (range 40-60 sec, n=6) from the start of copulation (ch 3). The pair then remain in copulation for a further 81 ± 9 min (range 35-105 min, n=7) (ch.3). As in *M.thalassinum*, there is no spermatophylax. The spermatophore represents 1.77 ± 0.18% (range 0.98-2.49% n=8) of male body weight (ch 3). During copulation, the male's unusually long cerci wrap around the end of the female's abdomen and cross over one another on the other side (ch 3). The female eats the spermatophore about 1 min after the end of copulation (ch 3). Males were observed to resume calling (ie."drumming") as little as 10 min after the end of copulation.
Cyrtaspis scutata	0	+	0	0	0	0	Ch.3	The spermatophore is transferred 143 ± 41 sec (range 92-223 sec, n=3) from the start of copulation (ch 3). The pair separate 91 ± 14 sec (range 70-118 sec, n=3) later (ch 3). A spermatophylax is present and the spermatophore represents 9.2 ± 0.4% (range 7.5-10.5%, n=7) of male body weight (ch 3). The female takes 1-2 hours to finish eating the spermatophylax, after which she eats the ampulla (ch 3).

Phasmodinae								
Phasmodes ranatriformis	?	+	?	?	?	?	80	A small spermatophylax is produced (80).
Zaprochilinae								
Gen.Nov.22,sp1.	0	+	0	0	0	0	92,93, 94,95	The spermatophore is transferred 20 min from the start of copulation (92). T process of spermatophore transfer takes 2.5 min and the pair separate 8.5 m later (92). The spermatophore represents 21% of male body weight (93). Th begins to eat the spermatophylax 4 min after the end of copulation and takes 80 min to consume it fully, after which she eats the ampulla (94). The male refractory period lasts for 5 days (95).
Hetrodinae								
Acanthoplus bechuanus	?	+	0	0	0	0	96	The female eats the large spermatophylax after the end of copulation (96).
A.armativerntris	?	+	?	?	?	?	97	A large spermatophylax is produced (97).
A.speiseri	0	+	0	0	0	0	98,99	The spermatophore is produced over 300 min from the start of copulation (98,99). The female takes 8 hours to finish eating the spermatophylax, after which she eats th ampulla (98,99).
Eugaster spinulosa	0	+	0	0	0	0	100, 101	Copulation lasts for 10-15 min (101). The spermatophore becomes visible 5 min from the start of copulation and the pair separate 5 min later (101). The female eats the spermatophylax a short while after the end of copulation (101).
E.guyoni	0	+	0	0	0	0	102	
Gymnoproctus sculpturatus	+?	0?	0	0	0	0	103	Copulation lasts at least 8 hours and no spermatophore is visible (103).
Ephippigerinae								
Ephippiger terrestris	0	+	0	0	0	0	Ch.3	Copulation lasts for less than 30 min (ch 3). The spermatophore represents 30.6 ± 0.3% (range 30.3-30.9 %, n=2) of male body weight (ch 3).

E.ephippiger	0	+	0	0	0	0	Ch.3, 104, 70,105	Copulation lasts for 26 ± 3 min (range 18-42 min, n=9) (ch 3). The sperm becomes visible 13.6 ± 1.2 min (range 11.3-17 min, n=4) from the start o copulation and the pair separate 6.1 ± 1.2 min (range 4-9.5 min, n=4) late The spermatophore represents 28 ± 1% (range 18.4-35.6%, n=20) of male weight (ch 3). The male sexual refractory period lasts for 3-5 days (104). Tl female generally takes 6.5-24 hours to finish eating the spermatophylax, aft which eats the ampulla (p.o.). In some cases, however, the female eats only of the spermatophylax and leaves the remainder to dry and fall away a day c later (p.o.).
E.cruciger	0	+	0	0	0	0	104, 105	Copulation lasts for 30 min (104). The spermatophore represents 32 % of m weight (104).
E.perforatus	0	+	0	0	0	0	Ch.3	The spermatophore becomes visible 3.5 ± 0.3 (range 2.8-3.9 min, n=4) from the start of copulation and the pair separate 23.4 ± 5.8 min (range 12-30.2 min, n=3) later (ch 3). The spermatophore represents 20.6 ± 2.1 (range 10-26%, n=7) of male body weight (ch 3). In the one case observed, the female began to eat the spermatophylax 2 min after the end of copulation and took 3.6 hours to finish it before eating the ampulla (p.o).
Ephippigerida taeniata	0	+	0	0	0	0	Ch.3	Copulation lasts for 3.0 ± 0.6 min (range 1.8-5.1 min, n=5) (ch 3). The spermatophore becomes visible 1.1 ± 0.3 min (range 0.6-1.5 min, n=3) from the start of copulation (ch 3). Copulation ends 1.5 ± 0.4 min (range 0.7-2 min, n=3) later (ch 3). The spermatophore represents 28 ± 0.9 % (19.3-32.9%, n=21) of male body weight (ch 3). Females generally take up to 24 hours to finish eating the spermatophylax, after which they eat the ampulla (p.o.). Occasionally, females only ate part of the spermatophylax and left the remainder of the spermatophore to dry and fall off one or two days later (p.o).
E.zapateri	0	+	0	0	0	0	Ch.3	In the single case observed, copulation lasted for under 30 min and the spermatophore represented 40.5% of the male's body weight (ch 3). The female took 13.5 hours to finish eating the spermatophylax, after which she ate the ampulla (p.o.).
E.saussureiana	0	+	0	0	0	0	Ch.3	In the single case observed, copulation lasted for less than 26 min and the spermatophore represented 28.1% of the male's body weight (ch 3).
Baetica ustulata	0	+	0	0	0	0	Ch.3	In the single case observed, the spermatophore became visible 23 min from the start of copulation (ch 3). Copulation ended 2 min later and the spermatophore represented 29.1% of the male's body weight (ch 3).

Steropleurus stali	0	+	0	0	0	0	Ch.7	Copulation lasts for 16.6 ± 0.9 min (range 10-24 min, n=19) (ch 7). The spermatophore becomes visible 13.9 ± 1 min (10-20 min, n=10) from the start of copulation and the pair separate 1.7 ± 0.2 min (range 0.5-3.15 min, n=[illegible] later (ch 7). The spermatophore represents, on average, 27 ± 0.7% (range [illegible] 36.7%, n=51) of male body weight (ch 7). The female begins to eat the spermatophylax 2.6 ± 0.5 min (range 1-5 min, n=10) from the end of copulation. It takes the female 7.49 ± 0.74 hours (range 2-14 hours, n=20) to finish the spermatophylax, after which she eats the ampulla (ch 7). The male sexual refractory period lasts for 3 ± 0.14 days (range 2-4 days, n=13) (ch 7).
S.asturiensis	0	+	0	0	0	0	Ch.3	Copulation lasts for 18.7 ± 2.7 min (range 12-25 min, n=4) (ch 3). The spermatophore becomes visible 10-16 min (n=2) from the start of copulation and copulation ends 2 min later (n=2). The spermatophore represents 27 ± 1.6% (range 14.2-30.9%, n=10) of male body weight (ch 3).
S.brunneri	0	+	0	0	0	0	Ch.3	In the single case observed, the spermatophore became visible 0.9 min from the start of copulation and the pair separated 1.4 min later (ch 3). The spermatophore represented 30.6% of male body weight (ch 3). The female began to eat the spermatophylax 2 min after the end of copulation and took 22 hours to finish the spermatophylax, after which she ate the ampulla (p.o.).
S.parezii	0	+	0	0	0	0	Ch.3	In the single case observed, the spermatophore became visible 2 min from the start of copulation and the pair separated 3 min later (ch 3). The spermatophore represented 24.7% of male body weight (ch 3).
S.martorelli	0	+	0	0	0	0	Ch.3	In the single case observed, the spermatophore was transferred 0.87 min from the start of copulation and the pair separated 2.13 min later (ch 3). The spermatophore represents 26.6 ± 3.3% (range 20.8-35.3%, n=3) of male body weight (ch 3).
S.catalaunicus	0	+	0	0	0	0	Ch.3	In the single case observed, the spermatophore became visible 15 min from the start of copulation and the pair separated 3.2 min later (ch 3). The spermatophore represented 40.3% of male body weight (ch 3).
S.andalusius	0	+	0	0	0	0	Ch.3	Copulation lasts for less than 30 min and the spermatophore represents 29 ± 1% (range 28-30%, n=2) of male body weight (ch 3).
Uromenus rugiscollis	+	+	0	0	0	0	Ch.3, 106	The spermatophore is transferred 104 ± 8.6 (range 68-164 min, n=10) from the start of copulation (ch 3). Copulation continues for a further 101 ± 10.2 min range 60-141 min, n=8) (ch 3). The spermatophore represents 11.5 ± 0.7% range 8.7-15.7%, n=12) of male body weight (ch 3). In the one case observed, the female began to eat the spermatophylax 4 min after the end of copulation and took 106 min to finish it, after which she ate the ampulla (ch 3). The male refractory period lasts for less than 1 day (Ch 3).

Callicrania monticola	0	+	0	0	0	0	Ch.3	The spermatophore becomes visible 26.5-30 min (n=2) from the start of copulation (ch 3). The pair separate 5-7 min (n=2) later and the spermatoph represents 28 ± 0.7% (range 26.8-29.2%, n=3) of male body weight (ch 3 female begins to eat the spermatophylax about 6 min after the end of copula and takes 12-17 hours (n=2) to finish it, after which she eats the ampulla (p
Platystolous pachygaster	0	+	0	0	0	0	102	
Pycnogastrinae								
Pycnogaster inermis	0	+	0	0	0	0	P.o.	The spermatophore is transferred 27.2 ± 2.5 (range 24.7-29.6 min, n=2) fr the start of copulation (p.o.). The pair separate 4-10 (n=2) min later and the spermatophore represents 22.4 ± 1.5% (range 19.5-26.4%, n=4) of male body weight (p.o.). The female takes 5-19 hours (n=2) to finish eating the spermatophylax and eats the ampulla immediately afterwards (p.o.).
Bradyporinae								
Bradyporus multituberculatus	0	+	0	0	0	0	107	Copulation lasts for 97 min (107). The spermatophore becomes visible 5-6 min befor the end of copulation (107). The female eats the large spermatophylax after the end of copulation (107).
Mecopodinae								
Mecopoda elongata	0	0	0	0	0	0	P.o.	The total duration of copulation is only 8 seconds (p.o.). There is no spermatophylax and the spermatophore represents only 0.74 ± 0.03% (range 0.71-0.76%, n=2) of male body weight (p.o.). In the 2 cases observed, the female left the spermatophore attached for about 6 hours before eating part of it. The remainder of the spermatophore was dropped (p.o.).
Euthypoda acutipennis	0	+	0	0	0	0	108	After the end of copulation, the female takes 2 hours to finish eating the spermatophylax (108).
Pseudophyllinae								
Zabalius apicalis	?	0	0	0	0	0	108	The female licks her genital area after the end of copulation, but invariably leaves the spermatophore attached for about 24 hours before eating it (108). There is no spermatophylax (108).

Adenes sp	?	+	0	0	0	0	108	The female eats the entire spermatophore within 30 min of the end of copulation (108).
Bliastes insularis	?	+	0	0	0	0	85	The spermatophylax consists of 2 separate lobes, each attached to one half the ampulla by a stalk (85). The female eats the spermatophylax at some poi after its transfer (85).
Pterophylla beltrani	+	0	0	0	0	0	109	The spermatophore is emitted 1 min from the start of copulation (109). copulation then continues for 24-37 min (109). There is no spermatophylax the female eats the spermatophore 6 min after the end of copulation (109).
Phaneropterinae								
Phaneroptera nana	0	+	0	0	0	0	P.o., 33,36	The spermatophore becomes visible 0.57 ± 0.1 min (range 0.3-0.8 min, n=4) from the start of copulation (p.o.). The pair sepatate 2.2 ± 0.3 min (range 1.63-3 min, n=4) later (p.o.). The spermatophore represents 5.5 ± 0.3% (range 3.9-7.4%, n=12) of male body weight (p.o.). The female begins to eat the stalked, leaf-like spermatophylax 5.3 ± 1.5 min (range 2.5-11 min, n=5) from the end of copulation (p.o.). In the one case observed carefully, the female took 2 hours to finish eating the spermatophylax, after which she ate the ampulla (p.o.). In some cases, however, the female appeared to eat only part of spermatophylax soon after the end of copulation and leave the rest in place for up to 11 hours (p.o.). One male was observed to resume stridulation 3 hours after the end of copulation (p.o). The female refractory period appears to be about 1 day (p.o).
P.falcata	0	+	0	0	0	0	P.o., 45	The spermatophore becomes visible 1 min (n=2) from the start of copulation (p.o.) The pair separate 3.2 ± 0.9 min (range 2.3-4 min, n=2) later and the spermatophore represents 16 ± 0.8% (range 14.2-18%, n=4) of male body weight (p.o.). In the single case observed carefully, the female began to eat the spermatophylax 2 min after the end of copulation and took 5 hours to finish it, after which she ate the ampulla (p.o.).
Tylopsis lilifolia	0	+	0	0	0	0	P.o., 33,36	The spermatophore becomes visible 0.4 ± 0.1 min (range 0.1-0.7 min, n=7) from the start of copulation (p.o.). Copulation ends 1.7 ± 0.1 min (range 1.3-2.3 min n=7) later and the spermatophore represents 25.7 ± 1.8% (range 16.7-33.4%, n=10) of male body weight (p.o.). In the single case observed carefully, the female began to eat the spermatophylax 15 min after the end of copulation and took 10.6 hours to finish it, after which she ate the ampulla (p.o.).

Amblycorypha parvipennis	?	+	0	0	0	0	110	The spermatophore represents, on average, 12% (range 10-20%) of male bo weight (110).
A.haustacea	?	+	0	0	0	0	72	
Arethaea ambulator	0	+	0	0	0	0	72	Copulation lasts for 2 min (72).
A.grallator	0	+	0	0	0	0	72	
Polichne spp	?	+	0	0	0	0	80	
Stiplonchlora marginella	0	+	0	0	0	0	85	Copulation lasts for 30 sec (85). The female eats the spermatophylax following the end of copulation (85).
Ctenophlebia spp	0	+	0	0	0	0	85	Copulation is "very rapid" (85).
Euthyrachis spp	0	+	0	0	0	0	85	Copulation lasts for a few min (85).
Barbitistes serricauda	0	+	0	0	0	0	P.o.	In the one case observed, copulation lasted for about 4 min and the spermatophore represented 28.6% of male body weight (p.o.).
B.berengueri	0	+	0	0	0	0	111	Copulation duration etc. as in *Isophya pyrenaea*, see below (111).
Metaplastes ornatus	0	+	0	0	0	0	113	Copulation consists of 2 distinct phases (113). In phase 1, which lasts for 10-60 min, the male introduces his specialised sub-genital plate into the female's genital chamber and stimulates the release of stored sperm (113). In phase 2, which lasts an average of 2.5 min, the spermatophore is transferred (113). This represents an averag of 22% (range 16-28%) of male body weight (113). The female takes several hours to finish eating the spermatophylax, after which she eats the ampulla (113).
Isophya pyrenaea	0	+	0	0	0	0	111, 112	Copulation lasts for 1-2 min (111). The female begins to eat the large spermatophyla a few minutes after the end of copulation (111). The female takes 3-4 hours to finish eating the spermatophylax, after which she eats the ampulla (111).
I.schneideri	0	+	0	0	0	0	33	A large spermatophylax is produced (33).

Leptophyes punctatissima	0	+	0	0	0	0	Ch.7, 45	The mean duration of copulation is 4.2 ± 0.2 min (range 1.7-8 min, n=27) Spermatophore transfer begins 1.18 ± 0.07 min (range 0.93-1.43 min, n=7 the start of copulation (ch.7). The pair separate 2.1 ± 0.27 min (range 0.73 min, n=7) later and the spermatophore represents 5.6 ± 0.26% (range 2.3- n=45) of male body weight (ch.7). The female detaches the small spermato and begins to eat it 11.5 ± 0.7 min (range 1.7-17 min, n=32) from the end copulation (ch.7). The female eats the ampulla directly after having finished the spermatophylax, 42.6 ± 1.8 min (range 22-74 min, n=37) from the end copulation (ch.7). Males resume stridulation 70 ± 4 min (range 60-91 min, from the end of copulation. One male was observed to transfer a spermatoph to a different female 217 min after a previous copulation (ch.7). The mean female refractory period is 185.4 ± 38.3 min (range 75-384 min, n=7) (ch.
L. laticauda	0	+	0	0	0	0	Ch.7	The mean duration of copulation is 3.9 ± 0.2 min (range 2.8-5.9 min, n=20) (ch.7). Spermatophore transfer begins 0.88 ± 0.04 min (range 0.67-1.2 min, n=16) from the start of copulation (ch.7). The pair separate 2.8 ± 0.2 min (range 1.8-4.9 min, n=18) later and the spermatophore represents 22.9 ± 0.7% (range 11.3-32.7%, n=60) of male body weight (ch.7). The female begins to eat the large spermatophylax 2.6 ± 0.8 min (range 0.1-9 min, n=12) from the end of copulation (ch.7). The female eats the ampulla directly after having finished the spermatophylax, 338.3 ± 20.4 min (range 225-462 min, n=10) after the end of copulation (ch.5). Males may begin "agressive song" (see 114) as little as 5 min after the end of copulation, but will not re-mate until at least the following day (ch.7). The mean female refractory period is 6.9 ± 1.0 days (range 1-15 days, n=15) (ch.7).
L. albovittata	0	+	0	0	0	0	P.o., 33,112	Spermatophore transfer begins 0.42 min (n=2) from the start of copulation and the pair separate 1.1 ± 0.2 min (range 0.9-1.2 min, n=2) later (p.o.). The the spermatophore represents 8.6 ± 0.4% (range 8.2-9%, n=2) of male body weight (p.o.). The female takes about 2 hours to finish the spermatophylax (112).
L. bosci	0	+	0	0	0	0	P.o., 36	The spermatophore is transferred about 1 min (n=1) from the start of copulation and the pair separate about 1 min (n=1) later (p.o). The spermatphore represents 7.3 ± 0.3% (range 6.5-8%, n=5) of male body weight (p.o.).
Poecilimon jonicus	0	+	0	0	0	0	P.o.	The spermatophore is transferred 0.98 ± 0.16 min (range 0.58-1.28 min, n=4) from the start of copulation (p.o.). The pair separate 1.0 ± 0.2 min (range 0.43-1.33 min, n=4) later (p.o.). The spermatophore represents 10.4 ± 1.2% (range 8-14.9%, n=5) of male body weight. In the single case observed carefully, the female took 4 hours to finish the spermatophylax, after which she ate the ampulla (p.o.).

P.schmidtii	0	+	0	0	0	0	P.o.	Copulation lasts for 2.2 ± 0.1 min (range 1.2-3.6 min, n=25) (p.o.). The spermatophore becomes visible 0.85 ± 0.07 min (range 0.53-1.65 min, n= from the start of copulation and the pair separate 1.3 ± 0.1 min (range 0.95 2.6 min, n=16) later (p.o.). The spermatophore represents 14.3 ± 0.33% range 7.2-19%, n=60) of male body weight (p.o.). The female begins to ea spermatophylax 7.6 ± 1.2 min (range 2.17-15 min, n=12) after the end of copulation (p.o.). The female takes an average of 243.0 ± 18 min (range 51-543 min, n=47) to finish the spermatophylax, after which she eats the ampulla (ch 6). Males have been observed to re-mate on the day following a previous mating (p.o). The mean female refractory period is 4.3 ± 0.8 days (range 1-8 days, n=11) (p.o.).
P.veluchianus	0	+	0	0	0	0	P.o., 115, 116	Copulation lasts for 2 ± 0.3 min (range 1.8-2.3 min, n=2) (p.o.). The spermatophore represents 26% of male body weight (115). The female begins to eat the large spermatophylax 13-16 min (n=2) from the end of copulation (p.o.) and takes about 9 hours to finish it, after which she eats the ampulla (116).
P.affinis	0	+	0	0	0	0	P.o., 115	The spermatophore becomes visible 1.0 ± 0.1 min (range 0.75-1.25 min, n=5) from the start of copulation (p.o.). Copulation ends 1.35 ± 0.2 min (range 0.78-1.8 min, n=5) later (p.o.). The spermatophore represents 16.4 ± 0.9% (range 12.6-22.1%, n=14) (p.o) or 15% (115) of male body weight. The female begins to eat the spermatophylax about 4 min (range 3-7, n=3) from the end of copulation and takes 205 ± 43 min (range 120-405 min, n=6) to finish it, after which she eats the ampulla (P.o.).
P.bidens	0	+	0	0	0	0	33	A medium-large spermatophylax is produced (33).
Polysarcus scutatus	0	+	0	0	0	0	P.o.	Copulation lasts for less than 10 min (p.o.). The spermatophore represents 15.9 ± 0.5% (range 15.5-16.4%, n=2) of male body weight (p.o.).
P.denticaudus	0	+	0	0	0	0	23	
Odontura stenoxypha	0	+	0	0	0	0	P.o.	In the single case observed the spermatophore represented 20.2% of the male's body weight (p.o.).
Dichopetala emarginata	+	0	0	0	0	0	72	Copulation lasts for several hours (72). There appears to be no spermatophylax (see 72 & 97).

References cited in table 2.1

1) Alexander & Otte 1967a; 2) Simmons 1988b; 3) Huber 1955; 4) Loher & Rence 1978; 5) Simmons 1986; 6) Alexander 1961; 7) Simmons 1990b; 8) Nagao & Schimozawa 1987; 9)Khalifa 1950a; 10) Sakaluk 1991; 11) Sakaluk 1987; 12) Sakaluk 1985; 13) Sakaluk 1984; 14) Evans 1988; 15) Loher & Dambach 1989; 16) Loher 19[illegible] 18) Otte & Cade 1983; 19) Boldyrev 1927; 20) Boldyrev 1928a; 21) Campan & Demai 1983; 22) Gabutt 1954; 23) Gerhardt 1921; 24) Mays 1971; 25) Bidochka & Snedden 1985; 26) Chopard 1951; 27) Walker 1980; 28) Walker 1983; 29) Alexander & Otte 1967b; 30) T.J.Walker & R.Love in Alexander & Otte 1967a; 31) Funk 1989; 32) Hoho[illegible] 1937; 33) Boldyrev 1915; 34) Fulton 1915; 35) Walker & Gurney 1967; 36) Gerhardt 1914; 37) Walker 1978; 38) Bell 1980; 39) Dambach & Lichtenstein 1978; 40) R.Rice Gwynne 1983a; 41) T.J.Walker in Alexander & Otte 1967a; 42) Love 1966 in Alexander & Otte 1967a; 43) Schimmer 1909 in Gerhardt 1921; 44) Boldyrev 1913; 45) Gerha[illegible] 1913; 47) Baumgartner 1910; 48) Walker 1984; 49) Boldyrev 1912; 50) Megusar in Gerhardt 1921; 51) Hubbell 1936; 52) Saltet 1969; 53) Boudou-Saltet & Capolongo 197[illegible] 54) Hubbell & Norton 1978; 55) Richards 1961; 56) Barrett 1991; 57) Ander 1939; 58) Tinkham & Rentz 1969; 59) S.Arnold in Field & Sandlant 1983; 60) Field & Sandla[illegible] 1983; 61) Cary 1981 in Field & Sandlant 1983; 62) Richards 1973; 63) Field 1980 in Field & Sandlant 1983; 64) Dodson et al 1983; 65) Morris 1979; 66) Gwynne 1985; 67) Wedell & Arak 1989; 68) A.Cherill, pers. comm.; 69) Wedell 1993a; 70) Fabre 1899; 71) Rentz 1972; 72) Isley 1941; 73) Gwynne 1981; 74) Gwynne 1984c 75) Rentz [illegible] 76) Kaltenbach 1970; 77) Kaltenbach 1990; 78) Sanger 1987; 79) Burr et al 1923; 80) Gwynne 1990a; 81) Gwynne 1982; 82) Gwynne 1983a; 83) Faever 1983; 84) Morris 1980; 85) Leroy 1969; 86) Gwynne 1987; 87) Whitsell in Gwynne 1987; 88) Meixner & Shaw 1986; 89) Gwynne et al 1984; 90) Bowen et al 1984; 91) Gwynne 1990b; 92) Simmons & Bailey 1990; 93) Gwynne & Bailey 1988; 94) Simmons & Gwynne 1991; 95) Simmons 1990a; 96) Power 1958; 97) Preston-Mafham 1990; 98) Mbata 1992a; 99) Mbata 1992b; 100) Bleton 1942; 101) Grzeschik 1969; 102) Vossler in Gerhardt 1914; 103) Schmidt 1990; 104) Busnel et al 1956; 105) Busnel & Dumortier 1955; 106) Rigalleau 1936; 107) Boldyrev 1928b; 108) Eluwa 1979; 109) Shaw & Galliart 1987; 110) Galliart & Shaw 1991; 111) Berenguier 1907; 112) Bellman 1988; 113) Helversen & Helversen 1991; 114) Hartley 1991; 115) Heller & Helversen 1991; 116) Reinhold & Heller 1993.