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Multiple paternity and first mate sperm precedence in the sierra dome spider, *Linyphia litigiosa* Keyserling (Linyphiidae)

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Abstract. Field observations of individually marked female sierra dome spiders, followed by electrophoretic paternity analyses, indicated that 60–70% of all eggs were fertilized by first mates. In this spider, first mates are determined via combat between males incited by females. They are larger than later mates, because the latter seldom fight other males prior to mating. However, a field experiment in which females mated first with a small male and then 1–2 days later with a large male (where neither male fought prior to mating), showed that first mate sperm precedence persists regardless of the physical qualities of first mates. Thus phenotypic attributes per se of first mates do not promote first mate advantage and females rely 'blindly' on the intrasexual process to determine their major sire. Consideration of five mating circumstances and associated patterns of paternity suggests that females control differential sperm use. For example, among secondary mates the duration of preinsemination phase copulation, when males might try to displace sperm, was negatively correlated with fertilization success. The copulatory behaviour of different secondary mates of individual females refuted the hypothesis that first mate sperm precedence is maintained by mating plugs. Male size was not correlated with quantity of sperm transferred or length of the intromittant organ, eliminating additional modes of sperm competition as explanations for first mate advantage.

Multi-male mating, when coupled with 'cryptic' female choice (sensu Thornhill 1983) or sperm competition (Parker 1984), may constitute an important foundation for sexual selection in a given species. Thus a necessary step in understanding what females gain from multiple mating involves determining the attributes of males that achieve fertilizations and the circumstances surrounding their matings. The data I present here are from field and laboratory studies of the sierra dome spider aimed at discovering the costs and benefits of polyandry. In this paper I have three main objectives. First, I document multiple paternity and first mate sperm precedence. Second, I examine via a field experiment whether phenotypic attributes of males determine the first mate's advantage. Third, I synthesize a range of observations that together suggest that females control sperm precedence. The data in this last part stem from studies of (1) male fertilization success in relation to five mating circumstances (the durations of preinsemination and insemination phase copulation, the number of intromissions during copulation, male position in the female's secondary mate order, and the female's final number of mates), (2) quantitites of sperm transferred in relation to male body weight and (3) the morphologies of male and female genitalia.

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Natural History

The sierra dome spider has an annual life cycle. At my study site 90% of males mature sexually within a 2-week period beginning in late June. Female maturation is less synchronous, beginning in late June and extending through mid-August. Mature males are nomadic, never build webs and provide no offspring care. They spend the breeding season (July–August) travelling between the dome-shaped webs maintained by females. Males fight for exclusive possession of any female's web they discover. When a male finds a web that he can control he usually pauses there for at least one period of daylight to forage, take refuge from predators and court the resident female.

Females cannot deter males from entering their webs and stealing prey. Males have larger fangs, longer legs and a more heavily built (for their overall body size) and armoured cephalothorax than

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females. However, females can avoid close contact with any male by withdrawing to the periphery of the web; courtship and copulation occur only within the central dome portion. Despite the male's dominance, I never observed forced copulations, probably because (1) the tight fitting genitalia require intromitting males to maintain precise orientation with respect to the female, (2) copulation must proceed for several hours to transfer sperm and (3) intromitting males can have their copulatory organs damaged by sudden movements of resistant females (Watson 1988).

The events leading to females' first matings are described in Watson (1990). Briefly, the intersexual behaviour of females within 2–5 days of maturation signals their proximity to sexual readiness and high reproductive value. The female's behaviour elicits attempts by males to guard her until her final moult; success virtually assures mating. Since several males typically discover a female over any 2–5-day period, the final male present at the time of female maturation is usually the victor of a series of fights between males. Secondary suitors are required to fight far less often before mating than first mates, because males attending non-virgin females depart on the evening following their arrival, with or without copulation. The prolonged guarding and enhanced intrasexual selection elicited by nearly mature females results in first mates, on average, being larger and more vigorous fighters than secondary mates.

METHODS

Data Collection

I studied a natural population of sierra dome spiders at the Flathead Lake Biological Station (Polson, Montana, U.S.A.), on the eastern shore of Flathead Lake. I observed free-living females during the 3–7-week period of sexual activity preceding their first oviposition. Females were individually marked on the tibia of the posterior pair of legs with coloured paint, and observed on their webs at the following times every day: 0300, 0600, hourly from 0800 through 2000 and at 2200 and 2400 hours. During each observation a standard protocol was used to record the female's pairing status, foraging success, attendance by males and intersexual behaviour. All complete (i.e. fertile) matings are diurnal and last over 2 h, so all were witnessed. After each pairing the male was captured. Field observations were followed by electrophoretic paternity studies.

The data used in regression analyses of paternity in relation to mating circumstances are based upon observations of 44 polyandrous females and their 113 mates (years 1982, 1984 and 1985). These 44 females mated with from two to five males (25 with two mates, 14 with three, three with four, two with five: $\bar{X} \pm \text{SD} = 2{\cdot}80 \pm 0{\cdot}94$ mates). Females were placed randomly in one of two categories: 24 mated ad libitum with spontaneously visiting males, while 20 'controlled mating' females were constrained to two matings with males I chose on the basis of body size. All matings were observed and males captured for 42 (95·5%) of the females, while one or more mates eluded capture for two females (0·5%). I determined electrophoretic phenotypes at one or more isozyme loci for an average of 20·4 offspring per female (SD = 10·1).

Controlled Mating Experiment

I used this experiment to determine whether first mate sperm precedence persists when females have a secondary mate that is much larger than their first. When it became evident from the behaviour of a penultimate instar female that she was nearing sexual maturation (Watson 1990), I began removing all males from her web during hourly observations. These females matured in the absence of a guarding male. Within a day of each female's maturation, I introduced the smallest male I could find in 1–1·5 h of searching on the study site. After copulation this male was captured. Within 48 h of the first mating, I introduced a second, but this time exceptionally large, male onto the female's web. Second matings took place within 3 days of the first ($\bar{X} \pm \text{SD} = 1{\cdot}63 \pm 0{\cdot}68$ days). No 'natural' matings occurred, because I continued evicting spontaneously arriving males during hourly scans.

Handling of Spiders

Males were captured after they finished mating. They were weighed to the nearest 0·1 mg, and their cephalothorax width was measured to the nearest 0·5 mm (Watson 1990). They were then frozen (−196°C) in numbered cryogenic tubes.

I captured females when they became fully gravid. They were housed at ambient temperatures and humidities in individual 1 quart (U.S.) mason jars with gauze lids. They usually produced cocoons within 1–4 days. I obtained a second clutch of eggs from some females by returning them to their jars or to a web on the study site for 2–4

weeks after they produced the first clutch. The majority of females were frozen on the day following their first oviposition; 85% of analysed progeny arose from first clutches. Females never accepted additional matings after the first oviposition.

The cocoons were transported to Cornell University and maintained at approximately 2°C until January or February of the year following collection, when they were brought to room temperature and opened. Broods were raised separately in 3-litre terraria with wild-type *Drosophila* as food. Spiderlings were raised to about 20% of adult size to ensure that homogenates of each individual would be concentrated enough to produce distinct staining patterns on the electrophoretic gels. Each spiderling that reached a suitable size was frozen in liquid nitrogen.

Sperm Counts

Sperm counts were done in 1983 using a separate set of 27 females. These females were collected haphazardly from the study site while in their penultimate instar. They matured in laboratory terraria in which they built webs, and mated once. Sperm counts were done 48 (± 8) h after mating.

To count sperm, the epigynum, which contains the spermathecae, was removed from the ventral surface of the female's abdomen and placed in a 10-µl drop of saline on a cool glass slide. The epigynum was pulverized in the drop with a glass rod. The drop was then drawn into a 10 µl capillary while being stirred. The recovered fluid (7–8·5 µl) was fed into a haemocytometer. Sperm were counted (in both free-swimming and encapsulated forms) at 200 × with a Nikon phase contrast microscope. The method was estimated to yield a count of 1% of the female's stored sperm.

Embolus and Leg Measurements

Whole pedipalps were soaked in 15% KOH at 40°C for 36–42 h, and then dissected under phosphate-buffered saline. The intromittant organ (the embolus) was separated from the radix without distortion and positioned on its side in the pool of saline (see Merrett 1962 for linyphiid pedipalp structure). An eyepiece micrometer was used to measure emboli from both pedipalps (to the nearest 0·0167 mm; magnification = 30 ×), from the distal opening of the sperm duct, to the midpoint of a 70–100° curve in the duct leading to the radix. Leg measurements were taken along the dorsal edge of the right anterior tarsus (to the nearest 0·2 mm).

Electrophoresis

Paternity analyses were based on isozyme data gathered via protein electrophoresis of females, their mates and offspring. Isozymes were separated using standard starch gel and staining techniques adapted from May et al. (1979) and Harris & Hopkinson (1976). Buffers were modified from Ridgeway et al. (1970), Ayala et al. (1973), Clayton & Tretiak (1972), Selander et al. (1971) and Markert & Faulhaber (1965).

Electrophoresis of adults and spiderlings was performed 6–9 months after each field season. Samples were maintained between −196 and −75°C until analysis. In a preliminary survey of 70 loci using four different gel and tray buffer systems, six polymorphic loci were resolved from homogenates of whole spiders (crushed in an approximately equal volume of 0·05 M Tris(hydroxymethyl) aminoethane–HCl; pH 7·1). Five isozymes were dialleic: esterase (EST), isocitrate dehydrogenase (IDH), triose phosphate isomerase (TPI), guanine reductase (GR) and adenosine deaminase (ADA). The sixth locus, 6-phosphogluconate dehydrogenase (6PGD), had three alleles. Of the 64 other systems surveyed, 30 were not active, 28 were monomorphic and six were active but poorly resolved.

The paternity analyses outlined below assume that the isozyme phenotype of an individual reflects its genotype. To test this assumption I determined whether isozymes were in Hardy–Weinberg equilibrium. Allomorph frequencies were consistent with alleles in Hardy–Weinberg equilibrium for five of the six loci (Table I). There was no evidence of generational or between-year shifts in allele frequencies (Table I), or that mating was non-random with respect to protein phenotypes. In analyses involving the mate and offspring of 13 females constrained to one mating, offspring genotype frequencies never deviated significantly from Mendelian expectations (all $P > 0{\cdot}05$).

Probably because of scoring problems, isomorph frequencies of ADA did not fit Hardy–Weinberg expectations (1982 data: $\chi^2 = 13{\cdot}17$, $N = 160$, $df = 2$, $P = 0{\cdot}001$; 1984 data: $\chi^2 = 9{\cdot}45$, $N = 86$, $df = 2$, $P = 0{\cdot}009$). Buffer systems yielding clearer ADA staining patterns were not developed until 1985 after samples from previous years had been depleted. The ADA locus was used to determine paternity in

Table I. The frequency of phenotypes at five isozyme loci and tests of Hardy–Weinberg equilibrium

Locus	Year	Allele frequency	Phenotype	Observed frequency	Expected frequency	Test result
EST	1982	1: 0·777	11	109	102·336	$\chi^2 = 2{\cdot}26$
(monomer)		2: 0·223	12	54	62·976	$P = 0{\cdot}323$
			22	12	9·689	
	1984	1: 0·706	11	45	52·630	$\chi^2 = 3{\cdot}59$
		2: 0·294	12	37	32·388	$P = 0{\cdot}166$
			22	8	4·983	
	1985	1: 0·765	11	30	29·824	$\chi^2 = 0{\cdot}02$
		2: 0·235	12	18	18·353	$P = 0{\cdot}990$
			22	3	2·824	
IDH	1982	1: 0·695	11	82	79·216	$\chi^2 = 1{\cdot}03$
(dimer)		2: 0·305	12	64	69·528	$P = 0{\cdot}597$
			22	18	15·256	
	1984	1: 0·733	11	47	48·356	$\chi^2 = 0{\cdot}57$
		2: 0·267	12	38	35·228	$P = 0{\cdot}753$
			22	5	6·416	
	1985	1: 0·716	11	27	26·145	$\chi^2 = 0{\cdot}37$
		2: 0·284	12	19	20·741	$P = 0{\cdot}833$
			22	5	4·113	
TPI	1982	1: 0·995	11	161	161·428	$\chi^2 = 0{\cdot}40$
(dimer)		2: 0·045	12	16	15·213	$P = 0{\cdot}819$
			22	0	0·358	
	1984	1: 0·956	11	82	82·254	$\chi^2 = 0{\cdot}20$
		2: 0·044	12	8	7·572	$P = 0{\cdot}905$
			22	0	0·174	
	1985	1: 0·990	11	50	49·985	$\chi^2 = 0{\cdot}01$
		2: 0·010	12	1	1·010	$P = 0{\cdot}998$
			22	0	0·005	
GR	1982	1: 0·943	11	156	156·508	$\chi^2 = 0{\cdot}64$
(monomer)		2: 0·057	12	20	18·920	$P = 0{\cdot}728$
			22	0	0·572	
	1984	1: 0·933	11	79	78·344	$\chi^2 = 1{\cdot}02$
		2: 0·067	12	10	11·252	$P = 0{\cdot}599$
			22	1	0·404	
	1985	1: 0·961	11	47	47·100	$\chi^2 = 0{\cdot}09$
		2: 0·039	12	4	3·823	$P = 0{\cdot}958$
			22	0	0·078	
6PGD	1982–1985	1: 0·987	11	310	310·037	$\chi^2 = 0{\cdot}05$
(dimer)		2: 0·005	12	3	2·952	$P = 0{\cdot}999$
		3: 0·008	22	0	0·007	
			13	5	4·961	
			23	0	0·024	
			33	0	0·020	

The abbreviated name and quaternary structure are given for each locus. Chi-squared tests have $df = 2$ except for the 6PGD locus which has 5.

only one 1985 family in which the phenotypes of the two prospective sires were identical at all other loci.

My statistical estimates of paternity assume that electrophoretic data from different loci enable independent tests of a putative sire's paternity. This assumption rests on there being little or no linkage amongst the loci that code for the isozymes under study. Linkage studies were carried out according to May et al. (1979). Unfortunately, of the 44 families available for paternity analyses, only four met criteria necessary for inclusion in the linkage assessments. These data provided marginal evidence for linkage between the EST and GR loci (Table II). Linkage of these two loci is unlikely to

Table II. Results of 2 × 2 linkage assessments

Loci	Number of offspring	χ^2	P	P_{sb}*
GR × IDH	23	1·10	0·29	0·87
IDH × EST	22	0·00	1·00	1·00
GR × 6PGD	11	0·82	0·37	0·87
EST × GR	9	5·44	0·02	0·08

*Pooled significance level calculated via the sequential Bonferroni method.

bias paternity analyses, because they were used in combination in only seven of the 44 families (16%), and in all but one of these a third locus (IDH or 6PGD) was also involved. No other evidence for linkage was discovered, but with this small sample evaluation of the no-linkage assumption is weak.

Statistical Paternity Estimation

Only 10% of all offspring could be assigned to a father by direct inspection of the isozyme phenotypes of the mother, potential sires and offspring. Therefore all mates had their levels of paternity assigned statistically. I estimated each mate's paternity using two different algorithms: Bipat and Empat. These estimates served as dependent variables in two parallel sets of regression analyses aimed at determining male attributes and mating circumstances associated with fertilization success.

Bipat is of my own design and is described in the Appendix. Empat provides formal maximum likelihood estimates of probabilities of paternity for each mate of a female via the EM algorithm (Dempster et al. 1977; Dickinson 1986; Dickinson & McCulloch 1989). Levels of Empat represent predictions of the proportion of a female's brood fathered by a given male (Kukuk & May 1988). I calculated Empat using a computer program provided by J. Dickinson.

I see Bipat and Empat as complimentary indices of paternity. Bipat conservatively allotted modest levels of paternity even to males expected to produce frequency distributions of offspring phenotypes quite different from those observed. Bipat is yielding to the hypothesis of mixed paternity. In contrast, Empat tended to give either high or low probabilities of paternity to the majority of males. For the 44 families I analysed, Empat often assigned a probability of paternity of 1·0 to one mate (i.e. 0·0 to all other mates) even when the others could easily have fathered a few offspring with phenotypes compatible with that of the statistically more likely sire. This all-or-nothing tendency of Empat was my major reason for also using Bipat in assessing each male's paternity. I did not use Bipat exclusively because of its less rigorous statistical interpretation. There is no a priori justification for concluding which algorithm provides a more realistic picture of sperm precedence in the sierra dome spider.

Regression Procedures

I used analysis of covariance to determine the relationship between five mating circumstances and male fertilization success. The analyses controlled for the number of pedipalps (one or two) used by a male during copulation; this covariate, denoted Numpalp, was known for all mates of the 44 females used in the paternity analyses.

Testing of ANCOVA assumptions revealed one violation: residuals of linear models with Empat as the dependent variable deviated from normality. Therefore in evaluating Empat-based models I performed both linear and logistical (logit) analyses (Anderson et al. 1980; Steinberg 1985). In cases where I report only the logit results, these agreed qualitatively with the corresponding linear analyses. In the logit analyses I used a trichotomous categorization of Empat as the dependent variable, denoted below as C\Empat (C\Empat = 1 when Empat $\leqslant 0{\cdot}33$, 2 when $0{\cdot}34 \leqslant$ Empat $\leqslant 0{\cdot}66$, and 3 when Empat $\geqslant 0{\cdot}67$). The significance of factors in logit models was determined via likelihood ratio tests (Anderson et al. 1980).

When some results of a set of statistical tests are significant, I report both the individual significance level and a pooled level calculated via the sequential Bonferroni method (Rice 1989). Throughout the text, P refers to the significance level of an individual test and P_{sb} to the Bonferroni adjustment. As a further aid to interpretation of selected linear regression coefficients, I report the percentage of the residual variance explained by individual factors ($\%R^2_a$), that is

$$\{[R^2_a(\text{full}) - R^2_a(\text{base})]/[1 - R^2_a(\text{base})]\} \times 100$$

where 'base' and 'full' refer to the reduced (i.e. covariates only) model and the model with the factor of interest added, respectively.

Statistics were calculated using version 4.0 of the SYSTAT computing package (Wilkinson 1988)

and the LOGIT supplementary module (Steinberg 1985). Bonferroni adjustments were calculated using a computer program written by W. R. Rice.

RESULTS

Multiple Mating and Paternity

During the 3–6 weeks between a typical female's first mating and first oviposition, she is visited by 7–14 males ($\bar{X} \pm SD = 1{\cdot}0 \pm 1{\cdot}0$ visit every 3 days, $N = 357$ female/days). During 1980–1988, 104 females observed consistently from maturation through to first oviposition copulated with one to four secondary suitors, for an average total number of mates of 2·3. (SD = 0·98).

Multiple paternity appeared to be common. Seven females (15·9%, $N = 44$) provided direct evidence of multiple paternity; their broods contained two or more groups of spiderlings having electrophoretic phenotypes that could not be explained by any single mate. Single paternity could not be excluded in the remaining 37 families. However, paternity estimates using the Empat algorithm yielded 22 families (50%) where two or more males had a probability of paternity $\geqslant 0{\cdot}05$, and 15 families (34·1%) where two or more males had a probability of paternity $\geqslant 0{\cdot}25$. Bipat indicated that multiple paternity was plausible in 43 families (97·7%).

Biased Sperm Use

Before testing hypotheses about sperm precedence, I examined whether there was evidence for non-random sperm use. For each electrophoretic locus that varied among the mates of a given female, I used chi-squared goodness-of-fit tests to compare isozyme frequencies in the progeny with those expected if fertilizations had been distributed randomly. When multiple loci were variable, separate tests were done and the resulting P-values combined (Fisher 1954).

Twenty-three of the 44 families (52·3%) provided data permitting rejection of the null hypothesis of random sperm use ($P \leqslant 0{\cdot}05$); 14 families (31·8%) yielded $P \leqslant 0{\cdot}02$. Eight families (18·2%) still gave significant results after a sequential Bonferroni adjustment of P-values ($P_{sb} \leqslant 0{\cdot}05$; 44 Ps included in this adjustment). The combined level of significance from all 44 tests of the null hypothesis of random fertilizations was below 0·001 ($\chi^2 = 316{\cdot}12$, $df = 90$).

Pedipalp Use: Covariate Numpalp

Secondary mates often copulated using a single pedipalp (81·9%, $N = 72$). One-pedipalp copulations were significantly less common for first mates (33·3%, $N = 45$; $\chi^2 = 19{\cdot}06$, $df = 1$, $P < 0{\cdot}001$). Sperm storage systems of female spiders consist of independent, bilaterally symmetrical halves. Each half is accessible to the male only via intromissions involving the corresponding pedipalp (Foelix 1980). Thus it would not be surprising if the use of one versus two pedipalps had an impact on male fertilization success.

In analyses holding first versus secondary mating status constant, first mate fertilization success did not differ according to the number of pedipalps used in copulation (Bipat: $t = 0{\cdot}378$, $P = 0{\cdot}707$; C\Empat: $\chi^2 = 0{\cdot}525$, $df = 2$, $P = 0{\cdot}974$). However, for secondary mates, using one pedipalp was associated with a reduction in mean fertilization success from 0·748 (SD = 0·358) to 0·536 (SD = 0·244) under Bipat, and from 0·493 (SD = 0·383) to 0·185 (SD = 0·306) under Empat (Bipat: $t = 3{\cdot}874$, $P = 0{\cdot}0003$; C\Empat: $\chi^2 = 11{\cdot}256$, $df = 2$, $P = 0{\cdot}0036$).

Male Fertilization Success

Mating status

First versus secondary mating status was the most important determinant of male fertilization success. Among the 44 families analysed, 27 (61·4%) first mates had higher paternity than any secondary mate of the same female. Of 69 secondary mates, 14 (20·3%) became the major sire of a female's offspring, and three (4·3%) had paternity equal to that of first mates.

Ad libitum versus controlled matings

The secondary mates of ad libitum mating females averaged 16·3% smaller than first mates (SD = 36·1, $N = 23$), but it was not rare for ad libitum females to have opportunities to mate with individuals that were larger than their first mate. Thirty-two per cent of all secondary suitors of ad libitum mating females were heavier than the female's first mate, and 15·3% were over 20% heavier ($N = 365$ secondary suitors of 122 females). The secondary mates of controlled-mating females averaged 99·6% larger than first mates (SD = 43·0, $N = 19$ females).

First mates of the 24 ad libitum mating females had higher mean fertilization success than secondary mates under all three paternity indices (Table III

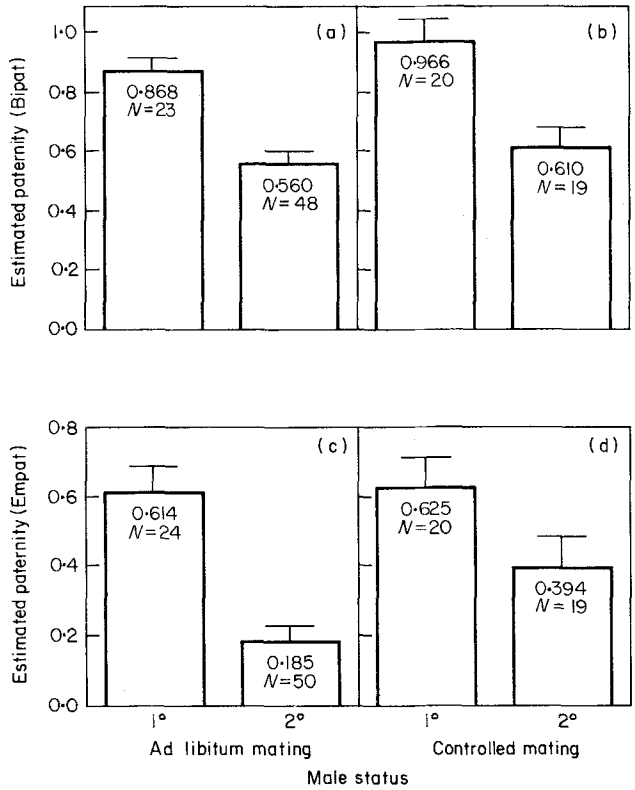

Figure 1. Mean estimated paternity for first (1°) and secondary (2°) mates of ad libitum and controlled mating females. Results of the Bipat (a and b) and the Empat (c and d) paternity algorithms are given. The figure displays raw means (+SE). (a) $t=3{\cdot}38$, $P=0{\cdot}001$; (b) $t=2{\cdot}57$, $P=0{\cdot}014$; (c) $t=4{\cdot}09$, $P<0{\cdot}001$; (d) $t=0{\cdot}89$, $P=0{\cdot}379$. The t-statistics are based on analyses that control for the effects of males using one versus two pedipalps (Table III).

and Fig. 1a, c). Bipat was also greater, on average, for first mates of the 20 controlled mating females, while Empat and C\Empat showed a statistically insignificant trend in the same direction (Table III and Fig. 1b, d). Pooled data for all 44 females also indicates significantly higher fertilization success for first mates (Table III).

The point of the controlled mating experiment was to uncouple the size of the first mate from the context of his being in control of the female's web at the time of her maturation. The frequency of first mate sperm precedence was the same in the ad libitum and controlled mating groups ($\chi^2=0{\cdot}299$, $df=1$, $P=0{\cdot}585$, $N=41$; three ties excluded). To determine whether the regression functions relating first versus secondary mating status and male fertilization success were the same in the ad libitum and controlled mating groups, a dichotomous variable indicating the group to which a male belonged, plus this variable's interaction with mating status, were added to the original model. The parameters of the model were then re-estimated using pooled data from both populations. By assessing the null hypothesis that the coefficients of the new indicator variable and its interaction with mating status were both zero (see Neter et al. 1985, pp. 343–345), I determined that the regression functions for the two groups were indistinguishable under Bipat ($F=1{\cdot}089$, $P=0{\cdot}340$), Empat ($F=2{\cdot}377$, $P=0{\cdot}098$) and C\Empat ($\chi^2=6{\cdot}642$, $df=4$, $P=0{\cdot}156$). Thus mating status per se, not the physical attributes conducive to becoming a first mate, is the direct determinant of first mate sperm precedence.

Mate number and order

The frequency of occurrence of first mate sperm precedence was not associated with the total number of secondary matings accepted by the female ($\chi^2=1{\cdot}076$, $df=1$, $P=0{\cdot}300$, $N=41$, three

Table III. The results of linear and logistical analyses of covariance assessing the influence of first versus secondary mating status on paternity

Matings considered	Paternity index	β*	SE	*N*	*P*†	P_{sb}	$\%R^2_a$
All females	Bipat	−0·275	0·061	110	2·0E−5	1·8E−4	15·05
	Empat	−0·306	0·078	113	1·7E−4	1·2E−3	11·28
	C\Empat	—	—	113	1·9E−3	7·6E−3	—
Ad libitum mating	Bipat	−0·261	0·077	71	1·2E−3	6·0E−3	13·09
Females‡	Empat	−0·391	0·096	74	1·1E−4	8·8E−4	17·93
	C\Empat	—	—	74	1·0E−3	6·0E−3	—
Controlled mating	Bipat	−0·271	0·105	39	0·014	0·042	13·02
Females‡	Empat	−0·121	0·137	39	0·379	0·758	−0·57
	C\Empat	—	—	39	0·759	0·759	—

*β is the regression coefficient for the mating status variable; negative values indicate that first mates had higher paternity. Numpalp (see Methods) was a covariate.

†Two-tailed *t*-tests (Bipat amd Empat) or likelihood ratio tests (C\Empat) were used to evaluate H_o: β = 0. *P* and P_{sb} are individual and table-wide levels of significance, respectively. Significance levels for Empat are suspect due to non-normality of residuals. $\%R^2_a$ gives the percentage of remaining variation in fertilization success explained by male mating status.

‡See also Fig. 1.

ties excluded). In analyses performed separately for first and secondary mates, the number of secondary mates accepted by a female was irrelevant to the mean fertilization success of first and secondary mates (all $P \geqslant 0{\cdot}128$). Among secondary mates, a male's position in the female's mate order had no relationship to fertilization success (all $P \geqslant 0{\cdot}727$). First mate sperm priority exists, but there is no evidence for a more generalized pattern of early mate advantage.

Copulation duration and intromission number

Copulations were diurnal and lasted 2–8 h ($\bar{X} \pm \text{SD} = 3{\cdot}6 \pm 1{\cdot}3$ h, $N = 111$). As in other linyphiid species (see Austad 1982; van Helsdingen 1983), every complete copulation was a two-phase process (84% of all copulations initiated are completed, $N = 200$). Copulations began with a 2–6 h ($\bar{X} \pm \text{SD} = 2{\cdot}63 \pm 1{\cdot}17$ h, $N = 107$) 'preinsemination phase', during which no sperm are present in the male's copulatory organs (pedipalps). During this phase, intromission rates (i.e. the rate at which the intromittant organ is inserted and withdrawn) were relatively high ($\bar{X} \pm \text{SD} = 19{\cdot}0 \pm 8{\cdot}0$ per min, $N = 94$). During the ensuing insemination phase of copulation, when males transfer sperm, intromission rates were lower (2–3 per min), because the intromittant organ was held within the female 10–20 times longer during each intromission than in the preinsemination phase.

The durations of preinsemination and insemination phase copulation did not affect the fertilization success of first mates (all $P \geqslant 0{\cdot}416$). The duration of preinsemination phase copulation had a surprising negative relationship with the fertilization success of secondary mates (Fig. 2; Bipat: $t = 2{\cdot}455$, $P = 0{\cdot}017$, $P_{sb} = 0{\cdot}120$, $\%R^2_a = 7{\cdot}88\%$; C\Empat: $\chi^2 = 12{\cdot}26$, $df = 2$, $P = 0{\cdot}002$, $P_{sb} = 0{\cdot}018$), but the duration of the insemination phase did not (Bipat: $t = 0{\cdot}701$, $P = 0{\cdot}486$, $\%R^2_a = -0{\cdot}85\%$; C\Empat: $\chi^2 = 2{\cdot}14$, $df = 2$, $P = 0{\cdot}343$). An estimate of the number of intromissions achieved during the preinsemination phase (i.e. duration multiplied by intromission rate) was unrelated to the fertilization success of both first and secondary mates (all $P \geqslant 0{\cdot}187$ and 0·633, respectively). (P_{sb}s were calculated using all eight *P*s reported in this paragraph.)

Sperm Quantity Versus Male Weight

First mates average larger than secondary mates (Watson 1990). If larger males transfer more sperm during copulation, then the tendency for ad libitum first mates to sire more offspring could be due to their sperm outnumbering those of other mates. Although first mates varied by a factor of 20 in how much of their sperm was stored by females (range = 1–19 sperm cells observed per count), no relationship existed between this quantity and male weight ($r = 0{\cdot}019$, $N = 27$, $P = 0{\cdot}927$).

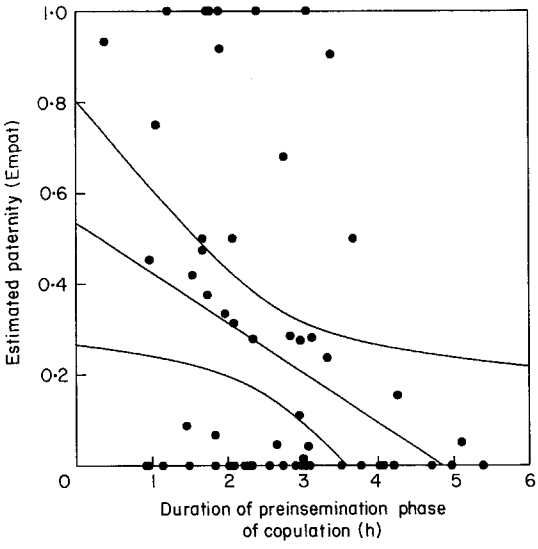

Figure 2. Relation between the duration of preinsemination phase copulation and the fertilization success of secondary mates as estimated using the Empat paternity algorithm. The least-squares best-fit line and 95% confidence band are provided.

Table IV. Pedipalps used in copulations by secondary mates in relation to those used by the same female's previous secondary mate

Palp(s) used by subsequent $(n+1)$th secondary mate	Palp used by nth secondary mate		
	Left	Right	Totals
Left	9 (7·8)	11 (12·2)	20
Right	8 (8·2)	13 (12·8)	21
Both	4 (5·1)	9 (7·9)	13
Totals	21	33	54

All observations involve different females and males. Numbers in parentheses are random expectations; $\chi^2 = 0{\cdot}69$, $df = 2$, $P = 0{\cdot}709$.

Embolus Length and Male Size

A longer embolus could be more effective in sperm transfer. This is another mechanism that could favour ad libitum first mates over secondary mates, since the former are typically larger. Alternatively, if longer emboli mainly help males remove the sperm of previous males, it could explain why size-related characters are associated positively with the fertilization success of secondary (Watson, in press), but not first, mates. However, this mechanism of competition between males was also unsupported; there was no correlation between male size (i.e. leg length) and length of the embolus ($r = 0{\cdot}031$, $N = 17$, $t = 0{\cdot}094$, $P = 0{\cdot}890$).

Mating Behaviour Contraindicates Blocks

Since secondary mates typically used only one pedipalp throughout copulation, I originally thought that first mate sperm precedence could be maintained by mating blocks deployed by a female's first mate. However, I later realized that different secondary mates of the same female varied randomly as to which pedipalp they used (Table IV). Fifty-nine per cent of secondary mates successfully employed the pedipalp that was not used by the same female's previous secondary mate ($N = 54$ females). Therefore pedipalp use is not dictated by static blockage of the female's epigyna.

DISCUSSION

First Mate Sperm Precedence

First mate sperm precedence in a linyphiid spider, *Frontinella pyramitela*, has previously been shown in a laboratory setting by Austad (1982) using a sterile male technique. Martyniuk & Jaenike (1982) found that final mates of free-living female filmy dome spiders, *Linyphia marginata*, could not have fathered all offspring, an observation in accord with first mate priority. In the sierra dome spider, although 60–70% of all fertilizations are by first mates, later mates sire a substantial proportion of many broods.

Female Choice or Sperm Competition?

The relative roles of intrasexual and intersexual selection in determining male fertilization success cannot be known without understanding the anatomical or physiological mechanisms involved. Moreover, both the male and female sides of any mechanism must be known; male adaptations for sperm competition may be functional only when females allow access to their sperm stores (see Villavaso 1975). For the sierra dome spider, however, four categories of indirect evidence indicate

that females exert substantial control over sperm precedence.

Mating and sperm plugs

First mates might achieve high fertilization success by using mating or sperm plugs. However, there was no association among different secondary mates of the same female as to which pedipalp(s) were used. Thus, patterns of pedipalp use are not dictated by mating plugs. Furthermore, dissections ($N=25$) and serial sections ($N=2$) of copulatory organs (i.e. epigyna) from multiply mated females ($N=25$ and 2, respectively) failed to reveal blockage of the epigynal openings or sperm ducts by broken parts of pedipalps or mucoid plugs. Austad (1982) looked for external plugs in *F. pyramitella* and also failed to find any. However, certain varieties of sperm plugs might dissolve in reagents used in dissection, dehydration or fixation. Moreover, sperm barriers might be subtle, consisting, for example, of no more than a membrane that hinders penetration of the sperm of competing males into favourable sections of the spermathecae. My work cannot rule out such mechanisms of sperm competition, but there is indirect evidence against their existence.

Female linyphiids possess a 'conduit' style sperm transport/storage system (Austad 1984; personal observation), in which there are separate tubes for entry and departure of seminal fluids on opposite sides of the spermatheca. Since earlier mates have an opportunity to position their sperm closer to the opening leading to the oviduct, a conduit system could encourage a 'first-in/first-out' bias in sperm precedence favouring early mates. Under these circumstances, if first mates install a barrier behind their sperm to prevent displacement away from the opening to the oviduct, secondary mates probably would also. This would result in a series of barriers gradually accumulating against the oviduct side of the sperm receptacle, decreasing the likelihood that ejaculates of later mates break through to fertilize eggs. However, this prediction is not in line with the observation that the fertilization success of secondary mates is unrelated to their position in a female's mating order.

Since secondary mates obtain some fertilizations, any sperm plugs deposited by early mates must occasionally be circumvented. The likelihood of a plug's failure should be positively related to the number of males that attempt to bypass it. The observation that a female's final number of mates does not influence the fertilization success of her first or secondary mates also contradicts sperm plug hypotheses.

Male size and sperm competition

Size and strength are likely correlates of a male's ability in several forms of sperm competition. For example, larger males might be better able to dislodge sperm plugs left by former mates, pump copious fluid through the female to flush out the sperm of previous mates, or deliver large numbers of viable sperm per ejaculate. However, physical stature and fighting ability per se do not govern first mate sperm precedence in the sierra dome spider. Extremely large secondary mates did not reduce the first mate's advantage any more than those of average size, even when the first mate was very small. Sperm counts add support to the conclusion that size-related advantages in fertilization success are not due solely to sperm competition; larger males did not deliver more sperm.

The lack of correlation between male size and embolus length eliminates another mechanism of sperm competition that would favour first mates. Moreover, species of linyphiid spiders fall into groups with long and short emboli (van Helsdingen 1983). Sierra dome spiders have a short embolus although females possess a long spiral sperm duct (personal observations). Thus a male's intromittant organ does not even come close to reaching the spermathecae.

Copulation duration and fertilization success

Studies of several species have shown copulation duration to be positively associated with amounts of transferred sperm, and have suggested that prolongation of copulation is a male tactic to enhance sperm displacement (Parker 1970; Fincke 1984; Waage 1984; Rubenstein 1989). For the sierra dome spider, sperm displacement seems a plausible function of lengthy copulation. However, among secondary mates fertilization success was unrelated to the duration of the insemination phase and the number of intromissions achieved during copulation, and negatively related to the duration of preinsemination phase copulation.

One explanation for the negative relationship between duration of the preinsemination phase and male fertilization success might be that males with

poor sperm competition ability attempt to compensate by prolonging their displacement efforts, but are seldom completely successful. However, secondary mates that copulate with one pedipalp, a behaviour that prevents access to 50% of the female's sperm stores, do not compensate with longer copulation (unpublished data). An alternative explanation, implying control of sperm precedence by females is that preinsemination phase copulation serves as an extension of courtship in which the male persuades the female to allow sperm displacement or insemination. Those males obliged to continue persuading for longer periods might, on average, be less likely to succeed.

Adaptiveness of First Mate Sperm Precedence

Data potentially germane to sexual selection are available to female sierra dome spiders in overlapping but non-identical forms for first versus secondary mates. Information on male size and weight, physical disabilities (e.g. deformed legs) and copulatory performance (e.g. intromission speed) is available for all mates. On the other hand, secondary mates are rarely as thoroughly tested in combat as first mates (Watson 1990) and, unlike first mates, they usually perform precopulatory courtship.

Precopulatory courtship consists of the male (1) maintaining orientation towards the female, (2) following her slowly about the dome portion of the web, (3) attempting to touch her gently with his anterior legs, (4) performing sporadic bouts of web strumming and (5) suddenly charging at the female (Watson 1988). Web strumming and charging can be energetic, but individual bouts last only 1–2 s and often occur (if at all) many minutes apart. In contrast, combat between males is intense. Fighting commonly escalates through an energetic display into a full-contact pedipalp-wrestling match, and then into frenzied biting and grappling. Fights often involve many minutes of continuous exertion and the risk of being killed. Precopulatory courtship and copulatory performance should hint at a male's strength and endurance, but females lack the physical capacity to probe the limits of a suitor's capabilities as effectively as another male.

First mates are larger than secondary mates (Watson 1990), but only the fact of their being first matters in their securing high fertilization success; female choice based on direct assessment of male size or other attributes plays no role. This is intriguing, because the fertilization success of secondary mates is modulated by male physical and behavioural attributes via female choice (Watson, in press). Thus, females have a capacity to discriminate directly between males. Many authors have suggested in agreement with Borgia (1979) that 'interactions between males provide perhaps the best composite indication of relative overall quality'. The sierra dome spider provides unusually strong support for this idea, because individual females regularly employ both modes of sexual selection at different times. First mate sperm precedence suggests that female sierra dome spiders 'recognize' first mates as their 'best bets' for principal sires.

Ostensibly there are genetic benefits for offspring fathered by males that have been tested via the intrasexual process, although work on actual fitness consequences is required before this can be confirmed. Control of sperm precedence also empowers female sierra dome spiders to time honest advertisements of their reproductive value. Such signals can be useful for manipulating male behaviour in ways that, for example, reduce foraging costs incurred by females while the male guards her (Austad 1984; Watson 1990).

Complex Genitalia and Sperm Competition

Complex 'lock and key' genitalia, such as those of linyphiid spiders, may promote a high degree of standardization of their various parts; nonstandard males might be forced into assortative mating or lose the ability to copulate. Furthermore, selection for standardization of genitalia could hinder evolution of specialized anatomical equipment for sperm competition. For example, to produce a longer intromittant organ while maintaining an ability to copulate, male sierra dome spiders would have to lengthen this single part of the pedipalp while maintaining the sizes, shapes and relative motions of the many other parts. This might be difficult if pleiotropy links development of the various parts of the pedipalp, as might be expected particularly under selection for standardization.

Eberhard (1985) proposed that complex male genitalia might evolve because of arbitrary female preferences. However, an adaptive component of intersexual selection for complex male genitalia could be that females thereby establish a 'developmental barrier' that hinders the male's ability to alter certain parts of their genitalia in ways favourable to

male control of sperm displacement. Individual females would be protected from males with new features conducive to sperm displacement, because other parts of the male's genitalia would probably have changed as well, making intromission difficult. If female genitalia remained relatively simple, as in spiders, females might secure a general advantage in conflicts over whether stored sperm are accessible to males during mating. Even if males did produce modifications conducive to sperm displacement without degrading their ability to copulate, females could respond with counter measures at a faster rate owing to lower resistance to evolutionary change to their genitalia.

APPENDIX

Calculation of the Bipat paternity index is described below. The algorithm is built around the large sample approximation of the binomial test statistic. The two major elements of Bipat are AMP and LLP, which are indices corresponding to a male's 'adjusted maximum' and 'lower limit' of paternity, respectively. Bipat is the arcsine transformation of the average of AMP and LLP.

The AMP component of the algorithm works as follows.

(1) By examining the multi-locus isozyme phenotypes of the mother, potential fathers and offspring, one determines how many progeny definitely were sired, might have been sired and definitely were not sired by each mate. The second, ambiguous class of offspring are the ones involved in statistical assignments. All subsequent steps are performed for one male and one locus at a time.

(2) The observed allele frequencies within the progeny are tallied at individual loci and the less common isomorphs at each locus are noted.

(3) Considering the phenotypes of the female and a particular mate, the expected proportion of the less common isomorph is calculated assuming exclusive paternity by that mate. Given a diallelic situation at a locus (always the case in the present study) this is a binomial proportion.

(4) The large sample approximation of the binomial test statistic

$$R = \text{abs}(N)P + \{ - Z[\text{abs}(N)P(1 - P)]^{0.5}\}$$

is solved iteratively for N, where R = the observed number of less common isomorphs (and so, alleles) in the total progeny, $Z = 1.96$ (the standard normal variate corresponding to a two-tailed significance value of 0·05), P = the expected proportion of less common alleles in the male's progeny and N = the maximum number of rare plus common alleles likely to pass into eggs (i.e. with approximate probability ≥ 0.05) during random fertilization employing the male's sperm, given the observed upper limit (i.e. R) on the total number of rare isomorphs. In other words, the calculated integer N is taken to represent the approximate maximum number of alleles that can be drawn from the male's sperm pool without the probability of observing a higher number of the less common allele falling below 0·05. (In the normal use of this equation to generate the binomial test statistic, the calculated quantity N represents sample size and R the test statistic itself. Note that the chosen level of the standard normal variate only crudely adjusts the probability limit at which further assignment of offspring to a male is halted.)

(5) N is divided by 2 to convert from alleles to diploid progeny, truncated so as not to exceed the number of offspring of ambiguous paternity produced by the female and added to the number of progeny that must definitely belong to the given male; the result is denoted N_2.

(6) AMP is calculated by adding to N_2 a fraction of the offspring whose phenotype was not known for the given locus, equal to the fraction of the offspring whose phenotype was known represented by N_2. This is required to balance multiple single-locus assessments of paternity in families where the number of offspring of known phenotype differs between loci. I did not use a particular locus in calculating Bipat unless at least 75% of all offspring were of known phenotype at that locus.

To calculate the LLP component of Bipat, the above calculations are repeated using input values derived by ignoring the focal male and assuming that paternity is distributed randomly among all the other mates of the female. This yields a maximum pooled paternity for the non-focal males. The difference between this quantity and the total offspring is interpreted as the lower limit of paternity for the focal male.

Each pair of AMP and LLP estimates are determined separately at each locus and for each member of the mate group. For example, in a mate group consisting of three males with two polymorphic loci among the males, six runs of the algorithm would be necessary. When a male's AMP or LLP scores differ between loci, the lowest AMP and the highest LLP

are chosen for final calculation of Bipat. This manner of choosing usually corresponds to emphasizing use of the most 'informative' locus (e.g. a locus at which the given male carries an allele not present in the mother or other possible fathers).

To calculate Bipat, AMP and LLP are divided by the total number of progeny, and these two proportions averaged. As is typical for proportions, the frequency distribution of these raw scores had slightly compressed tails relative to a normal distribution. Therefore the raw scores were subjected to arcsine transformation (Sokal & Rohlf 1981). This yielded the final Bipat score used as the dependent variable in regression analyses.

Note that the Bipat scores of all the mates of a female do not add up to 1, because Bipat is not a probability of paternity or a formal estimate of the proportion of offspring sired. Nor do the Bipat scores of all the mates of a female equal the female's total brood size; Bipat is not an estimate of the number of offspring sired by a male.

I can provide an annotated copy of the TK!Solver computer program I used for calculation on request, or see Watson (1988).

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REFERENCES

Anderson, S., Auquier, A., Hauck, W. W., Oakes, D., Vandaele, W. & Weisberg, H. I. 1980. *Statistical Methods for Comparative Studies: Techniques for Bias Reduction*. New York: John Wiley

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

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

Ayala, F. J., Hedgecock, D., Bumwalt, G. S. & Valentine, F. W. 1973. Genetic variation in *Tradacna maxima*, an ecological analog of some unsuccessful lineages. *Evolution*, **27**, 177–191.

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

Clayton, J. W. & Tretiak, D. N. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *J. Fish Res. Bd Can.* **29**, 1169–1172.

Dempster, A. P., Laird, N. P. & Rubin, D. B. 1977. Maximum likelihood from incomplete data via the EM Algorithm. *J. R. Soc., Ser. B*, **39**, 1–38.

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

Dickinson, J. L. & McCulloch, C. E. 1989. Use of maximum likelihood estimation to apportion broods among potential sires. *Anim. Behav.*, **38**, 719–721.

Eberhard, W. G. 1985. *Sexual Selection and Animal Genitalia*. Cambridge, Massachusetts: Harvard University Press.

Fincke, O. M. 1984. Sperm competition in the damselfly *Enallagma hageni* Walsh (Odonata: Coenagrionidae): benefits of multiple mating to males and females. *Behav. Ecol. Sociobiol.*, **14**, 235.

Fisher, R. A. 1954. *Statistical Methods for Research Workers*. 12th edn. Edinburgh: Oliver & Boyd.

Foelix, R. 1980. *The Biology of Spiders*. Cambridge, Massachusetts: Harvard University Press.

Harris, H. & Hopkinson, D. A. 1976. *Handbook of Enzyme Electrophoresis in Human Genetics*. New York: American Elsevier.

van Helsdingen, P. J. 1983. Mating sequence and transfer of sperm as a taxonomic character in Linyphiidae (Arachnida: Araneae). *Verh. naturw. Ver. Hamb.*, **(NF)26,** 227–240.

Kukuk, P. F. & May, B. P. 1988. Dominance hierarchy in the primitively eusocial bee *Lasioglossum (Dialictus) zephyrum*: is genealogical relationship important? *Anim. Behav.*, **36,** 1848–1850.

Markert, C. L. & Faulhaber, I. 1965. Lactate dehydrogenase isozyme patterns of fish. *J. exp. Zool.*, **159,** 319–332.

Martyniuk, J. & Jaenike, J. 1982. Multiple mating and sperm usage patterns in natural populations of *Prolinyphia marginata* (Araneae: Linyphiidae). *Ann. entomol. Soc. Am.*, **75,** 516–518.

May, B. P., Wright, J. E. & Stoneking, M. 1979. Joint segregation of biochemical loci in salmonidae: results from experiments with *Salvelinus* and review of the literature on other species. *J. Fish Res. Bd Can.*, **36,** 1114–1126.

Merrett, P. 1962. The palpus of male spiders of the family linyphiidae. *Proc. zool. Soc. London*, **140,** 347–467.

Neter, J., Wasserman, W. & Kutner, M. H. 1985. *Applied Linear Statistical Models*. 2nd edn. Homewood, Illinois: Irwin.

Parker, G. A. 1970. Sperm competition and its evolutionary effect on copula duration in the fly *Scatophaga stercoraria*. *J. Insect Physiol.*, **16,** 1303–1328.

Parker, G. A. 1984. Sperm competition and the evolution of animal mating strategies. In: *Sperm Competition and the Evolution of Animal Mating Systems* (Ed. by R. L. Smith), pp. 1–61. New York: Academic Press.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution*, **43,** 223–225.

Ridgeway, G. J., Sherburne, S. W. & Lewis, R. D. 1970. Polymorphism in the esterases of Atlantic herring. *Trans. Am. Fish. Soc.*, **99,** 147–151.

Rubenstein, D. I. 1989. Sperm competition in the water strider, *Gerris remigis*. *Anim. Behav.*, **38,** 631–636.

Selander, R. K., Smith, M. H., Yang, S. Y., Johnson, W. E. & Gentry, J. B. 1971. *Biochemical Polymorphism and Systematics in the Genus* Peromyscus *I. Variation in the Old-field Mouse* (Peromyscus polionotus). Austin, Texas: University of Texas.

Sokal, R. R. & Rohlf, L. F. 1981. *Biometry*. San Francisco: W. H. Freeman.

Steinberg, D. 1985. *Logit: a Supplementary Module for SYSTAT*. San Diego: Salford Systems.

Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *Am. Nat.*, **122,** 765–788.

Villavaso, E. 1975. Functions of the spermathecal muscle of the boll weevil, *Anthonomus grandis*. *J. Insect Physiol.*, **21,** 1275–1278.

Waage, J. K. 1984. Sperm competition and the evolution of odonate mating systems. In: *Sperm Competition and the Evolution of Animal Mating Systems* (Ed. by R. L. Smith), pp. 252–290. New York: Academic Press.

Watson, P. J. 1988. The adaptive functions of sequential polyandry in the spider *Linyphia litigiosa* (Linyphiidae). Ph.D. Thesis, Cornell University, Ithaca, New York.

Watson, P. J. 1990. Female-enhanced male competition determines the first mate and principal sire in the spider *Linyphia litigiosa* (Linyphiidae). *Behav. Ecol. Sociobiol.*, **26,** 77–90.

Watson, P. J. In press. Multiple paternity as genetic bet-hedging in female sierra dome spiders (*Linyphia litigiosa*: Linyphiidae). *Anim. Behav.*

Wilkinson L. 1988 *SYSTAT: the System for Statistics*. Evanston, Illinois: SYSTAT.