Spider Sperm Competition:

The Conduit / Cul-De-Sac Hypothesis -

A Route To Understanding Or A Dead End?

By

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For Amina

"Let the arachnologist watch his spiders in the life before he kills them to describe their carcasses, and the facts of structure will have a richer and more inspiring influence"

"This is the reason why mothers are more devoted to their children than fathers: it is that they suffer more in giving them birth and are *more certain that they are their own*".

Aristotle 4th Century BC

(my emphasis)

"...But this does not mean that the sperm from the first copulation is only instrumental in the fertilization of eggs. The sperms are retained in the female's spermathecæ, and ... they are kept alive for long periods.
Where several copulations take place the spermathecæ will contain the sperms from each copulation mixed together, so, as spiders are polygamous, the males which mate most frequently will leave the largest number of offspring".

Bristowe (1929)

0.0 Abstract

This thesis is an evaluation of the hypothesis that the spermathecae of spiders affects the sperm precedence patterns in a predictable way (Austad 1984). Spermathecae come in two varieties: cul-de-sac and conduit. Cul-de-sac spermathecae, according to the hypothesis, are supposed to lead to second male sperm priority and conduit to first male sperm priority .

The hypothesis was evaluated both directly and indirectly. Direct measurements were made of paternity in two species, *Pholcus phalangioides* and *Tetragnatha montana,* both of which are cul-de-sac species. It was found that *P. phalangioides* complies with the predicted precedence pattern and thus does not disprove the hypothesis. This second male priority pattern was despite a much shorter mating time by second mating males.

In *T. montana* no precedence pattern was found, with equal likelihood of first or second mating males of gaining paternity. There was in *T. montana* a possible influence of the duration of mating affecting the precedence pattern, with longer mating males gaining a higher paternity no matter what order they mated in. It is discussed whether or not this is due to sperm loading or genitalic stimulation (Eberhard 1985).

Indirect evaluation of the hypothesis included an analysis of mating behaviour in *Zygiella x-notata* which is a conduit species and was chosen as a comparison to the two cul-de-sac species. In *Z. x-notata* it was found that there was no difference between mating duration in first and second mating males. Mating persistence is thus the same in first and second mating males, suggesting that the males cannot detect that the female is a denuded

resource to second mating males. Hence first male priority may not be a factor in this species.

Other indirect methods of evaluating the hypothesis involved charting the incidence of mate-guarding and mating-plugs. The expected pattern of mate-guarding was for conduit species to pre-mate guard and for cul-de-sac species to post-mate guard, because of the predicted sperm precedence patterns associated with the spermathecae. The predicted pattern was not found. In the case of mating-plugs it was predicted that these should be deployed by cul-de-sac species because it is in these species that second males are able to usurp paternity to a large extent. The opposite pattern was found with mating-plugs of various design being utilized by conduit species. It is postulated that mating-plugs are the mechanism by which first male priorities are established in conduit species, where this pattern is found. The absence of plugs in cul-de-sac species is possibly the reason that second males can cuckold.

The additional data collected since 1984 reveal that patterns of paternity found in spiders seem to be more complex than was originally assumed by Austad (1984). Spermathecae are species-specific in character and this may reflect a species specificity in sperm precedence patterns. Thus the conduit / cul-de-sac dichotomy may not reflect a useful prediction of patternity patterns.

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0.1 Acknowledgements

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1.0 General Introduction[1]

1.1 Sexual Selection And Spiders: Early Enthusiasm, Later Neglect.

Within two decades of Darwin's '*The Descent of Man, and Selection in Relation to Sex*' (1871) the Peckhams were using spiders as study animals to investigate empirically sexual selection. Their work was summarised in two monographs on the Attidae (= Salticidae) (Peckham & Peckham 1889, 1890). In the years following this early enthusiasm, and up until the nineteen sixties, both araneology and sexual selection were relatively neglected. In the case of araneology the neglect was a result of a lack of commercial interest, for sexual selection it was a lack of a theoretical framework. Despite the neglect a number of strands in spider sexual selection research have become evident since the nineteen sixties.

1.1.1 Male-Male Competition

Darwin (1871) doubted the existence of male-male competition in spiders, on empirical and theoretical grounds:

"[I do].. not remember to have seen the males of any species fighting together for the possession of the female.. nor.. is this probable; ***for the males are generally much smaller than the females"*** (my emphasis). Later observations established that, even in spiders with extreme sexual dimorphism for size, male contests are a common phenomenon (eg. Wells 1988; Christenson 1990; Prenter 1992).

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[1] A glossary is provided in section 7.0, mainly of araneological terms.

Extreme small male size in comparison to the females in many spider species, as well as proving a taxonomic problem[2], has provided an active field of enquiry (York-Main 1990). This size asymmetry is the basis of most of the features of unique interest to students of sexual selection working on spiders and has attracted much speculation regarding its adaptive significance. However it is still undecided what the selection pressures are that have lead to micromales in spiders. Space is only available here to list some of the hypotheses proposed so far. For a fuller list see Vollrath (1980a) and York-Main (1990).

1. **Avoidance of cannibalism**: ironically, since it is the small size of males that makes them vulnerable to sexual cannibalism, a male may avoid predation by being smaller than the spectrum of prey items taken by the female. This cannot be a general explanation or, at best, can only explain the evolution of extreme size dimorphism because in many species males are of a size that is well within the prey sizes utilized by females (Nentwig 1983).

2. **Dispersal**: smaller males can make use of drop and swing and even aerial dispersal to seek out females. These options are not generally applicable to spider species where males are above a certain size.

3. **Decreased development time**:

(i) To avoid pre-reproductive mortality (Vollrath & Parker 1992).

(ii) To access virgin females and avoid sperm competition. This assumes that first male sperm priority is the norm in spiders.

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[2] Some male specimens have been placed in different families to the female of the same species (Coddington & Levi 1991).

(iii) To gain opportunistic matings with freshly moulted females (Robinson & Robinson 1980).

(iv) To avoid inbreeding - males in some species mature so much more earlier than the females of their own generation that they breed with females of the previous generation (Schæfer 1987). This hypothesis is thus not applicable to annual species.

4. **Kleptoparasitism:** Males may be able to feed off a female's web taking prey items she would miss (Vollrath 1987).

5. **Facilitation of copulation**: Large males in some species, where polymorphism for size exists, cannot copulate with females. This must be a secondary effect.

6. **Males are not really small**: it is the females that are large, in order to achieve a fecundity advantage (Darwin 1871; but see Shine 1988).

7. **Influence of marginal habitats**: small males from marginal habitats are more successful in obtaining reproductive output than are females because of their greater mobility (Jocqué 1983).

Thus no one explanation will cover all the situations under which small male size has evolved and the answer is likely to be a complex of many of these theories.

1.1.2 Female choice

Peculiarly the existence of female choice in spiders, in contrast to other animal groups, was never contested by students of sexual selection. This is because female behaviours regarding choice are too overt to be denied: sexual cannibalism of the courting male. Sexual cannibalism, for the most part, has been the subject of much morbid fascination and sensationalism[3] (Gould 1988) despite its potential importance (Elgar 1992) as a selection pressure upon spider mating behaviours. Few studies, however, have considered it in a rigorous way in spiders (Elgar *et al* 1990; Elgar 1992 and references therein). Most observations of sexual cannibalism, from laboratory studies, occur before the male has copulated, and are rare in frequency. Indeed, much of the evidence is anecdotal. Studies are usually conducted with the spiders housed in small containers and the male is killed after a struggle (Montgomery 1903). A rare exception to this pattern is *Latrodectus hasselti* where the males have a stereotyped sacrifice posture (Forster 1992). In this instance the male behaviour may have been selected for because it is advantageous for the male to provide himself to the female as a form of paternal investment (Buskirk *et al* 1984). When studies are field based there is a low incidence of sexual cannibalism (Robinson & Robinson 1980). Considerations concerning sexual cannibalism are related to those of micromales, of course, and have been suggested as a source of selection pressure maintaining adult males beneath the size of the prey taken by females (Bristowe 1958), as stated above.

More subtle forms of female choice are also possible in spiders: responses to the display of colours by male jumping spiders have already been cited (Peckham & Peckham

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[3] "...a sight which ... filled him with horror and indignation" (Kirklet & Spence 1818; Cited in Gould 1988).

1889, 1890). Also strumming of the web by web-building spiders can be included as a juncture at which female choice is exercised (Barth 1990).

1.2 The Expansion Of Sexual Selection Into The Realm Of Primary Sexual Characteristics: A Controversial Chapter Of Sexual Imperialism.

1.2.1 Primary And Secondary Sexual Characters

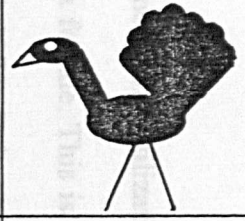
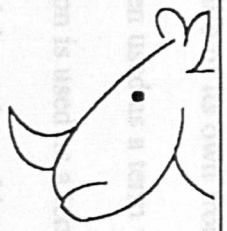
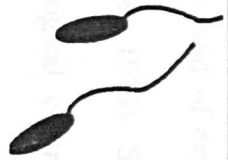
Darwin (1871) defined **primary sexual characteristics** in dioecious organisms as those in which "*the males necessarily differ from the females in their organs of reproduction*". These characters are directly involved in reproduction and are subject to natural selection to increase efficiency of conception and fertility. They contrast with **secondary sexual characters** which are "*where sexes differ in respects which are not directly connected with the act of reproduction*". These characters are not directly involved in insemination and are thus subject to sexual selection. These are the traits which Darwin hoped to explain in *The Descent of Man, and Selection in Relation to Sex* (1871) because they are often deleterious to an individual's survival.

Darwin recognized that the two halves of this dichotomy intergrade into each other and provided the example of prehensive organs such as those used by males of the freshwater crustacean *Asellus* to amplex females (Manning 1975) as one which fits into both classes. Prehensive organs are useful for keeping a pair together until insemination is successful but are also useful for preventing other males from taking over a female. This is one of the reasons Darwin wisely chose not to demarcate rigidly where sexual selection ends and natural selection starts (Cronin 1993). Indeed it was this flexibility that almost enabled

parties hostile to the theory of sexual selection (Huxley 1938) to subsume much of it (female choice) into natural selection. Huxley (1938) made matters worse by coining an avalanche of terminology, thankfully no longer used.

The scope of sexual selection has, however, expanded in the last 25 years (see Table 1.1) into traits which were previously universally recognized as primary sex traits. These new findings will probably obviate the need for a distinction between primary and secondary sexual characters in the future (Andersson 1994; Andersson & Iwasa 1996). This trend is in direct opposition to the intellectual onslaught which the theory of sexual selection underwent for almost a century in order to relegate it to the uncontroversial province of male-male competition and explain everything else in terms of natural selection (Huxley 1938; Cronin 1993).

Table 1.1 Overall Scope Of Sexual Selection Of Male Characters

Phenomenon:	Extended phenotype.	Display organs.	Combat organs.	Sexual cannibalism.	Copulatory organs.	Gametic competition.	Killing of conspecifics.
Timing of the phenomena relative to female reproductive cycle:	Precopulation.	Precopulation.	Precopulation.	Pre / post copulation.	At copulation.	Post copulation (around fertilization).	Post fertilization.
Level at which selection acts:	Artifact.	Individual.	Individual.	Individual.	Organ.	Cellular.	Individuals.
Considered by Darwin as due to:	Sexual selection.	Sexual selection.	Sexual selection.	Sexual selection.	Natural selection.	Natural selection.	-
Behavioural characters:	Web vibrations by courting spiders.	Courtship rituals to show off the display organs.	Fighting.	Male sacrifice and defence actions.	Copulatory courtship, sperm displacement	Kamikazi sperm.	Infanticide.
Morphological characters:	Bowers.	Plumage.	Horns.	Male defence organs.	Organs of intromission.	Sperm.	-
Spider example:	The web.	Salticid legs and palps.	*Linyphia* sp. male chelicerae.	*Tetragnatha* ssp chelicerae?	Pedipalps.	Sperm in sperm balls.	Trophic eggs?
General example of behavioural and morphological characters:							
References:	Dawkins 1990	Andersson 1994	Darwin 1871	Foster 1993	Eberhard 1985	Parker 1970	Birkhead & Møller 1992

Expanded Scope Of Sexual Selection

1.2.2 Sperm Competition

One of the most fruitful areas developed in the field of expanded sexual selection theory in recent years is **sperm competition** (Parker 1970). Sperm competition was originally found to be important in sterile-male biological control release programmes because non-sterilized males' sperm was a better competitor and so the programmes were not working effectively. Work on spiders came much later (Jackson 1980, Vollrath 1980b) because there was no impetus from pest control. More recent expansion of sperm competition theory and empirical work has been immense, although spider research has lagged behind bird and mammal studies, the traditional subjects for animal behaviour work (Smith 1984; Birkhead & Møller 1992).

The term sperm competition has taken on a life of its own from its original inception (Parker 1970), as evidenced by the fact that it is often used as a term without referencing the original paper (Parker pers. com.). Sperm competition is used as a term for many things so a new terminology is proposed here which will be used throughout this thesis:

Individual sperm competition (ISC) - refers to the mêlée of fertilization when two or more ejaculates are present in the sperm storage organ of a female. This is the most fundamental level at which sperm are said to compete (Sivinski 1984; Baker and Belis 1987, 1988; Warzinek 1993). Phenotypic expression can occur in sperm only if it is negative to the individual fitness of the sperm, and promotes the fitness of the ejaculate, through kin selection i.e. the kamikaze sperm hypothesis (Baker & Belis 1995). The individual sperm are not more athletic fitness because genes for faster sperm from a male would go to fixation in a population very quickly (Prout & Bundgaard 1976).

Ejaculate competition (EC). - refers to the situation when a **precedence pattern** is established. Where, for example, sperm stratification or sperm removal occurs then it is more appropriate to think of the competition as occurring at the ejaculate level because the phenotypic attributes of individual sperm are irrelevant and all act as equivalent tickets in a raffle. Continuing the analogy, a sperm priority pattern set up by sperm stratification is like cheating in a raffle by stuffing all your tickets at the top of the tombola and not spinning it. The contest is then going on outside the arena of the tombola and between who can cheat most effectively. A more effective strategy in this case would be to remove everybody else's tickets before putting your tickets in. These processes are often referred to in the literature simply as sperm competition.

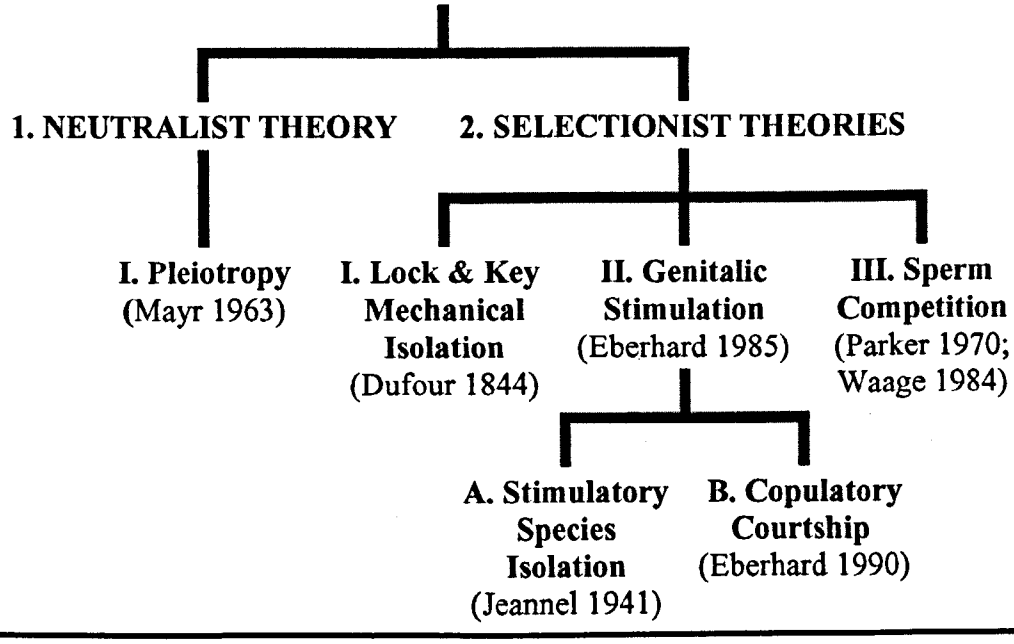
Sperm competition - an overall term for the study of adaptations to EC and ISC by males and females in the tripartite evolutionary game of female, first male and second male (Parker 1984).

1.2.3 Genitalia

Why are male genitalia so complex? A number of explanations have been forwarded to explain the complexity of genitalia (Fig. 1.1). These were all discussed by Eberhard (1985) who produced a new hypothesis that genitalic stimulation and female choice were at the root of why genitalic characters are so complex. This is as yet a fairly untested hypothesis, although there is some evidence against it in spiders (Huber 1993a, 1993b, 1995).

Evidence exists from the odonates that ejaculate competition is at the root of complexity in genitalia (Waage 1979 found that the aedagus is utilized in sperm removal). So far no evidence exists that spider genitalia can be used for this purpose.

Fig. 1.1 Alternative Hypotheses Relating To The Evolution Of Complex Genitalic Characters



1.3 Sperm Competition In Spiders: Sexual Selection Continuing After Copulation

Austad (1984) recognised the potential for sperm competition in spiders and the dearth of current knowledge of priority patterns (ejaculate competition) in the group. Most spiders fulfil all of Parker's (1970) conditions for high levels of sperm competition, *viz.*

(a) Multiple mating often occurs or is greater in incidence than the threshold for it to be of more than trivial importance (Parker 1984).

(b) Females store sperm for a long time, relative to their reproductive lifetime, though this is a not a necessary prerequisite, as shown by sperm competition studies in mammals.

(c) Long sperm life and viability in storage; encystment may facilitate this in spiders (Smith 1984; Alberti 1990).

(d) Efficient usage of sperm by females (not to the extent shown by *Drosophila* (Parker 1970) but the fertilization set is a high proportion of the capacity of the spermathecae generally as a result of the high output of eggs). However, females may use sperm wastage as a reproductive tactic (Higgins 1989) by expelling sperm of less favoured males as has been claimed for humans (Baker & Bellis 1995).

(e) Finally, high levels of sperm competition are expected because of a usual imbalance of effective sex ratios, often established as a result of unequal maturation times for males and females (Protandry - Fagerstrom & Wiklund 1982), i.e. the wandering males mature first and

seek out females (Darwin 1876). Thus an asymmetry in heterosexual encounters occurs, with females encountering more males than *vice-versa*. In some species male-biased sex ratios are also found, resulting from differential investment ratios (Fisher 1958; page 159), further increasing the chances of sperm competition. An example is provided by *Nephila clavipes* (Vollrath 1980b).

Originally Austad (1984) reviewed only three papers on sperm competition in spiders and formulated a generalization that conduit spider species had first male priority and cul-de-sac species had second male priority. Since the Austad (1984) review other studies have been undertaken but are inconclusive in support of the theory.

The influence of sperm plugs was underestimated in the original study (Austad 1984), though *Phidippus johnsoni* (Salticidae) definitely has a plug (Jackson 1980). This means that the spermathecal structure may not have been influential. It is merely that the two orifices of the complex epigyne in conduit spiders allow plugging whereas the cul-de-sac orifice does not. This then is an alternative hypothesis to the Austad (1984) "plumbing" hypothesis; that is, no phylogenetic constraint on sperm utilization stategies on the female's part but an ejaculate competition strategy on the male's part. Both of these theories will be discussed in the following thesis.

Masumoto (1993) investigated sperm priority patterns in *Agelena limbata* (Agelenidae) and here too the importance of plugging was stressed. Austad's (1982) study also showed the possibility of a cryptic plug being deployed in an entelegyne species

Frontinella pyramitela (Linyphidae) because remating was impossible in a female mated 24 hours previously despite a long second male preinsemination phase.

A study on another Linyphiid, *Linyphia litigiosa,* found that there was no influence of a mating plug in determining the first male priority shown by this conduit species (Watson 1991a, b).

1.4 Spermathecal Architecture And Sperm Precedence Patterns: The History Of An Idea.

The argument that spermathecal architecture[4] is an important determinant of the precedence pattern of a species has been suggested by a number of authors for a range of taxa. The exact arguments take a number of forms which are presented below with comments on their relevance to the situation in spiders.

1.4.1 Insects

Parker (1970) recognized that the females' sperm storage organ can have an influence on the outcome of sperm competition but did not expand upon this point. Walker (1980) gave the earliest detailed treatment concerning the influence contrasting spermathecal types can have on patterns of sperm precedence. Walker proposed that the shape of the insect spermathecæ could affect the outcome of sperm displacement: "*The gross morphology of the spermathecæ or other sperm storage sites may have an important influence on the amount of sperm displacement*". Walker recognized a dichotomy of spermathecal shape in insects: spheroid versus tubular (elongate). Building on the suggestions of a number of authors (Schlager 1960; Riemann & Thorson 1974; Brower 1975) Walker proposed that the more elongate the spermathecæ the more potential it presents to second mating males for displacing sperm to regions where they are unlikely to enter the fertilization set (see Fig. 1.2). This is because the ejaculates of the males are thought to be stratified on top of each other.

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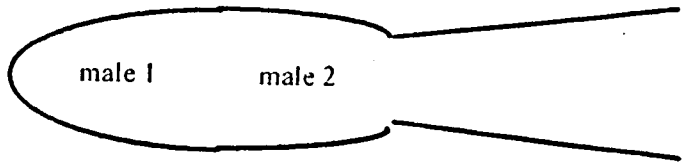
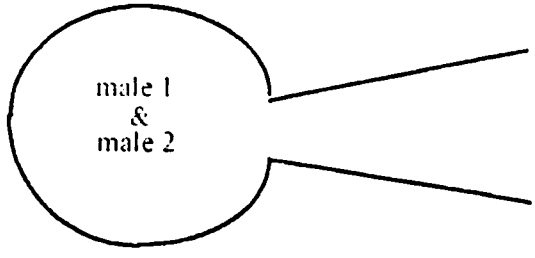
[4] I use the term spermathecal architecture to cover the construction, shape and tube configuration of the spermatheacæ

Fig. 1.2. Dichotomy Of Spermathecal Morphology Proposed For Insects (Walker 1980) And How The Structure Of The Spermathecæ May Influence Sperm Precedence Patterns.



A. Elongate / Tubular: The first male's sperm is pushed to the back of the spermathecae. The second male's sperm overlays it and is therefore more likely to be in the fertilization set, resulting in a high P_2. This is a common condition in the insects.



B. Ovoid / Spherical: The second male is unable to displace sperm to the back of spermathecæ and therefore a high P_2 is less likely than in A.

Hence higher P_2s are likely in species with tubular spermathecæ than those with spheroid spermathecae.

Data assembled by Walker seemed to fit this expected pattern fairly well but a few exceptions were also observed. As further evidence in support of his thesis, Walker (1980) observed that monoganous species tended to possess spheroid spermathecæ. The argument here was that if second mating males cannot displace sperm of prior mating males to gain paternity then they will not be selected to expend costly mating effort (Dewsbury 1982) on non-virgin females with the result that monogany is the prevailing mating system. If multiple mating does occur in these species then a high P_2 will not result because males are unable to displace the first male's sperm.

Contrasting with this analysis was that of Ridley (1989) who reanalysed the data with a more rigorous method which involved controlling for taxonomic artifacts (Ridley 1983). He found no evidence for the schema proposed by Walker (1980). Ridley raised important theoretical objections against Walker's (1980) suggestions, including the fact that the shape of the spermathecæ could not be objectively classified and that there is a lack of evidence for the operation of a sperm displacement mechanism.

1.4.2 Spiders

Arguments on both empirical and theoretical grounds suggest that spiders may prove a better candidate than insects as a group in which the spermathecal architecture influences P_2.

(i) The prevalence of protandry in the Entelegynae species studied so far (Linyphiidae: Austad 1982; Watson 1990, 1991a, b; Tetragnathidae: Vollrath 1980b; Christenson 1990; Salticidae: Jackson 1980) suggests that it may be important for males to mate early because

usurping of fertilizations is unlikely, or at low levels, by subsequent males. No data are available as yet on Haplogynae species.

(ii) Males in these studies pre-mate guard, thus complying with the predictions of the hypothesis. Rather weaker evidence is available for Haplogynes but preliminary experiments by Eberhard *et al* (1994) have shown the Haplogyne spider *Physocyclus globosus* does not pre-mate guard.

(iii) The most important criterion is P_2 values measured once mating is complete. These are low as predicted in the entelegyne studies so far (Linyphiidae: Austad 1982; Watson 1990, 1991; Tetragnathidae: Vollrath 1980; Christenson 1990; Salticidae: Jackson 1980; Agelenidae: Masumoto 1993). However until the present work no data were available on haplogyne species, except those on *P. globosus* (Eberhard *et al* 1994) which found that the P_2 did not differ from 0.5 which is consistent with random sperm mixing.

(iv) Unlike insects, a spider's intromittent organs are non-genital, being derived from the palps. The palps are loaded with sperm from a genital pore by the male prior to or during mating through a process of sperm induction (Bristowe 1958). The structure of the palp used in sperm transfer by the male may preclude sperm displacement by second males (Kraus 1978). In the spider spermathecal dichotomy proposed, sperm mixing would be of little importance because the encysted sperm of spiders are non-motile (Kanwar 1965; Foelix 1982; Alberti 1990; Alberti & Coyle 1990). However, it is not known how soon after they are transfered to the female the sperm become unencysted. For the proposed mechanism to work, it is important that they are non-motile so that sperm remain stratified within the spermathecae.

(v) The precedence pattern for spiders is more likely to be determined purely by the spermathecal morphology,. because it is a hard, chitinised structure. Therefore cryptic processes (Eberhard 1990) involving muscular contraction of the spermathecae (Siva-Jothy 1987) to redistribute the sperm by the female are unlikely. This factor also enhances the chances of sperm stratification taking place.

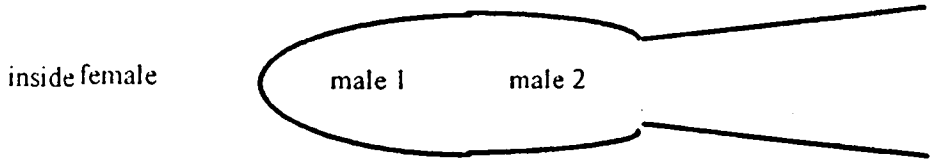
(vi) There is a more absolute dichotomy between the two types of spermatheca (Fig. 1.3): cul-de-sac and conduit rather than elongate and spherical which involves a degree of subjectivity in the designation of the two.

Blanket acceptance of the Austad (1984) hypothesis is premature however because significant doubts remain. The Austad (1984) spermathecal dichotomy may be false: a preliminary survey of the spermathecae of the main families of spiders reveals great diversity of morphology. The number of spermathecae also varies, from one to more than a hundred (Comstock 1940; Eberhard 1985, 1986).

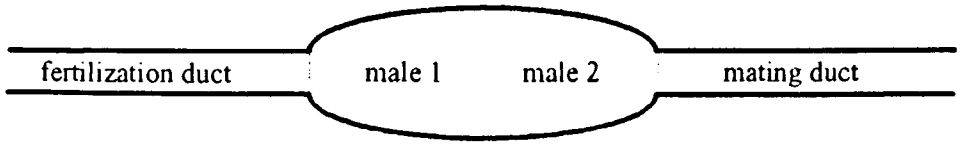
The taxonomy on which the spermathecal dichotomy is based (Kaston 1978) is now realized to be naive (Coddington and Levi 1991; Platnick, pers. com.) and is superceded by one which recognises widespread convergence of characters despite the parsimonious way in which it was constructed (based on Hennig 1966). These convergences include the spermathecae of spiders (Coddington 1990; Platnick *et al* 1991).

If adaptive convergence is as common as some workers claim (eg. Cain 1982, 1988) as is apparent in the Araneae, it follows that phyletic limitation of the female tract to given

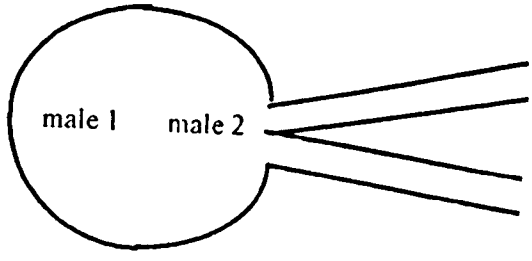
Fig. 1.3. Dichotomy Of Spermathecal Morphology In Spiders (Austad 1984) And How The Structure Of The Spermathecæ May Influence Sperm Precedence Patterns[5].



A. Cul-de-Sac: Second male's sperm overlays the first and as such is more likely to be in the fertilization set giving a high P_2.



B. Conduit: First male's sperm blocks entry into the fertilization set for any subsequently mating males giving a low P_2. However if the female uses up the supply of the first male's sperm one would predict a switch to a high P_2.



C. Influence Of The Fertilization Duct Position Relative To The Mating Duct: The spermathecæ is still of a conduit kind but the predicted priority pattern is different depending on the relative position of the mating and fertilization ducts. Here, despite being a conduit spermatheca, a high P_2 would be expected.

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5 Note the different mechanism of sperm precedence determination involved in spiders because the intromittent organ is thought to be incapable of sperm displacement.

specific P_2 levels is unlikely and evolution of the spermathecae to generate precedence patterns in line with female interests may be possible.

Such evolutionary processes may even be probable given the rapidity in the rate of evolution of characters that are subject to the influence of adaptive arms races, as the reproductive organs of polyandrous species have been claimed to be (Dawkins 1990; Waage 1984). This is because of the basic conflicts of interests that exist between male and female reproductive strategies, which makes the application of the theory of games to sexual selection processes (Maynard Smith 1982) so attractive. A case in point is that of *Nephila clavipes* (Christenson 1990) which has been shown to have a low P_2 (Vollrath 1980b) as expected in the entelegynae classification. However, this species is part of the Tetragnathid-Nephiline complex which seem to have converged back to the cul-de-sac spermathecal morphology (Levi 1980). The mechanism involved here is that the female's spermathecae harden after mating making subsequent insemination more difficult (Higgins 1989).

The "plumbing" hypothesis also ignores any influence of the male in determining precedence patterns, which even Walker (1980) in his original review does not state so dogmatically as Austad (1984):

"*..basic sperm precedence characteristics... have resulted primarily from the sperm utilization strategies of females...* ***not to deny the importance of selective pressures working on either sex...***" (Walker 1980; my emphasis).

For example, mating plugs secreted by the male exist in several spider species (Jackson 1980 and references cited therein).

A potential weakness of the "plumbing" hypothesis (Austad 1984) is the influence of cul-de-sac spermatheca shape variation on P_2. For instance, an ovoid cul-de-sac spermatheca could have a different influence on P_2 than a spherical one, as suggested by Walker (1980). Furthermore differences in the relative positions of ducts in the conduit species can influence the outcome of ejaculate competition if stratification occurs (Fig. 1.3C). The closer they are together the more they will favour a high P_2.

Additionally, as stated above, little or no data are available on the mating behaviour and P_2 figures of true Haplogynae spiders. Thus an equally valid hypothesis on the basis of the sparse data in the literature is that **all** spider species are constrained to a first male sperm priority pattern.

In conclusion, the proposed limitation to the sperm utilization strategies available to females as a result of spermathecal structure is a hypothesis awaiting evidence. The overall aim of this project is to supply appropriate evidence bearing on the matter.

1.5 The Entelegynae And Haplogynae: Shifting Definitions, Shifting Phylogeny.

Austad's (1984) original designation of the spermathecæ was cul-de-sac and conduit which is roughly analogous to Haplogyne[6] and Entelegyne[7] (Austad pers. com.). However they do not exactly map onto each other. Haplogyne is defined as a reproductive system lacking a fertilization duct, a condition considered to be primitive (Coddington & Levi 1991). Despite the fact that this definition utilises a negative character (and thus flies against Hennigian principles) Haplogynae and Entelegynae do seem to be monophyletic groups (Platnick *et al* 1991) despite what Cooke (1970) says. However, some Entelegyne groups, which normally have both the fertilization and mating ducts to their spermathecae, have secondarily lost the fertilization duct, *viz*: Tetragnathidae, Micropholcommatidae, Palpimonoides, Anapidae and Uloboridae. These convergences have caused confusion in the past leading to the erection of polyphyletic groups. For example, the Tetragnathidae have formerly been placed within the Haplogynes (Kaston 1978).

Simon (1892-1903 - cited in Bristowe 1929 and Cooke 1970) originally classified the Haplogyne group of families on the basis of genital morphologies. This was a subjective criterion, the Haplogynes being those with primitive, simple genitalia. This led to the anomalous position whereby the obviously primitive Pholcidae was placed within the entelegynes because of the complex male palps (Roberts 1985). In any case, Krauss (1978) showed that the so-called simple bulb structure in some of the palps of Haplogynes are in fact as specialized as that in the Entelegynes, ie. fundamentally the primitive bulbs do not have a different construction from the more complicated ones. Until recently Coddington (1990)

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6 From the Greek, *Haplous* meaning single or simple (Jaeger 1955).

7 From the Greek, *Enteles* meaning complete, full or perfect (Jaeger 1955).

believed that the Haplogyne condition: "*represents a grade and not a clade*" (my emphasis). However Coddington in his recent review changed his mind confirming the Haplogynes as monophyletic (Coddington & Levi 1991). It is important that the Haplogynes and Entelegynes are monophyletic because Austad (1984) proposed that the spermatheca places a phyletic limitation on sperm utilization strategies. Thus the secondarily derived loss of the fertilization duct in some Entelegyne families presents a problem for such a viewpoint because it means that no such phyletic limitation exists and the character is plastic enough for five groups to have lost the fertilization duct. The achitecture of the spermathecae may therefore be subject to adaptive forces suiting the female's sperm utilization strategy over evolutionary time, i.e. the priority pattern is modified according to the female's reproductive imperatives.

Henceforth the terms cul-de-sac and conduit will be used to refer to spermathecae with one and two ducts, respectively. The terms Haplogyne and Entelegyne will be reserved for the monophyletic groups which are slightly different because of the Tetragnathidae, Micropholcommatidae, Palpimonoides, Anapidae and Uloboridae which are Entelegynes but with cul-de-sac spermathecae.

Other characters confirm these five families as Entelegyne, for example male genital usage. In the Haplogynes simultaneous palp insertion is the norm, whereas in the Entelegynes they alternate their palps whilst mating. This alternation of the palps may be a mechanism enabling waxy secretions to be picked up from the oral area in order to plug the epigyne. In addition, entelegynes have a complex, chitinised epigyne whereas Haplogynes do not (this is a moot point in the Tetragnathids which have a much reduced epigyne).

1.6 Differences Between Spider And Insect Reproductive Systems: Importance To Sperm Competion And Spermathecal Influence Over Sperm Precedence

It is important to establish the differences between spiders and insects in their reproductive structures (Table 1.2) because the influence of the spermathecæ in determining the sperm precedence pattern has been undermined in insects (Ridley 1989). However, the mechanism proposed for insects was different and possibly part of its downfall was a result of the subjectivities involved in classifying the spermathecæ. It also required that sperm displacement was achieved by the male alone (Fig. 1.2). The mechanism in spiders is quite different (Fig. 1.3). A number of reproductive corollaries follow from the spermathecal architecture fixing the P_2 (Table 1.3).

In the Lepidoptera an analogous situation to the spiders exists in the construction of their spermathecæ (Drummond 1984). Some species of Lepidoptera have just a single duct serving each spermatheca and they represent a supposedly monophyletic, primitive minority of species (3% which still amounts to some 3000 species). These species are termed monotrysian and are analogous to the haplogyne condition in spiders. In contrast to these species the higher Lepidoptera (ditrysia) have separate reproductive ducts for mating and oviposition. The differences between the Lepidoptera and the spider reproductive systems are basically two: in the Lepidoptera the spermathecae are not paired and a spermatophore is used (Drummond 1984).

Drummond (1984) specified two processes determining sperm priority in the Lepidoptera: active and passive. Active processes are ones which influence the position of sperm in the spermathecae while the passive factors *"are 'passive' only in an ecological time*

frame; they are certainly subject to selection over evolutionary time because of their great potential for affecting evolutionary success".

Forster (1980) considered the evolution of the spermathecae from bursal sperm storage to the full entelegyne condition (Fig 1.4). Here the fertilization duct (B) is considered to be a novel structure, with the mating duct (A) opening out onto the epigyne. This suggests that the pathway from haplogyne to entelegyne is a smooth one and this means it can evolve back again easily with the loss of the mating duct as in the *Tetragnatha*. Duct configuration must be susceptible to selection over evolutionary time (Drummond 1984).

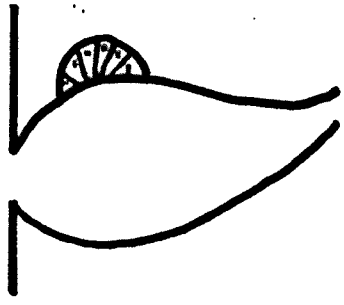
Table 1.2 Selected Reproductive Characters In Insects And Spiders Relevant To Sperm Competition.

Characteristics	Spiders	Insects	References[1]
Incidence of multiple mating.	Common.	Common.	*Jackson et al 1981* *Austad 1984* Parker 1970
Sperm.	Encysted. Non-motile until sperm capacitation occurs.	Free. Often in continuous movement in the spermathecæ.	*Alberti 1990* Richards & Davies 1977 Parker 1970
Sperm induction.	Indirect.	Direct.	*Foelix 1982* Richards & Davies 1977.
Sperm transfer organs.	Paired palps, sperm displacement unrecorded.	Single adægus, frequently used for sperm displacement.	*Savory 1928* Richards & Davies 1977
Sperm plugs.	Reported for some species.	Commonly utilized	*Jackson 1980* Parker 1970
Sperm transfer.	Free spermatozoa.	Free spermatozoa or enclosed in a proteinaceous spermatophore	*Alberti 1990* Richards & Davies 1977
Sperm storage organs.	Separate, usually paired spermathecae.	Usually single spermathecae[2]	*Foelix 1982* Richards & Davies 1977
Spermathecae shape.	Very variable, often spherical.	Generally ovoid or spherical, sometimes tubular.	*Coddington & Levi 1991* Walker 1980
Spermathecae ducts.	One or two.	One in most orders - but two in most Lepidoptera (97%).	*Austad 1984* Drummond 1984
Spermathecae construction.	Hard and chitinous - rigid.	Lined with chitin secreted from columnar epithelium on a basement membrane with a **muscular** coat.	*Austad 1984* Siva-Jothy 1987
Sperm stratification.	Not established, but likely for a number of reasons - see text.	Probably broken down due to a number of factors - see text.	
Fertilization.	Single sperm to egg?	Polyspermy common.	*Alberti 1990*, Parker 1970
Egg laying.	In cocoons - batched.	Singly or in batches.	*Turnbull 1973* Richards & Davies 1977

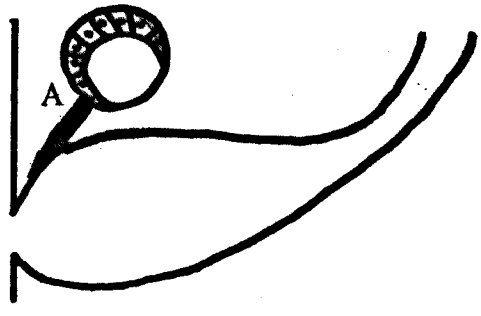
[1] Spider references first and in italics.

[2] Two spermathecae in *Blaps*, *Phlebotomus* and *Dacus*, three in *Culex* the Tabanidæ and most Calyptratæ (Richards & Davies 1977). Paired 'T' - shaped spermathecæ in libellulid dragonflies (Siva-Jothy 1987).

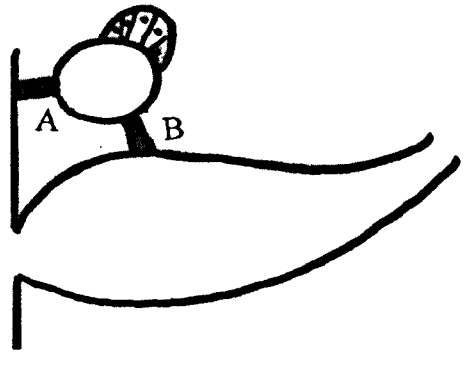
Fig 1.4. Evolution of the spider spermathecae.[8]



A. Bursal sperm storage



B. Haplogyne condition



C. Entelegyne condition

[8] Reproduced from Forster (1980). The homology claimed between haplogynæ and entelegynae is the "A" duct, the "B" is therefore a novel structure.

Table 1.3 Reproductive corollaries of spermathecal architecture fixing P_2 in a polyandrous system.

Factor.	Cul-de-sac	Conduit
Protandry[1].	No	Yes
Mate guarding.	Post-mating expected[2]	Pre-mating expected
P_2.	High	Low
Paternity assurance mechanisms.	Yes	No
Variation in mating success for males[3].	Low	Low

[1] Protandry is the earlier emergence or maturation of males compared with females.

[2] Mate guarding is ecologically dependant in cul-de-sac species because it is obviously not an economical use of time for a male to guard a female indefinately. An absence of mate-guarding is thus also likely in cul-de-sac species.

[3] This does not differentiate between the two types of spermathecae but because of sperm stratification the expected variation in P_2 for a particular species is low for both - assuming all males transfer enough sperm to fertilize all a female's eggs .

1.7 Project Aims And Introducing The Study Species

The principle aims of this study were to (i) augment the knowledge of P_2 estimations and consider the effect of sexual selection at different hierachical levels in spiders, (ii) to test the theory that the spermathecae have an influence on P_2 in the way predicted by Austad (1984), (iii) to investigate whether there are phylogenetic constraints on P_2 patterns as a result of spermathecal architecture and other behavioural or physiological mechanisms, and (iv) to observe the mating behaviour of all species studied, for three reasons. Firstly, to ensure that virgin females do indeed mate with both first and second males an obvious prerequisite for multiple insemination. Secondly, to asertain if the duration of mating has any effect on the paternity gained by the males involved. Finally, measure any differences in the first and second matings duration or behaviour.

Out of the 34,000 described species of spiders (Coddington & Levi 1991) clearly only a few could be studied here. The original intention of this study was to compare the P_2 values for two sympatric species within the same family (Tetragnathidae) that differed in their spermathecal architecture, *viz Metalina segmentata* and *Tetragnatha montana*. This approach was suggested by Austad (1984) as a test of his thesis, though it is unclear why the species should be so related[9], because his thesis should stand for any species of spider. The prediction is that because *M. segmentata* is a conduit species it should have a low P_2 and conversely *T. montana* should have a high P_2 because it is a cul-de-sac species.

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[9] This is unless the species are thought to only differ in the spermatheca and other variables remain constant. This proposition is unlikely however as they will differ in many other ways besides the spermatheca.

M. segmentata is designated a Semientelegyne spider (Brignoli 1978) because its mating duct and fertilization duct are next to each other and are connected to the spermatheca in adjacent positions. *M. segmentata* is an interesting test case for the Austad theory because, despite being a conduit species, this duct configuration should lead to a high P_2. This is because if sperm stratification is the reason for the P_2 levels predicted then a spermathecal architecture of a kind such as this should lead to the second male's sperm being deposited nearer the fertilization duct and hence gain the more paternity due to positional factors. This is discussed furthur in chapter 5.0.

Unfortunately *M. segmentata* was found to be difficult to raise in the laboratory because under the conditions used the eggsacs dry out before hatching. A higher humidity needs to be maintained in order to rear *M. segmentata* in the laboratory than can conveniently be generated (Prenter, pers. com.). Because *M. segmentata* matures in the autumn and the young do not hatch until the spring it is possible that they need a cold snap before hatching. This may also mitigate against them being a good laboratory animal because it would be difficult to decide when and how long to cool them for. The closely related species *M. mengei* may be a better subject for study as its young emerge soon after mating and do not overwinter in that stage. *T. montana* was found to be a suitable experimental animal so was maintained in the experimental protocol of P_2 measures.

Because few studies (Eberhard *et al* 1993) have looked at priority patterns in cul-de-sac species a second species of this type was selected for study. The primitive cul-de-sac species *Pholcus phalangioides* (Pholcidae) was selected to contrast with the advanced cul-de-sac species *T. montana*. Extensive investigation has been carried out on the sperm storage organ in *P. phalangioides*, by Uhl (e.g. 1992). This provided useful

background information on the mechanics of sperm storage in this species when interpreting the P_2 data. In both of these species P_2 was measured using electrophoretic markers to assign paternity in multiply mated females.

The third species selected, *Zygiella x-notata* (Araneidae), was chosen as an example of an advanced conduit species because it is easily reared in the laboratory (Vollrath pers. com.). P_2 was not directly measured in this species, instead the influence of a mating plug on mating behaviour was studied to ascertain its effect on mating duration. *Z. x-notata* was used to replace the gap left by the loss of *M. segmentata* from the experimental protocol. In this case *Z. x-notata* is a straight-forward conduit species with fertilization and mating ducts positioned at 180^0 to each other on the spermatheca.

Different reproductive traits in the species studied are summarised in Table 1.4.

Table 1.4 Contrast of study species in various reproductive traits

Species	Courtship	Palp application	Spermathecae		Mate Guarding
Pholcus phalangioides	No	Dual Insertion	Paired	Cul-de-sac[1]	No
Tetragnatha montana	No	Alternate	Single - functional spermatheca	Cul-de-sac	No
Zygiella x-notata	Yes	Alternate	Paired	Conduit	Premate guarding

[1] Really a *bursa copulatrix*.

2.0 Sperm Precedence Measurements In *Pholcus phalangioides* (Fuesslin) (Araneae, Pholcidae)

2.1 Introduction

Aspects of the biology of *Pholcus phalangioides* (Fuesslin) will be reviewed to put into context the population structure, mating habits, phenology and sperm precedence patterns investigated later in the chapter (sections 2.2 - 2.7).

2.1.1 Distribution And Population Structure

P. phalangioides is a cosmopolitan species (Bristowe 1958) most often found associated with human habitation. In the tropics *P. phalangioides* has been observed in open shady situations away from houses (Bristowe 1958), although in temperate areas it appears to be an obligate anthropophile favouring cellars especially at the northern limits to its range (Bristowe 1939 - 1941: Text Fig. 6 & 7).

P. phalangioides's latter-day association with humans is probably due to his residences being a close approximation to the spider's 'natural habitat', caves. Bristowe (1939 - 1941, 1958), from a survey, claimed that *P. phalangioides* was limited to an area where the average annual temperature exceeds 50^0F (approximately 10^0C). Hancock's (1984, 1992) observations of *P. phalangioides* in Scotland question these findings however. The present pattern could be a result of a range expansion, inadequacy of the original survey or both. The

presence of *P. phalangioides* in Scotland was established as early as 1953 (Richter 1953), however, so the latter explanation seems the more likely. The limiting factor to *P. phalangioides*' distribution on a local scale may be temperature. Egg production and development is often the most vulnerable stage in a life cycle. Platel (1989) and Schaefer (1976, cited in Nentwig 1987) found egg development to be temperature sensitive, with a zero point of development of about 14^0C. Egg development time is fairly lengthy at moderate temperatures so the temperature needs to remain above 14^0C for a significant period during the year. In this context, Bristowe's (1958) conclusion that a minimum annual average temperature of 10^0C for *P. phalangioides*' presence seems a reasonable one. If there has been a range expansion it could have been facilitated by the wider use central heating.

P. phalangioides may be cosmopolitan but its populations are not continuous. Discontinuities in its distribution occur naturally because of its cave or 'pseudocave' habitat and founder effects may play a large part in determining the genetic structure of populations (Porter & Jackob 1990). This is useful for studies, such as this, which are using genetic markers for paternity analysis, as long as specimens can be collected from a number of sites. One would expect populations to become fixed for particular alleles and the alleles to be different between sites, as a result of genetic drift. Thus if mating experiments to investigate precedence patterns are conducted with males from different sites one would be more confident of assigning paternity than if one was sampling individuals from a panmictic population. The sex ratios reported for *P. phalangioides* in the literature range from 3:1 (females: males) (Maughan 1978) to 17:1 (Montgomery 1908). One would expect this to depress the local genetic variation even further as a result of lowering the effective

population size. The expectation of local inbreeding with the consequence of greater homozygosity is especially useful in laboratory crosses as this will mean the expected F_2 genotypic ratios produced by each father are likely to be different.

2.1.2 Phenology And Life History

The sheltered environment in which *P. phalangioides* often lives usually means that very little seasonality can be detected in its activities and it is able to reproduce at most times of the year. However, phenological variation reflects local environmental fluctuations. Schaefer (1976) observed that *P. phalangioides* lays eggs throughout the year with a maximum in May in a location where the temperature remained above 20^0C. Montgomery (1903) also observed year-round egg production in Pennsylvania, U.S.A. Platel (1989), working in Holland, found females bearing cocoons in May to September. Bristowe's (1958) observations in southern England showed cocoon production to be constrained to the months June to August, although eggs can be laid in May if the female is inseminated the previous winter.

The variable length of time it takes female *P. phalangioides* to produce cocoons, has potential fitness consequences for males. This is because the longer sperm are stored the greater is the likelihood of cuckoldry by another male; thus the opportunity for sperm competition may vary in this species from locale to locale. Ambient temperature can also have fitness consequences for female *P. phalangioides*. Under the relatively benign conditions of captivity (20-28^{0}C) females are able to produce about 3 cocoons a year (Montgomery 1903; Miyashita 1988b; Platel 1989); up to 9 cocoons in 3 years with remating (Platel 1989). A similar level of cocoon production has also been reported from some studies of wild populations, and less under lower temperatures (Platel 1989). Observations of *P. phalangioides* phenology in the laboratory are probably a good representation of what

happens under most wild conditions. The aseasonal nature of this species is useful in studies of reproduction such as the present one, because mating experiments can be carried out the whole year round.

Once hatched, spiderlings can develop to maturity in 5-6 months (Schaefer 1976) usually through 5 instars, although 6 have been observed in captivity (Miyashita 1988a). This is the total number of instars in *P. phalangioides* because, unlike the more advanced families of spiders, no moult occurs within the cocoon. The young remain in their mother's web until about the third instar when they disperse by extending the web. As a result of such close living siblicide can be common (Bristowe 1958), but can be ameliorated in captivity by maintaining a high feeding regime. Aeronautical dispersal has not been reported in *P. phalangioides* young and little is known of how they colonize new sites other than by human transport. Males and females can already be separated at the penultimate instar when the male palpal organs are developing beneath a translucent sheath. Two primitive features of reproduction in *P. phalangioides* are noteworthy:

(i) Mature males sperm induce without a sperm web: they utilize a single thread held across the gonopore and then transfer the sperm to the chelicarae and finally to the palps.

(ii) Simultaneous palpal insertion occurs during copulation.

Adult lifespan is extended (3 years or more), in contrast to the case in the more advanced spiders, and therefore adult specimens can be collected throughout the year. This type of lifecycle is classified as eurychronous by Schaefer (1987).

2.1.3 Morphology Of Body And Gametes

(i) **External morphology:** *P. phalangioides* is very distinctive in appearance. The long spindly legs, giving a superficial resemblance to the Opilinids, are the most noticeable feature and are the basis of one of the common names for the species (Daddy long legs spider). Other names relate to their habitat and their fusiform body (Cellar spider / Long-bodied cellar spider).

(ii) **Internal morphology:** Pholcid genitalia are all morphologically similar (Maughan & Fitch 1976; Roberts 1985; Wunderlich 1987; Gertsch & Peck 1992). Detailed work by Uhl (1992, 1993a, 1993b, 1994a, 1994b) on *P. phalangioides* found the site of sperm storage to be the *uterus externus* and that no spermathecae (chitinised diverticulum of the reproductive tract specialized for sperm storage) as such exists. Austad's (1984) prediction of a high P_2 for this species still holds however. This is because the sperm storage organ has no separate fertilization duct and Austad therefore classified it as a cul-de-sac species based on the taxonomy of Kaston (1978).

(iii) **Sperm and egg structure:** Alberti & Weinmann (1985) studied the fine structure of the sperm of *P. phalangioides*. Mature sperm are 5μm in diameter and encysted when delivered to the female. When capacitated they are capable of movement based on glycogen metabolism; mitochondria are thought to be unimportant in mobilizing the flagellum. Sperm do not form agglutinated masses ('sperm balls'), a common occurrence in other haplogyne spiders. Whilst laying the eggs the female applies some liquid to the centre of the mass and

loosely binds the eggs together in the chelicerae (Platel 1989). It is not known if sperm are contained in this liquid and so it is unclear if fertilization is effected internally or after they are laid. Contrary to Bonizzi (1869), Maughan (1978) and Platel (1989) observed silk use in the construction of a rudimentary cocoon. The rudimentary cocoon may well be a reduction of a more elaborate cocoon (Foelix 1982, page 205) or the primitive condition within the araneids. Females hold cocoons for the duration of their development and help offspring emerge (Bonizzi 1869; Becker 1892; Montgomery 1903; Bonnet 1930; Kaston 1948; Gersch 1949; Wiehle 1953; Platel 1989). The cocoon holding habit of *P. phalangioides* means their eggs are less susceptible to damage either from predators or shifts in microhabitat conditions compared to spider species which deposit their eggs on the substrate.

2.1.4 Implications From The Biology Of *Pholcus phalangioides* For Sexual Selection

Little courtship occurs in *P. phalangioides* so little potential exists for sexual selection (especially female choice) prior to copulation. Sperm competition is potentially a powerful selective force in *P. phalangioides* because it fulfils all four of Parkers' (1970) conditions for pre-adaptation to a high level of sperm competition:

(i) **Female Receptivity:** Polyandry has been observed in *P. phalangioides* by numerous workers (Montgomery 1903; Miyashita 1988a; Platel 1989; Uhl 1993a). The presence of two or more ejaculates together in the sperm storage organ of the female is the most important precondition for sperm competition.

(ii) **Female's Sperm Storage Organs:** The long-term presence of sperm in the sperm storage organs increases the chances of the coexistence of multiple ejaculates and thus the opportunity for sperm competition. The sperm storage organ also has to be capacious enough to hold more than one ejaculate, for sperm competition to occur. Little evidence exists on this issue in *P. phalangioides*. Mature sperm are only 5μm in diameter and the sperm storage organ is relatively large (Uhl 1994a). Paternity measurements will be used in this study to establish if more than one male's sperm can father offspring after multiply mating a female. Another aim is to establish if ejaculates are separately stored with the first male's sperm being overlaid by subsequent male's sperm in the fashion suggested by Austad (1984) with the prediction therefore of a high P_2. If sperm stratification is established in this manner then sperm competition is reduced and the position of the sperm is more important than individual

sperm characteristics or relative ejaculate size in determining the proportion of the fertilization set accruing to a particular male.

(iii) **Sperm Longevity:** Viable sperm can survive for extended periods within the sperm storage organ of *P. phalangioides*. Uhl (1993a), using single mating experiments, observed the production of viable offspring up to one year after mating. The implication of this is that sperm can persist to compete with subsequent inseminations and potentially lead to high levels of sperm competition.

(iv) **Sperm Utilization And Wastage:** Sperm usage efficiency is not possible to establish as sperm counts have not been made for *P. phalangioides* ejaculates. However 183 offspring, spread over 6 clutches can be produced from a single mating (Uhl 1993a), so potentially efficiency is high. This condition of efficient sperm usage leading to a higher chance of overlapping ejaculates is not as important as was once thought (Parker 1984), a point reinforced by the growing body of evidence that sperm competition is of importance in the mating systems of vertebrates, which usually have very profligate sperm usage (Smith 1984, chapters 12 to 19; Birkhead & Møller 1992; Baker & Bellis 1995).

2.2 Specimen collection

Adult and subadult *P. phalangioides* were collected from various sites around Europe during 1992-1993, by a number of volunteers. Specimens from geographically disparate populations were used in order to maximize the genetic variation in the parents of laboratory crosses.

2.3 Mating Experiments

One hundred and thirty seven laboratory mating trials were necessary to produce 60 successful matings of *P. phalangioides*. A mating trial was abandoned if mating did not occur within 1 hour (N=77). Matings took place in the same cages in which spiders were reared (Fig. 2.3), and under the same conditions (Section 2.4). Economy of viewing time was achieved by setting up three mating trials simultaneously; even if all three successfully mated, accurate measurements of mating duration were possible. Four mating treatments were carried out on the collected *P. phalangioides*, classified on the basis of the mating status of the females, as shown in Table 2.1.

Further details of the mating treatments are presented in the following (see also Table 2.1):

(i) & (ii) Sub-adults were reared separately to ensure virgin status of the females used in both single and multiple mating experiments. Controlled matings were conducted eight days or more after females reached maturity because Uhl (1993a) showed that there was a refractory period of this duration within which mating was unlikely to occur. Mating experiments were conducted in the controlled temperature room in which the animals were reared. Females always made their web at the top of the cage (see Section 2.4, Fig 2.3 for cage description) so the males were introduced through the hole at the bottom, after removing the nylon mesh. After mating, the males were immediately removed to avoid the possibility of sexual cannibalism. In the case of the multiple matings a second male was introduced after the removal of the first (usually straight away but in the case of females 16/17 and 40/41, 10 and 1 days respectively after the first male was removed[1]). Second matings were usually made

[1] These delays have no biological significance they merely represent matings which were successfully achieved after a delay.

immediately after the first because of the difficulty of remating a female after a long duration. Records were kept of interactions between the mating pair and of the mating duration.

(iii) Details are as for (i) and (ii) except that the females used underwent their last moult in the wild. The main purpose of matings 53/54 to 59/60 was to ensure that multiple matings of females could be easily carried out under the laboratory conditions used. Uhl (1993b) and Platel (1989) observed multiple matings in this species in the laboratory, although under slightly different conditions and did not state how difficult it was to multiply mate females. These experiments also investigated if large numbers of offspring could be successfully raised under the rearing conditions used.

(iv) Twenty wild-collected, mature females were set up in cages (see Section 2.4, Fig 2.3) to lay eggs. Five additional females had egg batches when caught, but unfortunately none of the eggs successfully hatched, possibly due to mechanical damage during transit. Four of the twenty females did not lay eggs during the experiment so presumably did not have stored sperm to fertilize their eggs in captivity. Females were maintained in isolation throughout rearing so that all broods were fathered by the males with whom the female had mated in the wild before capture. Females were kept until they had laid up to three egg batches.

Table 2.1 Mating Treatments Used And Breakdown Of Numbers Of Broods Available For Paternity Analysis And Material Analysed

Mating Treatment.	Number Of Mating Observations. (number of females X number of times each mated).	Running Total Of Mating Observations.	Number Of Females In Treatment Producing Cocoons.	Number Of Females In Which Broods Were Analysed (see section 2.6).
(i) Single mating. (numbers 1 to 15)	15 X 1 = 15	15	10[1]	8
(ii) Multiple mating. (numbers 16/17 to 48/49)	17 X 2 = 34	49	16[2]	10
(iii) Wild collected and mated in the lab. (numbers 50 to 59/60)	3 X 1 = 3 (numbers 50 to 52)	52	3	0
	4 X 2 = 8 (numbers 53/54 to 59/60)	60	4	0
(iv) Wild mated, unmated in the lab. (N=20).	0 (numbers 61 to 80)	60	16	16

Nomenclatural Note:

Females are named after the accession number of the mating(s) they were involved in, hence the first female to be multiply mated is called 16/17, the next 18/19 etc. Thus a females' number unambiguously indicates what type of mating she was involved in. The females' broods are named after her with a lower case Roman numeral, thus the first brood of the first singly mated female is 1i, the second 1ii. A female's number does not imply her position in the temporal sequence of the matings, it is merely for labelling purposes; the different treatments were randomized.

[1] Females 11-15 did not produce eggs after a long incubation period in the lab and so the treatments were abandoned.

[2] Female 48/49 died before laying any egg-sacs.

2.3.1 Mating Observations And Data

Little or no courtship was observed in *P. phalangioides* in the successful matings observed in the lab (N=60/137 trials). The only preliminaries to copulation were when the male was observed to pull on the web, 'tugging' in the terminology of Robinson & Robinson (1980). This behaviour, when used (N=18/60), presumably informed the female of the male's presence. Copulation otherwise is entered into rapidly without courtship when the male and female align themselves so they are facing each other. These observations are in agreement with previous studies that involved free living *P. phalangioides* (Reagan & Reagan 1989; N.L.Reagan pers. com.). On the approach of the male the female is usually hanging in the web with the abdomen held above the cephalothorax, venter side up. If the female is receptive she remains in the abdomen up position and the male quickly inserts both palps (N=12/60). Usually (N=48/60), prior to insertion, a process here termed 'cephalothorax rubbing' occurred (Fig 2.1) during which the male swiftly brushed the female's sturnum with his chelicerae. The brushes were directed towards the epigyne, although they sometimes extended as far back as the spinnerets. When reaching the end of the forward brush the male pulls back away from the female to the starting position. This process can be repeated over 100 times, although about 20 is more usual. It is not clear whether this behaviour is of value in courtship or is merely the male attempting to locate the abdominal groove of the female. The female's abdominal groove must be located and clamped onto with the chelicerae before palpal insertion can occur (N.L.Reagan pers.com.). The grip on the abdominal groove is maintained throughout copulation.

Once the palps are inserted the female's abdomen is pulled over towards the male's abdomen until the male and female are almost venter to venter (Fig 2.2). It is impossible to tell if the female's abdomen is pulled by the male using his palps or the flexion is effected by

a movement of the female. This mating position is a variant of the typical haplogyne posture, known as Type 1, as shown in Foelix (1982; page 195, Fig. 143a).

There is some controversy about whether palpal inflation occurs during copulation and sperm transfer in *P. phalangioides.* Cooke (1966), generalizing from a study of the haplogyne spider *Dysdera crocata,* suggested that all haplogyne spiders are incapable of palpal inflation. Montgomery (1903) however, attested to the fact that they could inflate. I observed the phenomenon of palpal inflation in all copulations (N=60).

Parting from the female was as abrupt as the insertion, with the female often (N=37/60) chasing the male. The pursuit was usually not for any great distance and the male easily escaped despite the small size of the jar. Only one incidence of sexual cannibalism occurred in the 137 mating trials observed. Sexual cannibalism even in captivity does not seem to be a feature in the matings of *P. phalangioides.* This is despite the known araneophagic tendencies in *P. phalangioides* (Jackson and Brassington 1987). The venom of *P. phalangioides* is weak (Kirchner & Opderbeck 1990) and this may mitigate against a female's ability to cannibalize even if this were a favoured strategy. On balance, sexual cannibalism seems unable to explain the non-unity of the sex ratio contrary to the suggestions of Maughan (1978):

"*Perhaps the rapid attrition rate of males results from mortality associated with breeding*".

The mean duration for all observed matings was 3081 seconds (314 SE, Range = 85-7740, N = 60) or approximately one hour (51'21"). These findings are concordant with the findings of other workers (Bonizzi 1869; Montgomery 1903; Platel 1989; Reagan & Reagan

1989; Uhl 1993b). The observed matings (mating treatments (i) to (iii)) were classified on the basis of the mating status of both members of the mating pair. Males and females could be virgin or mated before a specific mating takes place leading to a 2 X 2 contingency table of mating types as shown in Table 2.2. For the purpose of the classification the wild collected females (numbers 50 to 59/60) were classed as already mated (K3 or K4), though the conclusions did not change if they were excluded from the analysis. The results are summarized in Table 2.3.

The matings involving a virgin female differed significantly in duration from those involving a mated female (K1 & K2 V^s K3 & K4) (Mann-Whitney, W = 1302, $p<0.0001$). However the mating status of the male did not affect the duration of a mating (K1 & K3 V^s K2 & K4) (W = 759, $p>0.05$). These findings are consistent with those of Uhl (1993), except she observed a narrower range of mating times for K3 & K4 mating types: 90-300 seconds as opposed to the 85-4294 seconds reported here (about half of the times are in the range observed by Uhl (1993b) and half are of greater duration).

Matings of type (ii) were independently tested for differences in first and second mating times because a female's first mating duration can be paired with the second to see if there is any relationship between the two. Again first mating durations are significantly longer in duration than second (paired t-test t = 4.32, df = 16, $p=0.0005$). The extent of the effect of the first mating duration on the second is shown in Graph 2.1. The graph shows no linear relationship between the two mating durations (r = - 0.548). It is interesting to note though that if the duration of the first mating exceeds 5000 seconds then the second mating never exceeds 300 seconds (N = 8). A long first mating time may be be a ploy to depress female receptivity at the second mating, and might constitute a form of 'contact mate

Table 2.2 Mating Treatments Classified By Mating Status Of Participants

K1 to K4 = Mating Kinds		Male	
	Mating status	Virgin	Mated
Female	Virgin	**K1**	**K2**
	Mated	**K3**	**K4**

Table 2.3 Mean Mating Duration For Different Mating Kinds

Mating Kind (number involved)	Mean duration of matings in seconds (mean; standard error; range)	
K1 (N=14)	4467; 575; 1168-7740	} 4450
K2 (N=18)	4437; 503; 1152-7540	
K3 (N=9)	1926; 764; 96-6348	} 1517
K4 (N=19)	1324; 339; 85-4294	
Total Mean (N=60)	3081; 314; 85-7740	

guarding' (Thornhill & Alcock 1983). The effectiveness of this strategy, in terms of reproductive success is assessed in a later section (2.6). Alternatively the major part of the first mating duration could be involved with overcoming female 'reticence' to mate and constitute a form of 'copulatory courtship' (Eberhard 1991). Palpal inflation occurred continuously through mating and implies sperm transfer (Foelix 1982), so this explanation appears unlikely.

Graph 2.1 First Mating Verses Second Mating Duration Of Multiply Mated Females

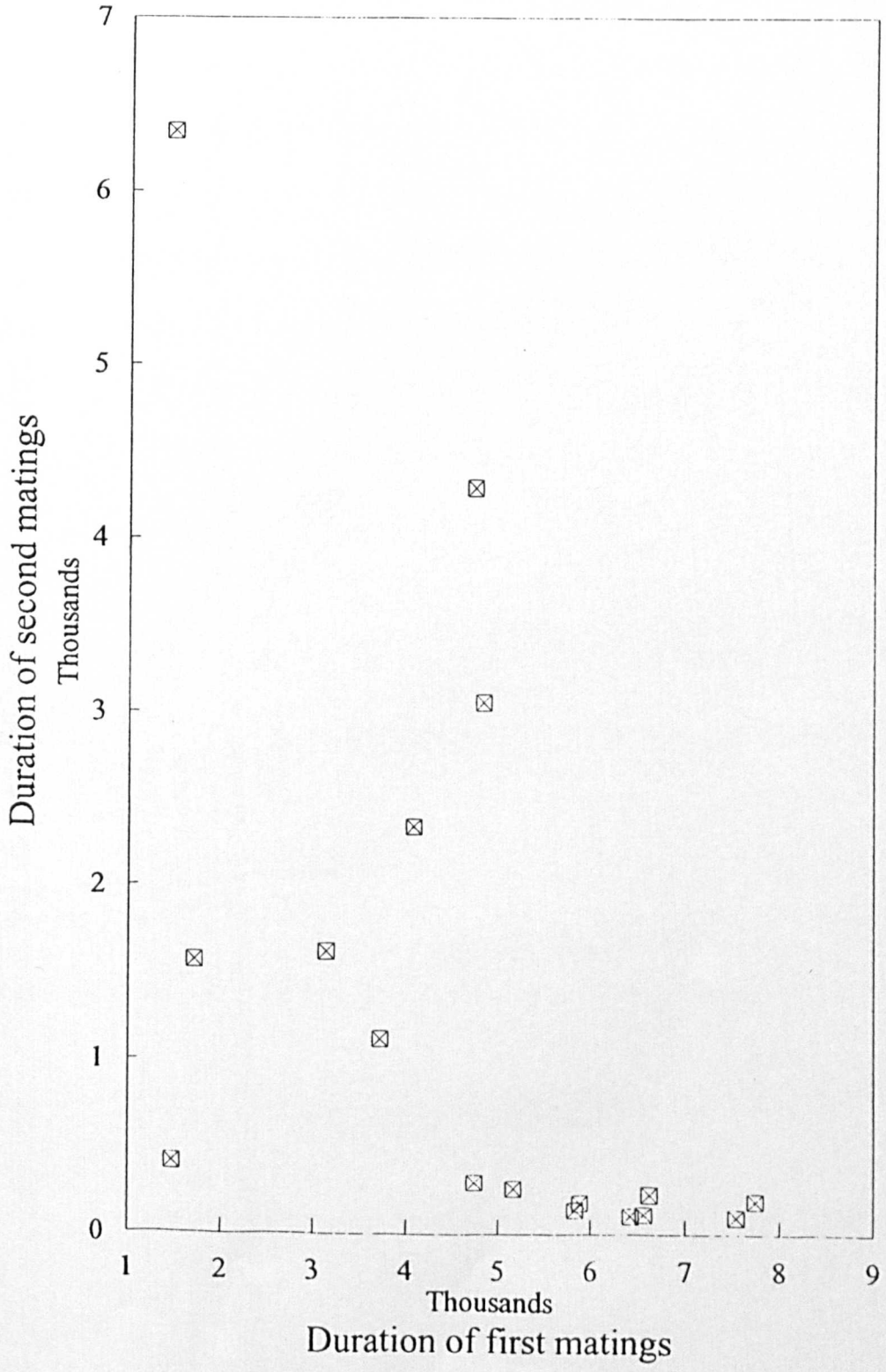
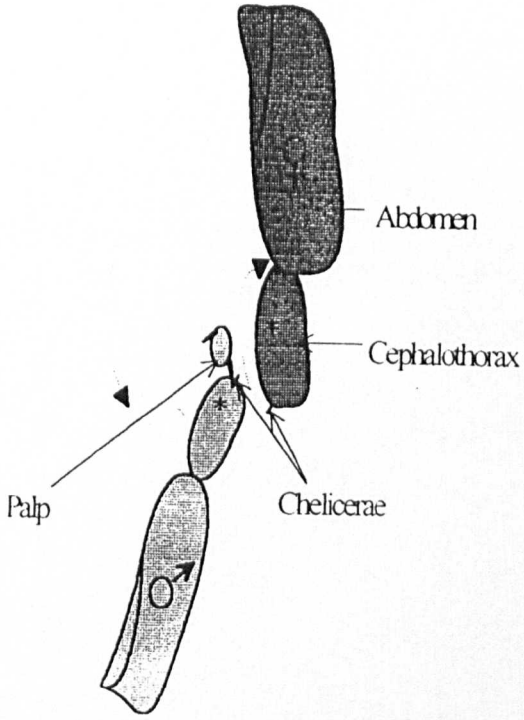


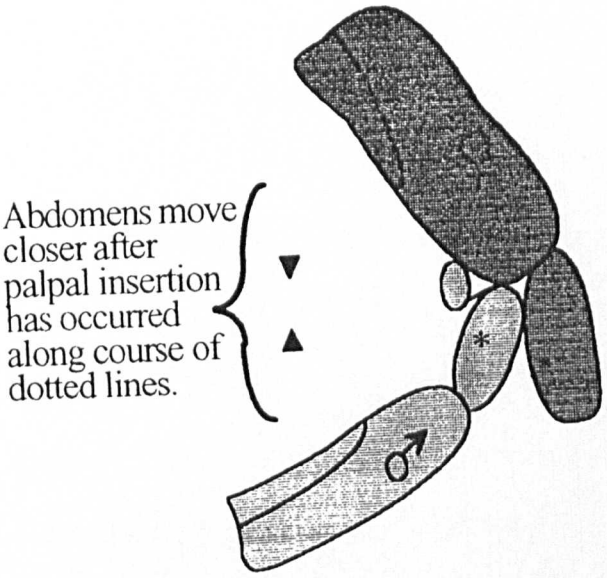
Fig 2.1 'Cephalothorax Rubbing' In *P. Phalangioides*



Legend For Fig 2.1:

* = Legs omitted in drawing; ·····▶ = Course of movement during 'cephalothorax rub'.

Fig 2.2 Mating Position of *P.phalangioides*



Legend for Fig 2.2:

During copulation the relative positions of the male and female were always as shown but can be in any absolute position rotated about the centre, although usually the female is above. *In copula* the abdomen of the female is pulled close to the male's abdomen and the male's chelicerae are inserted into grooves in the female's epigastric fold. The legs are omitted in the figure. The legs spread radially from the cephalothorax, the male and female tarsi, in life, are always opposed with the male's outside those of the female's.

2.4 Rearing Methods

P. phalangioides has been reared successfully by a number of authors (Bonizzi 1869; Montgomery 1903; Schaefer 1976; Nentwig 1983; Miyashita 1988a, 1988b; Platel 1989; Uhl 1993b). The rearing cages used in these studies varied but all basically consisted of transparent containers of moderate size. In the present studies 2 litre acetate sweet-jars were modified as shown in Fig 2.3. *P. phalangioides* is able to build its webs in the corners of walls (Kirchner 1986) so it was unnecessary to provide web supports.

Previous studies (cited above) successfully used a range of rearing conditions indicating that *P. phalangioides* has broad environmental tolerances. Miyashita (1988a), for example, found the growth of *P. phalangioides* to be unaffected by the duration of the photoperiod. In the experiments reported here, a photoperiod of 12L:12D was used throughout.

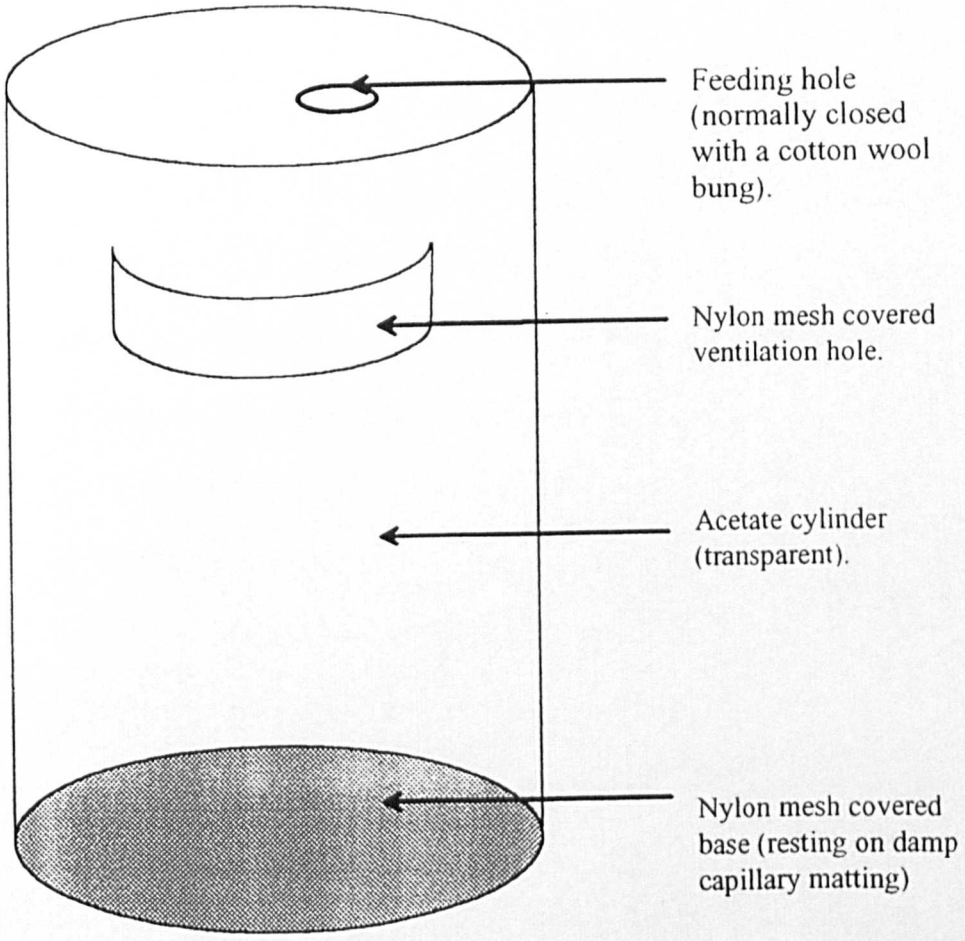
Nentwig (1983) found the spectrum of food taken by *P. phalangioides* to be very wide so again experimental convenience dictated the conditions used here. All young spiders were fed *ad libitum Drosophila melanogaster* and *Drosophila virilis* from maintained inbred stocks. *Lucilia* spp. ('greenbottles') and *Calliphora* spp. ('blowflies') were obtained from local fishing shops as larvae, pupated in covered jam-jars at room temperature and the emerging adults introduced with entomological tweezers to the adult spiders' cages. A range of prey was proffered to the spiders to maximize survival. In cages with many young the food was maintained at an especially high level (fed on a daily basis) to avoid sibling cannibalism. Though ordinarily arachnophagic (Jackson & Brassington 1987; Jackson & Rowe 1987) a female *P. phalangioides* will feed her young, (Bonizzi 1869; pers. obs.) so the mother was maintained with her brood until a further cocoon was laid. Different broods from a single

female were reared separately to allow an analysis of temporal change in P_2 patterns, and this also served to reduce cannibalism between broods.

Humidity was maintained by placing the rearing cages in plastic trays (60cm X 45 cm), floored with damp capillary matting. The nylon mesh across the open bottom of the jar ensured a high humidity inside. The spiders would also have been able to descend to the bottom of the cage to take liquid water if necessary. Drinking has been observed in the wild by Bristowe (1958). Maintaining all the cages in trays in the manner described allowed simultaneous watering by pouring a quantity of water into the tray once a week.

Females were maintained for up to 300 days after mating and observations were made daily to monitor when eggs were laid and hatched and thus establish a phenology of *P. phalangioides* under these conditions which can be compared with previous studies (Montgomery 1903; Platel 1987; Miyashita 1988a, 1988b; Uhl 1993a) utilizing different rearing environments.

Fig 2.3 Diagrammatic Representation Of The Spider Cages Used To Mate And Rear Spiders.



Summary of rearing conditions:
Temperature: 24 3°C
Humidity: Uncontrolled but high.
Feeding: Various Diptera *Ad libitum*
Light: 12L:12D.

2.4.1 Rearing Data

Wild-collected females' (numbers 50 to 59/60) phenology is not included in the following analysis because it is not known which cocoon it was they first presented in the laboratory nor the date of their first mating. Only multiply mated females (16/17 to 46/47) were maintained until they produced two or more cocoons. The phenology of the production of these cocoons is presented in Graph 2.2. On average 2.9 cocoons were produced by the females which allows an analysis of trends in sperm utilization with cocoon number (Section 2.6). Miyashita (1988b) maintained *P. phalangioides* for up to 800 days and observed females could produce up to 8 cocoons and Platel (1989) observed 9 in three years. In these previous studies females were remated between some of the cocoons. Uhl (1991) observed that female *P. phalangioides* lay up to 6 cocoons (mean= 3.7; range 1-6) after a single mating. A similar level of reproductive success could not be achieved from the captive females in this study though similar rearing conditions were used. Despite the desirability of a larger number of broods in assessing temporal trends in sperm utilization, time constraints and the necessity of harvesting live females meant egg production had to be truncated at 2-4 cocoons.

The mean time before a female laid her first cocoon after mating was 36.6 days (mode = 20.5, range 8-189; N=26, pooling single and multiply mated females (1 to 46/47)). This wide range reflects the situation in the wild (Bristowe 1958), where winter-mated females store sperm over winter and lay eggs in the spring but spring matings yield eggs shortly afterward. Platel (1989) reviewed the data available on the phenology of egg laying and concluded that temperature was the factor limiting when eggs could be successfully laid. All the spiders in this study were maintained at a constant temperature. Nutrition may also be a constraint on egg laying; most of the females in which a delay occurred before egg laying

were very thin at the time of mating compared to those laying eggs earlier. This means the duration for which sperm are stored in the *bursa externus* may not be less variable in tropical than temperate climes, if there is variation in patch quality. Overall there was a cocoon production interval of approximately 2 months on average (mean = 61.2 days; N = 46; range 8-243 days; pooled: mating 2 to cocoon 1 and cocoon n to cocoon n+1 (Graph 2.2)) .

The development time of eggs, once fertilized and laid, varied much less than the production period, with a mean of 22.8 days (range 10-34; N = 44[2]; SD = 4.5). This mean is slightly higher than the 16.7 obtained by Uhl (1993a), possibly a result of the marginally higher temperature her spiders were maintained at (23-28^{0}C). In future studies a higher rearing temperature may provide specimens more quickly.

The average figure for the number of progeny produced per cocoon (about 30-40; range 7-63) reported in the literature (Schaefer 1976; Miyashita 1988b; Platel 1989; Uhl 1993a) and the findings of this study (Graph 2.2) are in concordance. Previous workers counted the progeny at the hatchling stage, whereas in this study spiderlings were reared to the third or fourth instar. The implication of this is that differential survival of progeny is unlikely to disrupt segregation ratios of gametic markers used in paternity assignment. A caviat of this interpretation is that the females in this study may have been distributing reproductive resources differently from those in the other studies by laying fewer but larger cocoons.

In spiders generally, a negative trend is found between the number of progeny in a cocoon and its rank order in the laying sequence (Wise 1993). This relationship has been

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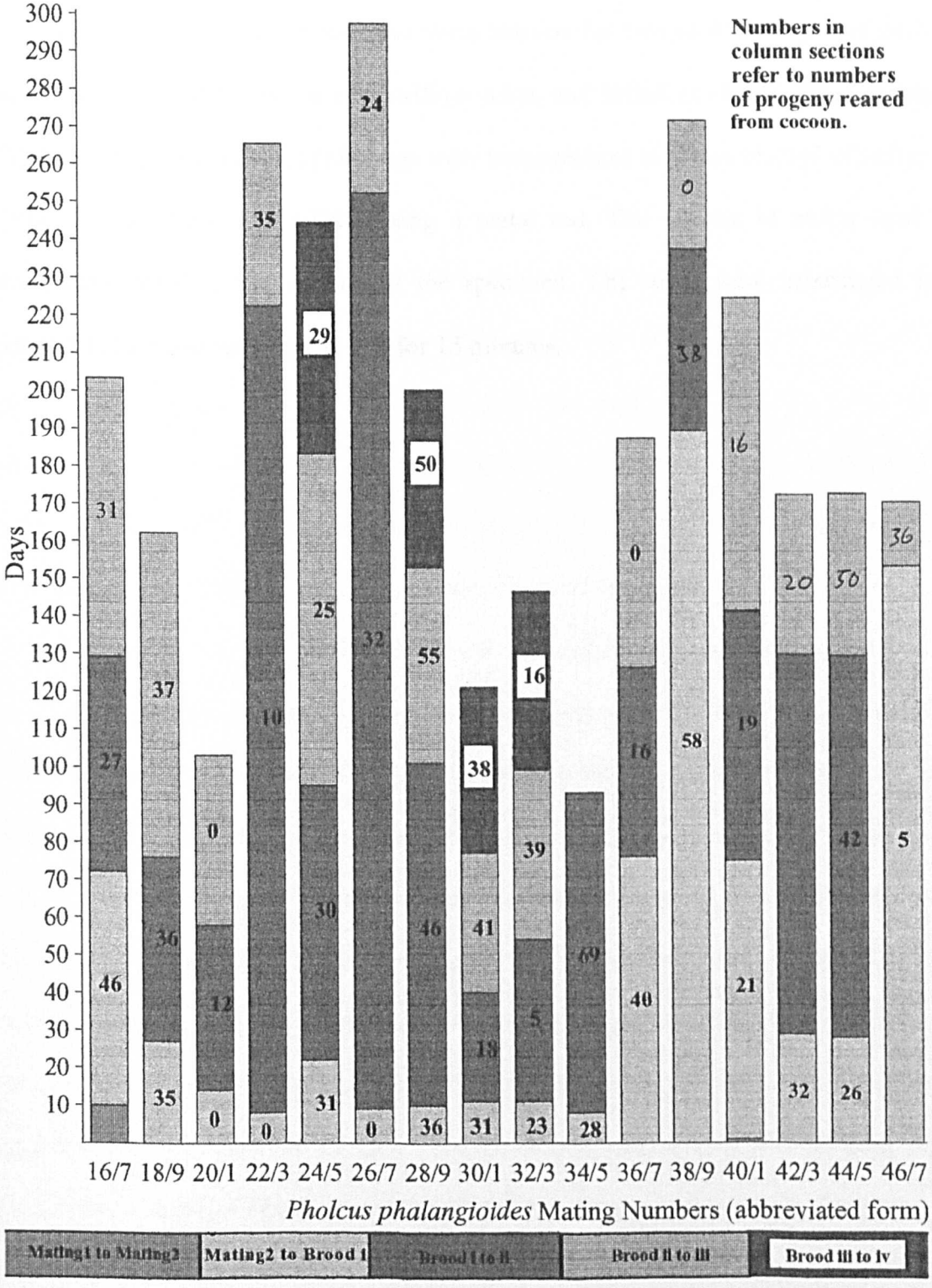
[2] 2 cocoons failed to hatch (20/21iii & 36/37iii) and were subject to öophagy by the mother. Four other cocoons (20/21i, 22/23i, 26/27i & 38/39iii) are recorded as having zero offspring - these cocoons did hatch but all offspring died before reaching 3rd/4th instar.

reported for *P. phalangioides* (Miyashita 1988a). No such relationship was found in the present study ($r = 0.024$; $N = 46$).

Graph 2.2 Phenology of Multiply Mated, Lab-Reared *P. Phalangioides*



2.5 Specimen Harvest, Storage and Preparation

Third to fourth instar spiderlings were starved for two to three days before being placed individually in 0.5ml microcentrifuge tubes and killed at -84°C. After storage at -84°C for up to ten months the spiderlings were homogenized at 4°C in 15-30µl of buffer (pH 7.0 tris / citrate (Den Boer 1978)) using a metal rod. The volume of buffer used was approximately equal to the volume of the specimen. The tubes were centrifuged in an Eppendorf 5414 microcentrifuge at 4°C for 15 minutes.

2.6 Electrophoresis

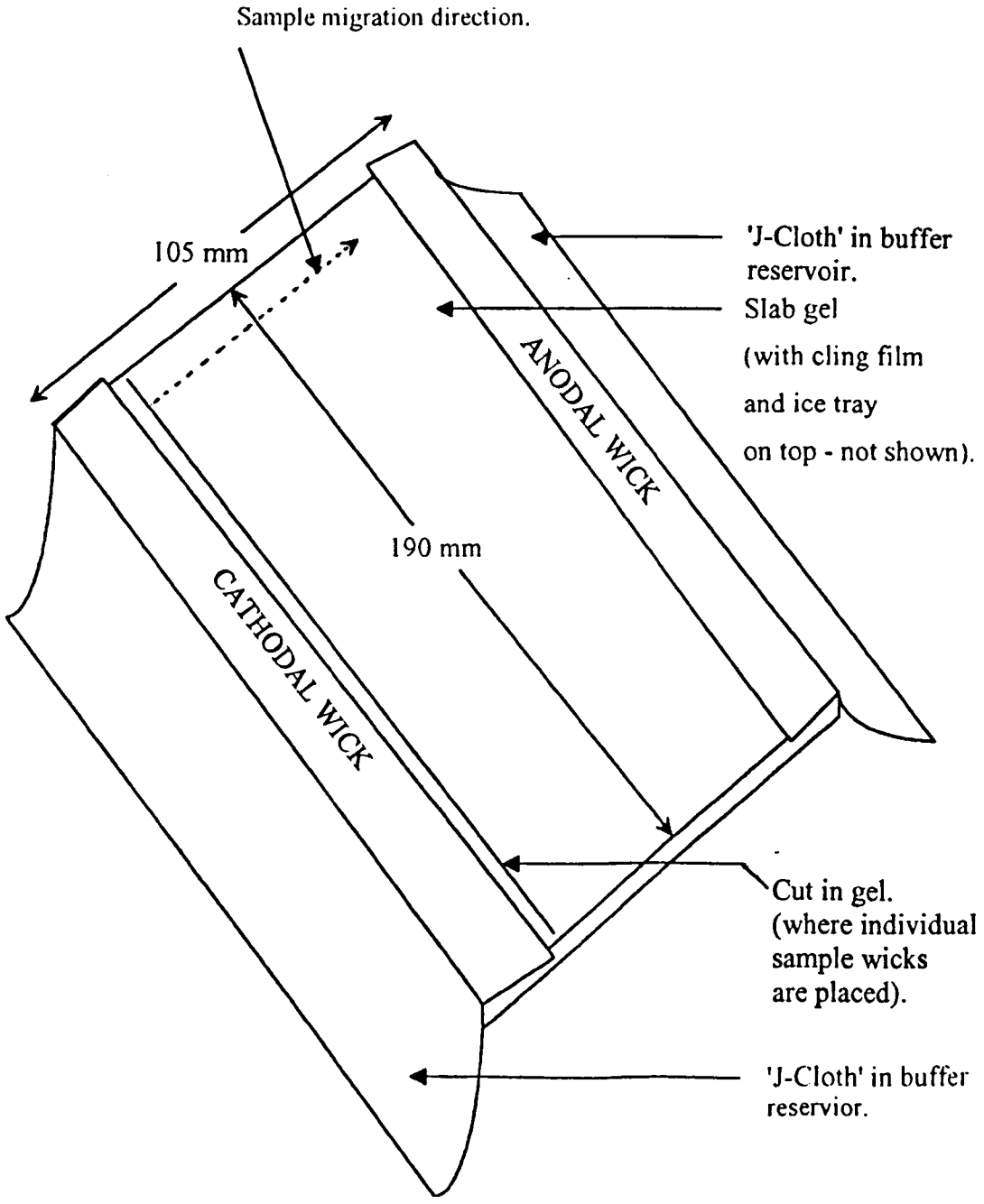
Preliminary experiments were conducted on adult specimens that were not involved in mating experiments to determine the gel running conditions which were most suitable to achieve consistently scorable bands.

Conditions eventually used were broadly similar to those of Oxford (1986) and are as follows. A 7.5% acrylamide (BDH product #44299 ('Electran')), 0.8% N,N' - methylene - bis-acrylamide (Sigma M-7279) monomer gel was prepared with the pH 7.0 tris (hydroxymethyl) methylamine (BDH product #10315) / citric acid (BDH product #10081) buffer of Den Boer (1978). The gel buffer in the preparation was 26.6 ml stock buffer and 0.4 ml TEMED (N,N,N',N - tetramethyl - ethylenediamine (Sigma T-8133)) diluted to 100 ml with water. Ammonium persulphate (0.14% w/v solution) (FSA product #A/6160) was used as a polymerization catalyst. The stock solutions were combined in the following amounts: 15ml acrylamide bis-acrylamide, 15ml pH 7.0 tris / citrate Buffer, 30ml ammonium persulphate. Once the solution was thoroughly mixed, it was poured into a 190 mm X 105 mm X 1.5 mm perspex frame and a glass plate top carefully applied, avoiding the formation of air bubbles. The gels were allowed to set for 15 minutes at room temperature, whereupon they were inverted and the perspex frame removed so the gel was left on the glass plate. The set gel was then covered with cling film. Gels were used on the same day as prepared; results using stored gels were inferior. The gels were run on a horizontal Shandon tank using the pH 7.0 tris / citrate buffer of Den Boer (1978) diluted X 2. The samples were soaked onto 1 X 6 mm pieces of Whatmans No. 5 filter paper, blotted and applied to a slit cut 15 mm in from, and parallel to, one long edge of the gel. The gel was connected to the reservoir buffer via four layers of 'J-cloth' (Fig. 2.4). To prevent dehydration, the gel and the sample slot were covered with cling film during electrophoresis. An ice pack was placed on top of the gel and

a constant voltage of 300 V applied (currents fluctuated between 45 to 60 mA) for 1 hour. During this time the whole apparatus was maintained at 4^{0}C.

After running, gels were incubated for 30-60 minutes in the reservoir buffer (7.0 pH tris / citrate) and stained for non-specific esterases, using alpha-napthyl acetate as a substrate (dissolved in one drop of acetone) and Fast Garnet GBC as stain. The Fast Garnet stain was prepared as a saturated solution in 10 ml of reservoir buffer 15 minutes before the completion of an electrophoretic run, made up to 150 ml and the substrate added just prior to use. After staining the stain solution was washed off with water and the gel fixed and dehydrated in 50% ethanol. The gel shrank down to approximately half its original size making the bands more definite by increasing the contrast between bands and the background. Gels were permanently stored in a heat-sealed plastic envelopes.

Fig 2.4 Diagrammatic Representation Of Electrophoresis Apparatus



2.6.1 Zymogram Interpretation

In paternity studies the markers used need to be demonstrably genetic to be of use. A detailed consideration of the esterase banding patterns of *P. phalangioides* and the genetic interpretation of them will be given in the following section. The full range of banding patterns is shown in Fig 2.5. Three spatially separate banding pattern systems were recognized. System I migrated between 110 mm and 120 mm, system II 100 mm to 105 mm and system III 40 mm to 50 mm from the origin. All combinations of banding patterns for the three systems were observed. As well as having quite different migration distances on the gel the systems always stained up in the same order with I appearing first and III last.

To ensure that none of the bands derived from ingested prey species, in addition to starving the young for a period before storing at -84^{0}C, control prey samples were run alongside *P. phalangioides* specimens (Plate 2.1). Esterases from prey species ran slightly more slowly than those of system III. A gel run for 2.5 hours confirmed the distinction of spider and prey enzymes and indicated that contamination from this source could be detected on gels run for 1 hour. The *Drosophila* prey used were from inbred stocks to try to ensure the range of variation of esterases did not exceed that visualized in these trial runs

No simple genetic interpretation could be found for system I and so it was not scored. Enzymes in systems II and III appear to be monomers. System II appears to be controlled by a simple diallelic locus with fast and slow bands. System III is slightly more complicated, with two interpretations possible. The first hypothesis is that the central band found in all individuals is the product of a monomorphic locus and should be ignored. This leaves the bands above and below it to be scored as a simple fast / slow system. The second hypothesis is that above each 'genetic' band there is a post-translational derivative, shown as a thinner

Fig 2.5 Schematic Diagram Of Esterase Banding Patterns

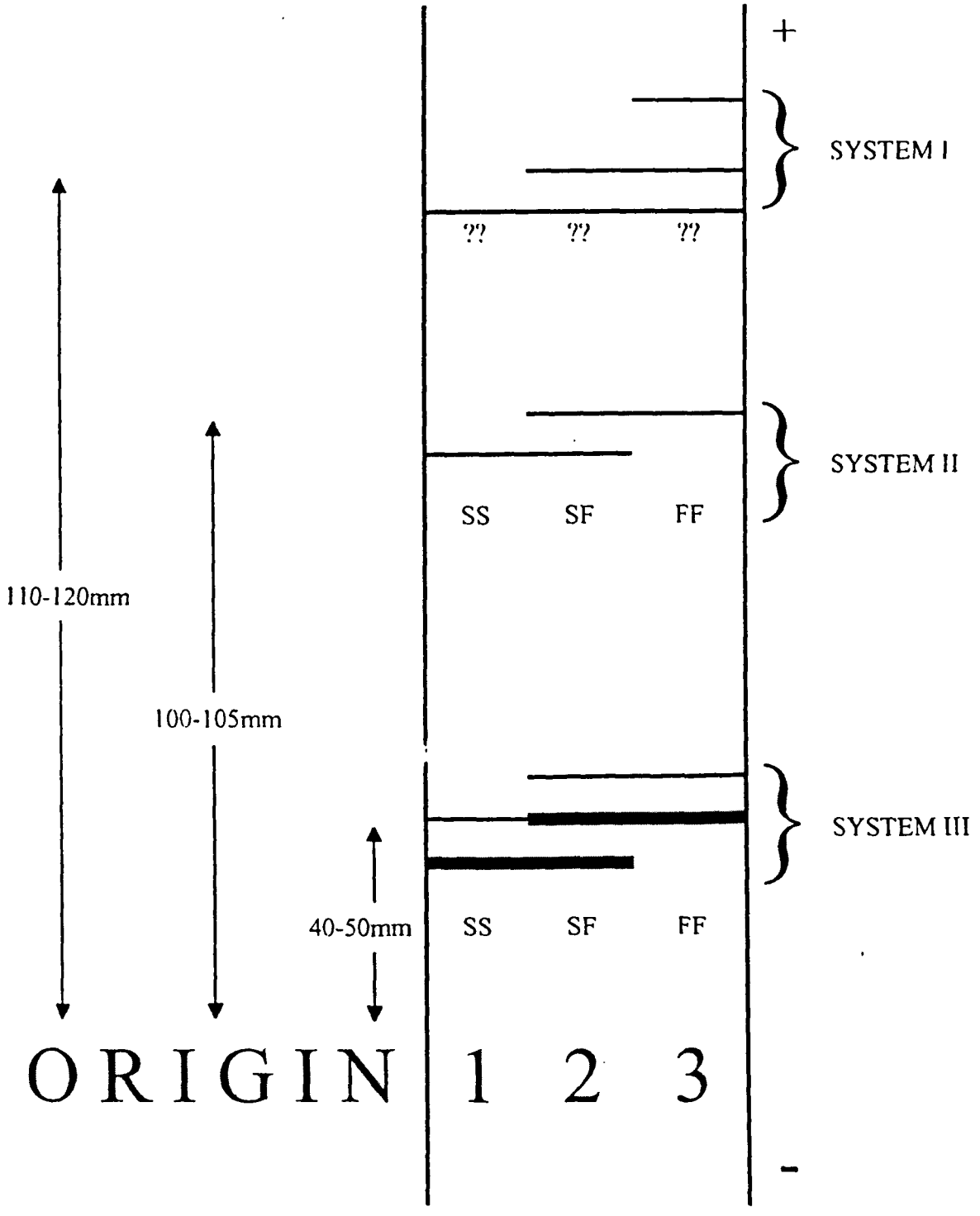
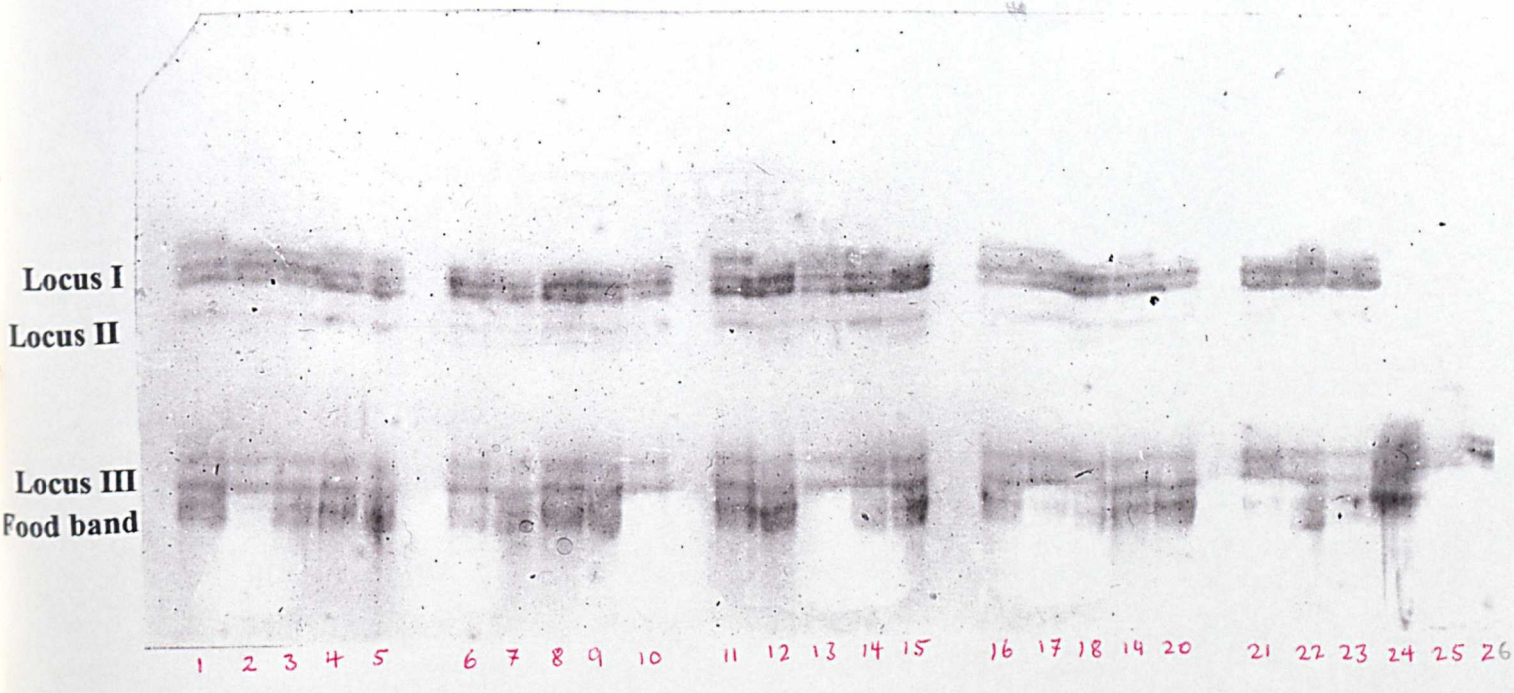


Plate 2.1 Zymogram Of *P.phalangioides* Esterase Banding Patterns With Control Food Esterase Patterns - 1 Hour Run.



Enterpretation of the gel is as follows:

Lane	1	2	3	4	5	6	7	8	9	10	11	12	13
System II	FF	FF	FF	FF	FF	FS	FS	FS	FS	FF	FF	FS	FF
System III	FF	FF	FF	FF	FF	FF	FF	FF	FF	FF	FF	FF	FF
Lane	14	15	16	17	18	19	20	21	22	23	24	25	26
System II	FF	FS	FF	FF	FS	FF	FS	FS	FS	FF	D[1]	D	GB
System III	FF	FF	FF	FF	FF	FF	FF	FF	FF	FF			

[1] D = Drosophila, GB = Green bottle

Table 2.4 Summary Of Single Matings: Parental And Offspring Allozyme Phenotypes At Loci II & III For Single Brood Reared

#	LOCUS II						LOCUS III				
	PARENTS & SCORE		OFFSPRING			Chi^2	SCORE	OFFSPRING			Chi^2
			SS	SF	FF			SS	SF	FF	
1	FEMALE	FF	0	12	18	1.20a	FF	0	36	0	N/A
	MALE	SF					SS				
2	FEMALE	SF	19	28	0	1.72a	SS	23	24	0	0.02a
	MALE	SS					SF				
3	FEMALE	SF	0	11	11	0.00a	FF	0	11	11	0.00a
	MALE	FF					SF				
4	FEMALE	FF	0	37	0	N/A	SS	0	37	0	N/A
	MALE	SS					FF				
5	FEMALE	SS	0	12	0	N/A	FF	0	8	4	1.33a
	MALE	FF					SF				
6	FEMALE	SF	13	24	0	3.27a	SF	10	21	7	0.89b
	MALE	SS					SF				
7	FEMALE	FF	0	0	34	N/A	SF	8	16	7	0.10b
	MALE	FF					SF				
8	FEMALE	FF	0	21	0	N/A	SF	0	10	11	0.05a
	MALE	SS					FF				

Explanatory Notes For Table 2.4

Score = Designation for allozyme band mobilities: SS = Slow; SF = Slow / Fast; FF = Fast.

Chi^2 = Chi^2 value for test of observed ratios against one of two *a priori* expected Mendelian ratios from parental crosses: a = 1:1; b = 1:2:1; N/A= Not applicable (when only one class of allozyme band mobilities found in offspring).

Note: All Chi^2 values are non-significant.

Explanatory Notes For Table 2.5

Score = Designation for allozyme band mobilities: SS = Slow; SF = Slow / Fast; FF = Fast.

Expected for male 1 and male 2 = Possible allozyme mobilities in offspring from crosses set up, given veracity of genetic interpretation (Section 2.6.1) of bands.

Contents of table are Chi^2 values for tests of observed ratios against one of two *a priori* expected Mendelian ratios from parental crosses: a = 1:1; b = 1:2:1; N/A = when only one class of allozyme band mobilities found in offspring. Raw data upon which Chi^2 scores are based are shown in table 2.6.

Shading indicates empty cells of table.

Note: All Chi^2 values are non-significant.

Table 2.5 Effective Single Matings Within Multiple Matings At Locus II[1]

#	PARENTS & SCORE		EXPECTED for male1 & 2	BROOD i	BROOD ii	BROOD iii	BROOD iv
16 / 17	FEMALE	FF	SF	All SF, as expected, in broods i, ii and iii			
	MALE1&2	SS					
18 / 19	FEMALE	FF	SF FF		2.00a		
	MALE1&2	SF					
22 / 23	FEMALE	SS	SS SF	Failed	Unscorable	0.26a	
	MALE1&2	SF					
24 / 25	FEMALE	SF	SS SF	0.13a	0.33a	1.5a	3.24a
	MALE1&2	SS					
32 / 33	FEMALE	SF	SF FF	2.9a	0.00a	1.29a	2.25a
	MALE1&2	FF					
34 / 35	FEMALE	SF	SF FF	0.36a	0.06a		
	MALE1&2	FF					
36 / 37	FEMALE	FF	FF	All FF, as expected, in brood i and ii.			
	MALE1&2	FF					

[1] There was only one effective single mating at locus III (brood 20/21ii) which did not deviate from its expected Mendelian ratio ($P<0.05$).

Explanatory Notes For Table 2.6

Score = Designation for allozyme band mobilities: SS = Slow; SF = Slow / Fast; FF = Fast.

Expected for male 1 & male 2 = Separately tabulated expected allozyme band mobilities for offspring of males involved in cross with female. Darkly shaded areas indicate where predictions for males are different and hence paternity diagnosis is possible from this locus for these crosses.

Main body of table contains numbers of offspring with allozyme band mobilities indicated above.

Subscript numbers in brackets indicate which male sired that group of offspring:

$_{(1)}$ = Male 1; $_{(2)}$ = Male 2. Light shading indicates an empty cell.

Table 2.6 Summary Of Multiple Matings: Parental And Offspring Allozyme Phenotypes At Locus II

#	PARENTS & SCORE		EXPECTED		OBSERVED OFFSPRING BAND MOBILITIES			
			MALE 1	MALE 2	BROOD i	BROOD ii	BROOD iii	BROOD iv
16 / 17	FEMALE	FF	SF	SF	34SF	25SF	24SF	
	MALE 1	SS						
	MALE 2	SS						
18 / 19	FEMALE	FF						
	MALE 1	SF	SF	SF		12SF		
	MALE 2	SF	FF	FF		20FF		
20 / 21	FEMALE	SF	SS					
	MALE 1	SF	SF	SF		08SF		
	MALE 2	FF	FF	FF		04FF		
22 / 23	FEMALE	SS	SS	SS			16SS	
	MALE 1	SF	SF	SF	Failed	Unscorable	19SF	
	MALE 2	SF						
24 / 25	FEMALE	SF	SS	SS	14SS	15SS	15SS	08SS
	MALE 1	SS	SF	SF	16SF	12SF	09SF	17SF
	MALE 2	SS						
26 / 27	FEMALE	FF						
	MALE 1	SS	SF		Failed	$27SF_{(1)}$	$10SF_{(1)}$	
	MALE 2	FF		FF		$05FF_{(2)}$	$14FF_{(2)}$	
28 / 29	FEMALE	FF						
	MALE 1	FF		SF	$15SF_{(2)}$	$11SF_{(2)}$		
	MALE 2	SF	FF	FF	18FF	35FF	$54FF_{(1)}$	$53FF_{(1)}$
30 / 31	FEMALE	FF						
	MALE 1	FF		SF	$06SF_{(2)}$	$13SF_{(2)}$	$36SF_{(2)}$	$24SF_{(2)}$
	MALE 2	SS	FF		$03FF_{(1)}$			$03FF_{(1)}$
32 / 33	FEMALE	SF						
	MALE 1	FF	SF	SF	15SF	01SF	17SF	11SF
	MALE 2	FF	FF	FF	07FF	01FF	11FF	05FF
34 / 35	FEMALE	SF						
	MALE 1	FF	SF	SF	11SF	31SF		
	MALE 2	FF	FF	FF	14FF	33FF		
36 / 37	FEMALE	FF						
	MALE 1	FF						
	MALE 2	FF	FF	FF	36FF	15FF		

Explanatory Notes For Table 2.7

Score = Designation for allozyme band mobilities: SS = Slow; SF = Slow / Fast; FF = Fast.

Expected for male 1 & male 2 = Separately tabulated expected allozyme band mobilities for offspring of males involved in cross with female. Darkly shaded areas indicate where predictions for males are different and hence paternity diagnosis is possible from this locus for these crosses.

Main body of table contains numbers of offspring with allozyme band mobilities indicated: SS = Slow; SF = Slow / Fast; FF = Fast. Subscript numbers in brackets indicate which male sired that group of offspring: $_{(1)}$ = Male 1; $_{(2)}$ = Male 2. Light shading indicates an empty cell.

Table 2.7 Summary of Multiple matings: Parental and Offspring Allozyme Phenotypes At Locus III

#	PARENTS & SCORE		EXPECTED		BROOD i	BROOD ii	BROOD iii	BROOD iv
			MALE 1	MALE 2				
16 / 17	FEMALE	SS	SF	SS	$19SS_{(2)}$ $15SF_{(1)}$	$02SS_{(2)}$ $23SF_{(1)}$	$24SF_{(1)}$	
	MALE 1	FF						
	MALE 2	SS						
18 / 19	FEMALE	SF	SS SF	SS SF FF		20SS 11SF		
	MALE 1	SS						
	MALE 2	SF						
20 / 21	FEMALE	SF	SF FF	SF FF		03SF 09FF		
	MALE 1	FF						
	MALE 2	FF						
22 / 23	FEMALE	SS	SS	SF	Failed	$10SF_{(1)}$	$35SF_{(1)}$	
	MALE 1	SS						
	MALE 2	FF						
24 / 25	FEMALE	SS	SF	SS	$28SS_{(2)}$ $04SF_{(1)}$	$21SS_{(2)}$ $7SF_{(1)}$	$19SS_{(2)}$ $06SF_{(1)}$	$25SS_{(2)}$ $03SF_{(1)}$
	MALE 1	FF						
	MALE 2	SS						
26 / 27	FEMALE	SS	SF	SS SF	Failed	$03SS_{(2)}$ 29SF	$05SS_{(2)}$ 19SF	
	MALE 1	FF						
	MALE 2	SF						
28 / 29	FEMALE	FF	SF	FF	$36FF_{(2)}$	$26SF_{(1)}$ $20FF_{(2)}$	$54SF_{(1)}$	$53SF_{(1)}$
	MALE 1	SS						
	MALE 2	FF						
30 / 31	FEMALE	SF	SF FF	SS SF FF	$03SS_{(2)}$ 06SF 01FF	$02SS_{(2)}$ 12SF 04FF	$09SS_{(2)}$ 17SF 10FF	$11SS_{(2)}$ 21SF 5FF
	MALE 1	FF						
	MALE 2	SF						
32 / 33	FEMALE	FF	SF	FF	$13SF_{(1)}$ $10FF_{(2)}$	$04SF_{(1)}$ $01FF_{(2)}$	$24SF_{(1)}$ $04FF_{(2)}$	$16SF_{(1)}$
	MALE 1	SS						
	MALE 2	FF						
34 / 35	FEMALE	SS	SF	SS	$11SS_{(2)}$ $17SF_{(1)}$	$54SS_{(2)}$ $10SF_{(1)}$		
	MALE 1	FF						
	MALE 2	SS						
36 / 37	FEMALE	SF	SS SF	SF FF	$07SS_{(1)}$ 14SF $15FF_{(2)}$	$04SS_{(1)}$ 05SF $06FF_{(2)}$		
	MALE 1	SS						
	MALE 2	FF						

2.6.2 Trends In P_2 Data

Locus III was the more diagnostic locus for paternity assignment being totally inconclusive for only one brood (female 20/21, table 2.7). Only those broods in which paternity could be assiged unambiguously were chosen to go into Table 2.8. In the case of brood ii for females 18/19 and 20/21, paternity would not be assigned because the offspring could have been fathered by either of the males, although the offspring of brood 18/19 are more likely to have arisen from the first mating male, so they were left out of the calculations of P_2. Table 2.8 shows P_2 values for all the multiple matings. The mean P_2 over all broods is 0.65 (N = 27). Although the variance is very high with values ranging from 0 to 1, the mean P_2 significantly differs from 0.5 (Sign Test - 1 tail: Median = 0.76; n = 27; p = 0.0015), the value expected if the spermatheca has no influence (ie. random sperm mixing) and ejaculate sizes are equivalent in first and second males. The same test was performed after excluding the broods where depletion of sperm from the second male could have occurred (see Table 2.8) and the same conclusion applied (Sign Test - 1 tail: Median = 0.78; n = 24; p = 0.0001).

An analysis of variance was performed to investigate if a trend in P_2 is detectable between successive broods using all matings pooled together. No trend was observable (One Way ANOVA p = 0.916).

For those cases in which there was significant heterogeneity in P_2 among successive broods the number with a rising P_2 equalled the number falling (table 2.8). For most there was no trend in P_2 levels between broods.

Explanatory Notes For Table 2.8

Main body of table on left hand side contains P_2 values for individual broods i-iv of each female, where it can be calculated unequivocally. Shaded regions indicate an empty cell.

Underlined P_2 values are highlighted because they are unusual observations in that they are below 0.5.

Italicized P_2 values are to indicate where sperm of one of the males involved in the crosses was depleted.

HET. II = Chi^2 heterogeneity test on numbers of offspring sired by males for locus II.

Bold Chi^2 figures are significant at the 5% level. 24/25II; 32/33II and 34/35II are not diagnostic of paternity and therefore heterogeneity Chi^2 test is not expected to be significant and this is the case at the 5% level.

HET. III = Chi^2 heterogeneity test on numbers of offspring sired by males for locus III.

Bold Chi^2 figures are significant at the 5% level.

df = degrees of freedom.

TREND = Indicates direction of trend in P_2 in broods where heterogeneity tests are significant:

Down = P_2 decreasing through broods, as predicted by Austad (1984) (Fig 2.6).

Up = P_2 increasing through broods opposite to predicted trend (Fig 2.6).

Level = No trend in P_2 through broods.

Table 2.8 P_2 Values Calculated from Diagnostic Loci

#	BROOD i	BROOD ii	BROOD iii	BROOD iv	HET.II	HET. III	TREND
16/17	0.56	0.08	***0.00***			**Chi^2=28.91 df=2**	Down
18/19							
20/21							
22/23		*1.00*	*1.00*				Level
24/25	0.88	0.75	0.76	0.89	Chi^2=5.17 df=3	Chi^2=1.63 df=2	Level
26/27		0.16	0.58		Chi^2=11.16 df=1	Chi^2=1.40 df=1	Level
28/29	1.00	0.57	***0.00***	***0.00***	**Chi^2=48.35 df=3**	**Chi^2=134.78 df=3**	Down
30/31	0.66	1.00	1.00	0.88	Chi^2=1.96 df=1	Chi^2=0.00 df=2	Level
32/33	0.57	0.80	0.86	1.00	Chi^2=0.35 df=2	**Chi^2=12.01 df=2**	Up
34/35	0.39	0.84			Chi^2=0.14 df=1	**Chi^2=19.01 df=1**	Up
36/37	0.68	0.60				Chi^2=0.35 df=1	Level

2.6.3 Incidence Of Multiple Paternity In The Wild And Genetic Variation At Collection Sites

In 6 out of 16 broods evidence exists that offspring genotypes are inconsistent with single male paternity (Table 2.9 and Table 2.10). This estimate of the incidence of multiple paternity comes from two sources, intra-brood ratios and inter-brood ratios. The former are broods differing significantly from any simple Mendelian ratio (63, 66, 73 and 74) and the latter where individual broods conform to a Mendelian ratio but where successive broods differ in the ratio to which they conform (64, 72 and 73). Even this high level of multiple paternity is probably an underestimate for several reasons:

(i) Only 2 loci were analyzed giving a small range of variation in the possible genotypes of parents and limiting the likelihood of males involved in matings being of different genotypes.

(ii) Compounding (i) is the fact that at some locations there is evidence that the fast allele has gone to fixation at one or both loci (shaded dark in Table 2.11). This means that in these populations the probability of males being of a different genotype is nil, and hence multiple paternity cannot be detected using these loci. Several of the females analysed came from these locations (females 61,62, 63,66, 67 and 68).

(iii) Limited sizes of broods means a high probability that by chance a brood does not significantly deviate from a Mendelian ratio despite a large deviation from the exact ratio (eg. brood 65i with a 3:10 ratio which does not deviate significantly from a 1:1 ratio at the 5% level). If large broods could have been reared from all females then the detection of multiple paternity may have been higher.

Explanatory Notes For Table 2.9

Bold female numbers highlight where some of a female's offspring cannot be explained by a single mating, as detected at one or both loci or between broods.

LOCATION = Where female was collected from, for key to locations see Table 2.11

Chi² = Chi^2 value for test of observed ratios against one of two *a priori* expected Mendelian ratios from parental crosses: a = 1:1; b = 1:2:1; N/A = when only one class of allozyme band mobilities found in offspring.

NOTE Female 74 Cannot be explained by genetic model proposed in section 2.6.1 - possibly due to misscoring of female.

Table 2.9 Summary Of Wild Collected *P. phalangioides* Gel Scoring At Locus II

			BROOD i				BROOD ii				BROOD iii			
#	LOCA-TION	PARENT SCORE	SS	SF	FF	Chi^2	SS	SF	FF	Chi^2	SS	SF	FF	Chi^2
61	H	FF	0	0	37	N/A	0	0	20	N/A				
62	H	FF	0	0	18	N/A	0	0	24	N/A				
63	W	FF					0	0	21	N/A	0	0	15	N/A
64	FF	SF					0	19	20	0.03a	0	22	23	0.02a
65	C	FF	0	3	10	3.77a								
66	L	FF	0	0	41	N/A								
67	L	FF	0	0	39	N/A	0	0	46	N/A	0	0	19	N/A
68	L	FF	0	0	20	N/A	0	0	41	N/A				
69	C	SF	6	10	5	0.14b								
70	HH	FF	0	10	9	0.05a	0	0	3	1.50a	0	8	5	0.35a
71	C	FF	0	16	0	N/A								
72	J	SF					0	10	17	1.81a				
73	WW	FF					0	0	21	N/A	0	1	10	7.36a
74	C	FF	0	10	22	4.50a								
75	C	SF	0	13	13	0.00a								
76	K	SS	16	9	0	1.96a								

Explanatory Notes For Table 2.10

Bold female numbers highlight where some of a female's offspring cannot be explained by a single mating, as detected at one or both loci or between broods.

LOCATION = Where female was collected from, for key to locations see Table 2.11

Chi² = Chi^2 value for test of observed ratios against one of two *a priori* expected Mendelian ratios from parental crosses: a = 1:1; b = 1:2:1; N/A = when only one class of allozyme band mobilities found in offspring.

NOTE Female 74 Cannot be explained by genetic model proposed in section 2.6.1 - possibly due to misscoring of female.

Table 2.10 Summary Of Wild Collected *P.phalangioides* Gel Scoring At

Locus III

			BROOD i				BROOD ii				BROOD iii			
#	LOCA-TION	PARENT SCORE	SS	SF	FF	Chi^2	SS	SF	FF	Chi^2	SS	SF	FF	Chi^2
61	H	SS	37	0	0	N/A	20	0	0	N/A				
62	H	SS	18	0	0	N/A	24	0	0	N/A				
63	W	FF					0	1	20	**17.1**a	0	0	15	N/A
64	FF	SF	12	16	0	0.57a	8	11	10	1.96b	11	21	12	0.14b
65	C	SF	0	8	5	0.69a								
66	L	FF	0	9	32	**12.9**a								
67	L	SF	0	20	19	0.03a	0	16	24	0.80a	0	18	22	0.40a
68	L	SF	5	17	5	0.90b	9	24	8	1.24b				
69	C	SF	6	9	7	0.82b								
70	HH	SS	20	0	0	N/A	3	0	0	1.50a	14	0	0	N/A
71	C	SF	7	9	0	0.25a								
72	J	SF	0	19	23	0.23a	14	17	0	0.29a				
73	WW	SF	5	20	9	2.00b	0	11	10	0.05a	0	5	6	0.05a
74	C	SF	5	27	0	**15.1**a								
75	C	SS	0	26	0	N/A								
76	K	SF	8	13	4	1.47b								

Table 2.11 Adult *P.Phalangioides* Scored By Location

	CODE #	LOCUS II	LOCUS III	COLLECTOR	LOCATION	ALLELE # & F_s	
C	C01 C03 C05 C06 C10 C12 C13 C14 C15 C16 C17 C21	SS SF FF SF FF -- FF SS FF SS FF	SF FF SF SF SF SF SF FF SF FF SF	A.E.Cooper.	Churchinford, Taunton, UK.	II S 08 F 12 F_s=0.40	III S 08 F 14 F_s=0.36
FF	FF20/3/1 FF100 FF103 FF104 FF105 FF106 FF108 FF110 FF111 FF201	SF FF FF FF SF -- SF SF FF SF	FF SS SS SF SS FF SF SF SF FF	F.Farr-Cox.	Burnham-on-Sea, Somerset, UK.	II S 09 F 11 F_s=0.45	III S 10 F 10 F_s=0.50
H	H01 H02 H03 H04 H05 H06	FF FF FF FF FF FF	FF SS SS SS SS SS	B.Harley.	Colchester, Essex, UK.	II S 00 F 12 F_s=0.00	III S 10 F 02 F_s=0.83
HH	HH01 HH02 HH04	FF SF SF	FF SS SS	E.Gardener.	Hemel-Hempstead Hertfordshire, UK.	II S 02 F 04 F_s=0.33	III S 04 F 02 F_s=0.66
J	J30 J21 J50 J100	SF SF SF	SF SF SF	D.Jones.	Waterlooville, Hants.,UK.	II S 03 F 03 F_s=0.50	III S 03 F 03 F_s=0.50
K	K01 K02 K03 K100	 FF SS SS	 SF SF SF	F.Katzer.	Mönchngladbach, Germany	II S 04 F 02 F_s=0.66	III S 03 F 03 F_s=0.50
KD	KD02	SS	FF	F.Katzer.	Mönchngladbach, Germany.		
KK	KK01 KK03	FF SS	SF FF	F.Katzer.	Bath, UK.		
L	L01 L02 L09 L10	FF FF FF FF	SF SF SF SF	P.Lee.	Lowestoft, Suffork, UK.	II S 00 F 08 F_s=0.00	III S 04 F 04 F_s=0.50

Table 2.11 Adult *P.Phalangioides* Scored By Location, Continued

	CODE #	LOCUS II	LOCUS III	COLLECTOR	LOCATION	ALLELE FI.	
M	M50 Mtest M001 M100 M101 M102	-- -- SF FF FF FF	SS SS SS FF SS SS	C.Merrett.	Llantwit Major, Glam, UK.	II S 10 F 02 F_S=0.83	III S 01 F 07 F_S=0.13
S	S10 S11	SS	SS	P.Smithers.	Plymouth, Devon, UK.		
V	V10 V13 V14 V16 V17 V18	FF SS FF SF FF FF	FF SS FF SS SF SF	F.Vollrath.	Oxford, UK.	II S 09 F 03 F_S=0.81	III S 06 F 06 F_S=0.50
VV	VV17 VV17/3/6 VV17/3/1 1 VV17/3/1 3 V17/3/14 V17/3/15	FF FF -- FF -- --	FF FF FF FF FF FF	F.Vollrath.	Basel, Switzerland.	II S 00 F 06 F_S=0.00	III S 00 F 12 F_S=0.00
W	W11 W16 W101 W105 W106 W108 W116 W119	FF FF -- FF FF FF FF FF	SS SF FF FF SF SS SF SF	Y.Western.	St.Davids, Pembrokeshire, UK.	II S 00 F 14 F_S=0.00	III S 08 F 08 F_S=0.50

The detection of multiple paternity in 'wild' populations of *P. phalangioides* means that sperm competition is an important factor in the mating system of this species. The laboratory mating paternity data are thus transformed from solely concerning the influence of the spermathecae on P_2 to implications about the peri-reproductive behaviours and mating strategies of *P. phalangioides* in the wild (Oxford 1993).

It is noteworthy that the variation revealed in this study is in contrast to the lack of variation in Porter and Jackob's (1990) study of allozyme variation in *P. phalangioides*. This could be for several reasons:

(i) Only one population was investigated by Porter and Jackob (1990) and that was small and isolated.

(ii) Starch gel electrophoresis was utilized by Porter and Jackob (1990), which may provide lower resolution of bands.

(iii) Esterases are very variable enzymes in general (Richardson et al 1986) and were found to be in *P. phalangioides* in this study but were not investigated by Porter and Jackob (1990).

2.7 Discussion

The original Austad (1984) theory of the spermathecal determination of P_2 in spiders is strictly an argument concerning the mechanism of fertilization. It is postulated that the fertilization set is a spatially distinct sub-set of the sperm in the spermathecae. In the case of *P. phalangioides* the prediction of a high P_2 follows from the prediction that the sperm in the region of the spermathecal duct (ie. the last in) is more likely to be the first out. Although *P. phalangioides* possesses only a *uterus externus* (Uhl 1992) and not a spermatheca proper, the former is functionally a sperm storage organ (Uhl 1993a,b) of the kind labelled cul-de-sac by Austad (1984). It is an effective sperm storage organ for extended periods of time (Present study; Montgomery 1903; Miyashita 1988a, b; Platel 1989; Uhl 1991, 1992, 1993, 1994a, b).

The prediction of a high P_2 has been borne out in the results of this study and contrasts to all the studies which have been made on entelegyne spiders which found a low P_2 (Jackson 1980; Vollrath 1980; Austad 1982; Christensen 1990; Watson 1990; Masumoto 1993 (although in *Agelena limbata* first male priority is a result of sperm plugs and not the spermathecal architecture)). Interestingly the only previous study on a cul-de-sac species (Eberhard *et al* 1993) found no evidence of a spermathecal influence on sperm precedence pattern which they viewed as a lack of a strong first male advantage, but is in fact compatible with no spermathecal influence and random sperm mixing of equally sized ejaculates.

A prediction of a high overall P_2 is not the only prediction from the logic of Austad's (1984) argument however. With successive broods, as the second male's sperm is depleted from the sperm stored then a decrease in P_2 should occur (Fig 2.6 A & B in contrast to C).

This point is obscured in Austads' review article (1984) and it is unclear if the high P_2 prediction refers to only the first or to all broods. If a high P_2 is to be maintained throughout the broods the situation needs to be as depicted in Fig 2.6A or B only with the gradient less steep and cocoon production finishing before the sperm of the second male is used up.

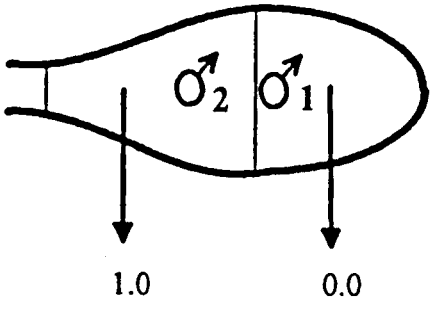
No downward trend in P_2 was found overall for the broods analyzed (Table 2.8). Factors other than the mechanism suggested by Austad (1984) could be involved in determining the paternity over broods, such as male traits like the quantity of sperm transferred. However, in two matings P_2 did decrease to zero (Table 2.8, 16/17 and 28/29), it is possible that these individuals passed over less sperm and sperm depletion occured, this is more likely given they are large broods.

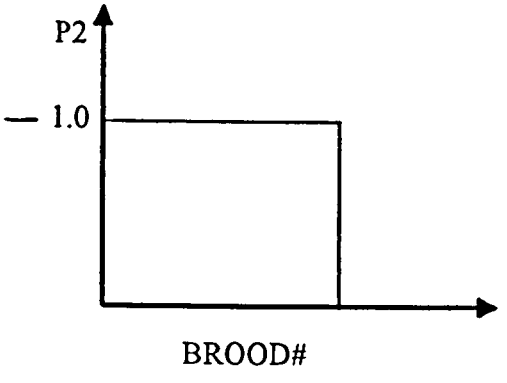
The most puzzling aspect of the results is the high reward to second copulations despite their very much shorter duration (table 2.8). The result is in contrast to previous workers' speculations as to whether sperm transfer from second matings occurred at all (Uhl 1993b). This begs the question of why first matings are so long if they secure little advantage to males in a mating system that appears to be promiscuous (table 2.9 and 2.10). Possible reasons are enumerated below:

(i) Laboratory artifact: the second matings were set up temporally close to the first mating and this may be uncommon in the wild. If this is the case then changes in the female reproductive tract after the first mating may make second copulations difficult and give second matings little reward. The long first matings would then be explained on the grounds

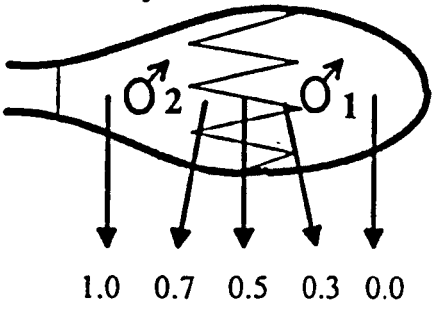
Fig 2.6 Diagramatic Representation Of Cul-de-sac Spermathecae With Consequences Of Varying Degrees Of Sperm Stratification On P2

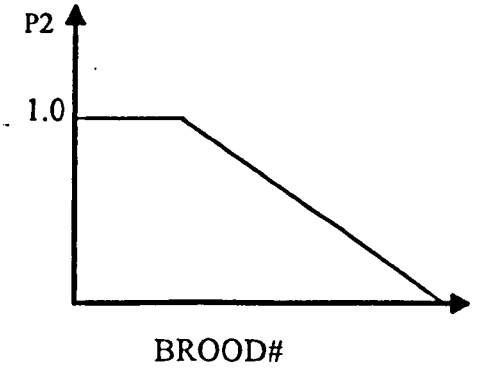
A. Absolute stratification of two males' ejaculates.



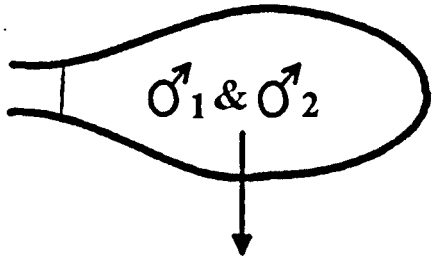


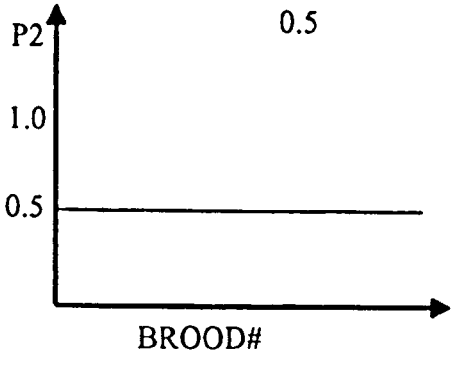
B. Sperm mixing at interface of two males' ejaculates.





C. Complete sperm mixing (no stratification).





of contact mate guarding for the duration over which most of these physiological changes take place. This would be a paternity assurance strategy. The incidence of multiple paternity in wild collected females might have only occurred when a female remated to refill slightly depleted sperm stores after already laying a cocoon. This suggests that the physiological mechanisms are reversible. Only one second mating experiment was carried out with some delay after the first mating (16/17) and this had a lower than average P_2 for all broods and accounted for 2 of the 5 unusual observations (table 2.8). Further experiments are required to determine if P_2 scores are depressed by a longer intermating period.

(ii) First males may be passing over a plug which only solidifies sometime after the mating. The laboratory matings in this case would have been performed at a point too early for the plug to solidify.

(iii) These explanations ((i) & (ii)) assume that the sperm storage organ provides an environment which favours second male priority. However this may not be the case and the spermatheca could have little or no influence on priority. Second males may gain priority not because of positional factors but by force of numbers - the outcome of sperm competition thus determined by the 'raffle principle' (Parker 1984, 1990). Second males may be able to secure more sperm in the female's sperm storage organ either by passing on more sperm at copulation or by passing a similar amount but removing sperm from the first male. This would not explain why second matings are shorter than the first, but interesting in these respects is the complex palp structure in *P. phalangioides* compared to most Haplogynes (Roberts 1985 volume 1) which may have a sperm removal function.

These alternative hypotheses to the Austad theory (1984) are not mutually exclusive. The current data set does not allow any conclusive choice between them. The results in isolation do not disprove the Austad theory (1984) and a verdict of not proven for the overriding influence of the spermathecae on P_2 levels must be returned at this stage.

3.0 Sperm Precedence Measurements In *Tetragnatha montana* (Simon) (Araneae, Tetragnathidae)

3.1 Introduction

The present chapter examines patterns of sperm precedence in the long-jawed orb-weaving spider *Tetragnatha montana* (Simon). The ecology and biology of *T. montana* will be reviewed, emphasizing those aspects relevant to sperm competition in order to place into context the results presented later in the chapter.

3.1.1 Phylogeny

According to Levi (1980, 1981) the Tetragnathinae represent one specialized branch derived from the Metinae, with the Aranaeid family another. A more recent review, however, (Coddington and Levi 1991) places the Araneidae as the most primitive, with the Tetragnathidae, including both the Metinae and the Tetragnathinae, being more specialized. However, opinions differ on the appropriate phylogeny to use, resulting in a confused nomenclature.

Throughout I shall adopt the taxonomic designations of subfamily status to the groups now considered to be included in the Tetragnathidae. This, as Levi (1980) points out, creates problems because of the intermediates that exist between them and the Araneidae. However, from the work conducted so far, it is clear that the Tetragnathinae are closer to the Metinae than to the Araneidae and this seems to be the simplest arbitrary designation to use.

3.1.2 Distribution, Habitat And Population Structure

The Tetragnathinae-Metinae-Nephila complex (Coddington & Levi 1991) is a successful spider group that is numerous in terms of individuals (Dabrowska-Prot *et al.* 1968), and species (Levi 1980, 1981; Okuma 1987) and distributed worldwide (North America: Levi 1980, 1981; Asia: Okuma 1988a; Africa: Okuma 1984). A relatively high proportion of *Tetragnatha* species is accounted for by representatives on Hawaii as a result of local explosive radiation (Okuma 1988b), a feature of many groups on this isolated archipelago. The genus is represented by six species in Great Britain and, in common with most Tetragnathids, they are morphologically homogeneous[1] with elongate bodies and legs, and large mandibles (Bristowe 1939; Roberts 1985).

The subject of this study, *Tetragnatha montana*, was chosen because dense populations were easily accessible at Askham Bog Nature Reserve (National Grid reference SE 570480) and Pocklington Canal Head (National Grid reference SE 799473). These sites were used because they were sufficiently distant for there to be no chance of the populations being panmictic. Differences in allele frequencies might therefore be expected which will help in paternity demarkation in the laboratory. The collection sites within these locations were typical of the sort of habitat usually occupied by *T. montana* and its kin: humid environs next to bodies of water (Bristowe 1958). It is by no means certain whether it is the humidity or the abundance of its mainly Nematocera prey which confines *T. montana* to these habitats (Bristowe 1929; Dabrowska-Prot & Luczak 1968; Dabrowska-Prot *et al* 1968a, b; Luczak & Dabrowska-Prot 1968; Nentwig 1987). Bristowe (1941) notes that drier areas can be tolerated by the species if they are sheltered.

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[1] With the notable exception of the many Hawaiian species (Okuma 1988b)

Ultimately temperature limits the range of a spider, although overwintering *T. montana* can tolerate extremely cold conditions. Schaefer (1976) for example, found that during the winter juveniles have a supercooling point of -18.4^{0}C).

The high density at which adult populations exist provides conditions conducive to high levels of sperm competition.

3.1.3 Phenology, Life History And Activity Periods

T. montana is a stenochronous species reproducing in spring and summer (Schaefer 1987). The number of moults undertaken is variable with adulthood reached in the 6th, 7th or 8th instar. This is a primitive feature, as is the fact that the adult males live for a relatively long time (3 to 4 weeks) (Vollrath 1987, citing Schaefer 1976).

The orb webs are flimsy and impermanent and females frequently move web sites even in prey-rich areas (Carico & Gillespie 1986). These factors prevent the long-term cohabiting of males and females. Monogamy is furthur precluded by: (i) the high density of spiders within populations, (ii) the longevity of males and (iii) the fact that females stay receptive after mating and even after laying cocoons. All these factors suggest that high levels of sperm competition in the wild are likely.

3.1.4 Morphology Of Body And Spermathecae And Palps

(i) **Body**: *T. montana* is similar to *T. extensa* in appearance (Plate 3.1); indeed Martin Lister's (1687) description of *T. extensa* (Parker & Harley 1992) probably applies to *T. montana* as well (plate 3.1). *T. montana* is well adapted to its habitat, and with its elongate body, and long legs set in a characteristic pose stretched out in front and behind along the shaft of the reed (Bristowe 1958) it is well camouflaged.

(ii) **Spermathecae**: The spermathecae are either the paired right and left seminal receptacles or the single median seminal receptacal (Fig 3.1). Sperm has been found in the central median seminal receptacal. It is by no means clear whether the two other seminal receptacles are used as well (Uhl pers. com.). From close mating observations the palp appears to be applied centrally, which is furthur evidence that it is the median seminal receptical which is used. This is contrary to the speculations of Kraus (1978). In any case, the spermathecae are all of the cul-de-sac type no matter which is used.

(iii) **Palps**: The palps are of an advanced complex design with a relatively long conductor and embolus of an arrangement unique to the species (Fig 3.2).

Plate 3.1 Male *Tetragnatha montana*

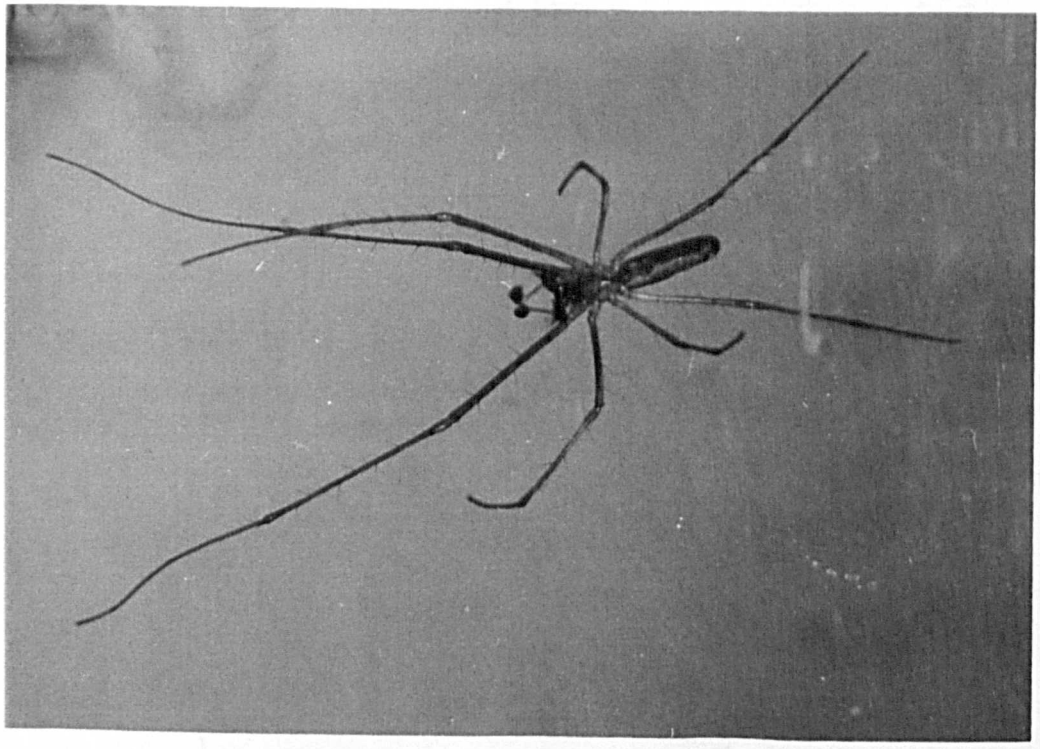


Fig 3.1 Spermatheca(e) of *Tetragnatha montana*

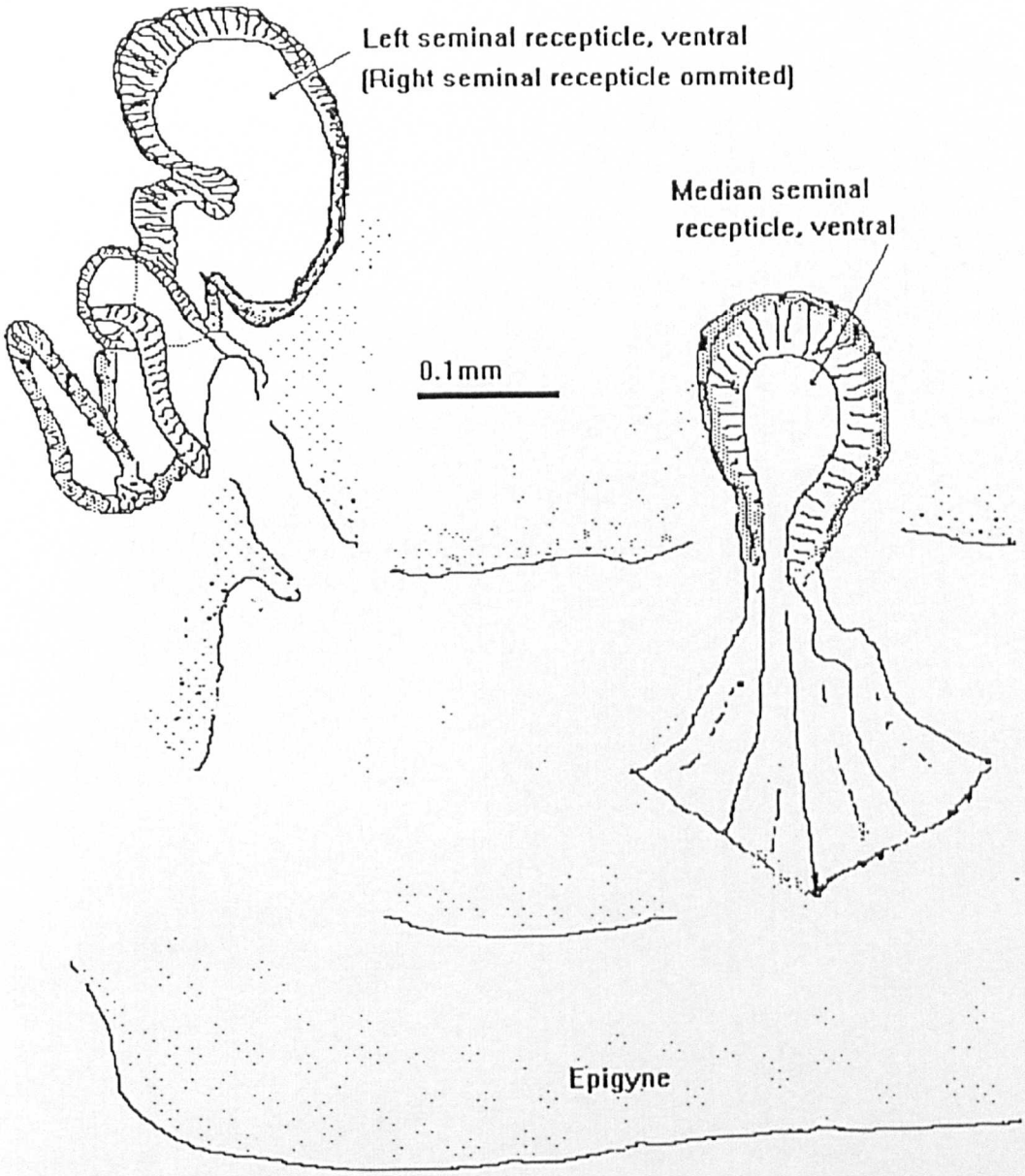
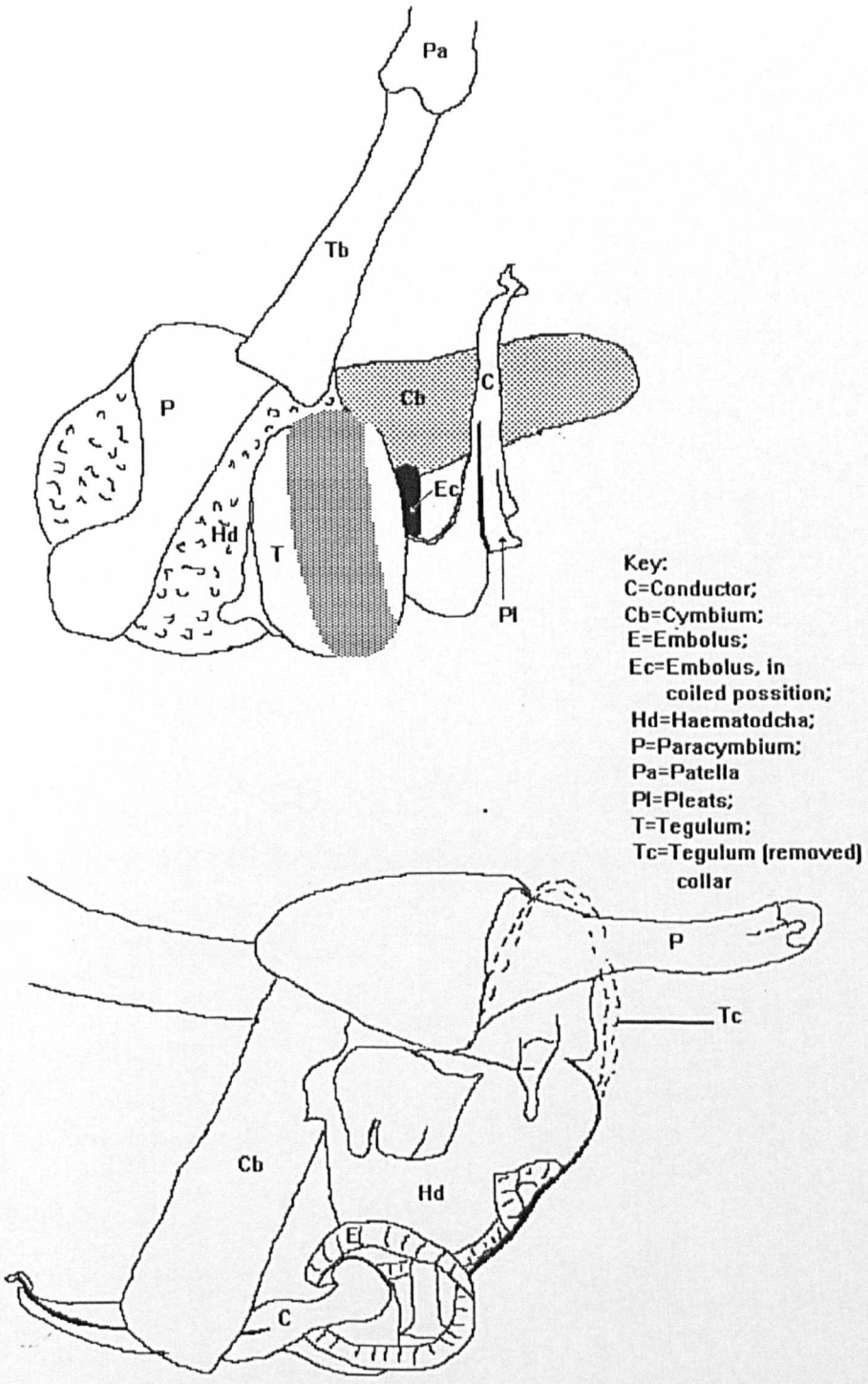


Fig 3.2 *Tetragnatha montana* Semi-Expanded Palps Viewed From Two Angles



3.2 Mating Experiments

Mating experiments were conducted in a controlled temperature room maintained at 20^0C and illuminated by a 60W fluorescent tube. Female spiders were placed in plastic cages (as depicted in Fig 2.3 and described in section 2.4) a day before the experiments were conducted, to make their orb webs. When a web was produced a male was introduced into the bottom of the cage and observations commenced. If mating did not occur within one hour from the introduction the mating trial was abandoned. Two hundred trials were required for 95 observations of matings to occur (47.5% success rate).

Three mating treatments were used, classified on the basis of how many times a female was mated:

(i) **Single matings**: virgin females were mated to a male of known mating history (n = 15). Ten of these matings resulted in viable eggs being layed.

(ii) **Multiple matings**: a virgin female was sequentially mated to two males of known mating history (n = 80). This produced matings of four kinds, classified on the basis of the mating history of the female and male as shown in Table 2.2. Ten of these matings resulted in viable eggs being layed.

(iii) **Wild matings**: Ten females were caught from the wild in a gravid condition and reared until they laid eggs to give an indication of the incidence of multiple mating in the wild. Three of these female's eggs hatched and were analysed.

Notes were taken of male and female behaviour during the observations and four classes of quantitative measure were made of male behaviour:

(i) **Total duration of coupling time** (time male and female are joined at the chelicerae). This represents the bulk of the time of male mating effort, since very little, if any, pre-coupling courtship occurs in this species.

(ii) **Total duration of palpal insertions** (total time in which both left and right palps are inserted in the epigyne). This represents the total time in which it is possible for sperm transfer to occur.

(iii) & (iv) **Duration of individual left and right palpal insertions**. The sum of left and right individual palpal insertion times equals (ii).

Each palpal insertion time was a small proportion of the total insertion time so an uneven number of palpal insertions did not in itself affect the symmetry of palpal usage time.

3.3 Rearing

The rearing protocol was as stated in section 2.4. Broods reared were small in number however because the number of offspring laid in each cocoon was small (around 25 offspring) and it was difficult to rear the offspring to a reasonable size for electrophoresis without a significant number dying. This led to small expected numbers for the Mendelian ratios when checking the genetics and exact tests were used in cases where this was appropriate.

3.4 Analysis Of Genetic And Paternity Data

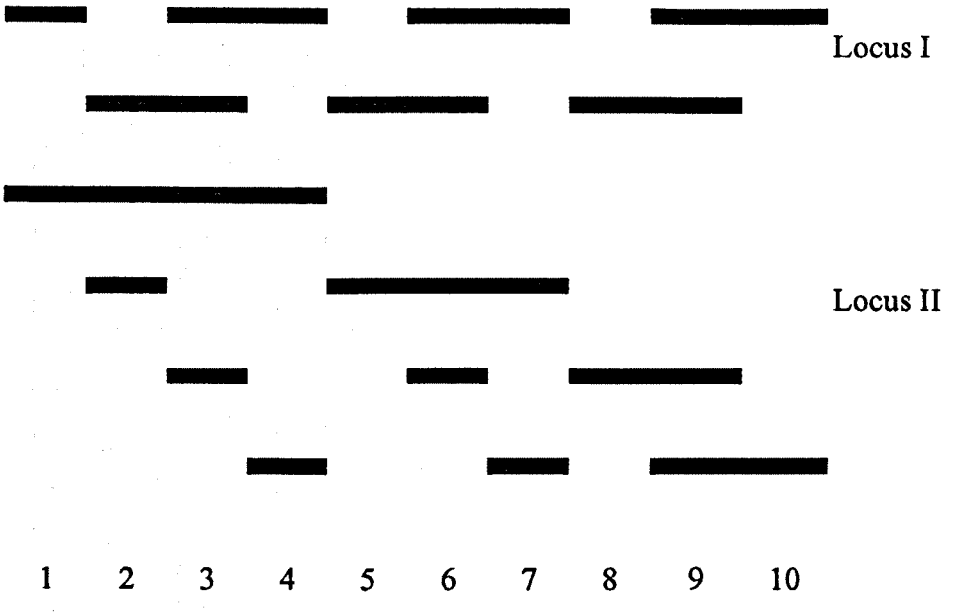
3.4.1 Genetic Data

Samples were electrophoresed to obtain isozyme patterns using the same conditions as in section 2.6 with the exception that 6.5% acryamide gels were used. The isozyme patterns suggest that only two esterase loci are present on the gel, designated I and II (Fig. 3.3); more than this is more common (Hebert & Beaton 1989). Locus II was more heat stable than locus I thus providing evidence that they are separate systems. This lack of heat stability led to the inability to score isozyme patterns at locus I in a number of samples.

For the single matings (Table 3.1) all 10 broods were consistent with predicted Mendelian ratios. The relevant Mendelian test was determined for locus I on the basis of the bands conforming to a simple diallelic system with homozygotes being SS and FF, and the heterozygotes SF. Locus II is more complicatedwith four alleles yielding ten combinations; four homozygotes and six heterozygotes (Fig 3.3). Additionally there is evidence for linkage (Table 3.2) with an estimated recombination frequency of 4.85% between the two loci.

Double matings 13/14, 15/16 and 27/28 (Table 3.3) are effectively single matings at locus I and provide further support for the genetic model. 15/16 and 27/28 are not inconsistent with a 1:1 relationship, with Chi^2 statistics of 1.67 for 15/16 and 0.00 for 27/28. 13/14 has only one phenotype so Chi^2 is not appropriate.

Fig 3.3 Allozyme pattern showing different combinations



1 = FF 11; 2 = SS 12; 3 = SF 13; 4 = FF 14; 5 = SS 22;

6 = SF 23; 7 = FF 24; 8 = SS 33; 9 = SF 34; 10 = FF 44.

Table 3.1 Summary For Single Matings: Parental And Offspring Allozyme Phenotypes

#	LOCUS I						LOCUS II												
	PARENT SCORES		OFFSPRING SCORES			Chi² / *	PARENT SCORES		OFFSPRING SCORES										Chi²
	FEMALE	MALE	SS	SF	FF		FEMALE	MALE	11	12	13	14	22	23	24	33	34	44	
1	SF	SF	4	5	3	0.66b	14	14	4	-	-	5	-	-	-	-	-	3	0.66b
2	SS	SF	6	4	-	0.40a	11	14	6	-	-	4	-	-	-	-	-	-	0.40a
3	FF	SF	-	10	15	1.00a	44	14	-	-	-	12	-	-	-	-	-	13	0.04a
4	SF	SS	9	6	-	0.60a	14	11	9	-	-	6	-	-	-	-	-	-	0.60a
5	SS	SF	3	3	-	**1.0a**	12	14	1	1	-	2	-	-	2	-	-	-	0.60c
6	SF	SF	1	3	1	**1.0b**	14	14	2	-	-	1	-	-	-	-	-	2	1.80b
7	SS	SF	5	3	-	**0.73a**	12	12	2	5	-	-	1	-	-	-	-	-	0.75b
8	SS	SS	11	-	-	N/A	14	11	7	-	-	4	-	-	-	-	-	-	0.82a
9	FF	??	??	??	??	??	14	23	-	3	5	-	-	-	3	-	4	-	0.74c
10	SF	SS	8	3	-	**0.23a**	24	11	-	3	-	8	-	-	-	-	-	-	2.27b

Explanatory Notes For Table 3.1

Score = Designation for allozyme band mobilities: Locus I - SS = Slow; SF = Slow/Fast; FF = Fast. Locus II - all combinations of the 4 alleles.

Chi² = Chi² value for test of observed ratios against *a priori* expected Mendelian ratios from parental crosses: a = 1:1; b = 1:2:1; c = 1:1:1:1;
N/A = not applicable (only one class of allozyme band mobilities found in offspring).

?? = Unscorable.

* = Exact probabilities where the sample sizes are too small for Chi² to be appropriate (emboldened). Some of the Chi² calculated have expecteds lower than is generally recomended. However the test is conservative, with low expecteds, only likely to inflate the Chi² score so it was unecessary to use an exact test in these cases because the result would have been the same.

Table 3.2 Linkage In *Tetragnatha montana* For Esterase Loci I and II

#	CODE NUMBER	PRESUMED PARENT GENOTYPES FOR LOCUS I & II		RECOMBINATION RECOGNISABLE IN PARENTS?	RECOMBINATION RECOGNISABLE IN OFFSPRING?	OBSERVED NUMBER OF RECOMBINANT PROGENY	ACTUAL NUMBER OF RECOMBINANT PROGENY
		FEMALE	MALE				
1	P12	S 1 / F 4	S 1 / F 4	Both	All	0	0
2	U02	S 1 / S 1	S 1 / F 4	One	All	0	0
3	P07	F 4 / F 4	S 1 / F 4	One	All	2	2
4	A12	S 1 / F 4	S 1 / F 4	Both	All	0	0
5	A04	S 1 / S 2	S 1 / F 4	One	All	0	0
6	P37	S 1 / F 4	S 1 / F 4	Both	All	2	1
7	A05	S 1 / S 2	S 1 / F 2	One	Half	1	2
8	P203	S 1 / S 4	S 1 / S 1	None	–	–	–
9	16	–	–	–	–	–	–
10	P11	S 2 / S 4	S 1 / S 1	None	-	-	-
						Recombination Frequency:	5 / 103 = 4.85%

Table 3.3 Summary Of Multiple Matings: Parental And Offspring Allozyme Phenotypes And Corresponding Mating Times

#	LOCUS I						LOCUS II												MATING TIME	
	PARENT SCORES		EXPEC-TEDS.*	OFFSPRING SCORES			PARENT SCORES		EXPEC-TEDS.*	OFFSPRING SCORES									MALE 1 (SECS.) J (I)	MALE 2 (SECS.) J (I)
	FEMALE	MALES		SS	SF	FF	FEMALE	MALES		11	12	13	14	22	23	24	33	34	44	
11/12	FF	SF SS	SF,FF SF	-	12	-	34	14 11	13,14,34,44 13,14	-	-	4	8	-	-	-	-	-	-	780 (708)	1020 (994)
13/14	SS	SS SS	SS SS	17	-	-	12	11 14	11,12 11,12,14,24	5	5	-	1	-	-	6	-	-	-	960 (836)	1440 (1392)
15/16	SS	SF SF	SS,SF SS,SF	10	5	-	14	14 ??	11,14,44 ??	4	-	-	10	-	-	-	-	-	1	1120 (1084)	360 (316)
17/18	??	?? ??	?? ??	2	7	2	24	12 44	24,44 12,14,22,24	-	-	-	-	-	-	5	-	-	6	950 (919)	778 (662)
19/20	SS	SS FF	SS SF	4	3	-	11	22 11	12 11	3	4	-	-	-	-	-	-	-	-	1378 (992)	798 (767)
21/22	SS	?? ??	?? ??	5	3	-	12	34 11	13,14,23,24 11,12	-	-	3	1	-	2	2	-	-	-	1271 (990)	900 (900)
23/24	SS	?? ??	?? ??	3	2	-	23	14 11	12,13,24,34 12,13	-	3	2	-	-	-	-	-	-	-	1132 (989)	1500 (1035)
25/26	SF	SS ??	SS,SF ??	3	7	-	14	44 14	14,44 11,14,44	3	-	-	7	-	-	-	-	-	-	1785 (1778)	990 (917)
27/28	SS	SF SF	SS,SF SS,SF	6	6	-	12	22 34	12,22 13,14,23,24	-	-	4	3	-	2	3	-	-	-	1340 (1275)	1110 (977)
29/30	SF	?? ??	?? ??	2	2	7	24	14 22	12,14,24,44 22,24	-	2	-	2	-	-	3	-	-	4	1040 (992)	525 (476)

Explanatory Notes For Table 3.3

*** Expecteds**-The emboldened expecteds are those where they are the same for the first and second male.

Score = Designation for allozyme band mobilities: Locus I - SS = Slow; SF = Slow/Fast; FF = Fast. Locus II - all combinations of the 4 alleles.

?? = Unscorable. **J** = Time during mating that the partners' jaws were locked. **I** = Time during mating that the palp was inserted in the female.

3.4.2 Wild Collected Specimens

As well as the adult specimens used in matings and electrophoresed in 1993 and 1994, wild collected male and female specimens were also run. Data are summarized in Table 3.4.

These specimens were analysed for Hardy-Weinberg equilibrium within collection sites and were found to be in equilibrium at locus I in Pocklington (Table 3.5B & D). However they were not found to be in equilibrium in Askham probably because of small expecteds for SS. This implies that random mating of the two populations exists and most importantly that there is no selection on the loci in question, at least in the wild.

3.4.3 Paternity Data

Data on the offspring of multiple matings are shown in Table 3.3 and paternity assignments given in Table 3.6. The outcomes of double matings are not consistent with any fixed priority bias. However, for the male most matings with the longest mating time gaining the greatest paternity (Table 3.6)[2] a pattern in agreement with a raffle principle with continuous sperm transfer during mating. Alternatively it is also concordant with female choice based on the genitalic stimulation theory of Eberhard (1985) - the longer the mating duration the greater the paternity assigned. Interestingly one of the matings (29/30) at locus I has a deviation from expected ratios ($Chi^2 = 9.0$ - significant for a 1:2:1 ratio) because the SF

[2] There is an 16% chance (using a one-tailed binominal test) of a deviation from a 50:50 ratio meaning it is not statistically significant.

score is under-represented or the FF is over-represented. The point is that whatever the female mated with, the progeny should not have an excess of homozygotes.

Table 3.4 *Tetragnatha montana* Compilation of Adult Scores by Year.

1994 (n = 64)								1993 (n = 40)							
#	CODE	II	I	#	CODE	II	I	#	CODE	II	I	#	CODE	II	I
1	$A01_M$	11	SS	34	$P01_F$	12	SS	1	$A01_F$	12	SS	34	$P202_F$	44	FF
2	$A04_F$	12	SS	35	$P02_M$	14	SS	2	$A02_F$	24	SF	35	$P203_F$	14	??
3	$A05_M$	11	SS	36	$P03_M$	14	SF	3	$A04_F$	22	SS	36	$P205_F$	24	??
4	$A06_M$	14	SF	37	$P04_F$	14	SF	4	$A05_F$	12	SS	37	$P206_F$	14	SS
5	$A07_F$	34	FF	38	$P05_M$	11	SS	5	$A06_F$	11	SS	38	$P208_F$	12	SS
6	$A08_M$	13	SF	39	$P07_M$	44	FF	6	$A17_M$	11	SF	39	$P302_F$	11	SF
7	$A10_F$	11	SS	40	$P08_M$	14	SF	7	$A18_F$	11	SS	40	$P311_F$	14	SF
8	$A11_M$	11	SS	41	$P09_F$	34	FF	8	$A600_F$	44	FF	KEY:			
9	$A12_F$	14	SF	42	$P10_M$	11	SS	9	$A700_F$	33	SS				
10	$A13_M$	11	SS	43	$P11_F$	24	SF	10	$A800_F$	14	SF	I = Locus I			
11	$A14_F$	12	SS	44	$P12_F$	14	SF	11	$P17_M$	11	??	II = Locus II			
12	$A15_M$	44	SS	45	$P13_M$	24	SF	12	$P24_F$	24	??	$_M$ = Male			
13	$A16_M$	44	FF	46	$P15_M$	44	FF	13	$P35_F$	44	SF	$_F$ = Female			
14	$A17_M$	11	SF	47	$P16_M$	11	SS	14	$P37_F$	11	FF	A = Askham sample			
15	$A18_M$	44	FF	48	$P17_M$	14	SF	15	$P48_M$	22	??	P = Pocklington sample			
16	$A19_F$	44	FF	49	$P18_M$	14	SS	16	$P53_M$	14	??	SS = Slow			
17	AI_F	14	SS	50	$P19_M$	14	SF	17	$P54_M$	11	??	SF = Slow / Fast			
18	AII_F	14	SS	51	PI_F	11	SS	18	$P56_M$	11	??	FF = Fast			
19	$AIII_F$	11	SS	52	PII_F	44	FF	19	$P60_F$	14	??	?? = Unscorable			
20	AIV_F	12	SS	53	$PIII_F$	14	SF	20	$P62_F$	11	??				
21	AV_F	44	FF	54	PIV_F	13	SS	21	$P64_F$	23	SS				
22	AVI_F	44	SF	55	PV_F	13	SF	22	$P79_F$	44	??				
23	$AVII_F$	11	SS	56	PVI_F	24	FF	23	$P86_M$	14	??				
24	$AVIII_F$	14	SF	57	$PVII_F$	11	SS	24	$P100_M$	11	SS				
25	AIX_F	14	SF	58	$PVIII_F$	44	SF	25	$P103_M$	22	??				
26	AX_F	11	SS	59	PIX_F	14	SF	26	$P104_M$	12	??				
27	AXI_F	14	SS	60	PX_F	44	FF	27	$P113_M$	11	??				
28	$WA01_F$	14	SS	61	PXI_F	14	SF	28	$P122_M$	34	SF				
29	$WA03_F$	12	SF	62	$PXII_F$	11	SS	29	$P125_M$	14	SF				
30	$WA04_F$	14	SS	63	$PXIII_F$	12	SS	30	$P126_M$	44	??				
31	$WA05_F$	11	SS	64	$WP01_F$	13	SF	31	$P127_M$	24	SF				
32	$A***_F$	11	SS					32	$P200_F$	12	SS				
33	$A20_F$	14	SF					33	$P201_F$	14	SF				

Table 3.5A Askham Genotype Frequencies Combined For Years 1993 And 1994

Locus I	Locus II										Totals
	11	12	13	14	22	23	24	33	34	44	
SS	0	0	0	0	0	0	0	0	1	5	6
SF	2	0	1	5	0	0	1	0	0	1	10
FF	10	5	0	3	1	0	0	1	0	1	21
Totals	12	5	1	8	1	0	1	1	1	7	37

Table 3.5B Pocklington Genotype Frequencies Combined For Years 1993 And 1994

Locus I	Locus II										Totals
	11	12	13	14	22	23	24	33	34	44	
SS	7	4	1	3	0	1	0	0	0	0	16
SF	1	0	2	12	0	0	3	0	1	2	21
FF	1	0	0	0	0	0	1	0	1	5	8
Totals	9	4	3	15	0	1	4	0	2	7	45

Table 3.5C Allele Numbers And Frequencies For Locus I[1] For Askham

	S (p)	F (q)	
	12	10	
	10	42	
Total	22	52	74
Allele Frequency	0.3	0.7	1.0
Genotype Frequency	p^2	2pq	q^2
Expected frequency	0.09	0.42	0.49
Expected number	3.26	15.54	18.28
Observed number	6	10	21
Chi^2	2.31	1.98	0.41
Total Chi^2	4.69 (df=1) SIG^2		

Table 3.5D Allele Numbers And Frequencies For Locus I For Pocklington

	S (p)	F (q)	
	32	21	
	21	16	
Total	53	37	90
Allele Frequency	0.59	0.41	1.0
Genotype Frequency	p^2	2pq	q^2
Expected frequency	0.35	0.48	0.17
Expected number	15.62	21.79	7.61
Observed number	16	21	8
Chi^2	0.01	0.03	0.02
Total Chi^2	0.059 (df=1) N/S		

1 There is an insufficient sample size for a calculation of a Hardy-Weinberg equilibrium for locus II.

2 This is significant but there are low expecteds for SS - this inflated the Chi^2

Table 3.6 Paternity Assignment Chart

Paternity More Consistent With			
First Male	Second Male	Mixed	?
17/18 Yes	11/12 Yes	19/20	15/16
21/22 Yes	13/14 Yes		
29/30 Yes	23/24 Yes		
	25/26 No		
	27/28 No		

Yes = Male gaining the paternity also mated the longer time
No = Male gaining the paternity did not mate for the longer time.

3.4.4 Wild Matings

None of the three matings was consistent with a single mating (Table 3.7). This implies that promiscuity is common in the mating system of *T. montana* and that sperm competition is or has been an important factor. Mating 3 has fewer SF than expected accounting for the high Chi^2. As in the instance of mating 29/30 at locus I, for every SS or FF there should be a SF no matter what the parents' genotypes were. Consequently, there may be some mistake in the scoring here, despite the fact that gels were double checked by an independent observer.

Table 3.7 Summary For Wild Mated females: Parental And Offspring Allozyme Phenotypes

#	LOCUS I					LOCUS II										
	FEMALE SCORES	OFFSPRING SCORES			Chi^2 / *	FEMALE SCORES	OFFSPRING SCORES									Chi^2
		SS	SF	FF			11	12	13	14	22	23	24	33	34	44	
1	SS	2	15	-	**$2.35X10^{-3}$a**	14	-	-	-	7	-	-	-	-	-	10	0.26a
2	SF	17	2	1	**$4.10X10^{-8}$b**	12	2	7	3	2	3	1	1	-	-	-	N/A
3	SF	7	2	4	**$6.35X10^{-3}$a**	14	5	-	-	4	-	-	-	-	1	3	2.69c

Explanatory Notes For Table 3.7

Score = Designation for allozyme band mobilities: Locus I - SS = Slow; SF = Slow/Fast; FF = Fast. Locus II - all combinations of the 4 alleles.

Chi^2 = Chi^2 value for test of observed ratios against a priori expected Mendelian ratios from parental crosses: a = 1:1; b = 1:2:1; c = 1:1:1:1; N/A = not applicable (*a priori* Mendelian ratios based upon a single mating do not exist).

?? = Unscorable.

* = Multinomial where the sample sizes are two small for Chi squared to be appropriate (emboldened).

3.5 Mating Behaviour

Mating behaviours observed were highly stereotyped. In agreement with the experience of previous workers (Subrahmanyam 1936; Bristowe 1941, 1958), little or no courtship was observed in this species; male and female engage directly in 'cheliceral joining'[3] (Plate 3.2, Fig. 3.4) without any preliminaries. Tetragnathid mating behaviour was first observed by Lister in 1678 (*Tetragnatha extensa*) who noted this characteristic cheliceral locking (Parker & Harley 1992, pp. 81-82).

The orientation of the pair within the web is variable and seems to depend on which direction the male approached the female (Fig 3.4). The positions of the pair in the 95 copulations observed were as follows: female above male (n = 49), side by side abdomens pointing upwards (n = 25), side by side abdomens pointing downwards (n = 1), male above female (n = 20). When joined a female posture is assumed which brings the abdomens together for copulation. This was assisted by the male who places his third pair of legs around the abdomen of the female (Fig 3.4).

Bristowe (1929) observed only single palpal insertion of the left and right palps in a *Tetragnatha* spp. in contrast to Gerhardt (1924) who observed multiple insertion of each palp. The present work (Table 3.8) supports the conclusions of Gerhardt (1924) in this species. Table 3.9 shows the average mating times for the four different mating combinations. The correlations of joined and insertion times for each of the first and second mates (all four categories combined) are very close (first male r = 0.897; second male r =

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[3] 'Cheliceral joining' is the term used here for the clasping of the female's chelicerae, by the male, between the fangs and a special apophysis (Fig 3.4, Plate 3.2; illustrated from a number of different angles: Subrahmanyam 1936; Bristowe 1929 (Text Fig. 11), 1941 (Text Fig. 92), 1958 (Fig. 109)).

0.941). Therefore only the insertion times will be analysed because as mentioned above, these correspond to the total time available for sperm transfer.

Before the males and females parted it was observed that the males would disengage their palps and hold them out of harms way above their cephalothorax for a few seconds. This indicates that the males may be the sex that determines the length of the mating duration, because the termination was anticipated.

Whilst collecting specimens in the field it was observed that males would line up around a female's web, never engaging in any aggressive behaviour with other males whilst waiting to mate with a female. Thus males did not monopolise access to a female to ensure paternity.

Plate 3.2 *Tetragnatha montana* Mating Posture

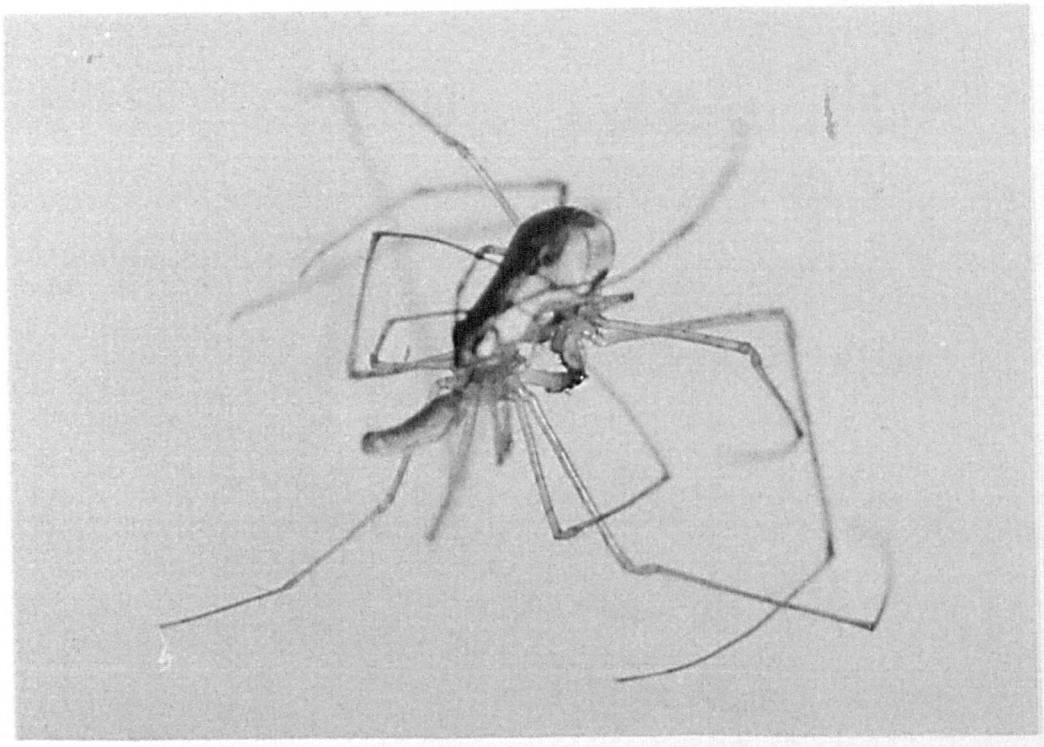
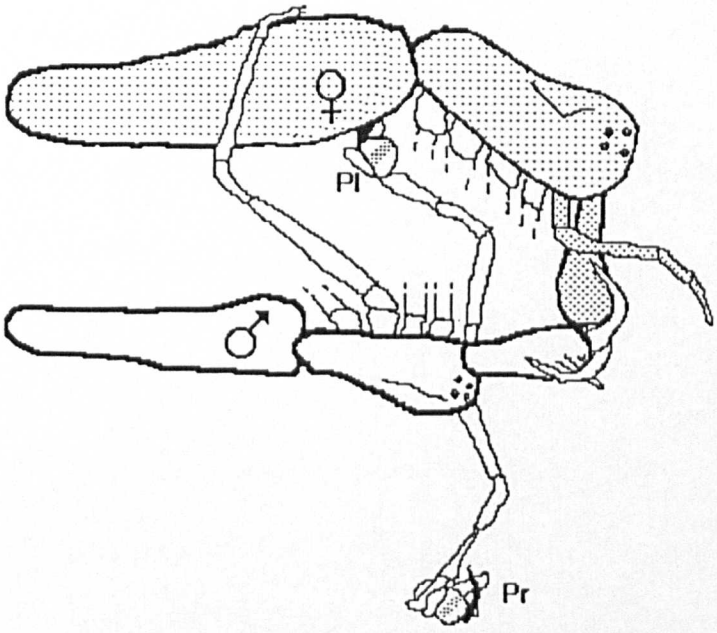


Fig 3.4 *Tetragnatha montana* Mating Posture



Legend for Fig 3.2

Tetragnatha montana in copula with left palp (Pl) about to insert into the female's epigyne and the right palp (Pr) deflating after insertion. Only the male's left leg III is shown above the trochanter to show its position above the female's abdomen.

The effect of the female's mating status on total insertion time was analysed by performing a t-test on the mating categories K1 + K2 versus K3 + K4: the difference was not significant (p = 0.096 t = 1.69, DF = 78). The effect of the male's mating status (virgin or mated) was analysed using a t-test in the same way using K1 + K3 verses K2 + K4. Again the difference was non-significant (p = 0.35 t = 0.94, DF = 78). This means that prior mating histories of males or females have no bearing on how long the pairs mate for. As previously discussed mating duration may have a bearing on the paternity of the brood so it is important to know what factors determine this.

The symmetry of palp usage may give the female an indication of the fitness of the male. In addition a male who fills the spermathecae more equally (if the median seminal recepticle is not used) may gain a higher paternity if it provides him with a longer duration of mating. Symmetry was measured by the equation:

absolute (Left - Right) / (Left + Right).

Also there is some indication that if fatigue occurs in the mating male then asymmetric palp usage will follow (Rovner & Wright 1975). This may lead to a shorter mating time if copulatory courtship is a factor. This was not found to be the case however (graph 3.1 and graph 3.2). Niether of the regressions of insertion time on palp use symmetry was significant, indicating that palp usage is not a contributing factor to the variability in insertion time, for either first or second males. Separate histograms (Graph 3.3 and Graph 3.4) of the first and second mating males reveal that the males were generally fairly symmetrical in their palp usage. Factors other than mating order may be influential in determining the symmetry of palp usage, for example, the female may not allow males she judges as being substandard from filling the spermathecae equally.

Table 3.8 Mating Durations Of First And Second Males Mated To A Single Female (Times in Seconds)

FEMALE #[1]	MALE #				JOINED		INSERTED		LEFT PALP		RIGHT PALP	
	1	K1[2]	2	K2	1	2	1	2	1	2	1	2
P32	P15	1	P13	3	255	897	216	791	109	387	107	404
P23	P16	1	P53	4	1,785	990	1,778	917	874	372	904	545
P208	P122	2	P100	3	1,271	900	990	900	540	400	450	500
P77	P08	1	A13	3	376	756	348	653	138	124	210	529
A06	P12	1	A07	3	1,230	3,109	1,119	2,428	605	991	514	1,437
P64	P53	1	P54	3	1,132	1,500	989	1,035	252	535	737	500
A16	P29	1	P57	3	1,380	660	1,224	600	589	217	635	383
P61	P57	2	P15	4	968	666	928	579	404	270	524	309
P82	A13	2	P16	4	1,082	550	1,030	481	636	318	394	163
P79	P08	2	P12	4	1,745	1,422	1,690	920	1,057	557	633	363
P24	A01	1	P48	3	1,040	525	992	476	322	188	670	288
P35	P09	1	A10	3	2,464	1,250	2,242	1,189	1,206	784	1,036	405
A12	A10	2	A09	4	1,260	725	1,213	639	428	379	785	260
A05	P117	1	P124	4	1,187	937	1,120	883	532	475	588	408
P205	P104	1	P126	3	950	778	919	662	339	268	580	394
P200	P122	1	A26	3	1,340	1,110	1,275	976	601	446	674	530
P209	P118	1	A23	3	1,798	843	1,739	721	739	365	1,000	356
P201	P127	1	P112	3	1,397	2,329	1,112	2,066	525	1,500	587	566
P202	A26	2	P118	4	1,140	741	890	697	443	406	447	291
P207	A23	2	P127	4	956	1,190	930	1,089	443	934	487	155
P304	P102	1	P103	3	1,330	1,127	1,270	770	543	241	727	529
P303	P113	2	P102	4	1,200	1,108	1,153	1,029	858	680	295	349
P302	P103	2	P113	3	1,378	798	922	767	455	483	467	284
P206	P86	2	P108	3	1,120	360	1,084	316	1,084	286	0	30
D03	D19	1	D18	3	1,380	1,140	1,165	1,089	569	617	596	472
U02	D16	1	D20	3	2,100	1,560	1,411	1,446	489	765	922	681
D05	D22	1	D21	3	1,593	2,700	1,504	2,029	831	1,377	673	652
D06	D20	2	D16	4	1,620	1,080	1,475	957	622	550	853	407
D08	D26	1	D15	4	780	840	468	711	349	294	119	417
D10	D19	2	U05	3	1,140	2,280	1,103	1,163	450	324	653	839
D11	D21	2	D17	4	1,380	2,040	1,349	1,840	1,036	1,124	313	716
D12	D27	1	D18	4	1,320	1,440	1,102	1,386	497	760	605	626
K01	S01	1	K02	3	960	1,440	836	1,392	434	907	402	485
K04	K13	1	K15	3	1,800	720	1,725	655	940	372	785	283
K09	K03	2	K05	3	780	1,020	708	994	240	519	468	475
U03	S05	1	K18	3	1,680	1,500	1,550	1,393	868	824	682	569
U04	K19	1	S06	3	2,340	720	2,091	615	707	179	1,384	436
S14	S15	1	S17	3	1,980	1,260	895	1,208	531	581	364	627
S19	S16	1	S18	3	900	1,020	889	645	532	463	357	182
S20	S17	2	S13	4	1,440	1,140	1,299	1,012	796	557	503	455

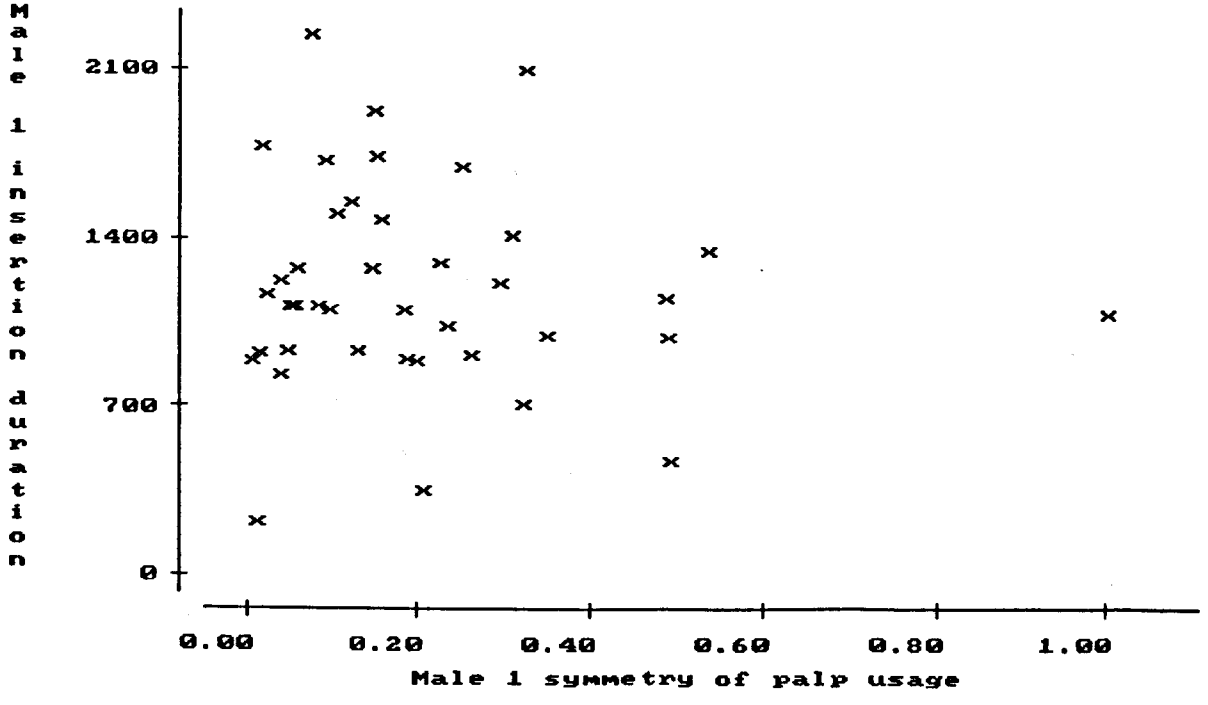
[1] Emboldened female numbers denote individuals who produced viable eggs, the progeny of which were analysed electrophoretically. Letter codes for individuals denote the year and location from which they were collected: P = Pocklington 1993, A = Askham 1993, U = University 1994, D = River Derwent (Kexby bridge) 1994, K = Pocklington 1994, S = Askham 1994.

[2] K1/K2 = mating kinds for first and second males respectively (see text). Joined = Time during which chelicerae are locked - total mating time. Inserted = Total time during which palps are inserted into the epigyne. Left palp / Right palp = Total time left / right palp is inserted during copulation.

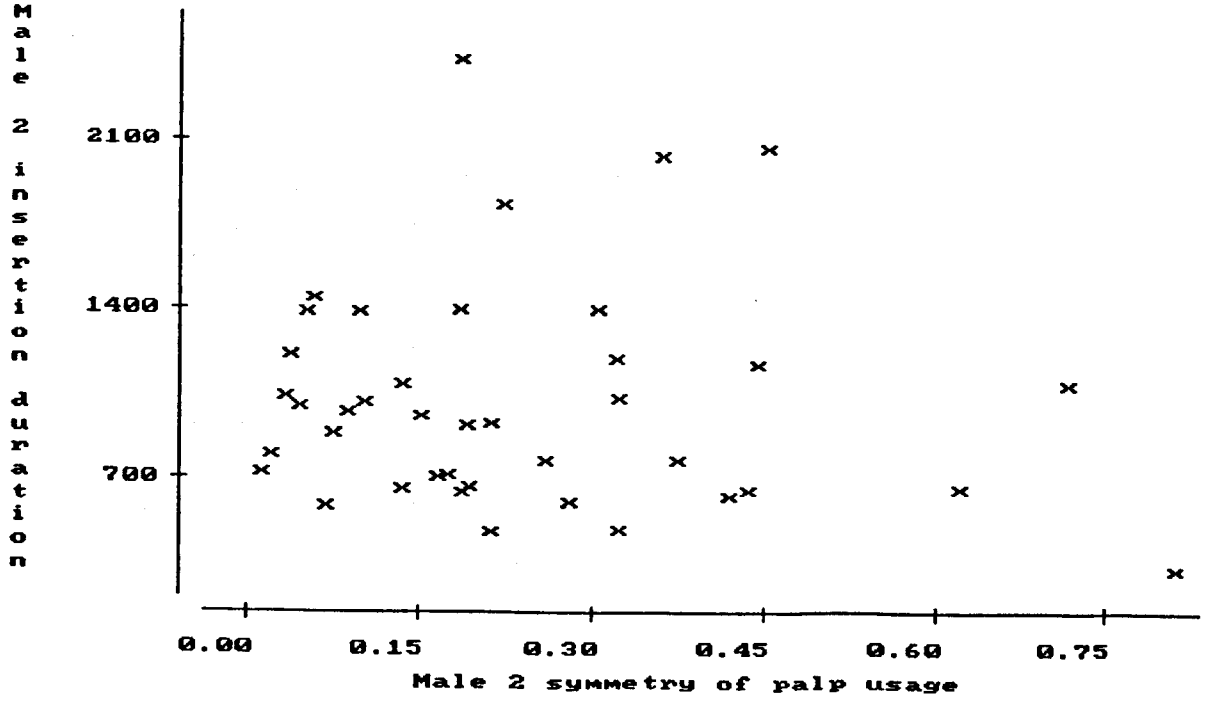
Table 3.9 Mean Mating Duration For Different Mating Kinds

Mating Kind (number involved)	Mean duration of matings in seconds (mean; standard error; range)	
K1 (n=25)	1199; 98; 216-2242	} 1169; 66; 216-2242
K2 (n=15)	1118; 66; 708-1690	
K3 (n=26)	1038; 101; 316-2428	} 1003; 73; 316-2428
K4 (n=14)	938; 94; 481-1840	
Total Time (n=80)	1086; 50 ; 216-2428	

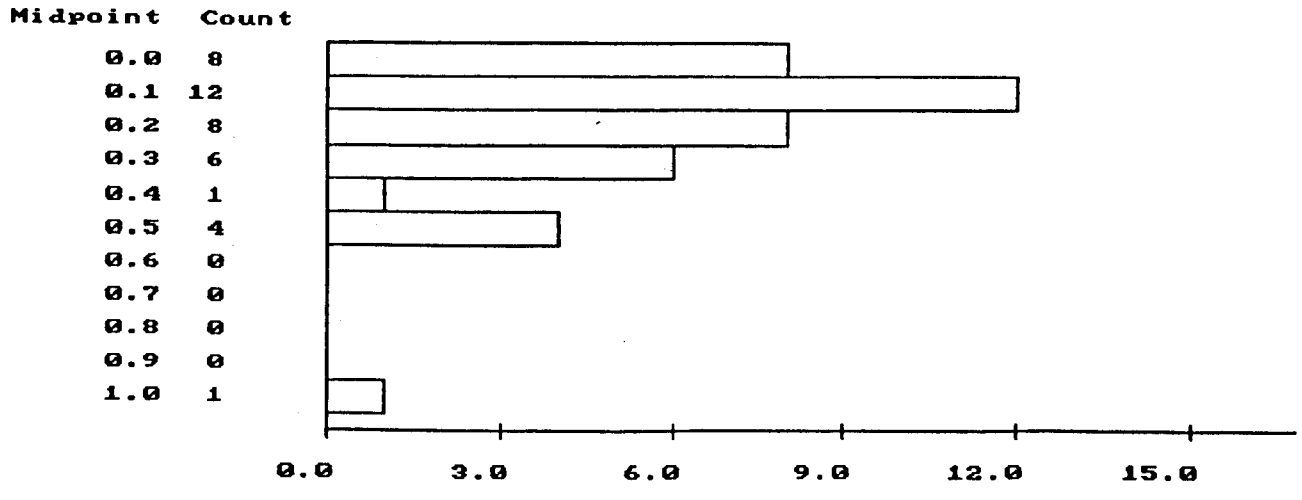
Graph 3.1 *Tetragnatha montana* Plot Of Mating Duration 1 Versus Symmetry Of Palp Usage 1 (First Mating)



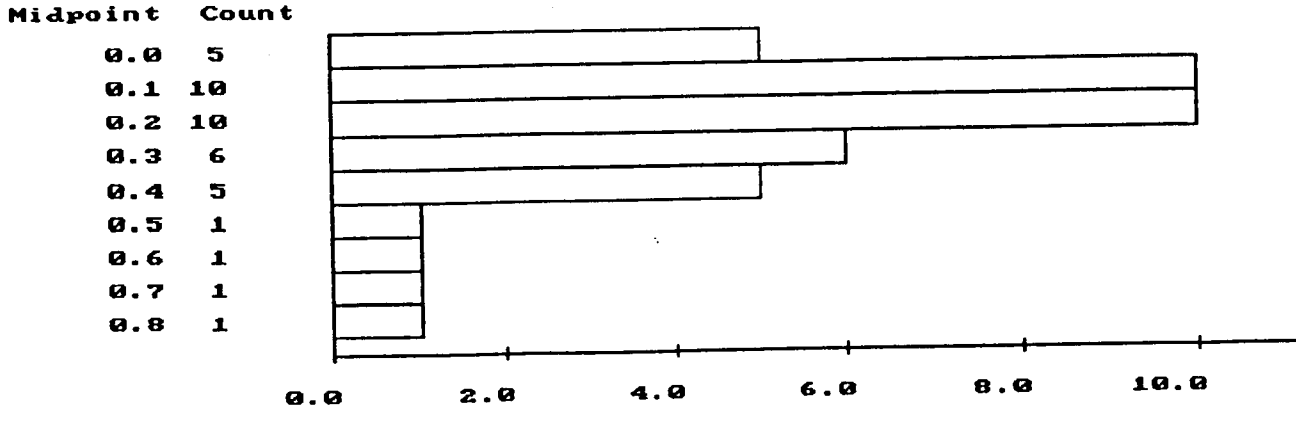
Graph 3.2 *Tetragnatha montana* Plot Of Mating Time 2 Versus Symmetry Of Palp Usage 2 (Second Mating)



Graph 3.3 Histogram Of The Symmetry Of Male 1 Palp Usage



Graph 3.4 Histogram Of The Symmetry Of Male 2 Palp Usage



3.6 Discussion and Conclusions

I have demonstrated that protein markers can be used for the detection of paternity in both laboratory reared and wild caught *T. montana* specimens. For this to be so two factors need to be met: that the proteins are heritable and that they are variable. Gels stained for esterase activity, in *T. montana*, meet these two criteria at both loci. Paternity assignment depends on the phenotypes of the two or more males involved in the matings being different. This is likely because there are in total 30 combinations of esterase phenotypes possible at the two loci. However, because of the close linkage between the two loci this effectively reduces the number of combinations. For example, S 1 and F 4 chromosomes are common in all populations studied. Nethertheless, the resolution of the system was sufficient to detect multiple paternity in all three wild collected female broods.

As predicted (Austad 1984) for a cul-de- sac species, there is no pronounced first male priority in *T. montana*. However there is no second male sperm priority pattern either. There is a hint that the longer mating male gains more paternity, but this was not statistically significant (Table 3.6). Thus the mechanism determining the outcome of sperm competition could be one of two ways:

(i) **A raffle principle**: whereby the transferral of sperm is continous throughout mating and the longest mating male thus manages to transfer the most sperm. The male transferring the most sperm gets the highest paternity.

(ii) **Genital stimulation theory** (Eberhard 1985): The male mating for the longest time stimulates the female the most and through cryptic processes the sperm of the most

stimulating male get to represent a higher proportion of the fertilization set than the least stimulating male.

This inability to separate the effects on mating behaviour of the two mechanisms is a central problem of the genital stimulation theory, which is only circumvented in Linyphiid species because they have an aspermic copulatory courtship period before sperm induction. It does make unique predictions pertaining to the structural morphology of the genitalia however. One prediction is that the genitalia are amply supplied with sensory apparatus. This prediction was not supported in spiders by the findings of Huber (1993b) however .

Males mating for a longer time would be expected to get a higher return in terms of paternity because mating is an energetically demanding exercise (Watson & Lighton 1994) and so should be minimised in duration. In addition, the pair are vulnerable during copulation (eg. to predation). It was found that the order in which the male mated had no effect on the mating duration. Nor was it found that the previous experience of the male in mating had any effect. Thus other factors must control the length of mating time.

In conclusion, I have found in a cul-de-sac species that there is no second male sperm priority patern. This is contrary to the hypothesis postulated by Austad (1984).

The symmetry of palp usage had no effect on insertion time for either first or second mating males. However, first mating males mate more symmetrically than second mating males (Graph 3.1, Graph 3.2). This may be due to the influence of a mating plug.

4.0 Mating Observations On *Zygiella x-notata* (Clerck 1757) (Araneae, Araneidae)

4.1 Introduction

Qualitative descriptions of mating behaviour are available for many species of spider (for example Robinson & Robinson 1980). However, quantitative measurements of copulation are rare in the literature (Fraser 1987). The mating ritual of *Zygiella x-notata* has been well described (Walcknaer 1841; Locket 1926; Bristowe 1929; Wiehle 1931; Smout 1976; Blanke 1986) but no detailed analyses of the number and duration of palpal insertions are available. Previous studies have often not taken into account the mating status of the participants observed. An unanswered question, therefore, is: are there any quantitative differences between first and second matings? It is important to control for this factor because of the influence of mating plugs, a chastity device which may prevent mating taking place or otherwise affect mating in already mated females. The prediction tested here is that second mating males spend less time inserting the palps, but mate for a longer time because of the influence of the mating plug.

A number of lines of evidence suggest that *Z. x-notata* has first male priority. First, despite the observations of Smout (1976), the males do mate-guard penultimate instar female spiders and mate with them 2-3 days after their final moult (Blanke 1986). They guard not at the edge of the web like *Z. atrica* (Smout 1976), but in the retreat with the female (pers. obs.). Penultimate females have obvious epigynes covered with a membrane and these are the females guarded by the already mature males. Protandry is thus a feature of this species.

Secondly I have observed mating plugs[1] in this species covering either one or both orifices of the epigyne. All other things being equal this would favour first male priority. Finally, Fig. 23 in Levi (1974) shows the spermathecae of this species to be of the conduit type with the ducts at 180^0 to each other, a feature consistent with first male priority acording to the Austad (1984) plumbing hypothesis. These lines of evidence lead to the conclution that *Z. x-notata* has first male priority. We shall see in the following if these lines of evidence have any effect on the mating times of first and second mating males because virgin females have a higher resource value; again, the prediction is for a longer first palpal insertion phase. This is unless the second male sperm loads, thus taking a longer time to mate. For this to happen the second male would have to be able to detect that the female was not a virgin.

The rationale behind mating times affecting paternity is based on the concept of copulatory courtship (Eberhard 1985): males spend time in copulation not only to transfer sperm but also to ensure paternity over a brood by achieving the greatest stimulation of the female; the longer mating male receives the lion's share of the brood. This hypothesis is yet to be confirmed using paternity measures. Sperm competition predicts the same pattern if the system is a lottery and sperm transfer occurs throughout the mating. The male who transfers the most sperm gets the highest paternity.

The duration of mating may be determined not only by mating order but also by the phenotype of the males, *e.g.* overall size. Recently the potential influence of fluctuating asymmetries in sexual selection has been addressed (Watson & Thornhill 1994). Spiders provide a rare opportunity to test if fluctuating asymmetry in the intromissive organs has an

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1 A waxy secretion in this case.

effect on mating time and mating sucess. This is because the intromissive organs are paired and can be measured easily.

4.1.1 Relevant Natural History

The genus *Zygiella* contains three species in the United Kingdom: *Z. x-notata*, *Z. atrica* and the rare *Z. stroemi* (Bristowe 1958; Roberts 1985). Fifteen species of *Zygiella* are known worldwide, all of which were described by Levi (1974). *Z. x-notata* is commonly found at high densities on windows and elsewhere around houses (Leborgne & Pasquet 1987a) which makes it an easy spider to sample. *Z. atrica* is found on gorse and other bushes whereas *Z. stroemi* has only been reported on pine trees at two locations in Scotland (Roberts 1985).

Z. x-notata only comes out during the night when it sits at the hub of an orb-web; during the day it hides in a silken retreat. The web is normally sectorial, ie. with a sector missing, but occasionally it is complete (Marples & Marples 1971; Smout 1976). The web is remade on a daily basis (Nielsen 1932).

Many studies have been undertaken on the foraging behaviour of *Z. x-notata* (Klärner & Barth 1982; Leborgne & Pasquet 1987a; Leborgne & Pasquet 1987b; Leborgne *et al.* 1991).

In England, mating in *Z. x-notata* normally takes place in September and October and this is followed by the production of 2 cocoons. The first one is the larger, as is common in spiders (Wise 1993). Nielsen (1932) found that up to 4 cocoons could be laid in captivity with an average of 43 (N = 8) eggs in each.

In nature, emergence from the cocoon takes place in April or May with the spiders maturing in September[2] (Smout 1976). The two months over which adult males are present enhances the chances for sperm competition to occur because it will skew the operational sex ratio in favour of males. In addition females readily multiply mate.

Smout (1976) described many other aspects of the ecology of *Z. x-notata* which are not directly relevant to the present study.

[2] On the Pacific coast of North America adult males are found from July to September (Levi 1974).

4.2 Mating Experiments

Sub-adult specimens of *Z. x-notata* were collected from the locations in the British Isles as shown in Table 4.1. The specimens were reared through to adulthood in isolation using the methods described in section 2.4.

Mating trials were prepared by serially introducing two males to the edge of the web of an initially virgin female housed in a standard container (Fig 2.3) under standard conditions (section 2.4)[3]. Only matings which resulted in fertile eggs were included in the data.

Observations were made on various aspects of reproductive behaviours. The following measurements were taken:

(i) **Courtship time:** time spent in active courtship prior to palpal insertion. This time was rounded to the nearest ten seconds.

(ii) **Mating time:** this is time for the copulation to take place including insertion and non-insertion periods[4]. This time was rounded to the nearest ten seconds.

(iii) **Left and right insertion times:** time of insertion of the palps into the epigyne. Also recorded was whether or not the haemadocha was inflated. In all cases where there was a successful insertion there was a haemadochal inflation.

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[3] Smout (1976) observed that mating will take place during both the day and night, so illumination levels may be of limited importance.

[4] These non-insertion periods include some courtship time during mating, for instance when the female temporarily goes back into the retreat.

Table 4.1 Mating Times Of First And Second Males Mated To A Single Female (Times in Seconds)

FEM-ALE #	MALE #		COURT. TIME*		MATING TIME*		LEFT PALP*		RIGHT PALP*		INTRO. NUMBER			
	1	2	1	2	1	2	1	2	1	2	L1	R1	L2	R2
U107	U821	U108	180	120	1,260	900	304	247	274	237	8	9	11	11
U212	U217	U821	720	420	1,020	900	171	260	253	276	5	8	6	6
U110	U211	U111	780	180	1,980	1,140	291	234	147	279	9	5	7	8
U504	U117	U211	1,260	1,620	3,180	1,560	111	376	85	192	4	3	8	5
U120	U211	U117	540	420	1,500	1,620	457	487	37	343	14	2	10	8
U119	U313	U106	180	300	2,520	2,220	235	220	179	274	8	8	7	8
U503	U110	U211	480	1,440	4,380	2,820	186	316	388	211	7	12	10	7
M03	U71	M05	300	180	2,100	3,600	225	340	226	321	10	9	10	10
C012	M07	U74	240	600	2,220	4,200	272	255	307	84	11	10	16	9
M08	C01	BY02	1,560	540	1,140	1,080	192	269	237	0	6	7	9	0
L06	C02	M07	1,080	2,280	1,620	1,320	108	148	74	145	4	3	6	6
C04	L04	M06	360	1,080	1,320	1,920	247	387	248	409	10	12	10	10
C03	L01	U73	180	180	1,020	1,860	33	214	107	292	2	4	8	9
BY01	BY02	U71	180	180	1,140	1,680	386	295	0	210	9	0	10	7
U01	C01	U72	420	0	840	1,140	142	321	195	273	9	7	10	9
L07	C02	L04	420	60	1,080	1,620	284	439	248	356	7	6	14	15
L08	M06	L01	660	360	2,460	2,340	385	287	467	400	11	11	9	10
U02	U71	M07	60	540	840	1,620	254	286	248	276	8	8	11	10
BY03	U73	L03	60	60	900	900	346	307	391	131	11	12	15	14

FEM-ALE #	MALE #		COURT. TIME*		MATING TIME*		LEFT PALP*		RIGHT PALP*		INTRO. NUMBER			
	1	2	1	2	1	2	1	2	1	2	L1	R1	L2	R2
U61	U63	U62	60	1,320	960	1,440	330	276	364	138	11	11	9	6
U64	U62	U63	240	1,200	1,980	1,620	257	350	311	271	9	10	7	10
B02	S02	B04	180	60	840	1,200	232	337	245	282	8	9	10	10
B06	S03	S05	60	120	1,020	1,500	237	264	229	310	9	9	8	9
B07	S04	S06	420	120	3,120	840	86	256	176	286	4	7	9	9
S07	B08	S01	180	240	1,080	1,440	207	234	209	3	7	8	7	1
B03	B17	S03	600	300	900	660	315	234	328	219	9	10	7	7
B18	S02	B08	540	1,320	1,260	1,920	190	120	219	125	6	7	6	6
B19	S05	B17	840	60	1,200	1,080	377	228	368	324	10	10	7	9
B22	S09	S10	120	120	1,320	1,020	175	280	178	360	7	7	8	10
B23	S06	S09	240	720	1,020	540	309	216	299	213	9	9	7	8
B24	B04	S04	120	300	1,320	600	375	17	397	57	11	12	1	3
S25	S12	S11	60	0	900	1,200	266	256	273	321	9	9	8	10
B21	S10	S12	240	120	1,080	1,080	376	176	353	248	10	10	6	8
B26	B17	S01	300	240	1,380	2,220	252	156	156	89	9	8	8	4
S14	S11	L01	420	420	1,380	720	245	245	246	246	8	8	8	8
B27	C022	L02	1,980	120	1,140	960	91	122	299	375	12	12	16	16
B28	C011	L05	120	300	960	1,140	287	208	260	225	9	8	9	9
S16	L04	C011	900	300	1,620	1,440	51	125	45	92	3	3	5	3

* = All times in seconds. **Court. time** = Time of courtship prior to copulation. **Mating time** = Total mating time i.e. Insertion + non-insertion time. **Left Palp** = Total insertion time of the left palp. **Right palp** = Total insertion time of the right palp. **Intro. number** = Number of intromissions of the right (R1, R2) and left palps of the first and second mating males (L1, L2). Locations from which specimens were collected: **U** = University of York concert centre; **M** = Millport marine biology station, Scotland; **C** = 38 Norfolk terrace, Cambridge; **L** = Moorfield, Grange avenue, Leeds; **BY** = 18 Wellington Street, York; **B** = Biology Department, York; **S** = University sports centre, York .

(iv) **Total Insertion time:** the sum of left and right insertion times.

(v) **Number of insertions:** the number of insertions of the left and the right palps.

To see if symmetry of palp usage affected the duration of the mating an index of symmetry was taken by: absolute [(L-R) / (L+R)] which gives a value from 0 to 1 with zero being absolute symmetry and one absolute asymmetry.

Morphological measurements were taken of the sternum width at its widest point (a reliable index of spider size) for a subsample of the males (N = 21) (Table 4.2) and females (N = 19) (Table 4.3) and the length of the left and right male palps (Table 4.2, Fig. 4.1). These measurements were made on a Reichert binocular microscope using an eye piece graticule (one eye piece unit = 0.0282 mm).

One hundred and seventy laboratory mating trials were necessary to produce 76 successful matings[5] of *Z. x-notata.* A mating trial was abandoned if a mating did not occur within 1 hour (N=94). Four mating treatments were carried out on the *Z. x-notata* classified on the basis of the mating status of the spiders involved (See table 2.2).

Blanke (1986) showed that females usually become unreceptive to matings 6-7 days after moulting although one was observed mating more than 25 days after the final moult. Multiple mating would take place during this window of time. In the experiments described

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[5] All of which produced viable eggs. In captivity cocoons could be forced to hatch at any time of the year in a controlled environment room maintained under conditions described in section 2.4.

here all first matings took place 2-3 days after the final moult and all second matings were completed within the next 5 days.

Close observations and notes were made during behaviour sequences and these form the basis of the following descriptions.

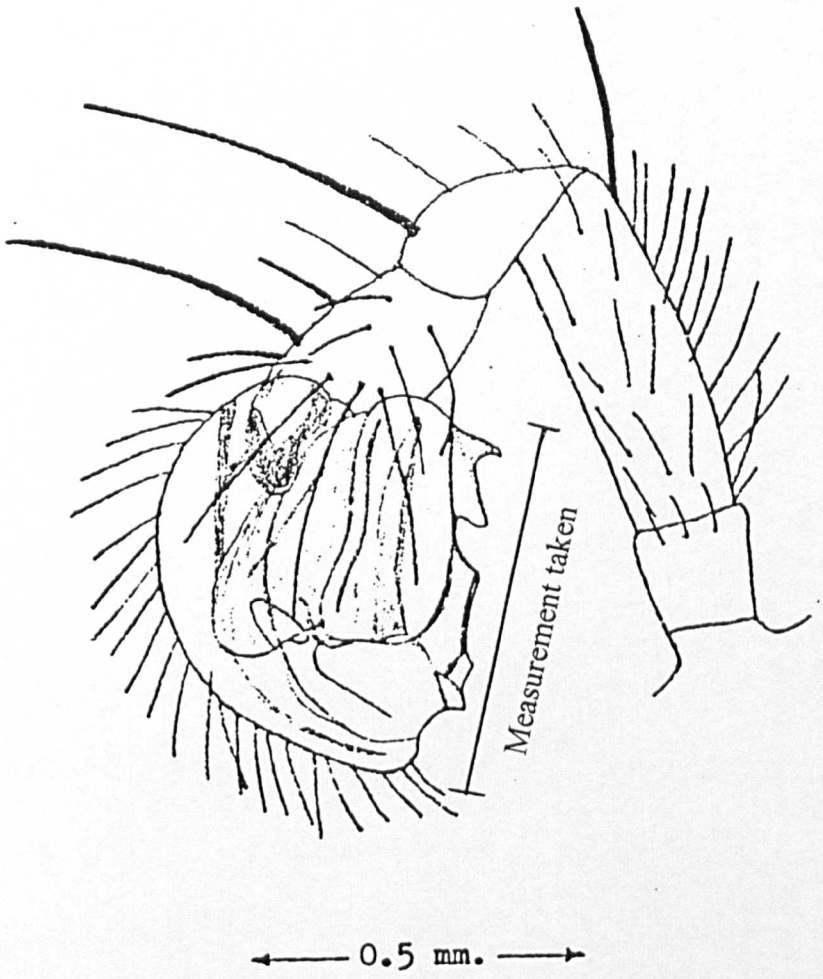
Table 4.2 Morphological Measures Of *Z. x-notata* Males

MALE #	STERNUM WIDTH		LEFT PALP LENGTH		RIGHT PALP LENGTH	
	EYEPIECE UNITS	mm	EYEPIECE UNITS	mm	EYEPIECE UNITS	mm
B04	28	0.79	22	0.62	21	0.59
B08	29	0.82	21	0.59	20	0.56
B17	31	0.87	24	0.68	25	0.71
C11	31	0.87	23	0.65	25	0.71
C22	29	0.82	23	0.65	24	0.68
L01	28	0.79	21	0.59	21	0.59
L02	31	0.87	20	0.56	20	0.56
L04	42	1.18	20	0.56	21	0.59
L05	30	0.85	20	0.56	20	0.56
S01	36	1.02	25	0.71	26	0.73
S02	30	0.85	23	0.65	23	0.65
S03	28	0.79	22	0.62	23	0.65
S04	31	0.87	18	0.51	19	0.54
S05	38	1.07	24	0.68	25	0.71
S06	29	0.82	22	0.62	22	0.62
S09	32	0.9	21	0.59	20	0.56
S10	30	0.85	22	0.62	22	0.62
S11	40	1.13	22	0.62	21	0.59
S12	30	0.85	22	0.62	25	0.71
U62	29	0.82	23	0.65	25	0.71
U63	30	0.85	20	0.56	20	0.56

Table 4.3 Morphological Measures Of *Z. x-notata* Females

FEMALE #	STERNUM WIDTH	
	EYEPIECE UNITS	mm
B02	36	1.02
B03	30	0.85
B06	35	0.99
B07	36	1.02
B18	41	1.16
B19	40	1.13
B21	35	0.99
B22	38	1.07
B23	41	1.16
B24	30	0.85
B26	36	1.02
B27	39	1.1
B28	40	1.13
S07	30	0.85
S14	40	1.13
S16	39	1.1
S25	38	1.07
U61	38	1.07
U64	39	1.1

Fig 4.1 The Male Palp - Measurement Taken



4.3 Results

4.3.1 Qualitative Results

The following laboratory-based observations generally agree well with those reported under more natural conditions by Smout (1976) and Blanke (1986). Males often laid down a mating thread on the female's web and proceeded to entice the female out of her retreat by strumming. This stage lasted from 0 to 2280 seconds. The mating thread was reinforced by back and forth movements along the initial guide thread by the male laying down more silk at each pass. Strumming by the male was in bouts with movements forward to touch the female in her retreat if she did not come out. Females may be reticent to emerge from their retreats because of the evolutionary influence of the wasp *Chalybion californicum.* This predatory insect entices the female from her retreat, in the same way as the male (Nentwig 1987) and so the female has to be sure of the identity of her suitor.

When the female did come out she gripped the mating thread with her third pair of legs forming the typical Aranaeid 'O' formation (Blanke 1986). At this juncture the male scraped the ventral surface of the female which was now revealed to him. Often the male went back and began to strum again. Eventually insertion occurred; the palps were usually applied alternately but sometimes one palp was inserted more times than the other. Between insertions the palp was applied to the oral area, perhaps to lubricate it or to apply some plug material.

It was possible to mate both males and females at least twice, as was observed by Smout (1976).

When the pairs parted no aggressive behaviour on the part of the female was observed, and thus no sexual cannibalism occurred. The male lingered on the web after mating to recharge his palps with semen (sperm induction (Montgomery 1903)). This whole process was completed within one minute. A triangular web was constructed within the web of the female, abdominal contractions deposited semen onto the sperm web and this was taken up by the palps, one at a time. The male then moved away and showed no signs of post-mate guarding.

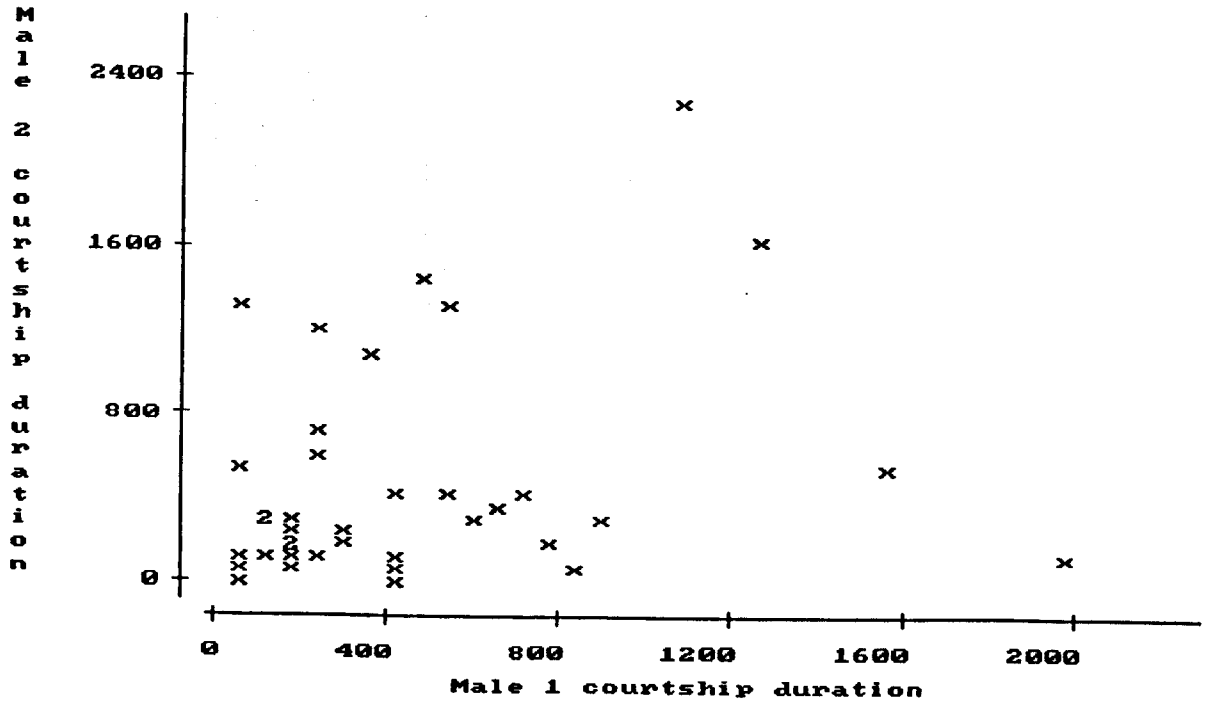
4.3.2 Quantitative Results

For a summary of the data for this section refer to Tables 4.1, 4.2 and 4.3.

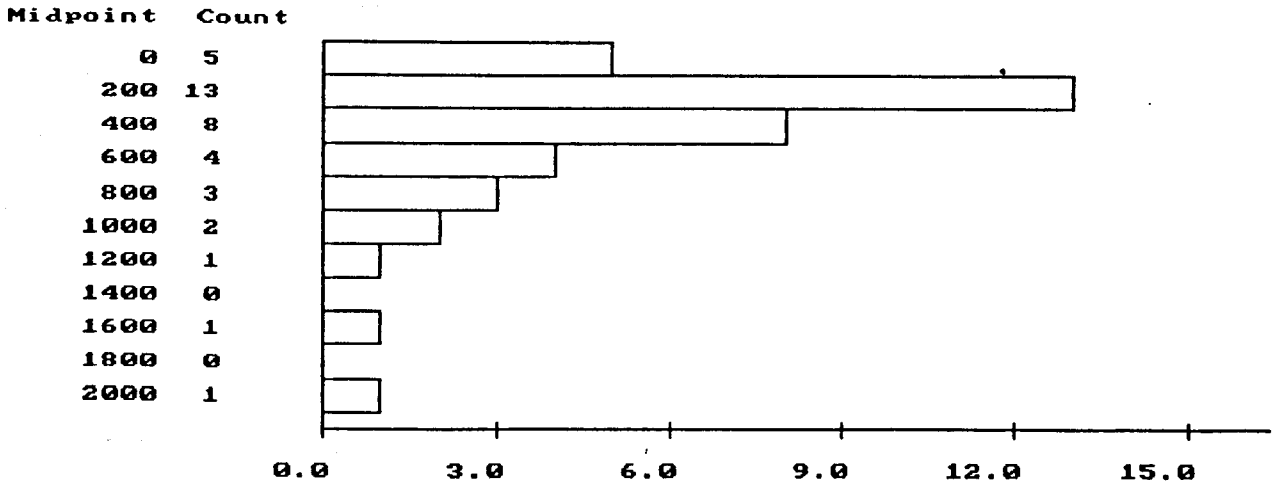
(i) Courtship Time:

This is the time spent by the male in pacifying the female prior to copulation. There seems to be no relationship between the duration of the first mating male's courtship and the second male's courtship (Spearman rank correlation coefficient = 0.265; n. s.) (Graph 4.1). Most of the courtships are of relatively short duration (generally below 800 seconds), but a few are considerably longer (up to 2280 seconds) (Graph 4.2 a & b). No significant difference between the mean duration of first and second courtships was found: first courtship (mean = 454.7 seconds; s. d. = 435.1; range = 60-1980 seconds) versus second courtship (mean = 472.1 seconds; s. d. = 531.0 range = 0-2280 seconds; paired sign test p = 0.73; N = 38), so courtship time is unaffected by the mating status of the female.

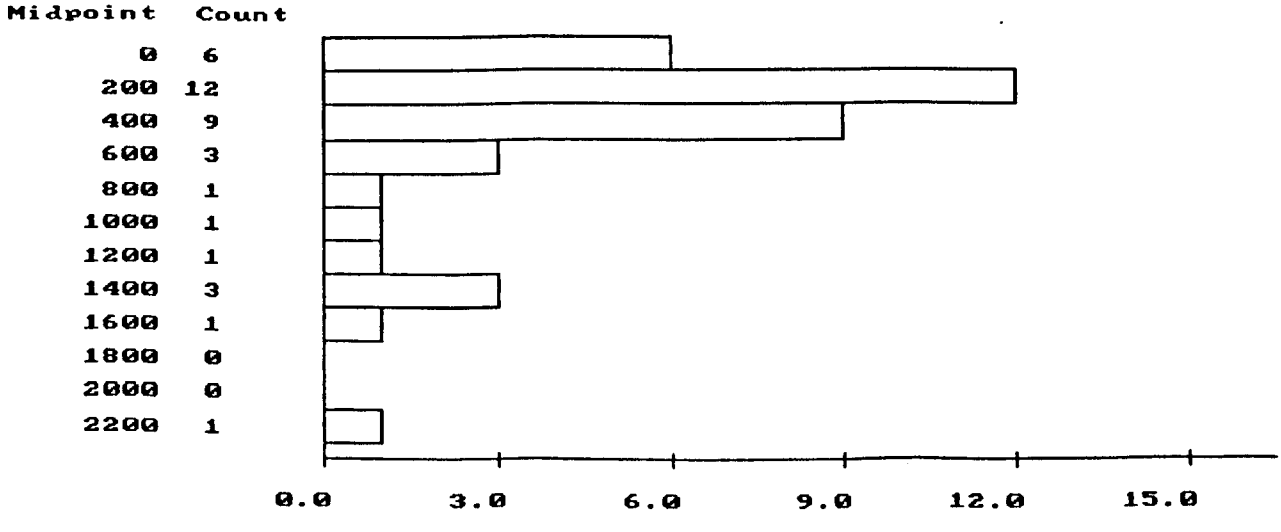
Graph 4.1 Total Courtship Time Of First Versus Second Males



Graph 4.2 a Histogram Of Courtship Duration Of Male 1



Graph 4.2 b Histogram Of Courtship Duration Of Male 2



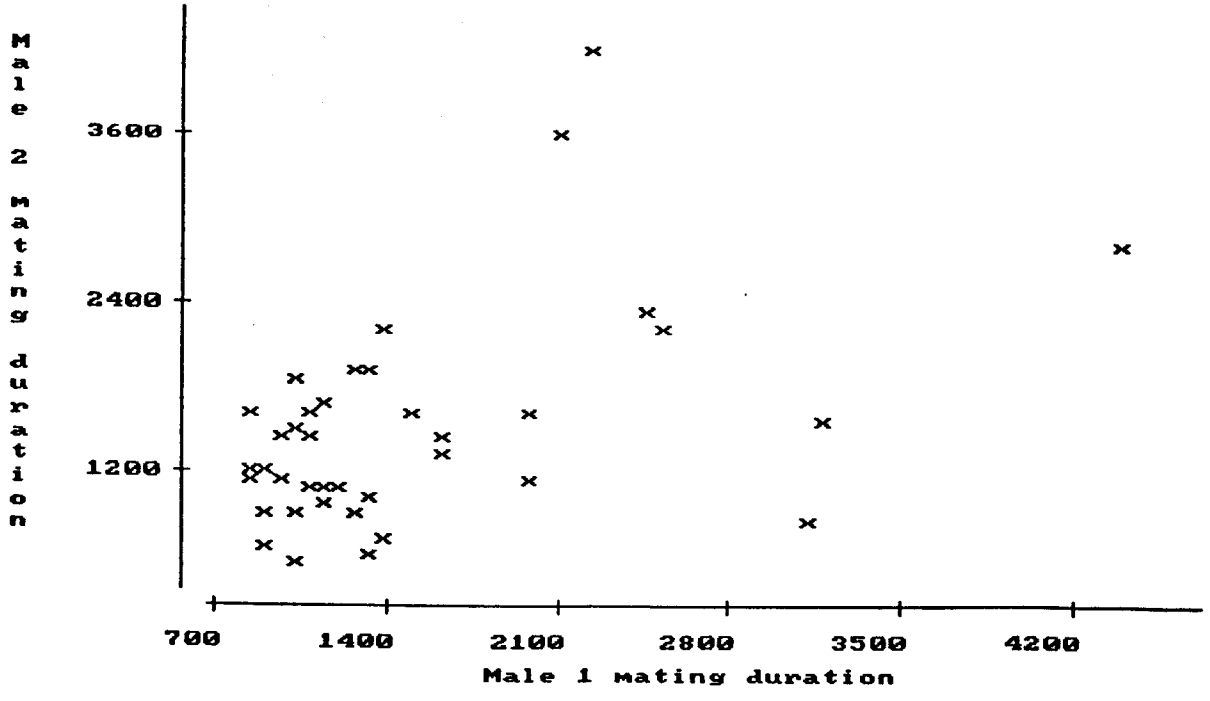
(ii) Mating Time:

Mating times (including insertion and non-insertion phases) were the same for first (median = 1230 seconds; sd = 775; range = 840-4380 seconds) and second (median = 1380 seconds; sd = 771; range = 540-4200 seconds; paired sign test $p = 0.87$ n. s.; N = 38) mating males, both showing a trend for many of short times and a few of longer duration (Graph 4.3, 4 a & b).

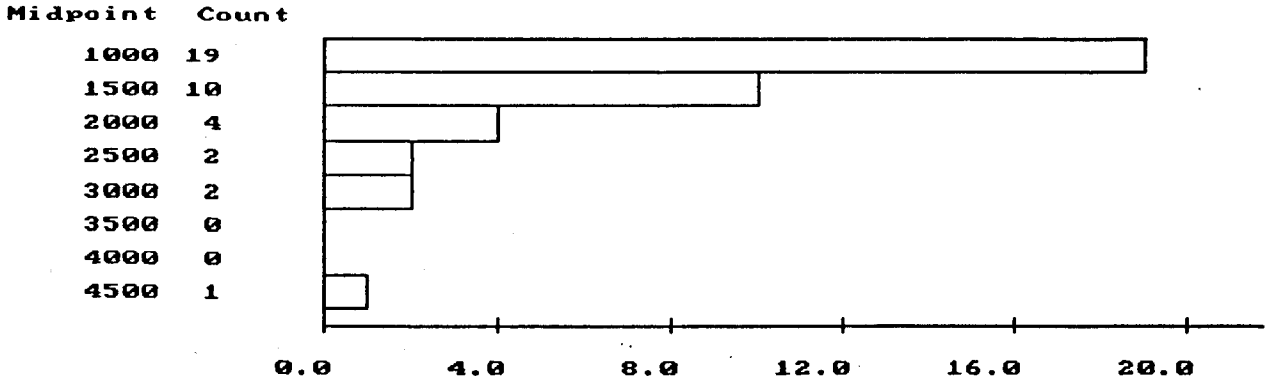
Smout (1976) found total mating duration to be from 60 seconds to 3600 seconds, for an undisclosed number of observations. Here with 76 observations it was found that the duration never went below 540 seconds with a maximum of 4380 seconds.

Courtship time and mating time have a low correlation for both first (Spearman rank correlation coefficient = 0.439, n. s.) and second (Spearman rank correlation coefficient = 0.291, n. s.) matings suggesting no relationship between these two aspects of reproductive behaviour.

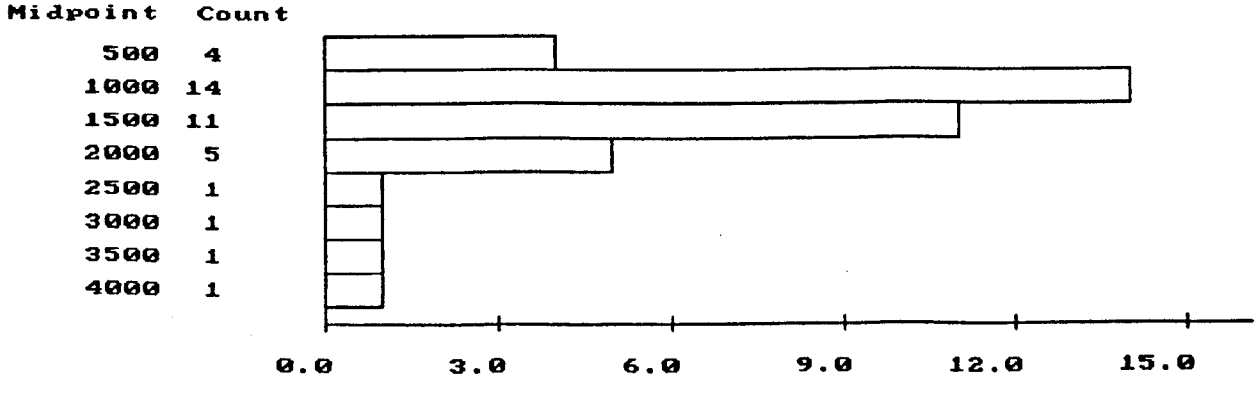
Graph 4.3 Total Mating Time Of First And Second Matings To A Single Female



Graph 4.4 a Histogram Of Total Mating Time Of First Mating Males



Graph 4.4 b Histogram Of Total Mating Time Of Second Mating Males



(iii) **Insertion Time And Symmetry Of Palp Usage:**

Four mating kinds were recognized as shown in Table 2.2 and Table 4.4. No significant difference was found between insertion times for these different categories (ANOVAR: F = 2.01; p = 0.12; df = 3, 72) (Table 4.4). This implies that the mating status of the spiders involved does not affect insertion time; the overall average was 487.3 seconds (N=76).

The order of mating had no effect on the proportion of time spent inserting the palp during the course of mating: (insertion time / mating time). For the first male the mean was 0.388 (S. E. = 0.032), and for the second, 0.387 (S. E. = 0.030). There was no significant difference between these means (paired sign test p = 0.87 n. s.; N = 38).

Another factor which does not affect mating time of the second mating male is the insertion time of the first mating male (Graph 4.5) (Spearman rank correlation coefficient = 0.012, n. s.).

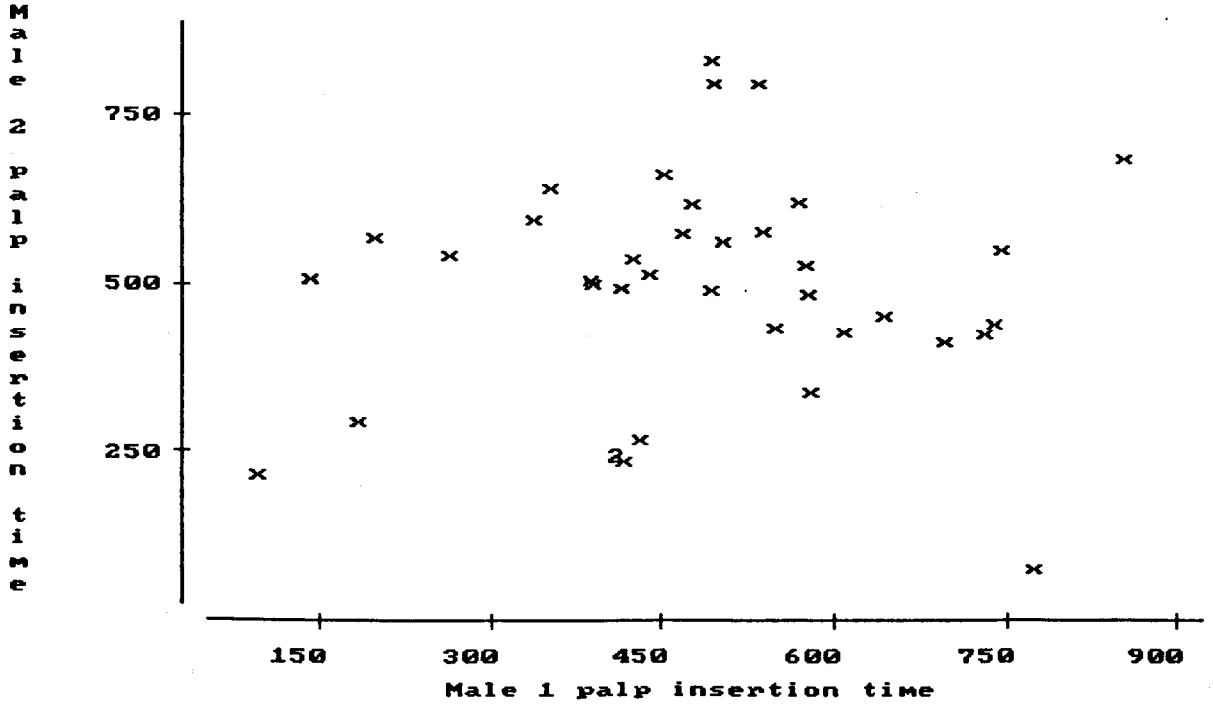
For both first and second mating males there are indications of an association between symmetry of palp use and duration of insertion time. The relationship is such that less symmetrical usage results in shorter insertion duration (Graph 4.6). However this relationship is not significant (Spearman rank correlation coefficient = -0.302, n.s.).

A subsample of ten individual matings was taken randomly from the 76 total. This was to investigate the mean palp insertion time. It was found, over the 166 palpal usages from the ten matings, that the mean palpal insertion time was 23.5 seconds (range 2-42 seconds). This compares with 25 seconds minimum and 70 seconds maximum of Locket (1926) for an undisclosed number of matings observed.

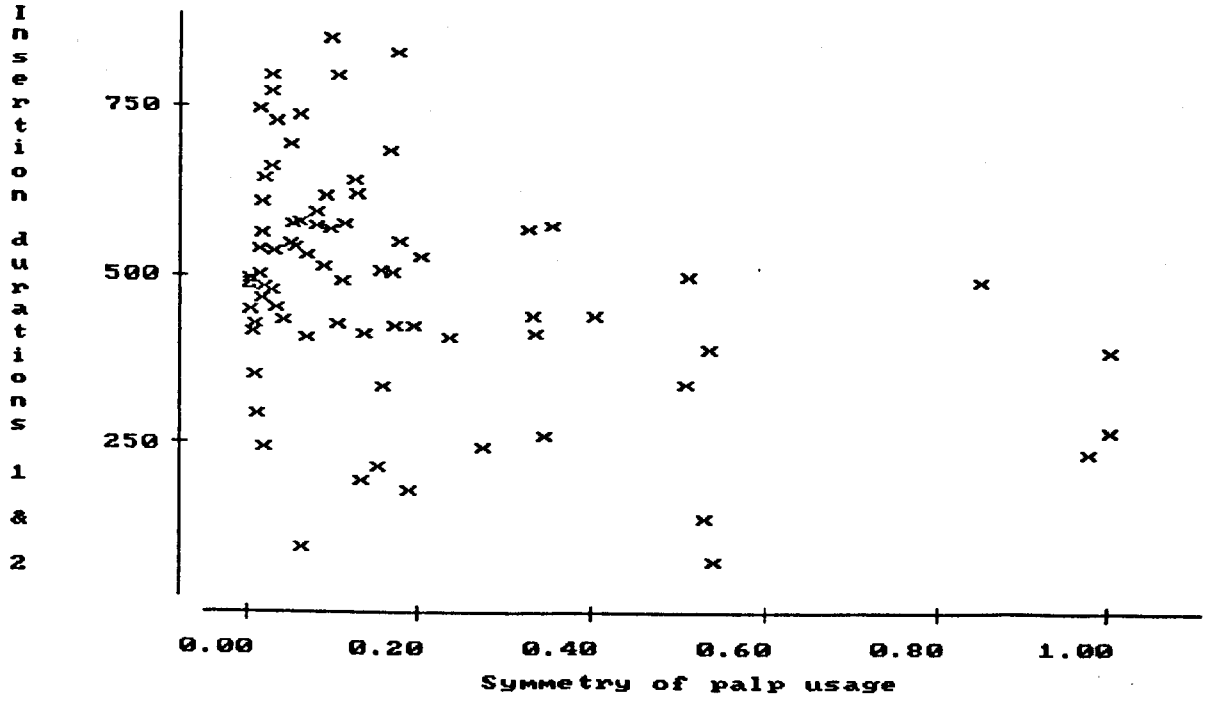
Table 4.4 Mean Insertion Duration For Different Mating Kinds

Mating Kind (number involved)	Mean duration of insertions in seconds (mean; standard error; range)
K1 (N = 22)	429.8; 31.5; 96 - 694
K2 (N = 16)	556.1; 46.2; 140 - 852
K3 (N = 14)	519.9; 24.1; 339 - 661
K4 (N = 24)	475.1; 40.7; 74 - 830
Total Mean (N = 76)	487.3; 19.5; 74 - 852

Graph 4.5 Total Palp Insertion Times For First And Second Matings To A Single Female



Graph 4.6 Plot Of Mating Times 1 & 2 Versus Symmetry of palp usage 1 & 2



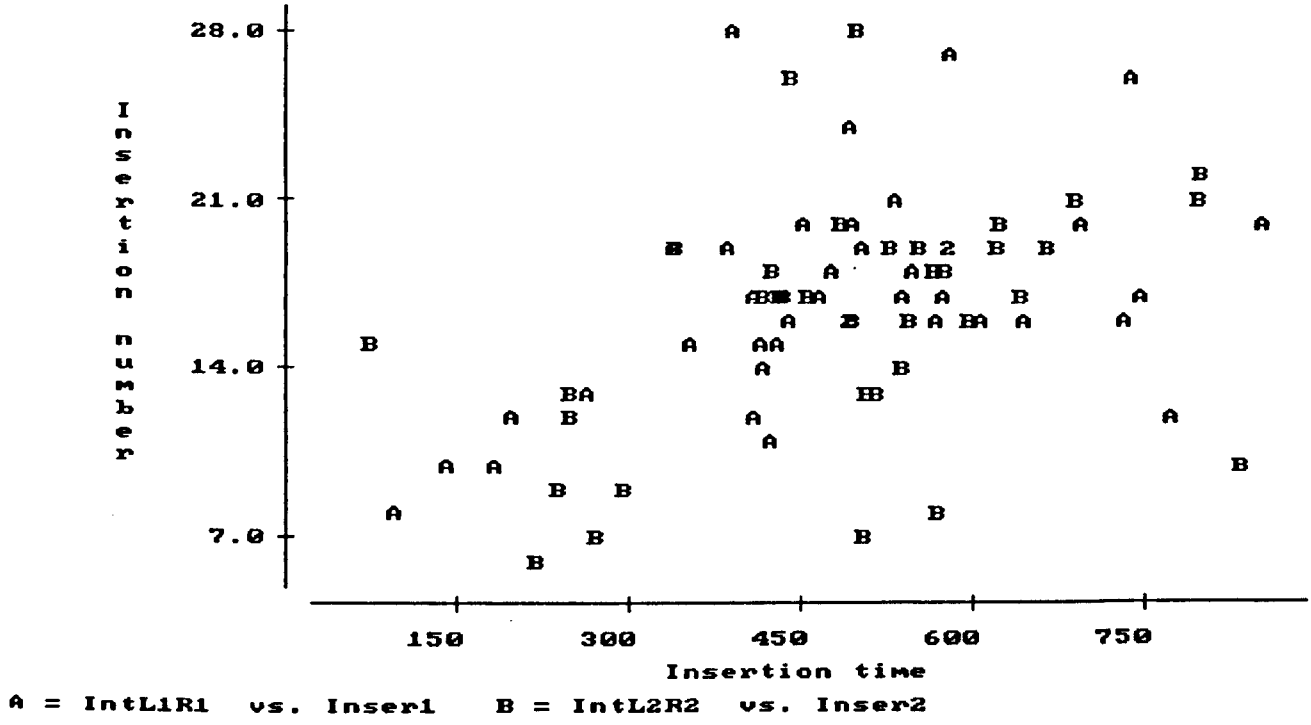
(iv) **Insertion Number:**

As would be expected the higher the number of insertions the longer was the insertion time (Graph 4.7) but the relationship was not strong (Spearman rank correlation coefficient = 0.431). Most matings had an approximately equal number of insertions (left and right) (Graphs 4.8, 4.9 a & b); only two individuals managed to insert only one palp (symmetry = 1), both of which were second mating males (Graph 4.9 b). A more symmetrical number of insertions did not result in a longer insertion duration (first mating males: Spearman rank coefficient = 0.056; second mating males: Spearman rank coefficient = 0.188 n. s.), nor was there any difference between first and second mating males in symmetry of palp usage (paired sign test $p = 1.00$; $N = 38$).

Graph 4.7 Plot Of Insertion Time Versus Insertion Number

A = Insertion number for first male versus insertion duration for first male.

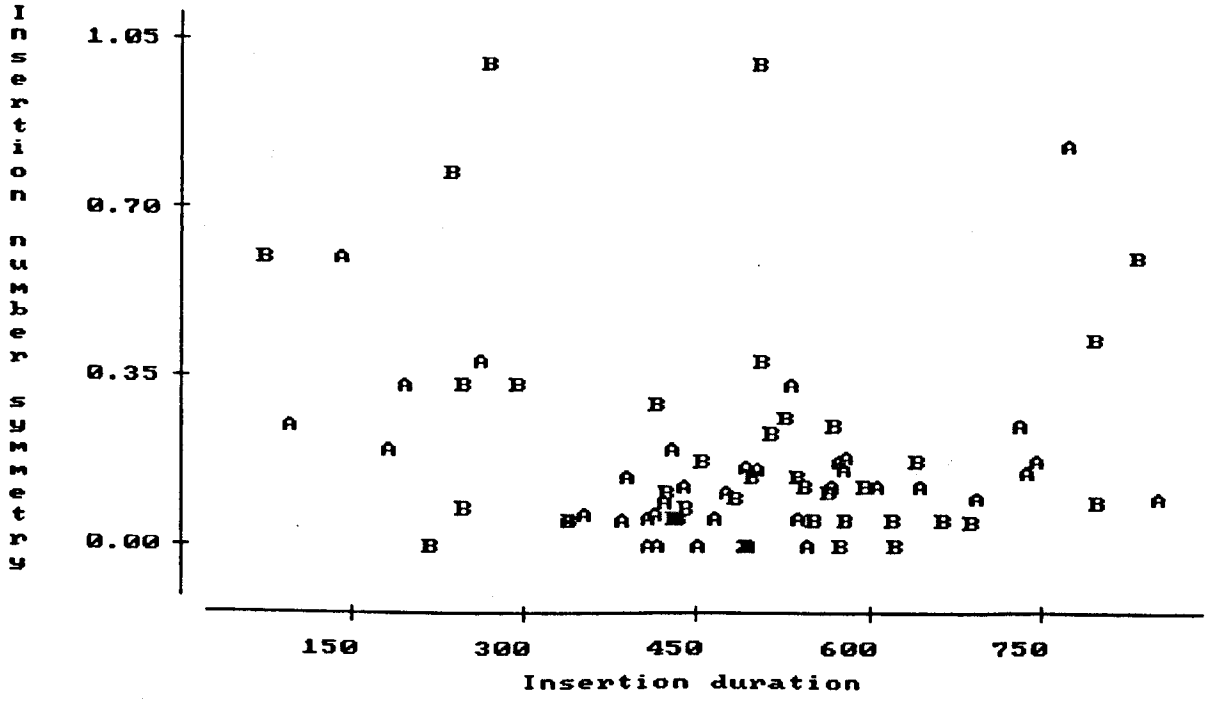
B = Insertion number for second male versus insertion time for second male.



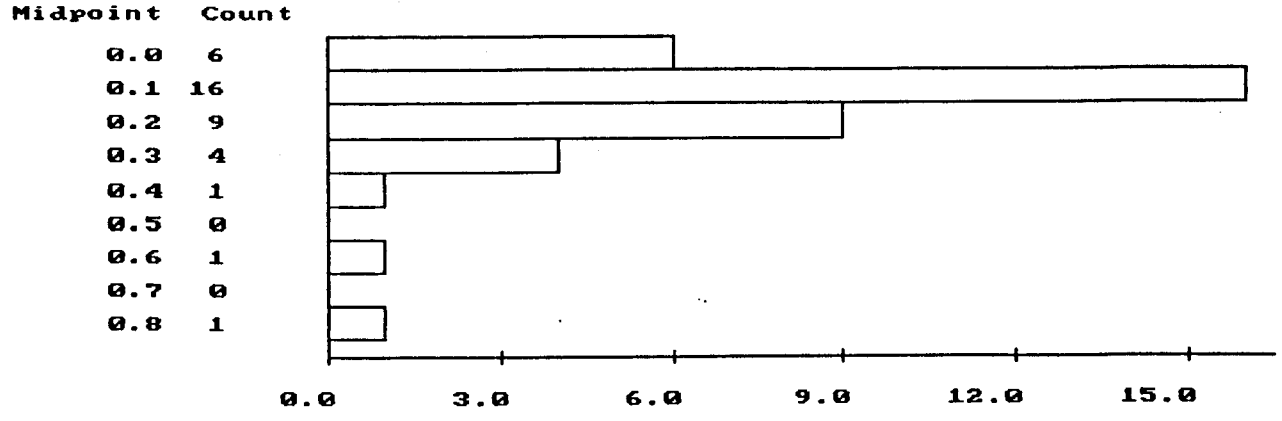
Graph 4.8 Symmetry Of Palp Insertion Number As A Function Of Insertion Time

A = Insertion number symmetry for first male versus insertion duration for first male.

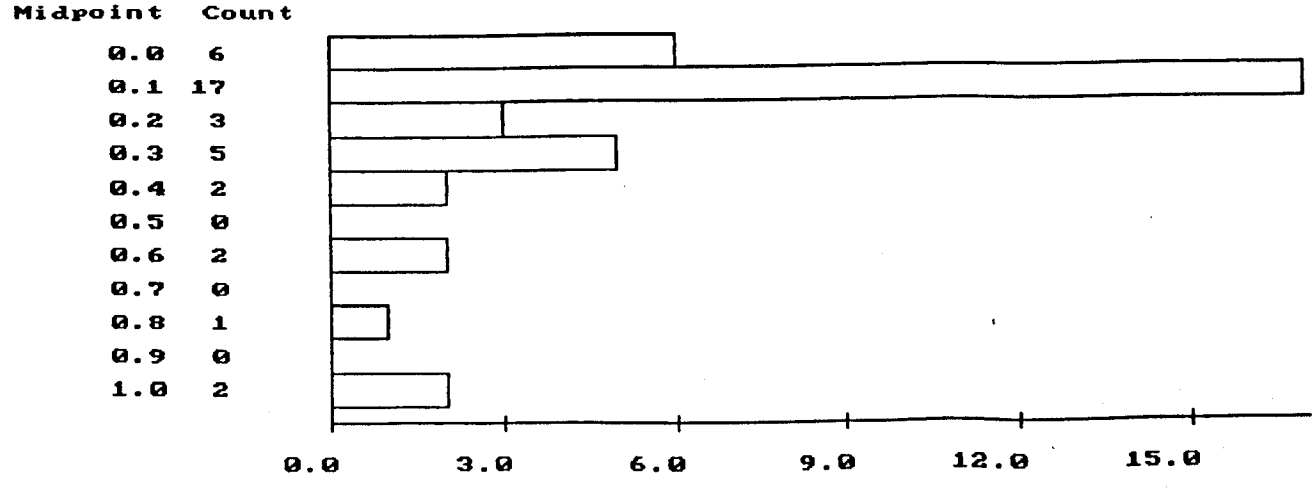
B = Insertion number symmetry for second male versus insertion duration for second male.



Graph 4.9 a Histogram Of The Symmetry Of Palp Insertion Number For First Mating Males



Graph 4.9 b Histogram Of The Symmetry Of Palp Insertion Number For Second Mating Males



(v) **Male Size:**

The males appeared to fall into two size classes for sternum width: 0.76 mm to 0.92 mm and 1.02 mm to 1.20 mm. Male size has no effect on courtship time (first mating male: Spearman rank correlation coefficient = 0.396 n. s.; second mating male: Spearman rank correlation coefficient = -0.284). Male size has no effect on mating time (first mating male: Spearman rank correlation coefficient = 0.269; second mating male: Spearman rank correlation coefficient = 0.236) (Graph 4.10) and has no effect on insertion time (first mating male: Spearman rank correlation coefficient = -0.272; second mating male: Spearman rank correlation coefficient = -0.092) (Graph 4.8). These statistics are summarized in Table 4.5.

Table 4.5 Spearman Rank Coefficients For Various Mating Attributes And Male Size

	Sternum Width Male 1	Sternum Width Male 2
Courtship Time	0.396	-0.284
Mating Time	0.269	0.236
Insertion Time	-0.272	-0.092

The measurements of the palps were all within a couple of graticule units and so it is impossible to test whether more symmetrical palps lead to longer mating times and they are probably due to errors of measurement. The very high degree of symmetry found in the palp measurements may be a result of past selection pressure. Alternatively the resolution of measurement might not have been sufficient to identify significant asymmetry. Future investigations may require a scanning electron microscope to perform the measurements.

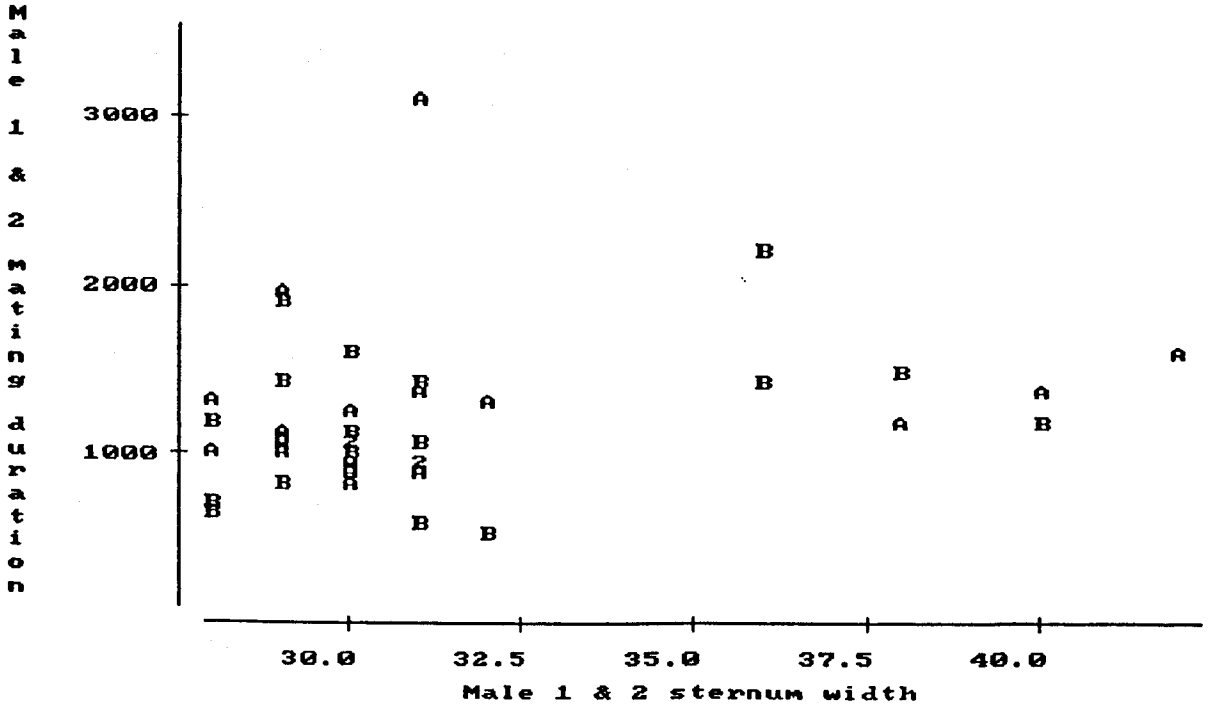
(vi) **Female Size:**

Female size (sternum width) did not affect the courtship, mating or insertion times for first and second mating males. For statistical evidence relating to these points see Table 4.6.

Table 4.6 Spearman Rank Coefficients For Various Mating Attributes And Female Size

Mating Attribute	First Or Second Mating Male	Spearman Rank Correlation Coefficient
Courtship Time	First Mating Male	0.328
	Second Mating Male	0.357
Mating Time	First Mating Male	0.146
	Second Mating Male	0.064
Insertion Time	First Mating Male	-0.073
	Second Mating Male	0.067

Graph 4.10 Plot Of Mating Time Versus Male Size



4.4 Discussion And Conclusions

Mating time in *Z. x-notata* ranged from 9 to 73 minutes. Identifying possible reasons for this variability was difficult; most associations tested proved to be non-significant.

No association was found between the following mating attributes:

(i) First male courtship time and second male courtship time.

(ii) First male mating time and second male mating time.

(iii) First male insertion time and second male insertion time.

(iv) Symmetry of palp usage and insertion time

This could be for one of several reasons:

(i) The female is cryptic about her mating status and therefore males behave as if they are always mating with a virgin female, or

(ii) The males in these experiments did not pre-mate guard as is usual in nature and therefore did not know the mating status of the female and mated with her as if she were already mated.

(iii) The female is judging the males on some criteria not considered here and is responsible for the termination of the matings. The females in this species are likely to be responsible for termination of mating as it can occur whilst the male still has a palp inserted, and seems to be unprepared for the end of the mating.

To separate these options observations need to be made on matings taking place between couples where guarding has taken place. These matings may be of a different duration if any part of the mating is not to do with sperm transfer but is copulatory courtship. The matings observed by Smout (1976) which were of a much shorter duration than observed here may have been ones where the male involved had mate-guarded.

In a recent paper, Hebets & Uetz (1995) found that copulation duration, in *Schizocosa ocreata*, was a function of the age of both the female and the male (post moult). Here age of the mating participants was randomized so it can not be investigated specifically as a factor. However second matings are, by definition, taking place with older participants so if age was an important contributing factor it would be expected to generate differences between first and second matings, yet none was found. This may be due to the lack of difference in time between first and second matings which could be as low as a few hours.

Austad (1982) found that the lengths of time in preinsemination and insemination phases were a function of the copulatory history of female *Frontinella pyramitela*.He observed that the preinsemination phase was longer for second mating males and the insemination phase was longer if the mating took place less than 24 hours after the first. This was in a species in which first male priority was the norm. Second mating males were therefore not getting a

return for their greater effort. This may also be the case in *Z. x-notata* where the guarding pattern of penultimate females is similar to *F. pyramitela.*

Jackson (1980) found a great deal of variation in mating duration in *Phidippus johnsoni* for any mating state (virgin, recently mated, gravid and postoviposition), but this was correlated with the site where copulation took place (inside or outside the nest). The longer the duration in this species the less likely was the female to mate with another male, so extended copulation duration has a function in this species.

One would expect that if a mating plug is placed on the epigyne, as has been observed in *Z. x-notata* (pers.obs.), this would affect the mating of the second male; however this is not the case unless it is stopping the passing of sperm. However, haematodochal inflation and fully inserted palps were observed in all four kinds of matings. A question remains regarding the purpose of the alternation of the palps in this species, and entelegyne species in general. It could be due to the picking up of plug material from the oral area to insert in the epigyne. Additionally however, the alternation of palps gives the female a way of assessing the athletic fitness of the male, a more athletically fit male being able to alternate the palps more regularly throughout the mating. It was found here that more regular palp insertion did not lead to longer mating time but it may lead to a higher paternity.

5.0 Sperm Competition And Its Evolutionary Consequences In The Spiders.

5.1 Introduction

The empirical chapters of this thesis (Sections 2.0 - 4.0) have failed to disprove the Austad (1984) hypothesis that the spermatheca in spiders has an effect on the sperm precedence patterns, although the *Tetragnatha* chapter (Section 3.0) is suggestive that the picture is not as simple as Austad suggests. There are insufficient data on P_2 values in spiders to test directly if they are determined by spermathecal architecture alone. However there are indirect methods which will be explained in the present chapter. These indirect methods include charting the incidence of mate-guarding and mating-plugs to see if they are in line with predictions that can be drawn from the Austad (1984) hypothesis.

5.2 Incidence Of Polyandry And Other Preadaptations Towards High Levels Of Sperm Competition.

Of fundamental importance among the factors which lead to sperm competition being a significant selection force is polyandry in females. If females are not receptive to further matings after their first one then sperm from more than one male will not be present in the sperm storage organ to compete for fertilizations. The breakdown of monogamy as a category in recent years, through close behavioual observations of animals in the wild, and the use of genetic fingerprinting and other molecular methods, has thrown the whole concept of mating systems into question (Crieghton & Hosie 1993). This would be as true for spiders as for any other group if it were not for the fact that it was recognized earlier. Montgomery

(1910), a turn of the century expert on spider sexual behaviour, was even then of the view that:

"*Monogamy is exceptional, and would appear to occur in cases where the male seizes immature females by force, and where the male lives in a mating nest with a female*".

Interestingly he notes that it is male competition that prohibits the mating options of females who have on the whole a high level of receptivity to male advances. The level of female receptivity is one of the main determinants of the importance of sperm competition recognized by Parker (1970) because if monogamy is the rule then male adaptations and counter adaptations towards sperm competition are unlikely to arise.

A compilation of the evidence for multiple mating in female spiders was undertaken by both Jackson *et al* (1981) and Austad (1984); a more extensive review is presented here (Appendix Table A5.1). The table does not represent an attempt to quantify the extent of multiple mating or its frequency in the wild because it clearly under-reports the extent of the phenomenon. This is because of the estimated 50,000 species of spiders only a small fraction has been examined and, in those that have, detection of polyandry is rarely a primary goal of the study. But in the spider species intensively studied, mating more than once is the norm. Monogamous spiders do exist though they are rare in the literature (van Helsdigen 1965; Pollard *et al* 1987, page 139; Huber 1995).

The data in the table were compiled using both behavioural and morphological information. Observations of a female mating more than once are straightforward enough, but how can morphology tell us that a multiple mating took place? In the case of *Latrodectus*

mactans, for example, the apical element of the male palp is shed into the spermatheca during copulation. More than one such apical element has been found in a single female's spermatheca, from which we can infer she mated more than once.

In iteroparous species (most spiders, Wise 1993) remating may occur before and / or after the first egg batch has been laid. Clearly the first egg batch, in the case of remating only after it has been laid, is immune to the effects of sperm competition.

Females need not be receptive for multiple mating to occur, as forced copulations can take place when the female is moulting and vulnerable to such mating tactics (Robinson & Robinson 1980). Because of the nature of the genitalia and the often larger female size, rape as a mating tactic in spiders, under normal circumstances, is unlikely. This implies that female spiders are generally receptive to matings over and above the first one.

Just because multiple mating occurs does not mean that multiple insemination (Parker 1970) is also occuring. Paternity data are required to confirm this. However multiple mating is a good indication that multiple insemination is also occurring and the latter certainly cannot occur without the former.

In conclusion, multiple mating in spiders is widespread and common.

5.2.1 Sex Ratios.

All other things being equal, a male-biased sex ratio will lead to more intensive sperm competition in a species. In *Latrodectus mactans* a male-biased sex ratio at birth was found by Montgomery (1908) with an average sex ratio of 8.19 : 1 (male : female). In the literature it is often cited that female-biased sex ratios are found in spiders in the wild state. This phenomenon is thought to be because of the longer lifespans of female spiders (Schæfer 1987). Examples of sex ratio values recorded are: *Clubiona robusta* 1: 2.3 (male : female) (Austin 1984) and the even higher sex ratio bias in a range of spiders in a Finnish forest of 1 : 3.5 (Huhta 1965). Rather lower figures to those above were found for *Cupiennius* species by Schuster *et al.* (1994); 1 : 1.2 in *C. salei*, 1 : 1.3 in *C. coccineus*, 1 : 1.6 in *C. getazi*. Consistently female-biased sex ratios are found in social spiders (Aviles 1986), but because the males are constantly in attendence there is ample opportunity for sperm competition.

However, such figures should be viewed with suspicion as regards their relevance to assessing the intensity of sexual competition. The number of males visiting a female's web will be determined by the operational sex ratio at the time of the female's receptive period, and this is not measured by the season averages that the figures above represent. In fact the operational sex ratio varies through the season and depends on when the census is taken (Oxford 1992).

5.2.2 Females' Sperm Storage Organs And Sperm Longevity

Virtually all spiders have specialized sperm storage organs, the spermathecae. Usually they are paired but there can be hundreds (Coyle 1983). In *Pholcus phalangioides,* despite there being no specialized spermathecae, fertile sperm can be stored for upwards of a year (Section 2.0). However, usually the sperm is stored in a special organ and presumably these are at least as efficient at sperm storage as is the case in *P. phalangioides.* In another primitive spider, *Atypus affinis,* sperm storage can also occur for a year (Bristowe 1958). In the more advanced spider, *Trite auricoma,* that has spermathecae, a similar sperm longevity has been reported (Forster & Forster 1973). Sperm storage efficiency of this order may be responsible for the reports of fatherless spiders in the literature (Valero 1970; Deeleman-Reinhold 1986; Gruber 1990).

As stated in the introductory chapter (Section 1.0), classically there are two types of spermathecae in spiders: cul-de-sac and conduit. The separation of the testes from the intromissive organ in spiders may affect the ability of the male to fill the spermathecae of the female. The charged palp represents a finite source on which to draw even if the testes could produce more sperm. If the palp reservoir volume is much bigger than that of the spermathecae then sperm competition could only happen if sperm removal occurs. We can therefore seek evidence regarding the ability of the male to fill the spermathecae of the female.

Huber (1995) found for *Anyphaena accentuata* that the volume of the sperm reservoir in the palp (6×10^6 μm^3) was four times larger than the volume of the spermatheca (1.5×10^6

μm^3) so one insemination could fill the spermatheca completely if all the available sperm was used. However he also showed that only 15% of the available sperm from the sperm reservoir was passed over to the female during a single copulation, thus leaving ample room for furthur inseminations. Interestingly, it was noted that *A. accentuata* did not multiply mate despite long second courtships (up to 3 hours), though this was only for a few observations. No evidence of sperm plugs to interfere with second matings was recorded either.

In *Clubiona pallidula* Huber (1995) observed that the respective sizes of the sperm reservoir and spermatheca were 2.2 x 10^6 μm^3 and 2.9 x 10^6 μm^3 which allows room for a second insemination even if all the sperm was passed over to the female during the first.

5.3 A Re-evaluation Of Current Measurements Of P_2.

Appendix Table A5.2 summarizes the data on P_2s for spiders that have been investigated so far. The data generally agree with the predictions of the Austad hypothesis. However a few anomolies are present; for instance, in *Agelena limbata* (Masumoto 1993) the sperm precedence pattern of a low P_2 was a result of sperm plugs and not spermathecal morphology. Indeed, when the plugs were absent P_2 could represent 60% or more of the brood.

Eberhard *et al* (1993) found in the Pholcid *Physocyclus globus* that the P_2 did not differ significantly from 50% and so random sperm mixing is not inconsistent with these data.

Oxford (1993) found in *Tegenaria saeva,* results not inconsistent with random sperm mixing despite the spermathecae being of a classically conduit type.

I also found in *Tetragnatha montana* (Section 3.0) that there was no second male sperm priority pattern as should be found if the hypothesis is correct. These observations are damaging enough to the hypothesis (Austad 1984). In the next sections we shall see what transpires from evidence gleened from mate-guarding and paternity assurance mechanisms.

5.4 Additional Sources Of Evidence Pertaining To Sperm Precedence: Peri-Reproductive Behaviours.

Because of the few direct P_2 measurements in spiders we are forced to use peri-reproductive behaviours to infer sperm priority and therefore assess the truth of Austad's (1984) hypothesis. One such behaviour is mate guarding. We would predict from the hypothesis that premate guarding should be associated with a conduit spermatheca, and postmate guarding associated with a cul-de-sac spermatheca, because of the predicted P_2s associated with these spermathecal types.

Another relevant behaviour is the application of mating plugs, which we would predict to be applied in cul-de-sac species and not in conduit species. This is because the ability to usurp paternity in the case of a cul-de-sac spermatheca is so much greater, and it would be expected that adaptations against this would be selected for. This is in opposition to the view of Austad (1984). Other peri-reproductive behaviours and their predicted distribution among cul-de-sac and conduit spermathecae are shown in Table 5.1.

5.4.1 Mate Guarding

Mate guarding in spiders has been analysed by Ridley (1983) from the perspective of precopulatory mate guarding being associated with moulting. However spiders also postmate guard. A range of definitions have been used in spiders as detailed below: these were the search terms used in compiling the data presented in Appendix Table A5.3:

Table 5.1 Reproductive corollaries of spermathecal architecture fixing P_2 in a polyandrous system[1].

Factor.[2]	Cul-de-sac	Conduit
Protandry[3].	No	Yes
Mate Guarding.	Post-mating	Pre-mating
P_2.	High	Low
Paternity Assurance Mechanisms.[4]	Yes	No
Variation In Mating Success For Males.[5]	Low	Low

[1] This table is repeated here from Table 1.3 for convieniance.

[2] Explanations of the statements made in the table are given in the text.

[3] Protandry is the earlier maturation of males compared with females, this is not discussed in the text because the data for protandry are essentially the same as that for mate guarding.

[4] Eg Mating plugs.

[5] This factor does not differentiate between the two types of spermathecal morphology but because of sperm stratification the expected variation in P_2 is low - assuming all males transfer enough sperm to fertilize all the female's eggs, at least in the first batch.

(i) **Cohabiting** (Jackson 1986a) **or the Suitor Phenomenon** (Robinson & Robinson 1980): When male and female non-social spiders coexist in close proximity around the mating season - up to and including 2 moults before the final moult.

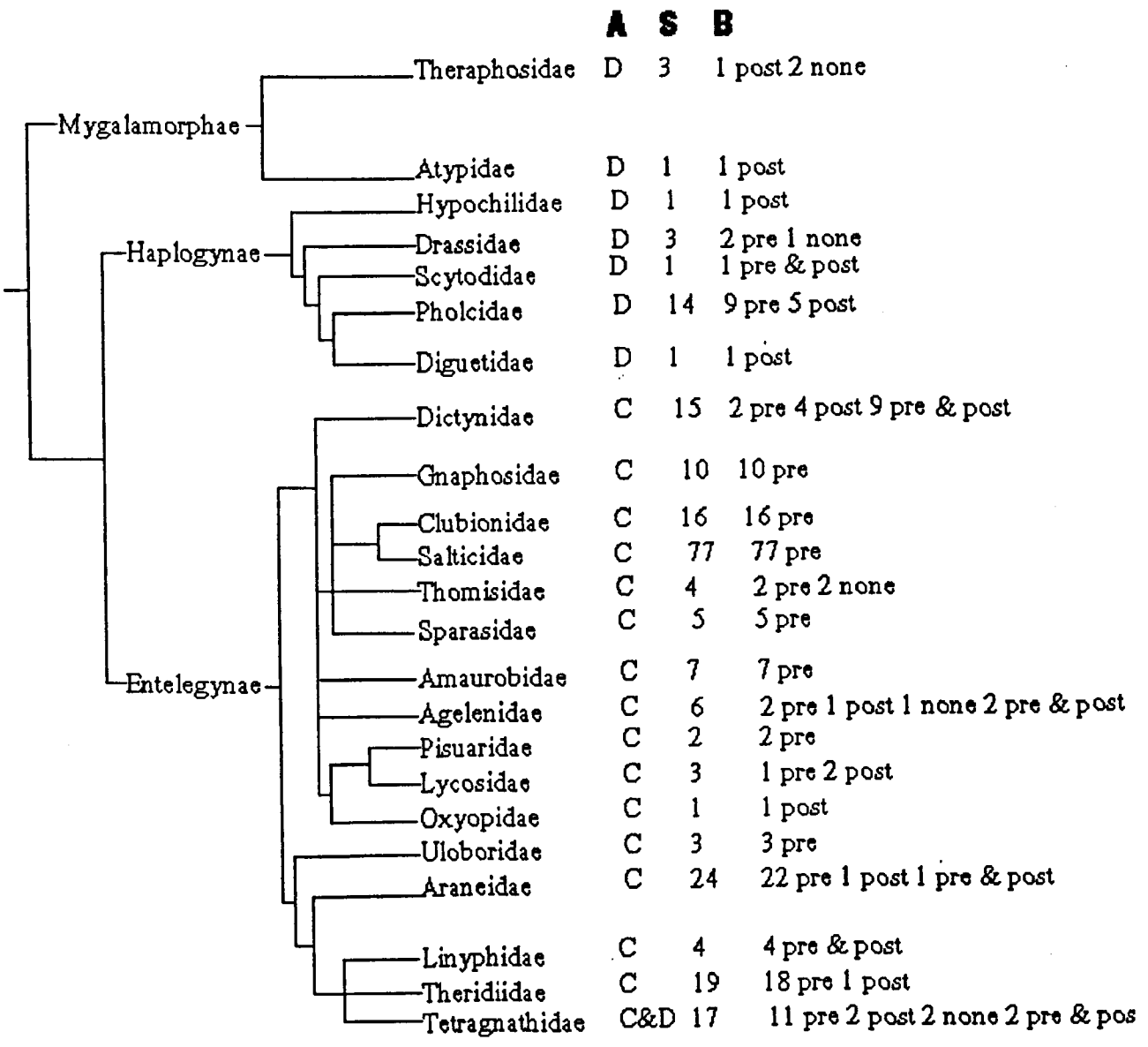
(ii) **Mate guarding**: When males stay with females and actively fight off other males seeking contact with the female.

(iii) **Premate guarding**: As (ii) but guarding only before the female matures.

(iv) **Postmate guarding**: As (ii) but guarding only after the female matures and the male has mated with her.

The hypothesis proposed by Austad (1984) would predict that conduit spiders premate guard because of the low P_2 that is assumed to be associated with this type of spermatheca. On the other hand cul-de-sac spiders should postmate guard, if they guard at all, to avoid cuckoldry from subsequent males. Ridley (1983) found that the primitive state in spiders was not to guard and that premate guarding evolved 4 times. Presented here is a much larger data set featuring some 236 species (Appendix Table A5.3). The phylogenetic distribution of mate guarding is shown in Fig 5.1. The figure reveals that the distribution of mate guarding is not as Austad's hypothesis would predict because all kinds of mate guarding types are associated with conduit species. The cul-de-sac species mostly postmate guard, if they mate guard at all, as predicted. However there are some anomalies even here.

Fig 5.1 Phylogeny of Families of Spider for Which Mate Guarding Data Exist



A = Spermathecal Architecture: D = Cul-de-sac; C = Conduit

S = Number of species

B = Mate-guarding behaviour: Pre, Post, Pre & Post or None

Phylogeny based on Coddington & Levi (1991)

5.4.2 Grasping Organs: A Novel Suggestion For Their Evolution

Prehensile organs have long been recognised as functioning during mating to keep the male and female together (Darwin 1871). Grasping organs for this purpose have evolved many times in the spiders (Appendix Table A5.4). Unlike the situation in amplexus[1] (Manning 1975) these organs are not used for extended periods of time but only at mating. They seem to function as a mechanical means of keeping the pair together so that palp insertion can occur. Thus these organs do not seem to be functioning in mate guarding.

The palp itself is often used in grasping the females' epigyne where it is present and primitively this may have been a means of pulling the female over a spermatophore, as is the case in scorpions (Cloudsley-Thompson 1968). From here the male palp evolved into an intromissive organ.

Bristowe (1958) observed a mating involving a freshly moulted male *Micrommata virescens* in which the male tried to apply his palp to the female and grip with the tibial spur in the chitinous pockets of the female's epigyne. The male had just moulted and thus the spur was soft and so the mating could not continue. In mature individuals the spur goes straight in and mating is completed.

In Appendix Table A5.4, 20 species are recorded as using grasping organs: 10 conduit species and 10 cul-de-sac species, all independently evolved[2]. Given the greater number of conduit species overall, this distribution suggests that grasping organs are

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1 A passive phase where the male remains attached to the female without true genital contact.

2 This is known because they all use different mechanisms to grasp the female.

probably more common in the cul-de-sac species which have a simpler epigyne, or none at all; thus they need the secondary purchase to hold the the palp in place during mating .

In addition, because the cul-de-sac species on the whole do not alternate the palps and thus there is no scope for copulatory courtship (Eberhard 1991), the male has to hold the palp in place using grasping organs to effect full transfer of sperm, which the female may not allow if not grasped. Thus this provides a selection pressure on the grasping organ to be perfected in the face of trial by the female.

5.4.3 Mating Plugs

Mating plugs in spiders have evolved at least 4 times and involve different mechanisms: secretion, embolus tip, embolus cap and scape[3] removal. The secretions may be further divided into sperm plugs, resinous material and waxy coating (Appendix Table A5.5). These latter distinctions may just reflect various descriptions of the same phenomena by different authors.

Plugging is a widespread phenomenon with 51 species in 14 families recorded here as utilizing them (Fig 5.2). This is almost certainly an underestimation of the frequency of plugging. Some plugs, as in *Latrodectus* species (Abalos & Baez 1963), are formed deep within the epigynal tubes and are not obvious externally. The occurrence of cryptic plugs of this sort has not been extensively studied. The cul-de-sac species might also utilize cryptic plugs although this has not been investigated.

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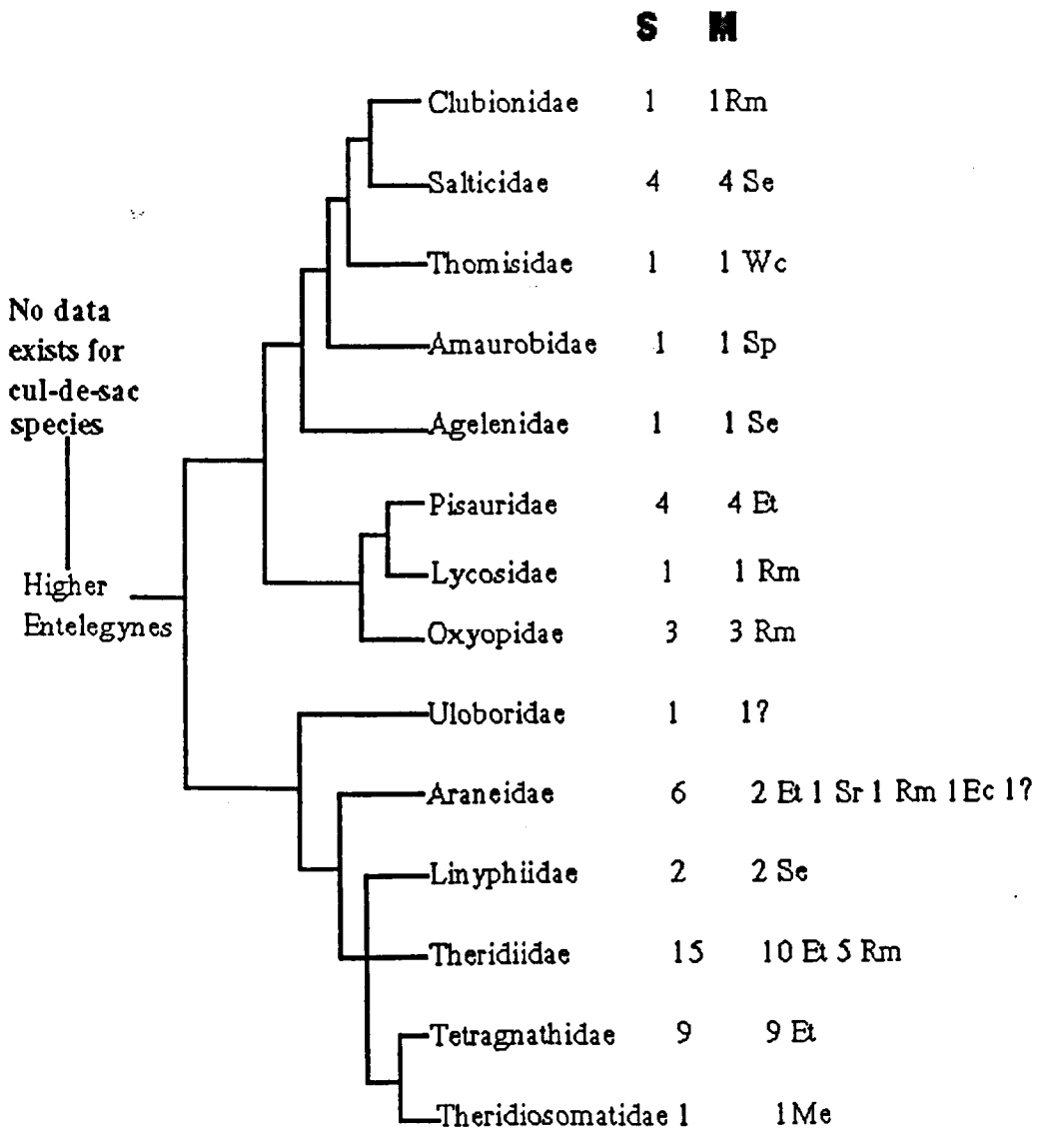
[3] A scape is a structure protruding from the epigyne that looks like an elephant's trunk (see glossary).

Fig 5.2 Phylogeny of Families of Spider for Which Mate-plugging Data Exist



S = Number of species

M = Mechanism of mating plug: Ec = Embolus cap, Et = Embolus tip, Me = Membrane over the epigyne, Rm = Resinous material, Se = Secretion, Sp = Sperm plug, Sr = Scape removal, Wc = Waxy coating

Phylogeny based on Coddington & Levi 1991

Fifty of the plugs were reported in conduit species and only 1 in cul-de-sac species. This pattern is opposite to that expected by the Austad (1984) hypothesis, despite what Austad (1984, page 235) claims about plugging in spiders. This is because in the conduit spiders first males are meant to be assured of a high level of paternity without the use of plugs. This is especially the case when one considers how costly some of the plugs are to make. For example, loss of the embolus tip may preclude, or reduce the efficiency of, further matings. When using plugs the males are often not assured of paternity (see Section 5.2) but presumably they enhance their chances.

The use of plugs may not be as expected because they are the **mechanism** whereby the first male priority pattern is established. This is certainly the case in *Agelena limbata* (Masumoto 1993). Another reason may be because a complex, chitinous epigyne is essential for the blocking of the copulatory pore. Of fundamental importance is the number of spermathecal ducts. In the case of the cul-de-sac architecture plugging may not be possible because the sperm have to come out of the same pore as they went in making plugging non-viable. For plugs to have evolved in at least some conduit species there must be a bias towards second male priority without them. Thus the spermathecal structure is having the opposite influence to that postulated by Austad (1984). Either this or a number of other mechanisms are functioning to lead to second male priority without the plug. These considerations mean that the picture is a lot more complicated than Austad suggests, with priority patterns constituting a species-specific characteristic just as the spermathecae are themselves.

Watson (1991a, b) found mating plugs not to be responsible for the first male sperm priority in the Sierra dome spider, *Linyphia litigiosa*, however this conclusion was not based on extensive morphological investigation but that the mating behavior was of a normal kind.

5.4.4 Single Palp Usage

Single palp usage is an interesting phenomenon because it means males are not totally utilizing the sperm storage space available and are therefore opening themselves to sperm competition (Appendix Table A5.6). This is because in spiders there are typically two spermathecae and one palp services one spermatheca and the other palp services the other spermatheca. There may be several reasons why males only insert one palp. Firstly, it could be a way of saving costly sperm for further copulations (Daly 1978; Dewsbury 1982) so that the male does not have to sperm induce again (Montgomery 1903). Secondly, it could be a way of conserving the integrity of the palp to ensure the possibility of a second mating in those species where a part of the palp is left in the epigyne or spermatheca of the female. Thirdly, it may be because it is impossible to insert the palp because of an existing block deposited by a prior mating male (Patel & Bradoo 1989), though this would only apply to matings after the first. Finally, in the case of *Tidarren fordum* it is because the two palps are too large to use and one is bitten off before sperm induction (Bristowe 1958).

5.5 The Logic Of Stratification

The rules translating copulations into offspring may not be as simple as Austad suggests if sperm stratification is the mechanism whereby a P_2 is established. This is because, logically, depending upon the spermathecal architecture high and low values of P_2 can be

found in cul-de-sac and conduit spermathecae (Table 5.2). As shown in the table, given the logical outcome of stratification, the shape of the spermathecae in the case of cul-de-sac (type I & II) and the relative positions of the tubes in the case of conduit (type III & IV) gives in the case of type II and type III the opposite P_2 prediction to that suggested by Austad (1984).

The epigyne of Entelegyne spiders is a structure which promotes stratification of the sperm from respective males because it does not permit the bulk of the pedipalp to enter the *bursa copulatrix,* never mind the spermathecae. This may provide a selection pressure on the embolus to be longer in length so that it can reach the spermathecae. This in turn may provide a selection pressure, in the form of an arms race, for the sperm duct to lengthen. This may be what has resulted in the very lengthy sperm ducts found in some members of the Theridiidae (Roberts 1985).

5.6 The Breakdown Of Stratification

Stratification as a sperm precedence mechanism is possible, as in birds (Birkhead & Møller 1992). Whether it happens in spiders is another matter (Table 1.2). Certainly the breakdown of stratification is possible given the extent to which the embolus (intromissive part of the palp) penetrates the spermathecae in some species[4]. For example the embolus penetrates deep into the spermatheca in the case of *Anyphaena accentuata* (Huber 1995). If this species multiply mated then sperm stratification would be broken down. Uhl *et al* (1995) also showed that in *Pholcus phalangioides* the palp can penetrate into the sperm storage

[4] Entry of the embolus into the spermatheca can still lead to stratification if sperm of the second male is layered under that of the first.

organ and therefore stratification cannot be the reason for the high P_2 in this species (Section 2.0). In some Theridiids the embolus tip is left in the spermathecae demonstrating that it penetrated thus far (Bhatnagar & Rempel 1962; Abalos & Baez 1963). Coyle & O'Shields (1990) also showed that within the mygalomorphs palp elements enter the spermathecae, again possibly ruling out stratification of the sperm. Bhatnagar & Sadana (1963) also found in the wolf spider *Lycosa chaperi* that the embolic tip penetrates into the opening of the spermatheca, meaning sperm displacement is a real posibility. Blest & Pomeroy (1978) found in the Linyphiidae that the length of the spiral mating ducts is correlated with the length of the filiform emboli and is consistent with the the emboli penetrating into the spermathecae as has also been proposed by van Helsdingen (1970).

Interestingly some of the above examples[5] have been of cul-de-sac species where, if Austad is correct, one would not expect there to be any advantage to second mating males of penetrating into the spermathecae because the fertilization set is meant to be near the entrance (= exit). Instead the entrance of the palps into the sperm storage structures in cul-de-sac species suggests a sperm removal or displacement function, as is common in the insects (Thornhill & Alcock 1983). The complex embolic elements which are hook-like in *Tetragnatha* (Roberts 1985) may function in this way. In spiders there is no empirical evidence for sperm removal.

Stratification may also be broken down if the pressure under which the sperm are ejaculated into the spermathecae is high. This would be advantageous to the second male in conduit type spiders because he would get his sperm nearer to the fertilization set.

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5 *Pholcus phalangioides* and the Mygalomorphs.

Finally stratification may be broken down if the sperm are stored for a long time (as is often the case, see Section 5.2.2) because the longer the sperm are stored the more likely it is that they jostle about, despite the probability of encystment.

5.7 Sperm Utilization Strategies: Phyletic Limitation Or Adaptation?

Even a casual inspection of the abundant literature assembled by taxonomists on the morphology of the spermathecæ reveals a wealth of structural variation (eg. Wunderlich 1987). The morphology of the spermathecæ is almost invariably species-specific, which is why the character has attracted the attention of taxonomists. Such variability is at odds with the theory postulated by Austad (1984), which claimed a phyletic limitation to sperm utilization strategies based on spermathecal architecture.

There are many ways in which the architecture of the spermathecae can influence the P_2 of a species besides being conduit or cul-de-sac:

(i) The volume of the spermathecae relative to the sperm reservoir: if a male inseminates all of the sperm from his sperm reservoir, and if the spermathecae does not have the capacity to allow more than one male's sperm in then, if sperm removal does not occur, this will lead to a low P_2 no matter what the spermathecal architecture is like.

(ii) If spermathecae are of type I (Table 5.2) then they should lead to a high P_2 if stratification is the determining mechanism in the ejaculate competition. However, the more

spherical the spermathecae the less likely it is that the sperm from a second male can monopolize the fertilization set, so this would lead to a lower P_2.

(iii) If the spermathecae are of type IV (Table 5.2) then they should behave like conduit spermathecae as Austad suggests. However, given stratification, then if the spermathecae are of type III (as is often the case *eg.* in Theridiids) then logically they should lead to a high P_2 which is the opposite prediction to that suggested by Austad.

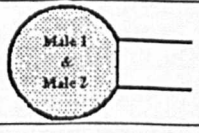
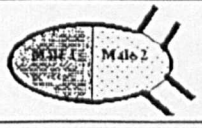
(iv) The shorter the mating duct relative to the embolus the more likely it is that the male has access to the spermathecae to remove the first male's sperm or displace it in appropriate ways. This would lead to a high P_2 no matter what the spermathecal architecture was like.

Thus there are many ways in which the spermathecae and associated ducts can be modified over evolutionary time to affect the P_2 in favour of the female's preference.

The various aspects of spermathecal morphology discussed above may suggest that they have undergone rapid and divergent evolution as a result of sexual selection. This hypothesis parallels that developed by Eberhard (1985) to explain why the intromittent organs of males are complex and species-specific. An example of the high species specificity of spermathecae is provided by the *Latrodectus* group, in which the females are indistinguishable except for their spermathecal structures, which are very different between species (Levi 1959).

The sperm storage organs of animals represent the last arena in which sexual selection can occur and the female cannot be regarded as an inert environment (Baker & Bellis 1995). However there are theoretical problems with a non-species-specific approach to sperm precedence patterns (Parker 1984). This is because there are two opposing forces in sperm competition, one favouring paternity assurance mechanisms and one favouring mechanisms for paternity usurping, and the strength of these may differ among species. Waage (1984) also pointed out the role of the female in this now tripartite evolutionary arms race (Dawkins & Krebs 1979). Thus one would expect rapid and divergent evolution of morphological characters and physiological mechanisms driven by competition between rival ejaculates.

Table 5.2 Proposed Theory Of Spermathecal Architectural Determination Of Sperm Precedence Patterns In Spiders.

Spermathecæ Type:	Cul-de-Sac[1]		Conduit[2]	
Description:	Elongate spermathecæ.	Spheroid spermathecæ.	Fertilization and mating tubes close.	Fertilization and mating tubes distant.
Diagram of spermathecæ:				
Predicted P_2:	High P_2	Low P_2	High P_2	Low P_2
Name:	Type I	Type II	Type III	Type IV

[1] This mechanism of spermathecal influence on the precedence pattern is analogous to that proposed by Walker (1980).

[2] This mechanism is novel.

Appendices

Appendix Table A5.1 Polyandrous Spiders.

This table is an expansion of table VI of Austad (1984) Those papers already cited by Austad are asterisked (*). If the original family designation by the author was different and according to an out of date classification it is included in brackets after the modern familial designation.

Species	Family	Spermathecæ	Nature Of Evidence	Notes	Authority
Geotrecha pinnota	?	?	Laboratory observations.		Montgomery 1910
Agelena limbata	AGELENIDÆ	Conduit	Observations of wild and captive females.	41 females, observed in the wild: 2 did not mate, 27 mated once, 12 mated more than once (30.8%).	Matsumoto 1991 Matsumoto 1993
Agelenopsis aperta	AGELENIDÆ	Conduit		After oviposition	Riechert unpublished*
Tegenaria derhami	AGELENIDÆ	Conduit		After oviposition	Montgomery 1903*
Tegenaria saeva.	AGELENIDÆ	Conduit	Electrophoretic.	Multiple paternity in the wild and the laboratory.	Oxford 1993
Ixeuticus longinuus	AMAUROBIDÆ	Conduit	Laboratory observations.	Repeated observations of matings with one male.	Gregg 1958
Araneus ssp. *A.cornutus* *A.patagiatus* *A.scopetarius* *A.umbraticus*	ARANEIDÆ	Conduit	Circumstantial.	Evidence from embolus cap displacement.	Levi 1975*
Araniella cucurbitina	ARANEIDÆ	Conduit	Circumstantial.	Species do not have an embolus cap and epigyne therefore does not get blocked.	Blanke 1973 (Cited in Levy 1975)
Argiope argenta	ARANEIDÆ (ARGIOPIDÆ)	Conduit - spheroid	Many male palpal apical elements may be found in one spermathecæ.		Abalos & Baez 1963
Gasterocantha cancriformis	ARANEIDÆ	Conduit -	Observation of female that mated a second time with a male reintroduced to her.	Before egg laying.	Robinson & Robinson 1980*
Zygiella x-notata	ARANEIDÆ	Conduit - Spheroid	Observations of captive individuals.		Yoward unpublished data
Castianeira longipalpus	CLUBIONIDÆ	Conduit		Before egg laying.	Montgomery 1903*

Dictyna calcarata	DICTYNIDÆ	Conduit			Jackson 1978c*
Dictyna sublata	DICTYNIDÆ	Conduit			Montgomery 1903*
Mallos trivittatus	DICTYNIDÆ	Conduit			Jackson 1978c*
Porrhothele antipodiana	DIPLURIDÆ	Conduit			Jackson *et al* 1981*
Thelechoris karschi	DIPLURIDÆ	Cul-de-sac	Laboratory study.		Coyle & O'Shields 1990
Ixceuticus martius	DISIDÆ (AMAUROBIDÆ)	Conduit	Observation of wild matings.	High incidence of multiple mating: 85% of 20 trials. This is despite mate guarding by males.	Willey 1992 Costa 1993
Dysdera crocata	DYSDERIDAE	Cul-de-sac	Laboratory study.	In Cloudsley-Thompson (1949) study - multiple mating was with same male. Females were always seen to be receptive to males in a study by Pollard *et al* (1987).	Cloudsley-Thompson 1949 Jackson *et al* 1981* Pollard *et al* 1987
Stegodyphyus sarasinorum	ERESIDÆ	Conduit	In wild many males court a female simultaneously.	The embolus does not break off and plug the female's epigyne. Low sex ratio (males to females) in this social species.	Bradoo 1979
Drassodes lapidosus	GNAPHOSIDÆ (DRASSIDÆ)	Conduit	Laboratory and field study.	Female will mate with a second male directly after the first.	Bristowe 1929 Bristowe 1958
Ceratinopsis interpres	LINYPHIIDÆ	Conduit	Laboratory observations.		Montgomery 1910*
Erigonioium gramincola	LINYPHIIDÆ	Conduit		6 generations per year. Females can lay and fertilize all their ~300 eggs from one mating but usually multiply mate before laying their eggs.	Zhao & Liu 1982
Frontinella pyramitela	LINYPHIIDÆ	Conduit	Laboratory study.		Austad 1982*
Linyphia triangularis *Linyphia hortensis*	LINYPHIIDÆ	Conduit	Laboratory study.		Stumpf 1990

Prolinyphia marginata	LINYPHIIDÆ	Conduit	Electrophoretic.	Evidence shows that the last male does not preempt previously stored sperm. From females which were caught with a mating partner in the wild.	Martyniuk & Jænike 1982*
Lycosa chaperi	LYCOSIDÆ	Conduit	Laboratory observations.	1 female was observed to mate with 10 males in one day.	Bhatnagar & Sadana 1965
Lycosa rabida	LYCOSIDÆ	Conduit	Laboratory observations.		Montgomery 1903*
Lycosa tarentula fasciiventris	LYCOSIDÆ	Conduit	Laboratory observations.		Fernandez-Montraveta & Ortega 1990
Lycosa stonei	LYCOSIDÆ	Conduit	Laboratory observations.		Montgomery 1903*
Oonops pulcher	OONOPIDÆ	Cul-de-sac	Observation of laboratory matings. Female mated twice in the same evening.	Multiple mating before egg laying.	Bristowe 1929
Philodromus rufus	PHILODROMIDÆ	Conduit		Before egg laying.	Dondale 1964*
Pholcus phalangioides	PHOLCIDÆ	Cul-de-sac spheroid	Observations of wild and captive individuals.	Montgomery 1903 (cited by Austad 1984) that this species would only mate with a second male after oviposition but this is not the case.	Yoward unpublished data Montgomery 1903* Miyashita 1988a Platel 1989 Reagan & Reagan 1989 Uhl 1993a, b
Pisaura mirabilis	PISAURIDÆ	Conduit	Females will remate up to a day before egg deposition.		Austad & Thornhill 1986
Corythalia fulgipedia	SALTICIDÆ	Conduit		Mating takes place up to 4 months after final moult of the female.	Crane 1949
Holoplatys sp.	SALTICIDÆ	Conduit			Jackson *et al* 1981*
Metaphidippus galathea	SALTICIDÆ	Conduit	Electrophoretic.	One female in a sample of three had a significant deviation from an expected ratio in one isozyme (scored for 8 variable isozymes).	Steiner & Greenstone 1991.

Myrmarachne lupata	SALTICIDÆ	Conduit	Laboratory studies.	Females mated with attending males but would also mate with subsequently presented males.	Jackson 1982a
Neon valentulus	SALTICIDÆ	Conduit			Wild 1969*
Phidippus johnsoni	SALTICIDÆ	Conduit	Laboratory studies.		Jackson 1980*
Phidippus purpuratus	SALTICIDÆ	Conduit	In laboratory females will multiply mate.		Montgomery 1910*
Portia ssp: *P.fimbriata* *P.labiata* *P.schultzi*	SALTICIDÆ	Conduit	Laboratory studies.	*P.labiata* and *P.schultzi* were more receptive to remating than *P.fimbriata.*	Jackson *et al* 1981* Jackson & Hallas 1986
Sicarus spp.	SICARIIDÆ	Cul-de-sac		Before egg laying.	Levi 1968*
Herennia	TETRAGNATHID Æ	Conduit	Wild observations.	Males mate only once and try to stop females from mating again.	Robinson & Robinson 1978
Nephila clavipes	TETRAGNATHID Æ	Conduit - tubes together	In wild.		Robinson & Robinson 1973 Vollrath1980b Christenson 1990
Nephilengys ssp.	TETRAGNATHIDÆ	Conduit		Males mate only once and try to stop females from mating again.	Robinson & Robinson 1978
Pachygnatha spp.	TETRAGNATHIDÆ	Cul-de-sac		Before and after egg laying.	McCook 1890*
Tetragnatha spp.	TETRAGNATHIDÆ	Cul-de-sac - tubular		Before and after egg laying.	McCook 1890*
Tetragnatha montana *Tetragnatha extensa*	TETRAGNATHIDÆ	Cul-de-sac - tubular	Observations of wild and captive individuals.	Males hold jaws of females so even if the females are unreceptive and try to bite males it is very difficult for them to avoid mating.	Yoward unpublished data West & Toft 1989
Aphonopelma hentzi	THERAPHOSIDÆ	Cul-de-sac		Before egg laying.	Bærg 1963*
Dugesiella hentzi	THERAPHOSIDÆ	Cul-de-sac		Before egg laying.	Petrunkevitch 1911*
Eurypelma californica	THERAPHOSIDÆ	Cul-de-sac		Before egg laying.	Bærg 1928*

Achœaranœ trepidariorum	THERIDIIDÆ	Conduit		Will remate at any time except when very gravid.	Montgomery 1910*
Argyrodes ululans	THERIDIIDÆ	Conduit	Field observations.	Short mating time.	Cangialosi 1990
Ceratinopsis interpres	THERIDIIDÆ	Conduit	Observations of wild individuals.	27 males were observed to mate with one female in one day.	Montgomery 1910
Latrodectus species: *L. mactans, L. varians, L. hesperus, L. curacaviensis L.geometricus L.hasselti.*	THERIDIIDÆ	Conduit	Many male palpal apical elements may be found in one spermathecæ (Abalos & Bæz 1963) .	As many as eight males have been observed in a single web of female *Latrodectus mactans.*	Abalos & Bæz 1963 Jackson *et al* 1981* Kaston 1970* Petrunkevitch 1911
Metepeira sp.	THERIDIIDÆ	Conduit	Many male palpal apical elements may be found in one spermatheca.		Abalos & Bæz 1963
Xysticus ferox	THOMISIDÆ	Conduit		Before egg laying.	Montgomery 1903*
Uloborus sp.	ULOBORIDÆ	Conduit - Poor diagram by Bradoo 1979	In wild many males court a female simultaneously.	Males do not fight over the females (social species).	Bradoo 1979

Appendix Table A5.2 Sperm Precidence Patterns in Spiders.

Species	Family	Spermathecæ	Evidence	P_2	Notes	Authority
Agelena limbata	AGELENIDÆ	Conduit	X-ray sterilization	Low	Mixed results because the observed precedence pattern seemed to be caused by mating plugs. When the plug was absent a second male could farther 60%+ of the brood.	Masumoto 1993
Tegenaria spp.	AGELENIDÆ	Conduit	Electrophoresis	Low	Evidence for complete sperm mixing in some cases.	Oxford unpublished data.
Frontinella pyramitela	LINYPHIDÆ	Conduit	X-ray sterilization	Low		Austad 1982
Linyphia hortensis	LINYPHIDÆ	Conduit	X-ray sterilization	Low	Second males fertilize on average 21% of the eggs of a female. (n= 23)	Stumpf 1990
Linyphia litigiosa	LINYPHIDÆ	Conduit	Electrophoresis	Low		Watson 1990 Watson 1991a Watson 1991b
Prolinyphia marginata	LINYPHIDÆ	Conduit	Electrophoresis	Low?	Study conducted on wild collected females collected with guarding males.	Martyniuk & Jænike 1982
Pholcus phalangioides	PHOLCIDÆ	Cul-de-sac	Electrophoresis	High		Yoward unpublished data.
Physocyclus globus	PHOLCIDÆ	Cul-de-sac	X-ray sterilization	Level		Eberhard *et al* 1993
Phidippus johnsoni	SALTICIDÆ	Conduit	X-ray sterilization	Low		Jackson 1980
Nephila clavipes	TETRAGNATHIDÆ	Conduit Spheroid	X-ray sterilization	Low	A large number of sterile eggs in the control treatments.	Vollrath 1980
Nephila clavipes	TETRAGNATHIDÆ	Conduit Spheroid	X-ray sterilization	Low		Christenson & Goist 1979 Christenson 1990
Tetragnatha montana	TETRAGNATHIDÆ	Cul-de-sac	Electrophoresis	Level		Yoward unpublished data.

Appendix Table A5.3 Cohabitation In Spiders

Species.	Family.	Spermatheæ type.	Pre / post mate guarding / suitor phenomenon / cohabiting / no mate guarding.	Notes.	Authority.
Agelena consociata	AGELENIDÆ	Conduit	Pre	Males are more aggressive to other males in the presence of penultimate females.	Krafft 1970a
Agelena labyrinthica	AGELENIDÆ	Conduit	Pre	Specialized retreat for cohabitation.	Sörensen 1880 Nielsen 1932
Argyroeta ?	AGELENIDÆ	Conduit	Post		Bristowe 1958
Tegenaria guyoni	AGELENIDÆ	Conduit	No mate guarding		Campbell 1883
Tegenaria ssp. *T. saeva* *T. gigantea*	AGELENIDÆ	Conduit	Pre & Post	The male and female cohabit in the same silk shelter.	G.S.Oxford pers. com.
Badduma longinguus	AMAUROBIDÆ	Conduit	Pre		Jackson 1986a
Baduma insignis	AMAUROBIDÆ	Conduit	Pre		Jackson 1986a
Baduma hygrophila	AMAUROBIDÆ	Conduit	Pre		Jackson 1986a
Cambridgea antipodiana	AMAUROBIDÆ	Conduit	Pre		Jackson 1986a
Cambridgea foliata	AMAUROBIDÆ	Conduit	Pre		Jackson 1986a
Desis marina	AMAUROBIDÆ	Conduit	Pre		Jackson 1986a
Ixectius martius	AMAUROBIDÆ (DISIDÆ)	Conduit	Pre	Males mate with females immediately after they moult. Females stay receptive to different males though they may eat incumbent males who try to ensure the success of their mating from other males.	Costa 1993
Araneus anatipes	ARANEIDÆ	Conduit	Pre		Jackson 1986a

Araneus cornutus	ARANEIDÆ	Conduit	Pre	Male and immature female cohabit all winter in a special chamber.	Savory 1928 Neilsen 1932 Locket & Millage 1953
Araneus diadematus	ARANEIDÆ	Conduit	Post		Gerhardt 1924
Araneus inustus	ARANEIDÆ	Conduit	Pre		Jackson 1986a
Araneus quadratus	ARANEIDÆ	Conduit	Pre		Savory 1928 Neilsen 1932
Argiope anasuja	ARANEIDÆ	Conduit	Pre		Jackson 1986a
Argiope aurantia	ARANEIDÆ	Conduit	Pre		Robinson & Robinson 1980 Jackson 1986a
Argiope katherina	ARANEIDÆ	Conduit	Pre		Jackson 1986a
Argiope sp.	ARANEIDÆ	Conduit	Pre		Jackson 1986a
Argiope spp. *A. æmula* *A. reinwardti* *A. argentata* *A. aurantia*	ARANEIDÆ	Conduit	Pre - "suitor phenomenon"	Males copulate with freshly moulted females (forced copulations / opportunistic matings). Possibly similar to crustacean situation.	Robinson & Robinson 1978 Robinson & Robinson 1980
Argiope trifasciata	ARANEIDÆ	Conduit	Pre		Jackson 1986a
Argiope trifasciata	ARANEIDÆ	Conduit	Pre & Post		Eberhard *et al* 1993
Argiope ætherea	ARANEIDÆ	Conduit	Pre		Jackson 1986a
Cyclosa bifida	ARANEIDÆ	Conduit	Pre		Jackson 1986a
Cyclosa trilobata	ARANEIDÆ	Conduit	Pre		Jackson 1986a
Deliochus zelivira	ARANEIDÆ	Conduit	Pre		Jackson 1986a
Epeira benigna *Epeira insularis* *Epeira trifolium*	ARANEIDÆ	Conduit	Pre		McCook 1890 Montgomery 1910
Gasteracantha minax	ARANEIDÆ	Conduit	Pre		Jackson 1986a

Gasterocantha cancriformis	ARANEIDÆ	Conduit	Pre	An extensive field study by Eberhard *et al* 1993 observed guarding only of females about to moult. Robinson & Robinson (1980) Claimed this species did not exhibit the "suitor phenomenon".	Eberhard *et al* 1993 Robinson & Robinson 1980
Metapeira intercrassata	ARANEIDÆ	Conduit	Pre	Large males have fighting advantage.	Forkner & Uetz 1993
Metepeira labyrinthea	ARANEIDÆ	Conduit	Pre		Jackson 1986a
Zygiella x-notata	ARANEIDÆ	Conduit - Spheroid	Pre		Yoward Unpublished
Atypus affinis	ATYPIDÆ	Cul-de-sac	Post	Throughout autumn and winter males cohabit in the retreat of the female.	Bristowe 1958 Clark 1969
Anyphaena accentuata	CLUBIONIDÆ	Conduit	Pre	Mating takes place soon after the females moult.	Bristowe 1958
Cheiracanthium ? gracile	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Cheiracanthium gilvum	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Cheiracanthium inclusum	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Cheiracanthium melanostomum	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Cheiracanthium sp.	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Cheiracanthium siedlitzi	CLUBIONIDÆ	Conduit	Pre		Berland 1927
Cheiracanthium stratioticum	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Clubiona cada	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Clubiona californica	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Clubiona cambridgei	CLUBIONIDÆ	Conduit	Pre	Pollard *et al* (1987) claim females are not receptive to second matings.	Jackson 1986a Pollard *et al* 1987
Clubiona clima	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a

Clubiona contrita	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Clubiona huttoni	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Clubiona papuana	CLUBIONIDÆ	Conduit	Pre		Jackson 1986a
Arangina cornigera	DICTYNIDAE	Conduit	Pre		Jackson 1986a
Dictyna ablopilosa	DICTYNIDAE	Conduit	Pre (post)	Field study of distribution of 5 males, males stayed with immature and mature females.	Jackson 1977b Jackson 1986a
Dictyna annexa	DICTYNIDÆ	Conduit	Pre (post)	Field study of distribution of 3 males, males stayed with immature and mature females.	Jackson 1977b Jackson 1986a
Dictyna arundinacea	DICTYNIDÆ	Conduit	?	Cohabit for 1 month plus.	Bristowe 1958
Dictyna calcarata	DICTYNIDÆ	Conduit	Pre (post)	Field study of distribution of 16 males, males stayed with immature and mature females.	Jackson 1977b Jackson 1986a
Dictyna coloradensis	DICTYNIDÆ	Conduit	Pre (post)	Field study of distribution of 16 males, males stayed with immature and mature females.	Jackson 1977b Jackson 1986a
Dictyna completa	DICTYNIDÆ	Conduit	Post	Single male observed with a female.	Jackson 1977b Jackson 1986a
Dictyna phylax	DICTYNIDÆ	Conduit	Pre (post)	Field study of distribution of 3 males, males stayed with immature and mature females.	Jackson 1977b Jackson 1986a
Dictyna tridentata	DICTYNIDÆ	Conduit	Pre (post)	Field study of distribution of 11 males, males stayed with immature and mature females.	Jackson 1977b Jackson 1986a
Dictyna volupis	DICTYNIDÆ	Conduit	Post	Mature males and females stay together on the web.	Montgomery 1910
Mallos niveus	DICTYNIDÆ	Conduit	Pre (post)	Field study of distribution of 21 males, males stayed with immature and mature females.	Jackson 1977b Jackson 1986a

Mallos trivittatus	DICTYNIDÆ	Conduit	Pre (post)	Field study of distribution of 62 males, males stayed with immature and mature females. Semi-social species.	Jackson 1977b Jackson 1978 Jackson 1986a
Paradictyna ilami	DICTYNIDÆ	Conduit	Pre		Jackson 1986a
Paradictyna rufoflava	DICTYNIDÆ	Conduit	Pre		Jackson 1986a
Dictina volumipes	DICTYNIDÆ	Conduit	Post	Male stays with female for a long time after mating.	Starr 1988
Diguetetia albolineata	DIGETIDÆ	Cul-de-sac	Post	Males did not preferentially mate with virgin females and males cohabitated with mature females. Sometimes (rarely) males cohabited with immature females, though they did not stay with females until they moulted.	Eberhard *et al* 1993
Drassus neglectus	DRASSIDÆ (DYSDERIDÆ)	Cul-de-sac	Pre	Males seal immature females into nests and mate just after they moult.	Montgomery 1910
Prosthesima atra	DRASSIDÆ (DYSDERIDÆ)	Cul-de-sac	Pre	Males hold subadult females and stay with them until they mature.	Montgomery 1910
Dysdera crocata	DRASSIDÆ (DYSDERIDÆ)	Cul-de-sac	Never cohabit		Cooke 1966
Anzacica gemmæ	GNAPHOSIDÆ	Conduit	Pre		Jackson 1986a
Drassodes lapidosus	GNAPHOSIDÆ	Conduit	Pre	The males cohabit with the females in the same silken cell when both are immatures. The males then mature before the females and stay with them until they mature. Males mate with females when freshly moulted. Females stay receptive after mating with first male.	Bristowe 1929 Bristowe 1958
Drassodes neglectus	GNAPHOSIDÆ	Conduit	Pre	Male and female live together in a nest.	Emerton 1890 Jackson 1986a

Gnaphosa brumalis	GNAPHOSIDÆ	Conduit	Pre		Jackson 1986a
Gnaphosa muscorum	GNAPHOSIDÆ	Conduit	Pre		Jackson 1986a
Herpyllus validus	GNAPHOSIDÆ	Conduit	Pre		Jackson 1986a
Lampona cylindrata	GNAPHOSIDÆ	Conduit	Pre		Jackson 1986a
Lampona sp.	GNAPHOSIDÆ	Conduit	Pre		Jackson 1986a
Prosthesima acta	GNAPHOSIDÆ	Conduit	Pre	Males amplex immature females.	Montgomery 1910
Scotophæus pretiosus	GNAPHOSIDÆ	Conduit	Pre		Jackson 1986a
Hypochilus pococki	HYPOCHILIDÆ	Cul-de-sac	Post	Males never associated with penultimate females in extensive study. Confirmed males stay with females in a lab study.	Eberhard et al 1993
Frontinella pyramitela	LINYPHIIDÆ	Conduit	Pre and Post (Jackson 1986a only observed pre-mate guarding but the sample size was only one example)	Males guard for very variable time and may guard a female before and after they have mated with her. Males will defend females by fighting off other males . Males will also feed off of the female's web. Males can assess virginity of females and only mate with virgin females in the wild.	Austad 1982 Austad 1983 Austad 1984 Jackson 1986a Suter 1990 Suter & Walberer 1989
Linyphia triangularis *Linyphia tenuipalpis* *Linyphia hortensis*	LINYPHIIDÆ	Conduit	Pre and Post (?)	Larger males guard females more effectively. Toft (1989) claims guarding pattern is indicative of first male priority like *Frontinella pyramitela*. Rovner (1968) showed that turnover of males on webs is high. Males are highly polymorphic in cheliceral size which may be indicative of alternative male strategies. Males fight for web possession. Emerton (1878) claimed only adults paired.	Emerton 1878 Rovner 1968 Toft 1989a, b, c Stumpf 1990

Geolycosa turricola	LYCOSIDÆ	Conduit	Pre	Males cohabit with penultimates only.	Miller & Miller 1987
Lycosa pullata	LYCOSIDÆ	Conduit	Post (?)	Males sit on backs of females for hours.	Bristowe 1958
Peucetia viridans	OXYOPIDÆ	Conduit	Post	Male stays with female for a long time after mating.	Whitcomb & Eason 1965.
Blechroscelis sp. *Madisimus* ssp A,B,C.	PHOLCIDÆ	Cul-de-sac	Pre	Males have longer legs than females and are dominant to them in interactions over prey within the web. Large male advantage in male -male contests. Males pair more readily with females that are ready to oviposit.	Eberhard & Briceño 1985
Pholcus ancoralis	PHOLCIDÆ	Cul-de-sac	Pre		Jackson 1986a
Pholcus phalangioides	PHOLCIDÆ	Cul-de-sac	Post		Yoward unpublished data. Bristowe 1958
Physocyclus dugesi *Haplopholcus æiæminoris* *Haplopholcus longipes*	PHOLCIDÆ	Cul-de-sac	Post	Groups are social but not only mother-offspring combinations.	Eberhard & Briceño 1985
Physocyclus globosus	PHOLCIDÆ	Cul-de-sac	Post	Males guarded mature females but rarely guarded immature females. Males fight when in female's web.	Eberhard *et al* 1993
Physocyclus sp.	PHOLCIDÆ	Cul-de-sac	Pre		Jackson 1986a
Psilochorus sp.	PHOLCIDÆ	Cul-de-sac	Pre		
Psilochorus sp.	PHOLCIDÆ	Cul-de-sac	Pre		
Smeringopus elongatus	PHOLCIDÆ	Cul-de-sac	Pre		
Inola amicabilis	PISAURIDÆ	Conduit	Pre		Jackson 1986a
Inola subtilis	PISAURIDÆ	Conduit	Pre		Jackson 1986a
Astia hariola	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Bavia æriceps	SALTICIDÆ	Conduit	Pre	4-8 days cohabiting	Jackson 1986c Jackson 1986a

Brevia jovialis	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Carrhotus viduus	SALTICIDÆ	Conduit	Pre	2-5 days cohabiting	Jackson 1986a
Corythalia fulgipedia	SALTICIDÆ	Conduit	Pre		Crane 1949
Cosmophasis micarioides	SALTICIDÆ	Conduit	Pre		Jackson 1986b Jackson 1986a
Cyllobelus rufopictus	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Cytæa? alburna	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Cytæa sp.	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Eris marginata	SALTICIDÆ	Conduit	Pre	2 Pairs stayed together for about 1 week before female moulted and mated.	Peckham & Peckham 1889 Jackson 1986a
Euophrys parvula	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Euryattus bleekeri	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Euryattus sp.	SALTICIDÆ	Conduit	Pre	5-8 days cohabitation.	Jackson 1986a
Hasarius adansoni	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Helpis minitabunda	SALTICIDÆ	Conduit	Pre	1-7 days cohabitation.	Jackson 1986a
Holoplatys planissima	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Holoplatys sp. 1	SALTICIDÆ	Conduit	Pre	2-10 days cohabitation.	Jackson & Harding 1982 Jackson 1986a
Holoplatys sp. 2	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Holoplatys sp. 3	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Hyllus plexippoides	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Hypoblenum sp.	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Lagnus kochi	SALTICIDÆ	Conduit	Pre	1-9 days cohabitation.	Jackson 1986a
Marpissa marina	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Menemerus sp.	SALTICIDÆ	Conduit	Pre	4-10 days cohabitation.	Jackson 1986a
Metaphidippus galathæ	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Metaphidippus protervus	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Metaphidippus æneolus	SALTICIDÆ	Conduit	Pre		Jackson 1986a

Mollika metallescens	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mopsus mormon	SALTICIDÆ	Conduit	Pre	3-12 days cohabitation. Males make a special nest next to the females in which to cohabit.	Jackson 1983 Jackson 1986a
Mymarachne ? luctuosa	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne constricta	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne lawrencei	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne lupata	SALTICIDÆ	Conduit	Pre	2-17 days cohabitation. Males been observed to fight.	Jackson 1982 Jackson 1986a
Mymarachne militaris	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne naro	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne plataleodes	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne striatipes	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne sp. 1	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne sp. 2	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne sp. 3	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne sp. 4	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Mymarachne sp. 5	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Phidippus ardens	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Phidippus audax	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Phidippus clarus	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Phidippus coccineus	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Phidippus johnsoni	SALTICIDÆ	Conduit	Pre	Males guard for up to 14 days and can detect how close a female is to moulting by nest pheromones.	Jackson 1977a Jackson 1978 Jackson 1986a
Phidippus paykulli	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Phidippus regius	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Phidippus sp.	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Philæus militaris	SALTICIDÆ	Conduit	Pre		Montgomery 1910

Plexippus opifex	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Plexippus dæmeli	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Plexippus sp.	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Portia africana	SALTICIDÆ	Conduit	Pre	Cohabitation of males with subadult females observed in wild. Males stay very close to females in their web. Males have complex fighting rituals.	Jackson 1986a Jackson & Hallas1986
Portia fimbriata	SALTICIDÆ	Conduit	Pre	Cohabiting pairs of subadult males and females observed in wild. Males have complex fighting rituals. 2-48 day cohabiting (median = 8.5). Males leave after mated.	Jackson 1986a Jackson & Hallas 1986
Portia labiata	SALTICIDÆ	Conduit	Pre	Cohabitation of males with subadult females observed in wild. Males stay very close to females in their web. Males have complex fighting rituals. 8-28 day cohabiting.	Jackson 1986a Jackson & Hallas1986
Portia schultzi	SALTICIDÆ	Conduit	Pre	Cohabitation of males with subadult females observed in wild. Males stay very close to females in their web. Males have complex fighting rituals. 13-19 day cohabiting.	Jackson 1986a Jackson & Hallas1986
Pseudicius sp. 1	SALTICIDÆ	Conduit	Pre	About 5 days cohabiting.	Jackson 1986a
Pseudicius sp. 2	SALTICIDÆ	Conduit	Pre	About 6 days cohabiting.	Jackson 1986a
Pystira orbiculata	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Simæa robustior	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Simætha pætula	SALTICIDÆ	Conduit	Pre	2-9 days cohabiting.	Jackson 1986a

Simætha thoracica	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Sobara bitæniata	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Synageles noxiosa	SALTICIDÆ	Conduit	Pre		Kaston 1948
Talavera minuta	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Thiania demissa	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Thiania sp.	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Thorellia ensifera	SALTICIDÆ	Conduit	Pre	Males will mate with mature females also but have to court more actively than males which cohabit.	Jackson & Whitehouse 1989
Thyene unflata	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Thyene sp. 1	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Thyene sp. 2	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Trite planiceps	SALTICIDÆ	Conduit	Pre	3-10 days cohabitation.	Foster & Foster 1973 Jackson 1986a
Trite auricoma	SALTICIDÆ	Conduit	Pre	4-13 days cohabitation.	Jackson 1986a
Trite bimaculosa	SALTICIDÆ	Conduit	Pre		Jackson 1986a
Scytodes fusca	SCYTODIDÆ	Cul-de-sac	Pre & Post	Social species. Males stay with females for a long time after mating.	Jackson 1986a
Oilos sp.	SPARASSIDÆ	Conduit	Pre		Jackson 1986a
Oilos sp.	SPARASSIDÆ	Conduit	Pre		Jackson 1986a
Olios ? diana	SPARASSIDÆ	Conduit	Pre		Jackson 1986a
Olios lamarcki	SPARASSIDÆ	Conduit	Pre		Jackson 1986a
Olios obesulus	SPARASSIDÆ	Conduit	Pre		Jackson 1986a

Herennia spp. *Herennia ornatissima*	TETRAGNATHIDÆ (*sensu* Levi 1980, 1981)	Conduit	Post - Robinson & Robinson (1980, Table 2) claim the species exhibits "suitor phenomena" but clear from the text that males mainly involved in post mate guarding.	Males will defend females by fighting off other males.	Robinson & Robinson 1978 Robinson & Robinson 1980
Leucage granulata	TETRAGNATHIDÆ (*sensu* Levi 1980, 1981)	Conduit (But see Levi (1980) for related ssp.)	Pre		Jackson1986
Leucauge mariana	TETRAGNATHIDÆ (*sensu* Levi 1980, 1981)	Conduit (But see Levi (1980) for related ssp.)	Pre	Males associated with penultimate females in an extensive study of a wild population.	Eberhard *et al* 1993
Metellina segmentata	TETRAGNATHIDÆ (*sensu* Levi 1980, 1981)	Conduit - Spheroid	Pre	Males wait on web with female until a suitable prey item is caught then mate. Mysteriously (Rubenstein 1987) did not observe this phenomenon. Males may be in attendence for a month before mating but leave directly after mating.	Bristowe 1929, 1941, 1958 Blanke 1974 Rubenstein 1987 Prenter *et al* 1994
Nephila clavata	TETRAGNATHIDÆ (*sensu* Levi 1980, 1981)	Conduit - Spheroid	Pre and Post		Miyashita 1994
Nephila clavipes	TETRAGNATHIDÆ (*sensu* Levi 1980, 1981)	Conduit - Spheroid	Pre and Post	Bigger males cohabit with females for a longer period of time.	Cohn *et al* 1988 Robinson & Robinson 1980 Vollrath 1980 Jackson 1986a

Nephilid spp. *Nephila maculata* *Nephila edulis* *Nephila madigascariensis* *Nephrilengys malabarensis* *Nephrilengys cruentata*	TETRAGNATHIDÆ (*sensu* Levi 1980, 1981)	Conduit - sheroid (*Nephila maculata* In: Levi 1980)	Pre - "suitor phenomenon"	Males of all species engage in agonistic interactions with conspecific males on the web. Males copulate with freshly moulted females (forced copulations / opportunistic matings). Possibly similar to crustacean situation. Males cohabit on webs at great risk to themselves.	Robinson & Robinson 1973 Robinson & Robinson 1980 Jackson 1986a
Nephrilengys sp. (From Kenya)	TETRAGNATHIDÆ (*sensu* Levi 1980, 1981)	Conduit - Spheroid	Pre		Jackson1986a
Nephrilengys spp.	TETRAGNATHIDÆ (*sensu* Levi 1980, 1981)	Conduit - Spheroid	Post	Males will defend females by fighting off other males.	Robinson & Robinson 1978
Tetragnatha montana *Tetragnatha extensa*	TETRAGNATHIDÆ (*sensu* Levi 1980, 1981)	Cul-de-sac	No guarding of subadult females, some males are associated with mature males.	Field survey	Yoward unpublished data. West & Toft 1989
Cteniza moggridgei	THERAPHOSIDÆ	Cul-de-sac	No cohabitation		Petrunkevitch 1911
Eurypelma californica	THERAPHOSIDÆ	Cul-de-sac	Cohabit - probably post mate guarding	Males stay with females for about 3 months and die after mating season - females live for 11+ years moulting each year. Male and female mate immediately upon introduction to each other in the lab.	Bærg 1928
Achæranea camura	THERIIDIDÆ	Conduit	Pre	5-21 days cohabitation.	Jackson 1986a
Achæranea decorata	THERIIDIDÆ	Conduit	Pre		Jackson 1986a
Achæranea extrilida	THERIIDIDÆ	Conduit	Pre		Jackson 1986a
Achæranea mundula	THERIIDIDÆ	Conduit	Pre		Jackson 1986a
Achæranea sp.	THERIIDIDÆ	Conduit	Pre		Jackson 1986a

Achæranea trepidariorum	THERIIDIDÆ	Conduit	Pre		Jackson 1986a
Achæranea ventricosa	THERIIDIDÆ	Conduit	Pre	1-16 days cohabitation.	Jackson 1986a
Achæranea veruculata	THERIIDIDÆ	Conduit	Pre		Jackson 1986a
Achæranea wau	THERIIDIDÆ	Conduit	Pre	Males copulate with freshly moulted females (forced copulations / opportunistic matings). Possibly similar to crustacean situation.	Lubin 1986 Lubin 1989
Archararanea (formerly *Theridion*) *tepidariorum*	THERIIDIDÆ	Conduit	Pre		Montgomery 1910
Argyroides antipodiana	THERIIDIDÆ	Conduit		Males are observed fighting more often in female than male webs.	Whitehouse 1991
Enoplognatha ovata *Enoplognatha latimana*	THERIIDIDÆ	Conduit - Tubular (Hippa & Oksala 1982)	Pre		Oxford,G.S. pers. com.
Latrodectus katipo	THERIIDIDÆ	Conduit	Pre		Jackson 1986a
Latrodectus mactans	THERIIDIDÆ	Conduit	Pre		
Latrodecus bishopi	THERIIDIDÆ	Conduit - Tubular (McCrone & Levy1964)	Pre? Male mature, does not mention status of female.	Sit in specially constructed retreats side by side. Males of larger size than is typical of the genus.	McCrone & Levy 1964
Latrodecus mactans	THERIIDIDÆ	Conduit - Tubular (McCrone & Levy1964)	Pre. Up to 8 males found in a females web.	No mention of agonistic interactions between males.	Petrunkevitch 1911 Jackson 1986a
Steotoda sp.	THERIIDIDÆ	Conduit	Pre		Jackson 1986a
Steotoda bipunctata	THERIIDIDÆ	Conduit	Post		Emerton1878 Nielsen 1932
Theridion ? theridioides	THERIIDIDÆ	Conduit	Pre		Jackson 1986a
Cymbacha saucia	THOMISIDÆ	Conduit	Pre		Jackson 1986a

Dioea dorsata	THOMISIDÆ	Conduit	None	Immature females drive off adult males.	Bristowe 1958
Misumenoides formosipes	THOMISIDÆ	Conduit	Pre	Males will defend females by fighting off other males.	Dodson & Beck 1992 Dodson & Beck 1993
Xysticus nervosus	THOMISIDÆ	Conduit	None	Immature females drive off adult males.	Montgomery 1909
Uloborus ? congregabilis	ULOBORIDÆ	Conduit	Pre		Jackson 1986a
Uloborus variabilis	ULOBORIDÆ	Conduit	Pre		Jackson 1986a
Zosis geniculatus	ULOBORIDÆ	Conduit	Pre		Jackson 1986a

Table A5.4 Grasping Organs Involved In Copulation In Spiders.

Species.	Family.	Grasping Mechanism[1].	Spermathecae.	Notes.	Authority.
All spiders	ARANEÆ	Apophyses on the palps are considered as an adaptation to hold onto the female's epigyne / vulva whilst *in copula*.	Conduit.		Bristowe 1929
Araneus, etc. (Eperids)	ARANEIDÆ	Stout spines on legs lock legs together.	Conduit		Bristowe 1929
Clubiona reclusa	CLUBIONIDÆ	Male holds the female's abdomen with chelicerae whilst mating.	Conduit		Bristowe 1958
Dictyna viridissima	DICTYNIDAE	Chelicerae - Holding the females chelicerae shut. Male's jaws are bowed to accomodate the females jaws between his own.	Conduit		Bristowe 1929 Berland 1912 (Cited In: Bristowe 1929, 1958)
Euagrus spp.	DIPLURIDÆ Mygalomorph	Male specific leg II structures, at the tibia-metatarsus joint, which grip the females legs II.	Cul-de-sac		Coyle 1986
Thelechoris karschi	DIPLURIDÆ Mygalomorph	Male holds the females pedipalps with first tibial apophyses.	Cul-de-sac		Coyle & O'Shields 1990
Porrhothele antipodiana	HEXATHELIDÆ Mygalomorph	Male has claspers on legs I to hold female. Females become more passive when clasped.	Cul-de-sac		Jackson & Pollard 1990
Baryphyma pratensis	LINYPHIIDÆ	Female grips the head of the male with her fangs.	Conduit	Female recieves blood meal from male's head during mating.	Blest & Taylor 1977

Hypomma bituberculatum	LINYPHIIDÆ	Female sticks her fangs into sides of ridges on males head turret.	Conduit	It is possible that the bulbous heads common in other Linyphiids and Theriidids are grasped in this manner also.	Bristowe 1958 Blest & Taylor 1977
Pardosa lapidicina	LYCOSIDÆ	Male hooks I, II and III legs around the proximal segments of the females legs.	Conduit		Eason 1969
Pholcus phalangioides	PHOLCIDÆ	Male holds the female's vulva with his chelicerae whilst palps are inserted.	Cul-de-sac	Male Pholcid species have species specific chelicerae.	Reagan pers. com. Eberhard & Briceno 1985 Levi 1968
Physocyclus simoni	PHOLCIDÆ	?	Cul-de-sac		
Scytodes thoracia	SCYTODIDÆ	Male holds special sockets located above the female's genital opening with his chelicerae.	Cul-de-sac	Simultaneous palp insertion.	Bristowe 1958 Levi 1968
Segestria spp: *S.florentina* *S.senoulata* *S.baverica*	SEGESTRIIDÆ	Male holds female's abdomen with fangs.	Cul-de-sac		Bristowe 1958
Metelina segmentata	TETRAGNATHIDÆ	Male holds female's leg with chelicerae.	Conduit		Bristowe 1958
Nephila ssp.	TETRAGNATHIDÆ	Male holds onto the female's sturnum with legs	Conduit	Males are very small in size compared to females.	Robinson & Robinson 1980
Pachygnatha spp.	TETRAGNATHIDÆ	Chelicerae.	Cul-de-sac		Bristowe 1929
Tetragnatha spp.	TETRAGNATHIDÆ	Chelicerae.	Cul-de-sac		Bristowe 1929
Ceropelma longisternalis	THERAPHOSIDÆ Mygalomorph	Tibial spurs grip the fang of the female.	Cul-de-sac		Costa & Pérez-Miles 1992

Argyrodes spp.	THERIDIIDÆ	Female grips the head of the male with her fangs.	Conduit	It is possible that the bulbous heads common in other Linyphiids and Theriidids are grasped in this manner also.	Blest & Taylor 1977
Xysticus cristatus *X.ulmi*	THOMISIDÆ	Silk to fasten female to ground and hold female's leg with chelicerae.	Conduit		Bristowe 1929 Bristowe 1958

[1] Many spider males fasten the female with a bridal veil. It is possible that this is used to immobilize her, not for avoidance of sexual cannibalism but to ensure insertion of the palps, because the epigyne does not have securing apophyses. However this process does not fix the male and the female together so is not considered a grasping device in the sense used here.

Appendix Table A5.5 Mating Plugs: Taxonomic Incidence And Type In Spiders.

Species.	Family.	Spermathecæ.	Plugging Mechanism[1].	Notes.	Authorities.
Agelena limbata	AGELENIDÆ	Conduit	Amorphous secretion over females epigyne.	20 females were collected from the wild: 13 had complete plugs 7, incomplete. Complete plugs cannot be penetrated by second males. Larger males are better at overcoming incomplete plugs.	Masumoto 1993
Amaurobious fenestralis	AMAUROBIDÆ	Conduit	Sperm plug?	The mating plug originates from the palpus.	Suhm & Alberti 1993
Amaurobious ferox	AMAUROBIDÆ	Conduit	Sperm plug?	A white droplet is deposited on the female's vulva some of which the female removes. Gerhardt (1923) speculated that the hardening of this droplet would prohibit furthur matings.	Gerhardt 1923 (cited in Bristowe 1929)
Araneus (Epeira) redii	ARANEIDÆ	Conduit	?	It was observed that it is impossible for females to mate a second time.	?
Argiope argentata	ARANEIDÆ	Conduit	Embolus tip breaks off, but many may be found in the epigyne.	Costly behaviour as it is postulated this prevents further use of palp - disputed point.	Levi 1975
Argiope argenta	ARANEIDÆ (ARGIOPIDÆ)	Conduit - spheroid	Embolus tip wedged in spermathcæ and granular mass around the apical element.	No distinct fracture plane exists where the tip breaks off unlike some other species Eg. *Latrodectus* spp.	Abalos & Baez 1963
Argiope braennichi	ARANEIDÆ	Conduit	Embolus tip breaks off.		Roberts 1995
Larinioides cornutus	ARANEIDÆ	Conduit	Scape removed from female during mating.	Mature, mated females never have this structure, but it is not known if the scapes absence interferes with further matings. Also known in many other Araneids.	Wright, J. pers. com. Roberts 1995

Numerous *Araneus* spp. eg. *A.diadematus*	ARANEIDÆ	Conduit	Embolus cap breaks off - left in the epigyne.	Costly behaviour as it is postulated this prevents further use of palp - disputed point.	Levi 1975
Hypsosinga spp.	ARANEIDÆ	Conduit	Flat transparent scale on the palp breaks off during mating and lodges on epigyne competely obscuring it.		Roberts 1995
Zygiella x-notata	ARANEIDÆ	Conduit	Resinous material applied to one pore of epigyne.		Yoward unpublished data
?	CLUBIONIDÆ	Conduit	Resinous material applied to epigyne.		Forster 1967
Florina coccinea	LINYPHIIDÆ	Conduit	Male secretes a white liquid onto the females epigyne during copulation. White liquid came from the mouth region.		Willey Robertson & Adler 1994
Leptyphantes sanctivincentii	LINYPHIIDÆ	Conduit	Males have massive gnathocoxal glands.	It is hypothesised that the function of these glands is to plug the epigyne during mating.	Juberthie-Jupeau & Lopez 1991
Linypia triangularis	LINYPHIIDÆ	Conduit	During copulation a drop of whitish fluid appears in the epigynal openings of the females. Fluid hardens later, pluging the epigyne.	Liquid probably secreted by the male.	Stumpf 1990
Porrhomma egeria	LINYPHIIDÆ	Conduit	Male secretes a white liquid onto the females epigyne during copulation.	Sperm induction occurs before the male encounters the female in this species.Copulation lasts up to 14 hours.	Bourne 1978
Pardosa oncka	LYCOSIDÆ	Conduit	Both pits of the epigyne are filled with a conspicuous mating plug.		Kronestedt 1987
	MESOTHELÆ LIPHISTIDÆ (HEPTATHECA FUE)	Cul-de-sac			Changmin *et al* 1988

Oxyopes sertatus	OXYOPIDÆ	Conduit	Amorphous mass over epigyne.	Amorphous mass possibly formed from the well developed maxillary epidermal cushions in this species	Lopez 1987
Peucetia viridans	OXYOPIDÆ	Conduit - spheroid	"Resinous" material applied over the epigyne with the two pronged portion of the male's palpal paracymbium embedded in the resin.	Examination was of preserved and live material - almost all mated females had epigyne covered with "resinous" material (with paracybium contained) sometimes covering the whole of the epigynum.	Whitcombe & Eason 1965 Exline & Whitcombe 1965 Brady 1964
Peucetia longipalpis	OXYOPIDÆ	Conduit - spheroid	"Resinous" material applied over the epigyne, the paracymbium does not snap off in this species.	Examination was of preserved and live material - almost all mated females had epigyne covered with "resinous" material, sometimes covering the whole of the epigynum.	Brady 1964
Dolomedes spp: *D.scriptus* *D.triton* *D.vittatus*	PISAURIDÆ	Conduit	Embolus tip is left in the epigyne sometimes.		Carico 1973
Heliophanus sp.	SALTICIDÆ	Conduit	Plug	Plug makes spiders unidentifiable	D.Jones pers. com.
Phidippus johnsoni	SALTICIDÆ	Conduit	Amorphous mass over epigyne. sometimes with "wedges " inserted.	Sometimes one pore was more thouroughly covered than the other.	Jackson 1980 Jackson *et al* 1981
Portia ssp: *P.fimbriata* *P.labiata* *P.schultzi*	SALTICIDÆ	Conduit	Epigynal plug as *Phidippus johnsoni.*		Jackson 1986a
Herennia ornatissima	TETRAGNATHIDÆ	Conduit	Embolus tip breaks off and lodges in the female's epigyne.		Robinson & Robinson 1980
Herennia spp.	TETRAGNATHIDÆ	Conduit	Embolus tip breaks off and lodges in the female's epigyne.		Robinson & Robinson 1978

Nephila maculata	TETRAGNATHIDÆ	Conduit	Embolus tip breaks off and lodges in the female's epigyne.	Males mate with freshly moulted females and then guard them.	Robinson & Robinson 1978 Robinson & Robinson 1980
Nephila clavipes	TETRAGNATHIDÆ	Conduit	Embolus tip breaks off and lodges in the female's epigyne.	Males mate with freshly moulted females and then guard them.	Robinson & Robinson 1978 Robinson & Robinson 1980 Vollrath 1980 Christenson 1990
Nephila madagascariensis	TETRAGNATHIDÆ	Conduit	Embolus tip breaks off and lodges in the female's epigyne.		Robinson & Robinson 1978 Robinson & Robinson 1980
Nephilengys cruetata	TETRAGNATHIDÆ	Conduit	Embolus tip breaks off and lodges in the female's epigyne.		Robinson & Robinson 1978 Robinson & Robinson 1980
Nephilengys Malabarensis	TETRAGNATHIDÆ	Conduit	Embolus tip breaks off and lodges in the female's epigyne.		Robinson & Robinson 1978 Robinson & Robinson 1980
Nephilengys spp	TETRAGNATHIDÆ	Conduit	Embolus tip breaks off and lodges in the female's epigyne.		Robinson & Robinson 1978
Pachygnatha clerckii	TETRAGNATHIDÆ		Sperm plug?	A white droplet is deposited on the female's vulva some of which the female remouves. The droplet contained sperm. Gerhardt (1923) speculated that the hardening of this droplet would prohibit furthur matings.	Gerhardt 1923 (cited in Bristowe 1929)
	THERIDIIDÆ	Conduit			Levi 1959 (Cited in Masumoto 1993)

?	THERIDIIDÆ	Conduit	Embolus tip wedged in spermathcæ.		Gertsch 1979 (Cited by Austad 1984)
?	THERIDIIDÆ	Conduit	Resinous material applied to epigyne.		Forster 1967
Achœaranœ tepidariorum	THERIDIIDÆ	Conduit - Speroid	Embolus tip wedged in spermathcæ. However blockage of the mating duct is not affected.	Specialized constriction at embolus tip this is the site of breakage during mating.	Abalos & Baez 1963
Achœaranœ wau	THERIDIIDÆ	Conduit	No evidence of plugs in this social spider.		Lubin 1986
Argyrodes spp: *A.argyrodes* *A.syriaca*	THERIDIIDÆ	Conduit	Amorphous mass / thick clot of resinous material over epigyne.	Amorphous mass possibly formed from from the palp which is very large in this species.	Levy 1985 Lopez 1987
Latrodectus species: *L.mactans,* *L.varians,* *L.hesperus,* *L.curacaviensis* *L.geometricus* *L.hasselti.*	THERIDIIDÆ	Conduit - Tubular (Abalos & Bæz 1963)	Embolus tip (apical eliment) is broken off during mating (and is specialized for this function, Abalos & Bæz 1963). Kaston (1970) found in *L.hesperus* that a second (?) set of apical elements are deposited not as far in the spermathecae as the first (?) apical eliments, thus plug may be entirely effective.	1. Taxonomically difficult group, all with very similar genitalia. eg. Levi (1964) suggested the *L. curacaviensis* of Bhatnagar & Rempel (1965) may be *L.variolus* or *L. hesperus* in reality. Therefore these species do not represent independant data points. 2. Many apical elements may be found in one spermatheca so not totally effective in preventing multiple mating (they may prevent multiple insemination). 3. The apical element is not required for males to mate. They can mate with up to 3 females (Breene & Sweet 1985).	Abalos & Bæz 1963 McCrone & Levi 1964 Bhatnagar & Rempel 1965 Ross & Smith 1979 Breene & Sweet 1985
Metepeira candida *Metepeira* sp.	THERIDIIDÆ	Conduit	Embolus tip wedged in spermathcæ.	Mated females always have an embolus tip in the spermathecæ.	Abalos & Baez 1963

Steatoda triangulata	THERIDIIDÆ	Conduit	Amorphous mass over epigyne.	Amorphous mass possibly formed from the well developed maxillary epidermal cushions in this species. Extremely variable spermathecal structure.	Braun 1956 Levi 1957
Steatoda sp.	THERIDIIDÆ	Conduit	Amorphous mass over epigyne.	Amorphous mass possibly formed from the well developed maxillary epidermal cushions in this species.	Lopez 1987
Teutana triangulosa	THERIDIIDÆ	Conduit	Plug and embolus breakage.		Braun 1956
Theridion pictum *T.Pinastri* *T.varians*	THERIDIIDÆ	Conduit	Plug.		D. Jones pers. com. Roberts 1995
Theridiosoma gemmosum	THERIDIOSOMATIDÆ	Conduit	Mature females are always found with a membrane over their epigyne.		Roberts 1985
Misumenops celer	THOMISIDÆ	Conduit	Waxy / oily coating formed over the atrium of the epigyne after mating.	Females do not mate more than once in the lab and males nibble palps between the chelicerae.	Munniapan & Chada 1970
?	TOXOPIDÆ	?	Resinous material applied to epigyne.		Forster 1967
Uloborus ferokus	ULOBORIDÆ	Conduit	Waxy plug is applied over the epigyne.	The waxy secretion possibly comes from a gland in the palp.	Muniappan & Chada 1970 Patel & Bradoo 1989

[1] Mating plugs are defined here as any epigynal obstruction around the mating orifice occurring after mating. Alternative names include sperm plugs, embolus cap, plug, epigynal plug, chastity belt, apical element etc. Forster (1967) also mentions use of antiaphrodisiacs and lack of receptivity of mated females but does not state the species involved.

Appendix Table A5.6 Single Palp Usage In Spiders.

Species	Family	Spermathecæ	Notes	Autority
Argyronata sp.	AGELENIDÆ	Conduit	Sometimes one palp is used in mating, sometimes use two.	Bristowe 1958
Tegenaria derhami	AGELENIDÆ	Conduit	Very short mating time.	Montgomery 1903
Dictyna arunducea	DICTYNIDÆ	Conduit	Only observed a single mating.	Bristowe 1958
Dictyna volupis	DICTYNIDÆ	Conduit		Montgomery 1903
Thelechoris karschi	DIPLURIDÆ	Simple epigyne Cul-de-sac	Only sometimes uses one palp.	Coyle & O'Shields 1990
Acrosoma gracile	ERESIDÆ	Conduit		Montgomery 1903
Eresus niger	ERESIDÆ	Conduit	Single palp inserted many times.	Bristowe 1958
Xerolycosa miniata	LYCOSIDÆ	Conduit		Bristowe 1958
Oonops pulcher	OONOPIDÆ	Cul-de-sac	Bristowe (1958) observed the mating under a microscope and claimed both palps were used alternately. Montgomery (1903) observed the use of one palp only.	Montgomery 1903 Bristowe 1958
Oxyopes heterophtholmus	OXYOPIDÆ	Conduit		Bristowe 1958
Heliophanes cupreus	SALTICIDÆ	Conduit		Montgomery 1903
Metellina segmentata	TETAGNATHIDÆ	Conduit		Prenter Pers. Com.
Asagena serratipes	THERIDIIDÆ	Conduit		Montgomery 1903
Eucharia bipunctata	THERIDIIDÆ	Conduit		Montgomery 1903
Theridion lunatum	THERIDIIDÆ	Conduit	Very short mating time, but enough sperm were transferred to fertilize many eggs.	Bristowe 1958

Theridion tepidarium	THERIDIIDÆ	Conduit	Very short mating time, but enough sperm were transferred to fertilize many eggs. Montgomery (1903) claims both the palps were applied simultaneously.	Bristowe 1958 Montgomery 1903
Tidarren fordum	THERIDIIDÆ	Conduit	Male bites off one of his palps (left or right) long before mating. Palps are huge - each as big as his cephalothorax.	Bristowe 1958
Philodromus aureolus	THOMISIDÆ	Conduit		Bristowe 1958
Philodromus dispar	THOMISIDÆ	Conduit	Very short mating time.	Bristowe 1958
Xysticus stomachosus	THOMISIDÆ	Conduit	Variable mating: sometimes inserts one palp sometimes two.	Montgomery 1903
Uloborus ferokus	ULOBORIDÆ	Conduit	Very short mating time.	Patel & Bradoo 1989

6.0 General Discussion

The Austad (1984) hypothesis has been a stimulus to a considerable body of work (for example, Watson 1990, 1991; Eberhard *et al* 1993; Masumoto 1993 and the present thesis). What progress has been made as a result of these new findings? Can it still be claimed that the spermathecal architecture represents a phyletic limitation to sperm utilization strategies? In chapter 2.0 in *Pholcus phalangioides* it was found that a trend in P_2 with broods did not comply with that expected by Austad (1984), and in chapter 3.0 it was shown that another cul-de-sac species, *Tetragnatha montana*, does not necessarily have the predicted second male priority. I raise additional empirical and theoretical objections to the Austad (1984) hypothesis in Chapter 5.0

I will review in the following theoretical objections, and findings from comparative work, pertaining to Austad's theory, arising from my thesis. Although female interests in the outcome of sperm competition have been stressed by various workers (Smith 1984; Birkhead & Møller 1992; Baker & Bellis 1995) and by Austad (1984) himself, the fixing of sperm precedence patterns at such a deep node[1] in the phylogeny of spiders does not take into account these interests in the tripartite evolutionary game of sperm competition. Female interests will differ among species and would be expected to lead to different outcomes, in relation to sperm precedence, which are species-specific. The presence, and nature, of mating plugs, for

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[1] Between the Haplogyne and the Entelegyne, the first dichotomy drawn within the true spiders (Coddington & Levi 1991).

example, are also expected to be species-specific. The rewards to plugging differ given the prevailing mating system e.g. whether it is monogamous or polyandrous. The more monogamous the mating system the less plugs are selected for, but the more plugs are utilized by males the more monogamous the mating system is forced to be. This feedback should result in a coevolutionary cycle of plugging and non-plugging or an equilibrium level of plugging based on risk of cuckoldry assessments made by individual males: in some cases plugging in other cases not (Parker 1984). It is expected that plug usage will be found stochastically in the phylogeny of conduit species. In species where plugging is found low P_2s are expected as a result of the plug, but without the plug the spermathecal architecture is expected to favour a high P_2, otherwise the plug would be redundant (Masumoto 1993). In the conduit species at least there is expected to be a cycle of adaptation and counter adaptation. This is contrary to Austad's (1984) claim for the incidence of mating plug usage, as discussed in chapter 5.0. He expected there to be mating plugs in conduit species because the eggs could be laid without the removal of the plug. The plug would remain functional, preventing cuckoldry after the first and subsequent egg sacs. However this ignores the effect of spermathecal 'plumbing'; Austad expected a low P_2 in all conduit species and so a plug would be of low utility in protecting the egg sacs from insemination from males subsequent to the first. This is especially the case when one considers how expensive mating plugs are to produce: they often prevent further matings taking place for the male (Levi 1975).

Variation in P_2 may be a result of the partial application of plugs, the degree of plugging determining the P_2 found. Further work on mate-plugging in spiders could concentrate on documenting the incidence of plugs and their effectiveness. Most observations of plugs have

been by taxonomists and no concerted effort has been expended to seek them out as a phenomenon in their own right. Thus many subtle plugs may have been overlooked, and the importance of them in spiders underestimated. Indeed, plugs may be the mechanism whereby sperm priority patterns are established. For some plugs it is obvious as to where they originate from (e.g. parts of the palp which break off in the epigyne or the spermatheca entrance) but others, such as secretions, could be from the female or the male and originate from various parts of them. Radioactive labels could be used to determine from which sex the plugs come from, in those cases where it is not obvious. Further work could concentrate on the phyletic distribution of plugs to ascertain if there is a pattern (for instance, certain mechanisms of plugging found in some families and not others) or if, as predicted, it is stochastic.

The association of mate-guarding type with spermathecal morphology was found not to be consistent with the predicted pattern of Austad (1984). He suggested that conduit spiders should premate-guard because of the low P_2 assumed to be associated with this type of spermatheca. Cul-de-sac spiders, conversely, should postmate-guard if they guard at all to avoid loss of paternity to subsequent males. However all kinds of mate-guarding were found to be associated with the spermathecal categories (Chapter 5.0). No formal test of association was applied to these data because information was available for only one evolutionary independant derivation of spermathecal morphology. Only once in the phylogeny of spiders, for which mate-guarding data exist, is there a branching point from cul-de-sac to conduit. Additional data are required on those species with secondarily derived cul-de-sac spermathecal organisation before further analyses are possible. However, these preliminary findings are inconsistent with the Austad (1984) hypothesis.

Sperm stratification as a mechanism for the determination of sperm precedence patterns is a phenomenon found in some birds (Birkhead & Møller 1992). However whether or not it is important in spiders is another matter. It certainly has to be the case if the patterns of sperm precedence expected by Austad (1984) are those found in nature, because within the spermatheca it is the relative positions of the sperm pools from different males that determines the P_2. As explained in Chapter 5.0, sperm stratification in spiders can be broken down in a number of ways. Firstly, if palpal elements penetrate the sperm storage organ. Secondly, if the pressure under which the sperm are ejaculated into the spermathecae is high. Thirdly, the longer the sperm are stored the less is the chance that any sperm stratification is maintained. Some species of spider store sperm for a very long time. Finally, if the spermathecae is not large enough to hold more than one male's ejaculate then sperm stratification will not be established in the first instance. Further avenues for investigation are suggested by these factors which tend to break down stratification. Firstly, measurements of the relative sizes of the mating duct and the corresponding embolus, in a number of species, to assess how common it is for the embolus to penetrate into the spermatheca. Secondly, measurements of the relative sizes of the spermatheca and the sperm reservior of the male's palp, in a number of species. This will indicate if a male has the ability, if he passes over all the sperm contained, to fill the spermatheca with sperm and hence mitigate against sperm stratification, unless sperm removal can occur. Finally, to directly investigate spermathecae, excised from multiply mated females to see if sperm stratification occurs. Molecular markers could be used to establish if sperm from opposite ends of the spermatheca are from the different males.

The literature search uncovered a curiosity in the spider world worth investigation. In *Tidarren fordum* the males have such huge palps that they bite one off before courting commences. The cost of removing a palp is that it is not utilizing all the insemination opportunities available to it, because when it mates it can fill only one of the female's spermathecae. It would be interesting to establish if there is a genetic basis to the removal of the palp - either left or right, and also what frequencies of which palp is removed in the wild at different sites. In an attempt to explain the past evolutionary forces leading to palpal excision the first step could be to measure the relative sizes of the spermathecae and the palps. The evolutionary forces leading to the massive size of the palps may have been an attempt on behalf of the male to fill the spermathecae of the female, in a climate whereby the sperm could not be removed by subsequent males, so avoiding sperm competition. The evolutionary resonse of the female could have been to enlarge the size of the spermathecae, with a counter-reponse by the males to enlarge the palps, until the males could not maintain both palps and seek out females and mate. Thus I am suggesting a case of run-away selection. I would expect the size of the spermatheca and the palps to be about the same, if the above is true, because males with a single palp should be able to keep track with any size increase the female may make in her spermathecal size.

The major thrust of this thesis has been an examination of precedence patterns in cul-de-sac spiders, *viz Pholcus phalangioides* and *Tetragnatha montana*. I have demonstrated that esterase markers can be used to assign paternity in multiply fathered broods and can detect multiple paternity in the wild. To function in these ways the markers have to be variable, demonstrably genetic and selectively neutral. This I was able to do in the case of both *P. phalangioides* and

T. montana for the first two factors. In *P. phalangioides* mortality of the young, in the lab, was low so selection could not be acting on the markers in this species. In *T. montana* mortality was higher so I can only assume selection was unimportant.

Using esterase markers it was found that *P. phalangioides* complied with the predicted precedence pattern of Austad (1984), which is a high P_2, despite a much shorter mating duration for second males. Indeed, Uhl (1993b) believed, without the benefit of paternity measures, that the second males did not inseminate the female because they mated for such a short time. This shows how invaluable it is to observe those matings in which the P_2 measurements are to be taken, not only to ensure that both males mate with the female, but also the duration of mating, otherwise this paradox would not have been unearthed. We can attempt to explain this paradoxical result of a shorter mating duration leading to higher paternity in a number of ways:

(i) **The finding is a laboratory artifact**: multiple matings in the wild would not be so close together. The interpretation taken here is that the sperm of the first male, like that of the second male, is passed over in the first few minutes of copulation. The rest of the mating duration is in fact contact mate-guarding, whilst a physiological process is undergone in the female's sperm storage organ ensuring the first male paternity over at least the first brood. Alternatively, a substance is passed over which reduces the receptivity of the female which only comes into activity some time after the mating. However, the first male does not mate-guard for the full duration of this physiological process, but only until the arrival of another male (in the wild) is reduced below some threshold. Second males must be able to detect that the female is mated

and so mate for a short time, just sufficient for sperm to be passed over, because the first male's activities will suffice in preventing further matings and is assured of the bulk of the paternity over the brood.

These hypotheses can be tested in a number of ways:

(a) Staging matings between virgin females and males, interupting the matings after the average time it takes second mating males to mate and measure if as much sperm is transfered as for first mating males mating for the much longer duration. The fecundity of single mated females mated for these two durations could also be assayed.

(b) If males are contact mate-guarding to ensure paternity the duration the male mates should be affected by his socio-sexual surroundings. The presence of other males whilst the male is mating a virgin female should affect mating duration. This could be investigated in a number of ways. Firstly, the presence of silk from a male could be introduced to a female's cage by excising the palps of a male (to ensure the virginity of the female) and leaving him in the female's cage overnight to produce silk. Then the duration of a mating to that female could be recorded to see if it is affected by this treatment. If the hypothesis is correct then the male should mate for a longer period because there is a greater likelihood of males being in the locale and usurping his paternity. Secondly, a male could be placed in a virgin female's cage, in a vented phial to see if the direct presence of a male affects the duration of a subsequent mating. In this case the mating should be, if the hypothesis is correct, of an even longer duration than found in the experiments of Chapter 2.0. Evidence of the utility of long first

mating times to reduce female receptivity, which only comes into effect some time after the matings, comes from the fact that it is very difficult to get non-virgin females mated again some time after the first mating. This is why most matings were set up so that the two matings were close together.

(ii) **Cryptic mating plug**: It may take the first male some time to pass over a cryptic mating-plug which takes time to harden. The second matings in this case were set up too close to the first for the plug to become effective. This hypothesis could be tested easily by a microscopic search for the plug in a singly mated female, using radioactive labels if necessary.

(iii) **Sperm removal**: The second mating males may be getting the bulk of paternity because they are removing sperm deposited by the first male. It is interesting in this context that the male's palp in *P. phalangioides* is very complicated, especially when compared to other Haplogyne species. Complex intromittant organs are found in Damseflys and these are associated with sperm removal (Waage 1979); it is possible that this is the case also in *P. phalangioides*. Sperm removal by males mating after the first male has not been observed in spiders. This can be investigated by stoping sperm induction, in a male, by blocking the gonopore and then mating him to a mated female. If any sperm is found on his palp then it must have been removed from the female. If however males will not behave normally if their gonopore is blocked then sperm labeling must be used to ensure the source of the sperm on a second mating male's palp is from the first male. Sperm removal potentially explains why the second male gets most of the paternity but it does not explain why he mates for such a short

time relative to the first male. Indeed, one would expect sperm removal to make the second male's mating time longer than the first.

(iv) **Female control**: Females may control the length of the mating duration and have interests which are as yet cryptic.

None of these arguments are mutually exclusive and some combination of these factors may be the truth behind the paradox.

If stratification of sperm within the sperm storage organ influences the sperm precedence pattern then a trend in P_2 across broods would be expected. In the case of *P. phalangioides* this would be expected to be a decrease in P_2 among broods with time as the sperm of the second male is used up. This is because, according to Austad (1984), the sperm are stratified in the sperm storage organ and so as the second male's sperm is utilized to fertilize eggs then the displaced first male's sperm should come into play. No such trend was observed. It is possible that there were not enough broods analysed to show any trend which may be there. This is because of time restrictions; the number of broods laid by a female was truncated at up to four broods; if more were allowed to be laid and analysed then a trend may be found.

In *T. montana* it was found that the P_2 was very variable between broods and no overall sperm precedence pattern was found. There was a suggestion[2] that males mating for the longest

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[2] The suggestion was not significant so further experiments need to be carried out to see if this trend is real.

time, on the whole, received a higher share of the paternity. Maybe a difference between the Haplogyne cul-de-sac and the Entelegyne cul-de-sac has been uncovered here.

Intraspecific variations in precedence patterns are a common phenomenon. Parker (1970) found intraspecific variation in precedence patterns in his review of the insects, though the variation was usually constrained to a range which did not span the 0.5 level. Here in *T. montana* the variation is higher and entails the full spectrum of precedence patterns, from first male to second male priority. Intraspecific variation in P_2 can come from a number of sources. These sources can be convieniently divided into male and female determinations.

(i) **Female**: Processes beyond the shape of the spermatheca can go on within it. These may include secretions which can partition seminal pools so separate access is made to them at the time of fertilization. Bias in the male chosen to fertilize the eggs, based on characters established during the intimate contact of *coitus*, where the female is able to assess the male, is then possible. Ejection of sperm is a phenominon known in mammals (Baker & Bellis 1995) and could potentially occur in spiders whereby sperm from an unfavoured male could be expelled prior to fertilization. I would expect this form of sperm manipulation to be restricted to highly polyandrous species because it would be too risky a strategy in species where access to more than one male could not be depended upon.

(ii) **Male**: The males have more strategies available in terms of maximising their reproductive success and they can be a source of P_2 variation. I shall list them and appraise them in terms of relavance to *T. montana*:

(a) Plugging: Masumoto (1993) found mating-plugs to be a source of P_2 variation in *Agelena limbata*. They are an unlikely source of P_2 variation in *T. montana* because there is a single entry and exit point to the spermatheca and the female lays her eggs soon after mating (approximately one week in lab observations), when the female is still receptive to matings. This means that the plug would be operational for a short time and would have to be removed by the female. If the female can remove it then a subsequently mating male presumably could.

(b) Mate-guarding: Mate guarding can produce variation in P_2, if there is a window of time in which it is most effective to mate with a female, after which matings are less successful i.e. a physiological process, such as the spermathecal morphology changing (Higgins 1989), alters the chances of insemination. This does not seem to be the case in *T. montana* because males do not guard the females (observations in the field) but leave after mating.

(c) Kamikazi sperm: Studies have shown that the ejaculate is not a homogeneous mass of sperm but they differ and each sperm type potentially plays a different rôle (Baker & Bellis 1995). If the composition of the ejaculate differs between males engaged in individual sperm competition, within the spermatheca, then males with the most coordinated ejaculate will win out and this victory will be independent of the mating order and highly variable.

(d) Sperm removal: The extent to which sperm is removed from a spermatheca may be highly variable resulting in a P_2 like that found in *T. montana*.

(e) Extended mating time: The extention of mating time above that necessary to transfer sperm may function as a copulatory stimulus (Eberhard 1985) resulting in higher paternity for the individual stimulating the female the most. This is possibly the case in the Linyphiids (Watson & Lighton 1994). It may be the reason why there is an indication of a longer mating time leading to higher reproductive sucess in *T. montana*, which does not seem to be the case in *P. phalangioides*. Longer mating times also mean all the other male paternity assurance mechanisms (a-d) have longer to take place. For example, extended mating times can function as a form of contact mate-guarding. The times involved for mating in *T. montana* do not seem long enough for this to be the reason for any variation in P_2. There will be a trade-off for the males' mating duation for each unit of mating time they are forgoing travelling time to their next mating partner. As *T. montana* has been observed to live in very dense populations the chances of finding another female are high and the chances of cuckoldry are also high so a male's reproductive interests may be best served by mating with as many females as possible, rather than ensuring paternity with a single female. Crucial in determining the outcome of this trade-off may be the weight of the female, as this is an indication of her reproductive worth (fecundity), independent of how many times she has mated. Further experiments could assess if males mate for a longer time with heavier females than lighter. This is because it seems that males determine the length of mating duration in *T. montana* (the males, before disengagement, secure their palps above their cephalothorax, see chapter 3.0).

The conclutions about P_2 in the lab may be artifacts as far as mating success in the wild is concerned, of course, because the females may be more promiscuous than only mating twice there. There is an indication of this because observations have been made in the field, whilst

collecting specimens, of many suitors successfully courting the females who were mature. The fact that all three wild collected females, whose broods were reared in the lab, were found to have multiply mated seems to confirm that insemination also occurred.

Further experiments in *T. montana* could use cellulose acetate electrophoresis to measure paternity, because the banding patterns produced on this medium have been found to be identical to those on polyacrylamide (pers. obs.). This would have the advantage that smaller specimens can be electrophoresed. Thus one would be able to harvest the broods virtually after emergence from the cocoon, so mortality resulting from rearing would be lower and the broods analysed would thus be bigger. Further experiments measuring P_2 values in spiders, wishing to assess the Austad hypothesis, should concentrate on species which are primarily cul-de-sac and have a spermatheca (*T. montana* is secondarily cul-de-sac and *P. phalangioides* has not got a spermatheca). It would be interesting to see if such species conform to the predictions made by Austad (1984).

In *Zygiella x-notata* none of the factors investigated was found to be at the root of the observed variation in mating duration. This could be because in *Z. x-notata* the female is using cryptic criteria not related to those investigated to determine the duration she is willing to allow a male to mate for.

In the thesis I unsuccessfully attempted to look at the influence of symmetry of palp size on mating duration in *Z. x-notata*. If there is any mileage in theories about copulatory courtship (Eberhard 1991) then the effect of palp symmetry on mating sucess is a potentially interesting

field of enquiry. Achieving bilateral symmetry in complex morphological reproductive structures - the palps - must be developmentally demanding and potentially afford the female an indication of the male's genetic worth. This is important in spiders because males do not provide any parental care except in very rare instances. Avoidance of detection of palpal size asymmetry may be one reason why Entelegyne spiders alternate the palps during mating as opposed to Haplogyne which simultaneously apply both palps. The Entelegynes pay a penalty for this avoidance of assessment in lower potential sperm transfer rates. There may originally have been a trade-off of these factors (sperm transfer rate and female assessment avoidance) but now is phyletically fixed with Haplogyne spiders simultaneously inserting the palps and Entelegyne spiders alternating. It is clear, however, that females can assess Entelegyne males by the symmetry of their palpal usage.

Information on the symmetry of palp usage in both *Z. x-notata* and *T. montana* suggested no effect of this on mating duration, though it may affect mating success in other species. The reasoning behind this is that the symmetrical use of palps is energetically demanding (Watson & Lighton 1994) and may give valuable information about the athletic fitness of the male to the female.

As well as data internal to this thesis there is evidence in the literature that Austad was wide of the mark in postulating his theory. When the project was started no data regarding sperm precedence patterns in cul-de-sac spiders were available. During the course of the work a paper on sperm precedence patterns in a Haplogyne cul-de-sac species was published (Eberhard *et al* 1993). This species too showed the lack of a strong first male advantage in

sperm precedence as predicted by Austad (1984). However this is not good enough to support the hypothesis because it should have also shown a distinct second male advantage, but did not.

In conclusion, many avenues for further investigation exist in the arena of sexual selection in spiders, but the Austad (1984) hypothesis is no longer a paradigm within which to frame sperm competition investigations. The modified version outlined in chapter 5.0 may be a more promising theory; only more P_2 estimates will show if it fairs any better than that of Austad's which has lasted unchallenged for 12 years.

7.0 Glossary[1]

Bursa copulatrix: A pouch-like structure of the epigyne which houses the copulatory pore.

Cephalothorax: The anterior of the two major divisions of the body of a spider, also called the prosoma.

Chelicera (pl. **chelicerae**): One of a pair of jaws, each comprising a large basal portion (paturon) and a fang.

Conductor: A semi-membranous structure in the male palp which, when functional, serves to support and guide the embolus in copulation.

Dimorphism: The presence of one or more morphological differences that divide a species into two groups. Many examples come from differences between the sexes (sexual dimorphism) but others represent different forms within one sex (e.g. males of *Oedothoral gibbosus*).

Embolus (pl. **emboli** adj. **embolic**): The structure containing the terminal portion of the ejaculatory duct and its opening in the male palp; it may be very small in some species, or a long coiled, whip-like structure in others.

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1 Taken from the British Arachnological Society Members' Handbook.

Entelegyne: When the female has external genitalia in the form of an epigyne having two symmetrical halves.

Epigyne (or **epigynum**): A more or less sclerotized and modified external structure associated with the reproductive openings of the adult females of most spider species.

Fertilization ducts: Ducts leading from the female's spermathecae through which stored sperm are passed to fertilize the eggs.

Haematodocha (pl. **haematodochae**): A balloon of elastic connective tissue between groups of sclerites in the male palp, which distends with blood during copulation causing the palpal sclerites to separate and rotate. There may be up to three haematodochae - refered to as proximal, middle, and distal, separating three groups of sclerites.

Haplogyne: When the females have little or no external genetalic structure or epigyne. (cf. **Entelegyne**).

Monophyletic group: A group of taxa descended from a single ancestral species.

Palp (or **palpus**): The second appendage of the cephalothorax, originating behind the chelicerae but in front of the legs; its coxa also forms the maxilla; it lacks a metatarsal segment. in adult male spiders it is modified, often greatly, for sperm transfer.

Pedipalp: The correct term for the second appendage of the cephalothorax, but in spiders usually shortened to palp or palpus.

Scape: A finger, tongue or lip-like appendage, free at one end, arising from the midline of the female epigyne.

Sperm duct: A duct in the female epigyne through which sperm travels from the copulatory pore to the spermatheca.

Spermatheca (pl. **spermathecae**): A sac or cavity in female spiders, used for the reception and storage of sperm.

8.0 References

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