

Multi-male mating and female choice increase offspring growth in the spider *Neriene litigiosa* (Linyphiidae)

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Abstract. The purpose of this controlled-breeding study was to investigate the viability consequences of female choice and sequential polyandry for offspring in a way that would separate the influences of these two aspects of female sexual behaviour. Female sierra dome spiders, *Neriene litigiosa* (= *Linyphia litigiosa*) typically mate two to three times before production of their first batch of eggs, although some females (ca 16%) mate only once. Strong fighters are preferred as first mates and principal sires. Large males that give a vigorous performance during copulatory courtship are preferred as sires among a female's secondary mates. In this study, the number of matings by free-living females was experimentally controlled and the size and copulatory vigour of all the females' mates were recorded. At the end of the breeding season, the females were collected and their broods were obtained in captivity. Randomly chosen subsets of 28 female's offspring were reared under standard conditions during the following spring. The spiderlings were reared for 27 days in the company of siblings under conditions that would encourage the expression of genetic variation in viability, and growth and survivorship were monitored. After controlling for maternal effects (i.e. female size and oviposition date) and variance in feeding opportunities among rearing groups, both mate number and mate size were positively and independently related to offspring growth rates and the size of offspring attained after emergence from the natal cocoon. The results support the hypotheses that the preference for large males yields viability benefits to offspring and that polyandry can augment the benefits of selective mating.

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Clarifying the adaptive functions of female mating preferences and multi-male mating remain enticing problems for behavioural ecologists (Andersson & Iwasa 1996; Reynolds 1996). The challenge is most pronounced in the many species whose males make little or no material investment in females or their offspring. In these breeding systems, 'good genes' hypotheses offer an important class of explanation for the evolution and maintenance of selective polyandry (Andersson 1994). The good genes hypothesis states that selective mating indirectly improves female reproductive success by increasing the probability that males of high genetic quality will pass viability-enhancing traits to the female's offspring. Females experimentally allowed access to preferred males should, under this hypothesis, produce more viable offspring than females denied such access.

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Polyandry may function, in part, to facilitate females' pursuit of good genes (Walker 1980). The good genes perspective on polyandry predicts that the offspring of females assigned to multiple mating treatments in controlled-breeding studies will, on average, be more viable than the offspring of females restricted to single matings. One way that polyandry may potentiate good genes sexual selection is by ameliorating perceptual or energetic constraints on the efficacy of pre- or post-copulatory selection. Such constraints might impose severe consequences under strict monogamy (Watson 1991a, b; Møller 1992; Stockley et al. 1993).

Controversy surrounds the hypothesis that the adaptive functions of female reproductive behaviour include the sorting of prospective sires according to viability-related traits they will pass to offspring (Kirkpatrick & Ryan 1991). Although plausible mechanisms for the maintenance of heritable fitness variation among potential mates

have been proposed, such as mutation bias (Pomiankowski et al. 1991), the antagonistic coevolution of hosts and parasites (Hamilton & Zuk 1982) and the unmasking of relevant additive genetic variance by directional selection (Pomiankowski & Møller 1995), it may still be difficult to envisage how genetic benefits of selective multiple-male mating could routinely outweigh direct and immediate costs associated with these behaviours (Arnqvist 1989; Watson 1993). Costs of selective mating include (1) loss of time and energy by females as they assess and attempt to reject unsuitable males (Watson 1990, 1993), (2) increased vulnerability to predators or other dangers during mate searching and inter-sexual interactions (Watson 1993; Reynolds & Cote 1995; Godin & Briggs 1996) and (3) increased risk of injury from sexually aggressive males. Multiple mating compounds the costs of selective mating and adds the cost of increased exposure to sexually transmitted disease (Watson 1993).

Costs of selective mating may not always be so high, however. Gibson & Bachman (1992) provided an example of apparently very low costs of mate choice in a lekking sage grouse, *Centrocercus urophasianus*, and argued that even small indirect fitness benefits could be sufficient to maintain mate choice. Moreover, even in reproductive systems where the male's sole contribution is sperm, direct benefits may follow from mate choice (Petrie et al. 1992) or multiple-male mating (Alcock et al. 1977; Ridley 1988; Arnqvist 1989; Watson 1993). Post-copulatory mechanisms of sexual selection enable females to pattern their use of sperm to improve offspring genetic quality even when cues other than male quality influence mating decisions per se (Watson 1991b, 1993). Determining whether enhanced offspring viability can provide a general explanation for complex patterns of female reproductive behaviour awaits studies that investigate diverse sources and episodes of selection on mating preferences and mate number and jointly analyse a wide range of costs and benefits.

In this paper, I present data from a field-based, controlled-breeding study of the spider *Neriene litigiosa* Keyserling 1886 (= *Linyphia litigiosa*). The two-generation study was designed to measure (1) the influence of experimentally imposed multiple versus single mating on the growth rates of offspring (i.e. rates of increase in body mass) during the 27 days following their

emergence from the natal cocoon, (2) the mechanism whereby polyandry may enhance offspring viability and (3) the genetic covariance between offspring growth rates and the body mass of their known or most likely father (i.e. the single mate of monogamous females or the first mate of polyandrous females, respectively; Watson 1991a). I also examined the relationship between the courtship vigour of the known or likely sires and offspring growth. I discuss how sequential polyandry and the female preference for large males may work together to enhance offspring viability in a breeding system that might render the use of either strategy alone less effective.

Female *N. litigiosa* prefer large males as sires for their offspring and express this preference in two temporally separate contexts. First, females late in their penultimate instar actively elicit a sequence of male-male fights. Each fight winner attempts to guard the female until she matures, often a matter of several days (Watson 1990). Since size is a strong predictor of fighting ability, this tactic usually leads to the female being attended by a larger than average male at the time of her final moult and concomitant sexual maturation. The newly mature female always mates with this male. First mates typically sire 60–70% of a female's offspring (range=0–100%; Watson 1991a). Females appear to rely completely on the outcome of male-male competition for selection of their first mate and major sire, because experimentally replacing the 'champion' present at the time of female maturation with a very small male does not diminish female sexual receptivity or the average magnitude of first male sperm precedence (Watson 1991a).

The second context in which female preference for large males is expressed involves direct cryptic female choice by non-virgin females. Polyandrous females, which made up 80–90% of the population in the 10 summers I have monitored the species' breeding behaviour, preferentially use sperm of the larger members of their typical pool of one to two secondary mates ($\bar{X}=1.3$, range=0–5). This preference is expressed independently of male mating order and in a setting largely devoid of male-male competition (Watson 1991b).

Non-virgin females express a second, independent cryptic preference for males that perform more vigorous copulatory courtship, measured as the maximum intromission rate achieved during

pre-insemination phase copulation (Watson 1991b). Since this male character may be associated with another aspect of fighting ability, namely, aggressiveness (Watson & Lighton 1994), this second female preference may also find indirect expression in the sub-adult female's tactic of provoking male-male competition before her first mating. Thus, it seems likely that the preferences expressed in the male-male competition and direct choice components of the mating system are in agreement for both male size and vigour/aggressiveness.

Female reliance on male-male competition to choose their first mate and principal sire leaves them slightly vulnerable to a genetically small male becoming the principal sire of their offspring. Multiple mating may promote offspring growth in the relatively uncommon instances where, through sampling error, the final winner of the series of fights that typically precede the first mating will merely be the best of a bad lot. In nature, 22.7% of free-living females ($N=254$) have a secondary mate that is larger than their first mate, so a sizeable number of females may benefit from polyandry in this manner.

My earlier analyses of the genetic consequences of polyandry in *N. litigiosa* compared three alternative good genes hypotheses. Although re-mating seems to have direct benefits in terms of decreasing male foraging interference (Watson 1993), I have interpreted the sperm use patterns of polyandrous females as a form of genetic 'bet-hedging' (Watson 1991b). Under the bet-hedging hypothesis, females mix the sperm of two or more mates, all of whom have been judged by identical or concordant criteria to be of high quality, to reduce the number of offspring negatively affected either by perceptual errors by the female (Johnstone 1994), or other imperfections in the mate selection system (e.g. weak male-male competition or short-term restrictions on the female's ability to compare and properly choose mates) that occasionally lead to inflated estimates of the quality of a given mate. This sort of bet-hedging is not designed to increase the genetic diversity of the progeny per se, although this may often result. Instead, it is an adaptation to reduce the deleterious genetic effects of less than totally reliable sexual decision rules. I shall argue that the current study supports this bet-hedging interpretation of the genetic benefits of polyandry in *N. litigiosa*.

METHODS

Field Research

The controlled-breeding component of the study was done in the field during the spider's breeding season in June-August 1989 at the Flathead Lake Biological Station, Polson, Montana, U.S.A. Use of wild parental genotypes should provide more realistic estimates of genetic benefits of preferential and multi-male mating than may often be possible to obtain with laboratory stocks. Allowing inter-sexual encounters to occur in nature also helped to ensure that (1) female sire selection criteria were expressed normally and (2) any fitness consequences of multiple mating, which should depend on the proper expression of preferences, would typify those in nature. Although controlled on an individual female basis, the number of matings per female and the variation in male body size ranged as would be expected for unmanipulated females (see below).

First, I randomly assigned each individual from a haphazard sample of 88 free-living females to single and multiple mating treatments. I successfully monitored 62 of these females through to completion of their mating treatment. Of these, 43 survived to oviposit, and 34 had fully or partially viable broods that I attempted to rear in the laboratory.

All females were penultimate instar virgins at the time I assigned them to treatments. For identification, they were temporarily marked with a dab of enamel paint on the tibia of one of their hindmost legs and then permanently re-marked on both hind legs immediately after completion of their final moult and first mating. I used scan sampling (10-12 scans per day between 0800 and 2300 hours) to note each female's final moult and first mating as well as any secondary matings. Based on hundreds of hours of round-the-clock scanning during several previous years, and regular examination of female webs for new sperm webs (indicative of mating) during morning scans in the present study, it is known that no matings occurred late at night or early in the morning. Thus, scans were omitted between 2300 and 0800 hours with virtually no risk of missing matings. When a female matures near dusk, however, she may mate that same evening. I performed focal observations on all such females beyond the

scheduled scanning times to ensure that their first mating was recorded.

I also made focal observations of each mating to gather data on male copulatory performance and to verify completion of insemination phase copulation. Copulatory performance was measured by taking a 3-min count of the male's pre-insemination phase intromission rate no less than 40 min after the initiation of copulatory courtship; this provides an adequate 'warm-up' period for the male, after which performance reaches an asymptote and stabilizes. The inception of insemination phase copulation is marked by the construction of a conspicuous sperm web and charging of the palpi with ejaculate. I observed one or both of these for every mating in the analysis. Insemination phase copulation is also marked by much lower intromission rates than in the pre-insemination phase (Watson 1991a, b). Insemination was observed in every mating after noting evidence of sperm induction. Thus, there is no doubt that every mating recorded resulted in the male injecting sperm, although since females may reject sperm this is no guarantee that the female was functionally inseminated (Watson 1991b). Each insemination phase lasted a minimum of 30 min (usually 40–60 min), after which the male showed no signs of trying to re-initiate mating. Sexually satiated post-insemination males can be behaviourally identified: after withdrawing from the copulatory position they either begin to depart from the web, capture and consume prey, or settle into a prolonged bout of unhurried grooming.

Males usually depart within 1–3 h after completion of mating. To avoid losing mates, I routinely collected them no more than 20 min after cessation of insemination phase copulation. Each male in the study mated only once and was weighed to the nearest 0.1 mg within 3 h of capture. (Throughout the text, 'size' formally refers to mass, although in male *N. litigiosa* mass is highly correlated with a more accepted size measure for spiders, i.e. prosoma width; $r=0.81$, $N=97$, $P<0.00001$.) Controlling the mate number of the females was readily achieved in the field using the scan procedure described above, because insemination is always preceded by pre-copulatory courtship (Watson 1993) and copulatory courtship (Watson 1991b), each usually lasting over an hour. Males that I did not wish to mate with a given female were usually removed before

pre-insemination phase copulation began, and always well before insemination phase copulation. Removed males were released on the study site, but far enough from relevant focal females so that re-entry of the web would be unlikely. Those few cases in which a short period of pre-insemination phase copulation occurred should not have biased results for those females, since this phase of mating does not appear to result in sperm replacement or removal (Watson 1991b). Male removals were done quickly and with little disturbance to the female or her web.

I randomly chose one-half of the females in both the polyandrous and single mating treatments to accept as their first mate a non-serial, fighting-winning male collected (just several minutes beforehand) from the web of an avoidant penultimate or not-recently-matured non-virgin female. The remaining females had their first mate decided normally via serial male–male fighting (Watson 1990). Non-fighter males were collected from a trail near the study site, the first male encountered was taken. Once their exoskeleton dries for 1–2 h, newly mature females are highly receptive (both overtly and with respect to average sperm usage patterns) to virtually any male in possession of their web. Thus, these introductions of non-fighters to newly mature virgin females of the experimental group, performed immediately after evicting the natural guarding male that otherwise would have become the first mate, resulted in the females having males of random quality with respect to fighting capacity as their first mates at a frequency far higher than that of the females allowed to keep their natural first mates (Watson 1990, 1991a). The purpose of this manipulation was to increase the proportion of females in the study having at least one secondary mate as the largest potential sire of their offspring, thus increasing the number of opportunities within the sample for polyandry to result in the hypothesized growth rate benefits. Subjecting single-mating females to the same treatment ensured that the consequences for offspring growth of having male–male competition fail to determine the first mate would be equal between the single and multiple mating treatment groups. Nine of the 16 polyandrous females (56%) whose offspring I reared in this study had at least one secondary mate that outweighed their first mate and, as expected, natural first mates were significantly heavier than those of the females assigned

Table I. Results of repeated measures ANCOVA testing for correlates of offspring body mass and growth rate during the 27-day rearing period

Source	Sum of squares	df	Mean square	F	P (one-tailed)
Spiderling body mass (between-brood effects)					
Single- versus multiple-male mating	0.09	1	0.09	6.87	0.008
Body mass of first or sole mate	0.19	1	0.19	14.35	0.006
Oviposition date	0.11	1	0.11	8.52	0.004
Female post-oviposition mass	0.33	1	0.33	2.48	0.065
Prey availability per spiderling	0.06	1	0.06	4.52	0.023
Total number of cannibalisms	0.17	1	0.17	12.90	0.009
Number of spiderlings (day 10)	0.03	1	0.03	1.92	0.091
Error	0.27	20	0.01		
Spiderling growth rate* (within-brood effects)					
Day of spiderling weighing	0.08	2	0.04	9.73	<0.001
Single- versus multiple-male mating	0.04	2	0.02	5.60	0.006
Body mass of first or sole mate	0.09	2	0.05	10.43	<0.001
Oviposition date	0.04	2	0.02	4.54	0.012
Female post-oviposition mass	0.01	2	<0.01	0.52	0.300
Prey availability per spiderling	0.12	2	0.06	15.79	<0.001
Total number of cannibalisms	0.05	2	0.03	4.94	0.009
Number of spiderlings (day 10)	0.03	2	0.01	2.71	0.045
Error	0.18	19	<0.01		

See text for variate and covariate descriptions.

*The main within-brood effect is time: the day of spiderling weighing. All other within-brood effects denote interactions with time. *F*-statistics and *P*-values are based on multivariate repeated measures analysis.

random first mates ($\bar{X} \pm \text{SD} = 17.39 \pm 1.72$ and 15.16 ± 2.58 , respectively; two-sample *t*-test: $t = 2.68$, $P = 0.013$). There was no significant difference in the weights of the first mates of single- versus multiple-mating females (16.76 ± 2.68 and 15.91 ± 2.26 , respectively; two-sample *t*-test: $t = 0.88$, $P = 0.385$).

The body mass of males ranged from 11.4 to 20.4 mg for first mates and from 13.2 to 22.6 mg for secondary mates (in nature, range = 6.7–29.6; 10–28 mg is not uncommon). The proportion of this size variation that is genetic is unknown, but it is plausibly enough to make female choice and genetic bet-hedging vis-à-vis male body size genetically beneficial.

Although most females in my study population mate two to three times during the 4–6-week period between sexual maturation and oviposition, the propensity of females to re-mate varies (Watson 1990, 1993). I was usually able to obtain a female's pre-assigned level of two or three matings, regardless of her resistance, by introducing sexually active males on to her web nearly daily, thus increasing the pressure to re-mate (Watson 1993). Three females originally assigned

to the multiple mating treatment never accepted a secondary mating, however; these individual's families are included in the analyses of offspring growth as single-mated females. Their exclusion from the entire data set increases the statistical significance of the relationship between mate number and offspring growth, so their inclusion is conservative relative to the research hypothesis. Two females scheduled to mate three times, but that would mate only twice, also are included in the polyandrous female group; these individuals did not affect the statistical outcomes.

In nature, a female leaves her web and hides her egg sac in the forest litter. To obtain broods, I captured heavily gravid females and maintained them individually in 100-cc containers under ambient conditions of temperature and humidity. Females built small webs in these containers and were regularly watered and fed natural wild-caught prey (assorted midges, caddisflies and mayflies). Females varied in the time they spent in captivity prior to oviposition, but this did not influence offspring growth rates. When added to the base model (Table I) in multivariate repeated measures ANCOVA, neither the number of days

females spent in captivity prior to oviposition nor the proportion of weight gain by the egg mass after the female was brought into captivity (calculated as [(egg mass weight) - (female weight immediately after oviposition) - (female capture weight)]/(egg mass weight)) bore a relation to offspring or growth rates (all $P > 0.51$).

Laboratory Research

Rearing of spiderlings

Egg sacs were placed individually in sterile 20-cc glass tubes with moist cotton plugs and kept in dark, cool (4–6°C), humid (80–90% relative humidity) conditions until rearing began. As occurs in nature, eggs hatched 12–14 days after oviposition and after an additional 2 weeks the spiderlings underwent one moult within the cocoon, remaining in the cocoon to overwinter as second instars. I reared spiderlings from 34 broods, each from a different female. Three broods could not be used in the analyses of offspring growth, because I lacked information on a covariate-female post-oviposition body mass (see below). In two other broods, all the offspring died before the rearing period ended. An additional brood was excluded because I lacked data on the body mass of the female's first mate. Thus, 28 broods were included in the analyses relating female mate number and male mass to offspring growth rates. Of these, 12 broods were of single-mated females and 16 were of multiple-mated females. Subsets of each female's second instar offspring were chosen for rearing by tearing open cocoons and placing the first spiderlings encountered in the rearing boxes up to the required number (i.e. 10 spiderlings per rearing box, up to two boxes per female); this event marked day 1 of rearing for the spiderlings of that brood. All selections of spiderlings were performed blind to the characteristics of the mother and potential sires, including experimental treatments.

Three sets of 27-day rearings were performed (12 April–7 May, 15 May–11 June and 23 May–18 June) during which I raised single-mated and polyandrous females' offspring concurrently. Rearing set was not related to offspring size or growth rate when added to the multivariate model of Table I ($F=0.54$, $P=0.47$ and $F=1.29$, $P=0.30$, respectively), and did not reduce the significance of mate number or sire mass; it was left out of the

final model. To generate competition, I raised F1-generation family members (i.e. full and half-siblings) together. Each sibling-group inhabited a clear plastic box, $28 \times 14 \times 12$ cm, with a tight, removable lid and a 1.5-cm diameter screened ventilation port. All sibling-groups started with eight to 10 second-instar brood mates per container (day 1). The rearing containers were assigned randomly with respect to treatment groups and first mate weights. Each contained a network of twigs arranged to allow up to 10 spiderlings to locate a site for placement of a functional web. Spiderlings within a box tended to spread themselves out evenly throughout their container. Their spatial distribution was further influenced by idiosyncrasies in the arrangement of web supports in each container. Locations and orientations of the rearing containers in the environmental chamber were randomized every 2 days.

The lid of each box had a hatch through which prey were added. All sibling groups were fed simultaneously with chill-anaesthetized winged *Drosophila*. Since the webs of *N. litigiosa* are non-sticky, prey dumped into one location, even if they fell on to a web positioned directly below the feeding port, quickly revived and distributed themselves throughout the box. The spiderling with its web beneath the hatch was not able to capture more than one fly at feeding time. Attacks usually occurred after the flies revived and began movements, so prey capture typically involved a struggle. *Drosophila* are two to three times as large as second instar *N. litigiosa*, so it was generally difficult for a spiderling to make a capture until after one to two flies had been consumed. To avoid unintended bias, I performed all feedings blind to the number of mates of the mother and the body sizes of potential sires. Feedings occurred every 2–4 days, ensuring that a few living prey were always available for capture in every box. The number of living spiderlings in each box was determined prior to each feeding, and the number of *Drosophila* provided were adjusted to maintain similar per capita prey availability across boxes. I made a more exact determination of the number of flies placed into each rearing box by counting fly carcasses during the spiderling weighings on days 20 and 27. The average \pm SD number of flies fed per capita over the course of the 27-day rearing period was 14.1 ± 5.0 . On average, polyandrous females' offspring received slightly more

flies (15.0 ± 4.8) than did those of single-mated females (13.0 ± 5.1), but the difference was not significant (pooled variance *t*-test: $t=1.04$, $P=0.31$) and this was statistically controlled.

Since other characteristics of each brood's rearing box were standardized across broods, the environmental contribution to within- and between-brood resemblance should be the same across families and treatments. Increased family resemblance arising from rearing brood mates in a common container, however, could inflate the statistical significance of estimates of between-brood and inter-group differences in offspring growth. Several observations suggest that this potential bias was not a problem in this study. First, the large variance in growth rates seen within broods sharing the same rearing container ensures that slight relative similarities of rearing environment did not erase variation potentially attributable to genetic differences among family members. Furthermore, the offspring of five females were split across two rearing containers. To help assess the extent to which within-brood variance was suppressed by raising siblings together, I used data from these five families in a nested ANCOVA with (1) brood and (2) rearing batch nested within brood as variates, prey availability as a covariate, and mean offspring weights on days 20 and 27 as the dependent variables. There were no significant within-brood differences in offspring mass between rearing containers ($N=56$ offspring, $P=0.585$). Finally, competition for food within broods should have reduced within-brood resemblances (Falconer 1989), which would tend to make estimates of inter-family differences conservative. So, other than differences in prey availability among rearing containers, a source of variation explicitly controlled in relevant analyses, sharing versus not sharing a container should have had little influence on variation in the growth of siblings.

Studies of the indirect fitness effects of mating behaviour risk generating spurious negative results when the offspring generation is raised in a laboratory environment (Boake 1994). Conditions in the laboratory, which often offer copious feeding and a lack of competition, may alter the expression of genes between free-living parental and laboratory-reared offspring generations and lead to modes of expression unrepresentative of the result of non-random mating in nature (Howard et al. 1994; Nicoletto 1995). In a non-

stressful rearing environment, genetically compromised individuals may produce fit phenotypes. To detect fitness-related genetic variation, the rearing environment must make realistic demands on developing individuals by imposing relevant, nature-mimicking stresses.

To impose moderate stress on developing spiderlings, I began rearing rather late, forcing offspring to live on yolk reserves about as long as they would during a relatively cold wet spring at the site of parental origin in northwest Montana. Thus, individuals with higher metabolic efficiency may have had a better residual store of yolk to help them through the first days outside the egg sac. Moreover, I raised siblings communally in containers that provided enough space and structural support for all individuals to have adequate webs, but that also caused territorial competition throughout rearing as individuals jockeyed for better web positions or more building space. Since I observed the spiderlings frequently and took notes on mortality, I could often distinguish between non-traumatic deaths and fight-related deaths; the latter always result in cannibalism and a deformed carcass. I saw little evidence of moult-specific mortality during rearing: it was similar to the frequency seen in nature among fifth and sixth instar sub-adults. I estimate that approximately 30–40% of all mortality within the rearing chambers resulted from territorial fights. Territorial competition decreased individual foraging efficiency and increased average energy expenditures both in competing and in building new webs when supplanted by a rival. Competition should also result in disproportionate survivorship of the best individuals from each brood, as in nature. The third stress factor came from restricted feeding. Although a few flies were almost always present in the rearing box, all spiderlings had to fast sometime. The wild type flies I used were evasive and often fewer than one living fly were available per spiderling. Thus, less competitive individuals frequently had multi-day spans without prey capture. The smallness of the prey required spiderlings to make multiple captures to grow substantially.

Weighing of spiderlings

I used rates of increase of live body weight during the 27-day rearing period as my measure of spiderling growth rate. Because of their elastic abdomen, spiders can gain mass within instars,

but moults are required at some point to release further growth potential. Measures of mass include both within- and between-instar growth. All the spiderlings that survived the entire rearing period underwent at least one moult. The fastest-growing spiderlings underwent three to four moults.

Because of their small size and high tolerance of close proximity, newly emerged spiderlings from each brood were weighed en masse on day 1 of rearing, before any feeding. The total mass for each brood was divided by the number of spiderlings weighed to obtain day 1 mean spiderling mass within each brood. Sibling-groups experienced differential mortality during rearing due to uneven rates of siblicide, disease and unknown causes. The number of spiderlings present in each rearing box ranged from 3 to 10 (median=9) on day 10 post-emergence, and from 2 to 10 (median=7) and 2 to 9 (median=6) at the weighings on days 20 and 27, respectively. The mean number of living spiderlings per box did not differ significantly between single and multiple mated females on days 1, 10, 20 or 27 (*t*-tests, all $P > 0.355$). I weighed 212 spiderlings on day 20 and 178 on day 27. Surviving spiderlings were weighed individually on days 20 and 27. For all weighings, I placed spiderlings in weighing vessels using a fine sable hair brush while an assistant who was blind to the identity of each brood performed the weighings on a six-place Mettler balance. Weighing vessels consisted of tiny gelatin capsules handled with latex gloves and tweezers throughout the weighing process. Care was taken to neutralize the effects of static electricity using an anti-static gun.

Data Analyses

I used repeated measures ANCOVA to determine effects on offspring size and growth rates of two main factors: (1) multiple-male versus single-male mating by females; and (2) the body mass of the offsprings' known or most probable sire (i.e. the sole mate of single-mated females and the first mate of multiple-mated females, respectively; Watson 1991a, b). I performed additional analyses (1) to examine the importance for offspring growth of the female gaining a secondary mate that was heavier than her first mate and (2) to identify the mate(s) of females whose body mass best explained variance in offspring growth regardless of any a priori likelihood (i.e. based on

mating order) of their siring offspring. In all analyses the dependent, repeatedly measured variable was the mean live body mass of the spiderlings from each of 28 broods. Mean spiderling mass was determined three times for each brood: on days 1, 20 and 27 post-emergence.

In the main analyses, there were seven independent variables: two variates and five covariates (see below). Each variable's influence on the variation among broods in average mass is denoted by the between-brood effects from the repeated measures ANCOVA (Table I). The corresponding significance levels are based on single-*df* tests that are valid under the usual assumptions of ANCOVA, which the data satisfy. Offspring growth rates are equivalent to the slopes of the statistically adjusted (i.e. multivariate) linear relationship between the main within-broods factor, time (i.e. the day of spiderling weighing; Table I) and the repeatedly measured spiderling weights. Each factor's influence on variation in spiderling growth rate is given by its interaction with time, the significance of which I tested via two-*df* tests. For these tests I report 'multivariate' *F*-statistics and *P*-values (Statistica 1993; SYSTAT 1996), which are robust to non-fulfilment of special data assumptions termed 'compound symmetry' and 'sphericity', which my data do not fulfil (Mauchly's sphericity test: $\chi^2_2 = 15.53$, $P = 0.0004$; Statistica 1993).

Measurements of body mass for offspring, sires and dams were transformed by taking their cube roots to render their dimensionality equal to that of the other variables in the model (Lande 1977; Lande & Arnold 1983); this transformation slightly increased *P*-values for most tests, so it was a conservative procedure. The main hypotheses that I tested were directional. Thus, where appropriate, I report one-tailed significance levels. One-tailed significance levels are denoted $P_{\alpha/2}$ and two-tailed levels as *P*.

Variates

In the main analysis, the first variate was female mating status, represented by a dummy categorical variable that specified whether a female had been subject to the single or multi-male mating treatment (six females pre-assigned treatment could not be fulfilled; see above). The second variate was the live body mass of the most likely major sire. Although all of a female's mates were

weighed, exploratory analyses (see Results) indicated that only the mass of first mates was statistically associated with the growth of offspring; this is consistent with first mate sperm precedence as revealed by previous electrophoretic paternity analyses (Watson 1991a). Therefore, the variate 'mate mass' in the final model (Table 1) represents a female's first mate (polyandrous females) or single mate (monogamous females).

Covariates

The main model's five covariates controlled for (1) estimated per capita availability of prey during spiderling rearing, (2) residual effects of variance in spiderling survivorship and thus density among rearing containers, (3) female oviposition date, (4) female post-oviposition body mass and (5) the number of siblicidal cannibalisms that occurred during rearing. The covariates were chosen on the basis of a priori theoretical considerations and were included in the model irrespective of their univariate or multivariate significance levels, or whether partial *F*-tests indicated that their inclusion yielded significant improvement in the model.

The covariate adjusting for variation in per capita prey availability was calculated as the total number of *Drosophila* added to each rearing box between days 1 and 27 (fly carcasses were counted on days 20 and 27) divided by the mean number of spiderlings present during rearing. Living spiderlings were counted on days 1, 10, 20 and 27, and these counts were summed and divided by 4 to obtain the mean number present during the rearing period.

There could be effects of differential density on spiderling growth that were independent of prey availability. These were controlled by a covariate based on the number of spiderlings still present in each rearing container on day 10 post-emergence. In exploratory analyses, I analysed spiderling growth in relation to exact spiderling counts taken on days 10, 20 and 27 post-emergence. None of the three possible count covariates led to substantial reductions in the significance level of the two variates, female mate number and sire mass. Since prey availability is a per capita measure, it is highly correlated with the count data, especially for day 20 and 27 counts ($r=0.65$ and 0.71 , respectively). The spiderling count taken on day 10 was least correlated with prey availability ($r=0.30$, $P=0.11$) and reduced the significance of the mate number variate the most.

The female oviposition date covariate was intended to control for maternal developmental rate and other maternal effects on offspring quality. For example, eggs that are produced earlier may have been formed in a less senescent and more disease-free mother; many females apparently succumb to disease before they can oviposit, and later broods tend to have more inviable eggs (Watson 1993, unpublished data). Controlling for female growth rates and possible non-genetic maternal effects was also the purpose of the female post-oviposition body mass covariate. Female body mass was measured to the nearest 0.1 mg immediately after completion of oviposition and thus should provide a measure of female mass largely unaffected by inter-individual variance in the mass of developing eggs. The final covariate, the frequency of cannibalisms within a rearing box, helped to adjust for changes in the growth of surviving spiderlings due to feeding on siblings and to the ensuing reduction in competition for food and web space.

Testing the bet-hedging mechanism

Watson (1991b) proposed that polyandry may enhance one or more components of offspring viability, such as growth, by reducing the genetic impact of a sub-optimal choice of first mate. This genetic bet-hedging hypothesis predicts that polyandry should be most beneficial to offspring when their mother has had a low-quality (e.g. small, light) first mate and at least one high-quality (e.g. large, heavy) secondary mate. To test this hypothesis, I first developed a multivariate linear model consisting of the five covariates and the variate first mate mass (see above) using single-mated females only ($N=12$). I estimated the model once with the day 20 offspring weights as the dependent variable and again using the day 27 weights. I then used the resulting model's parameter estimates to generate expected offspring weights for the polyandrous females ($N=16$) had they mated only once. Finally, I regressed the difference between the observed and expected offspring weights for each polyandrous female against the weight difference between those same females' heaviest secondary mate and their first mate. The bet-hedging hypothesis predicts a positive relationship.

Weighted least squares

Weighted least squares is a method of adjusting the relative influence of observations on the

outcome of an analysis of variance according to their expected error variance. The number of offspring weighed on days 20 and 27 post-emergence varied from two to 15 among broods. Within-brood variances ranged from 0.013 to 13.061 on day 20, and from 0.290 to 15.789 on day 27 (Levene's test for homogeneity of variances: $F=4.11$, $P<0.00001$ and $F=3.11$, $P<0.00001$, respectively). To help ensure that statistics were not influenced by a few small broods, I augmented the main repeated measures analysis described above with a weighted analysis (SYSTAT 1996).

I rejected the use of weighting factors directly derived from the within-brood variance in offspring body mass. Multiple mating by females is expected to increase the variance in genetically influenced traits of offspring when there is mixed paternity within broods, as in *N. litigiosa*. Thus, using the inverse of the within-brood variance in body mass as the weighting factor would degrade the analysis by reducing the leverage of a key experimental group. Variance in offspring body mass on days 20 and 27 was much greater for multiple-mated than for single-mated females in this sample (see below). I decided that weighting the analysis using the number of offspring weighed per brood (the mean number on days 20 and 27), although related to the variance, would be more directly related to the quality of information embodied in each brood-specific mean spiderling weight.

Male-male competition and offspring growth

I tested for effects on offspring growth of having a natural fight-winning first mate versus a non-fighter introduced on to the female's web immediately after her sexual maturation by adding a bivariate indicator variable to the main model (Table I), as well as an interaction term between this factor and female mate number. I referred to the results for the main effect to determine whether the treatment of having a non-fighter had an overall negative effect on offspring size or growth, and to the interaction with female mate number, to see whether untested first mates differently affected the offspring of single- versus multiple-mated females; the genetic bet-hedging hypothesis for polyandry predicts a greater negative effect of the treatment on single-mated females.

Multiple mating, egg viability and offspring survivorship

I examined potential negative fitness effects of polyandry in the forms of reduced egg viability and offspring survivorship (Watson 1993). I analysed the proportion of each female's eggs that hatched and the proportion of her offspring that survived the 27-day rearing period. Survivorship analyses compared females with respect to the total proportion of their offspring that survived and the proportion of offspring known not to die traumatically as a result of siblicide. All broods were the females' first. I used multivariate analyses to control for female oviposition date and post-oviposition body mass. For the survivorship analysis, I also controlled for which of the three periods each brood was reared. Since the fertility and survivorship data were proportional, I performed arcsine transformations prior to analysis to promote homogeneity of error variance and normality of error effects.

RESULTS

Multiple Mating and Egg Mass

The freshly laid whole egg masses of polyandrous females weighed slightly less than those of single-mated females ($\bar{X} \pm \text{SD} = 16.10 \text{ mg} \pm 3.01$, $N=16$ and 18.40 ± 3.20 , $N=12$, respectively; two-sample t -test: $t_{26}=1.95$, $P=0.062$). There was no difference between single- and multiple-mated females in the total number of eggs produced (34.94 ± 7.72 and 39.75 ± 8.02 , respectively; $t_{26}=1.61$, $P=0.120$) or the average mass of individual eggs (i.e. total mass divided by egg number: $0.469 \text{ mg} \pm 0.070$ and 0.468 ± 0.064 , respectively, $t_{26}=0.029$, $P=0.977$).

Multiple Mating and Offspring Growth

Of all the offspring in the upper quartile of the sample distribution of body mass, 73.2% belonged to multiple-mated females on day 20 ($N=56$ offspring) and 66.0% on day 27 post-emergence ($N=47$). Multivariate repeated measures ANCOVA (Table I) corroborated the univariate result (Fig. 1) that polyandrous females had offspring that gained weight significantly faster and that, on average, attained significantly greater body mass than the offspring of single-mated females.

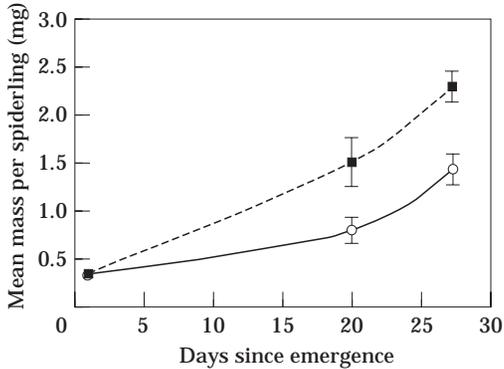


Figure 1. Average body mass of the offspring of multiple-mated females (---■---) and single-mated females (—○—) on days 1, 20 and 27 after emergence from the natal cocoon. Groups of 8–10 broodmates were weighed together on day 1, and their combined mass was divided by the number of spiderlings to obtain the brood means for that day. Individual spiderlings were weighed on days 20 and 27 to calculate brood means. Plotted data are categorical within-brood means (± 1 SE) unadjusted for covariates. Differences in growth between the offspring of polyandrous and monogamous females are significant (see Table I).

To ensure that the finding of higher growth rates in offspring of multiply mated females was not driven by a few small broods with especially large or small offspring, I re-estimated the model via weighted least squares. The influence of each brood on the analysis was weighted by the mean number of spiderlings weighed on days 20 and 27 post-emergence. The value of the weighting factor varied from 2.0 to 13.5, giving more influence to brood means based on the body masses of more spiderlings. The difference in the masses and growth rates between the offspring of multiply versus singly mated females retained significance in the weighted analysis ($F_{1,20}=5.32$, $P_{a/2}=0.016$ and $F_{2,19}=4.25$, $P_{a/2}=0.015$, respectively).

Sire Mass and Offspring Growth

I tested the good genes prediction that the female preference for heavier mates and sires in *N. litigiosa* (Watson 1990, 1991a, b) leads to greater mean offspring growth rates by including the body mass of known sires or most likely sires (i.e. the mates of singly mated females or first mates of multiply mated females, respectively) in the repeated measures ANCOVA. As predicted,

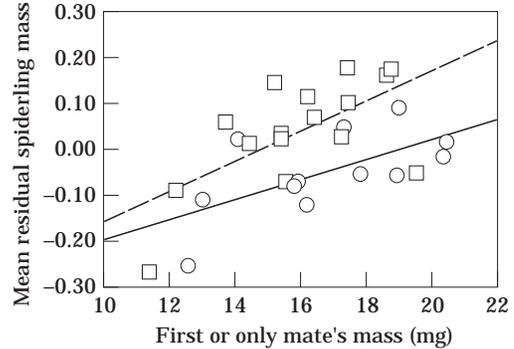


Figure 2. Residual variation in mean within-brood body mass of offspring versus the mass of the single mate of single-mated females (i.e. known sires: —○—; $r=0.657$, $N=12$ broods, $F_{1,10}=7.59$, $P_{a/2}=0.010$) and the first mates of multiple-mated females (i.e. likely major sires: ---□---; $r=0.663$, $N=16$, $F_{1,14}=11.01$, $P_{a/2}=0.0025$). Residuals are plotted instead of raw data, because no trend is visible without adjusting for covariates. Residuals are adjusted via repeated measures ANCOVA for inter-brood variation in prey availability, the number of surviving offspring at day 10 (i.e. density), the number of cannibalisms, oviposition date and dam post-oviposition body mass (i.e. the Table I model, but with the sire mass and female mating status variates omitted). The positive relationship between sire mass and offspring growth is apparent only in residuals adjusted for prey availability. Adjustment for the other covariates further strengthens the positive relationship, except post-oviposition body mass, which has no effect. The vertical separation between the two lines is eliminated if the residuals are also adjusted for female mating status, so the plot illustrates again the effect of polyandry on offspring growth. The slopes illustrate growth rates in relation to sire mass, since the Y-axis plots multivariate residuals of offspring mass. The slopes of the two lines are not significantly different ($F_{1,14}=1.07$, $P=0.319$). The regression equation for the pooled data is: $Y = -1.182 + 0.468X$, where X = the cubic root of sire mass ($N=28$ broods, $r=0.526$, $F_{1,26}=9.96$, $P_{a/2}=0.002$).

the body mass of known or suspected major sires was positively related to offspring mass and growth rates (Fig. 2; Table I). These results held up in the weighted analysis ($F_{1,20}=17.42$, $P_{a/2}<0.001$ and $F_{2,19}=14.27$, $P_{a/2}<0.001$, respectively).

As predicted on the basis of first mate sperm priority shown by *N. litigiosa* (Watson 1991a), the body mass of the first mate of each polyandrous female ($N=16$) was a stronger predictor of offspring mass (unweighted: $F_{1,9}=8.21$, $P_{a/2}=0.009$)

and growth rate ($F_{2,8}=6.19$, $P_{\alpha/2}=0.012$) than (1) the mass of the heaviest mate ($F_{1,9}=0.53$, $P_{\alpha/2}=0.241$ and $F_{2,8}=0.67$, $P_{\alpha/2}=0.268$, respectively), (2) the average mass of all mates ($F_{1,9}=0.50$, $P_{\alpha/2}=0.249$ and $F_{2,8}=1.17$, $P_{\alpha/2}=0.177$), or (3) the mass of the 'best' mate having the highest summed rank for body mass and copulatory courtship performance ($F_{1,9}=0.12$, $P_{\alpha/2}=0.367$ and $F_{2,8}=0.54$, $P_{\alpha/2}=0.300$). Weighted analyses gave similar results.

Copulatory Vigour and Offspring Growth

The pre-insemination phase intromission rate of the first mate, which I obtained for 26 females, was not a strong predictor of offspring growth. Addition of intromission rate to the list of seven independent variables in the base model (Table I) indicated no relationship to offspring mass ($F_{1,17}=0.184$, $P=0.67$) and a weakly positive but non-significant relationship to growth rate ($F_{2,16}=2.875$, $P=0.086$). Inclusion of copulatory vigour did not appreciably change the significance of the other variables in the model. In the weighted version of this analysis, the relationship of intromission rate to offspring growth rate neared two-tailed significance ($F_{2,16}=3.448$, $P=0.057$).

Genetic Bet-hedging and Offspring Growth

Nine of the 16 polyandrous females in the study (56%) had a heavier secondary mate than their first mate; secondary mates averaged 3.5 ± 2.5 mg heavier for these females. Thus, there was some opportunity for bet-hedging in this sample. However, the difference in mass between the heaviest secondary mate and the first mate of polyandrous females was not a significant predictor of the difference between the observed mass of polyandrous females' offspring and that expected had those females mated only once (day 20: $r=0.145$, $F_{1,14}=0.301$, $P_{\alpha/2}=0.296$; day 27: $r=0.127$, $F_{1,14}=0.228$, $P_{\alpha/2}=0.320$; Fig. 3). Results of a weighted analysis were similar.

Natural fight-winning first mates were larger than the non-fighting assigned first mates ($\bar{X} \pm \text{SD}$: $17.26 \text{ mg} \pm 1.73$ and 15.14 ± 2.68 , respectively; separate variances t -test: $t_{20}=2.45$, $P=0.024$; see also Watson 1990). However, whether the first mate was a fight-winner per se was not a predictor of offspring growth. After controlling for all the

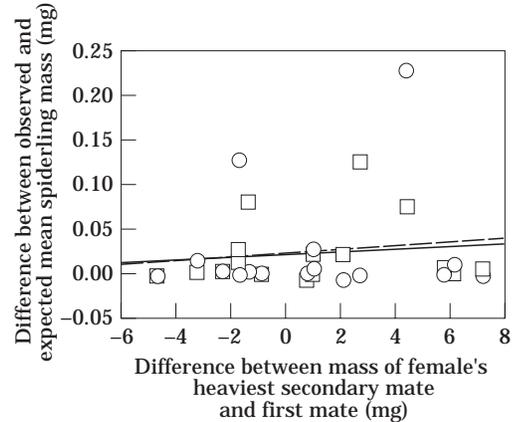


Figure 3. The difference between mean observed and expected body mass of the spiderlings of polyandrous females ($N=16$) versus the difference in mass between each female's heaviest secondary mate and her first mate. Data are plotted from day 20 (—○—) and day 27 (---□---). Expected mean body mass was calculated from data on single-mated females (i.e. non-bet-hedgers; $N=12$) using the multivariate equation relating offspring mass to six independent variates including mate mass (i.e. all those in Table I except female mate number). Thus, the Y-axis represents the anticipated results of multiple versus single mating vis-à-vis offspring growth, and the X-axis measures the expected opportunity to profit from bet-hedging vis-à-vis male body mass. The bet-hedging hypothesis predicts that larger differences in mass between the heaviest secondary mate and first mate should be associated with larger differences between the observed and expected body mass of a polyandrous female's offspring.

factors from the main repeated measures model (Table I), there was no evidence that the fighting status of the first mate affected offspring growth ($N=28$, all $P_{\alpha/2}>0.40$), nor did it have a differential effect on the broods of monogamous versus polyandrous females (all $P_{\alpha/2}>0.35$ for the interaction term: female mating status \times first mate fighting status).

Survivorship, Density and Offspring Growth

Covariates based on spiderling counts taken either 10, 20 or 27 days post-emergence all failed to indicate an influence of spiderling survivorship on spiderling mass (all $P \geq 0.41$) or growth rate (all $P \geq 0.20$) beyond that accounted for by the per capita prey availability covariate. Using the covariate based on the day 10 spiderling count,

which was the version least correlated with prey availability and which most reduced the significance of female mate number (see Methods), all the main results were strongly positive (Table I), making it unlikely that the reduced survivorship within broods of polyandrous females (see below) caused the increased growth observed in their surviving offspring or, more generally, that variance in spiderling density among rearing containers per se was a factor in offspring growth.

Multiple Mating and Variance in Offspring Growth

Within-brood variance in offspring mass was greater for multiply mating females (Mann-Whitney *U*-test: day 20, $U=54.0$, $\chi^2_1=4.52$, $P=0.034$; day 27, $U=53.0$, $\chi^2_1=4.71$, $P=0.030$). Similar results were obtained using ranges ($P=0.019$ and 0.046 , respectively). By virtue of the way variance is calculated, the number of offspring weighed per brood was negatively correlated with within-brood variances. Single- and multiple-mated females did not differ, however, in the number of offspring weighed per brood (Mann-Whitney *U*-test: day 20, $U=113.0$, $\chi^2_1=0.24$, $P=0.623$; day 27, $U=125.5$, $\chi^2_1=1.11$, $P=0.292$), so sample sizes should not be responsible for the differences in variance between these groups. Within-brood variances in spiderling body mass were unrelated to the absolute difference between the mass of the mother and (1) her first mate, (2) her heaviest mate, (3) the mean mass of all her mates or (4) her 'best' mate (day 20, all $r \leq 0.24$, $P \geq 0.33$; day 27, all $r \leq 0.18$, $P \geq 0.34$). I also found no significant correlations between the range of body masses represented across all of a female's mates and the within-brood variance in offspring mass (all $P \geq 0.261$).

Multiple Mating, Egg Viability and Offspring Survivorship

No fitness decrements were associated with polyandry in this sample of females. I found no association between polyandry and female survival through oviposition: 11 single-mated and nine polyandrous females died before laying eggs ($\chi^2=1.36$, $N=62$ females, $P=0.24$). Moreover, although 15 of the 43 females (seven of the 34 whose broods I attempted to rear) that survived to oviposit were sub-fertile ($\leq 75\%$ hatch), singly

mated and polyandrous females did not differ in the proportion of their eggs that hatched (64.5%, $N=18$ and 78.4%, $N=25$, respectively). In a multivariate analysis that controlled for oviposition date ($F_{1,29}=6.04$, $P=0.020$) and the average weight of individual eggs (i.e. fresh weight of the entire egg mass divided by egg number; $F_{1,29}=0.043$, $P=0.84$), the proportion of a female's eggs that hatched was unrelated to single- versus multiple-mating status ($F_{1,29}=0.031$, $P=0.86$; $N=33$ first broods).

I have no data on the cause of 136 (60.7%) of the 224 deaths that occurred during the study's 27-day rearing period (i.e. no carcass was found). For the remaining 88 spiderling deaths, I determined whether they were due to siblicide/cannibalism ($N=47$, 21%) or a non-traumatic cause (probably disease; $N=41$, 18.3%) by closely examining every carcass with a hand lens. Cannibalism results in a much more mutilated carcass than does non-traumatic death. I also directly observed a number of siblicides and non-traumatic deaths. In multivariate analyses ($N=33$ first broods) controlling for rearing set ($F_{1,28}=11.71$, $P=0.0019$; early set survived better), oviposition date ($F_{1,28}=0.85$, $P=0.37$) and prey availability ($F_{1,28}=2.62$, $P=0.12$), the overall survival of spiderlings was unrelated to female mate number ($F_{1,28}=0.45$, $P=0.51$). In a model with identical independent variables (and similar patterns of statistical significance as above), but with the non-siblicidal death rates as dependent variable, mating status was again a non-significant predictor ($N=33$, $F_{1,28}=0.61$, $P=0.44$).

DISCUSSION

Genetic Benefits of Female Preferences and Polyandry

In multivariate analyses that controlled for a wide range of maternal and environmental effects, experimentally imposed variation in female mate number and the body size of the known or most likely sire were independently and positively related to offspring growth (Table I). Offspring of polyandrous females (Fig. 1) and larger males (Fig. 2) grew faster and achieved greater mass during their first several instars outside the natal cocoon than did the offspring of single-mated females or smaller males.

In *N. litigiosa*, male body size is important in determining the fertilization success of both primary and secondary sires (Watson 1990, 1991a, b). The genetic covariance between paternal mass and early instar growth found in this study supports 'good genes' interpretations of selective mating. It is not surprising that the covariance is stronger for single-mated female offspring (Fig. 2), because every offspring's father is known, reducing noise in the analysis.

There has been little work on the effects of multiple-male mating on progeny viability (but see Kasuya 1992). The present study appears to be the first to document indirect fitness benefits of polyandry after partitioning the effects of female preferences for male traits from mate number preferences. Suter & Parkhill (1990) found in another Linyphiid spider, *Frontinella pyramitella*, that copulation duration was positively correlated with egg and hatchling weight. In *N. litigiosa*, multiple mating means increased total copulatory duration before the female's first oviposition, but in this case more mating does not affect the weight of eggs or newly emerged second instar spiderlings. The present study's finding that egg and newly emerged spiderling weights did not differ between single- and multiple-mated females bolsters the interpretation that the increasing difference in spiderling mass between treatment groups after emergence is due to genetic differences, not simply the burgeoning of a phenotypic headstart for the offspring of multiple-mated females.

This study failed to support a hypothesis for the evolution of polyandry based on avoidance of embryonic mortality stemming from intragenomic conflicts (Zeh & Zeh 1996). The lack of difference between single- versus multiple-mated females in the viability of eggs (measured as the percentage of eggs that hatched) and offspring survivorship indicate that monogamous females, which were experimentally prevented from using post-copulatory mechanisms to reduce fertilizations by incompatible sperm, did not have more young that failed to develop.

In nature, the potential of post-copulatory female choice to enhance offspring fitness may be greater than is suggested by some controlled-breeding experiments in which females are restricted to smaller numbers of matings than they typically would accept, often only two. In this study, matings occurred in nature, and females in the multiple mating treatment were allowed to

mate with the number of males that is typical of free-living females (Watson 1991b), so sperm precedence patterns are likely to be representative of those in nature (Zeh & Zeh 1994).

The random assignment of single versus multiple mating treatments was another advantage of this study; this should have randomized maternal effects on offspring between treatments, making it unlikely that multiple mating was secondarily associated with offspring growth (e.g. via a higher frequency of multiple mating by healthier females). Moreover, offspring rearing environments were standardized. Residual variation in prey availability, which slightly favoured offspring of polyandrous females, was removed statistically. Three covariates in the main model (Table 1) touched upon sources of variance in feeding rates among broods: (1) prey availability; (2) the number of spiderlings present at day 10; and (3) the number of cannibalisms. Thus, it is unlikely that systematic differences in climatic conditions, levels of competition for food, space or other environmental factors can explain the observed patterns of offspring growth. Finally, another interpretational problem is avoided because (1) the majority of females in my study area (ca 85%) produce only a single brood and (2) few females that do produce a second brood have an opportunity to re-mate after their first oviposition (<5%). Thus, it seems unlikely that female *N. litigiosa* are adapted to adjust investment in eggs in response to mate quality.

Viability and early growth

An expectation of genetic benefit hypotheses for non-random or multiple mating by females is that sexually persuasive male traits are indicators of genetic endowments that, on average, increase offspring viability. For example, Halliday & Verrell (1988) predicted that females biasing fertilization of their eggs towards large males would benefit by having faster-growing offspring. In *N. litigiosa*, speedy growth, especially in early life, is the basis for increasing resource holding power, the range of useable prey types and decreasing vulnerability to predators. In the dense populations in which *N. litigiosa* typically occur, intra- and interspecific competition over web sites is common (e.g. with the filmy dome spider, *N. marginata*) and large spiderlings have an enormous advantage. Early gains in size probably translate non-linearly into increased survival prospects.

Under competitive conditions in captivity, size differentials among spiderlings in their first week of life outside the cocoon typically increase over time (personal observation).

Genetic benefits of selective mating do not depend strictly on the heritability of the favoured traits. In a given sexual selection system, a wide array of genetic covariances could figure in the cost/benefit balance of mate selection. The point is illustrated by this study, which did not measure the heritability of adult male size or competitive ability, but rather its covariance with the growth of early instar progeny of both sexes. In *N. litigiosa*, much of the adaptive significance of female sexual behaviour could be connected to its effect on the competitive ability of developing offspring. Although larger adult males are sexually more successful and larger adult females are more fecund, the positive effect of female choice and polyandry on the growth of early instar spiderlings may make a larger contribution to offspring fitness.

Only controlled-breeding studies of heritabilities or genetic covariances among preferred male traits and components of offspring viability can provide strong evidence that natural selection acts on female sexual preferences via their viability consequences for the next generation. The positive effect of the female's preference for large male body mass on the growth rates of offspring in *N. litigiosa* add a new taxon to a growing list of such studies, involving, for example, field crickets, *Gryllus bimaculatus* (Simmons 1987*); house mice, *Mus musculus* (Lenington 1983); guppies, *Poecilia reticulata* (Reynolds & Gross 1992*); barn swallows, *Hirundo rustica* (Møller 1994), cockroaches, *Nauphoeta cinerea* (Moore 1994*) and peacocks, *Pavo cristatus* (Petrie 1994*). Studies with an asterisk after the year of publication, like the present study, reported associations between preferred male traits and offspring developmental rate. Of course, other studies have obtained negative results in a range of taxa (Boake 1986; Korzeniak & Jasiński 1990; Nicoletto 1995).

Physiological Implications of Female Preferences

In *N. litigiosa*, heavier males spend less energy to attain a given level of courtship performance than smaller males, even after compensating for the expected positive allometric relationship between mass and efficiency (Watson & Lighton

1994; unpublished data). In general, greater efficiency allows prey to be transformed into body mass and offspring production at a higher rate, so it is expected to be fundamental in determining differences in viability and sexual competitiveness among individuals of both sexes (Hall et al. 1986; Brown et al. 1993). The mechanism whereby the female's preference for large sires promotes rapid offspring growth may involve genetic factors affecting metabolic efficiency.

A trade-off exists, however, between efficiency and another physiological trait, maximum metabolic rate (MR_{max}), which is negatively correlated with efficiency in the male population (unpublished data). The physiological basis for this trade-off, although unknown, could arise if more rapid energy use increases the number of oxygen free-radicals produced per unit of energy consumption. Free-radical clean-up mechanisms also require energy, which would decrease overall metabolic efficiency at higher metabolic rates. Cryptic female choice in *N. litigiosa* positively selects for two uncorrelated male traits, body mass and copulatory courtship performance (Watson 1991a, b). Although mass is linked to efficiency, courtship performance is related to MR_{max} (Watson & Lighton 1994; unpublished data). Selecting simultaneously for both traits may amount to selecting for maximum metabolic 'power': the maximum rate of performing useful work (i.e. the product of efficiency \times MR_{max}). Selection on females to favour fitness traits like power, which express an amelioration of the trade-off between two or more simpler traits (e.g. efficiency and MR_{max}), may help explain the evolution of multi-component female sexual preferences.

Polyandry and Genetic Bet-hedging

Polyandry enables a female to distribute fertilizations among several well-tested mates, thus reducing the impact of occasional flawed evaluations of mate quality, that is, genetic bet-hedging (Watson 1991b). Bet-hedging can reduce the risk of genetically handicapping entire progenies when indirect choice mechanisms (e.g. male-male competition) fail to produce high-quality mates, as can occur in *N. litigiosa* just due to sampling error (Watson 1990, 1991b). Bet-hedging can also provide such protection in sexual selection systems where errors are occasionally made during direct sexual assessments. For example, when there is

much environmentally induced variance in a viability-related sexual signal that also varies due to heritable genetic factors, multiple mating may provide more reliable genetic benefits than highly selective monogamy. Body mass is probably a trait in which important genetic variance (e.g. in metabolic efficiency) is often partially obfuscated by environmental influences. Even if most variance in body size is environmental, however, the costs of choosing a genetically small mate may be high. The genetic bet-hedging hypothesis for the evolution of polyandry predicts that some component of offspring fitness genetically correlated with preferred male traits will increase, on average, when females have the opportunity to multiply mate. The results of this study support this prediction, because female *N. litigiosa* prefer heavier mates as sires, and polyandry promotes offspring growth even after adjusting for the effects of sire mass.

An incidental increase in within-brood genetic variance is expected under bet-hedging. However, its magnitude should be smaller than if the female's sire preferences were specifically designed to increase the genetic diversity of her progeny, as expected under the genetic diversity hypothesis for polyandry. Indirect support for a bet-hedging interpretation of polyandry comes from earlier work showing that the design of female sexual preference does not follow expectations of the diversity hypothesis. Unlike the expectation of the genetic diversity hypothesis, the male trait(s) sought by bet-hedging females should remain the same across all instances of mate assessment, even if the mechanisms of mate assessment change over the female's life history; this occurs in *N. litigiosa* (Watson 1990, 1991a, b).

Stockley et al. (1993) reported that many successful copulations occur between close relatives in high-density populations of the common shrew, *Sorex araneus*, and argued that polyandry and multiple paternity reduce the risk of all of a female's offspring being weakened by inbreeding depression, an explanation of polyandry conceptually similar to that provided by the bet-hedging hypothesis. The inbreeding compensation hypothesis interprets polyandry as an adaptation to being unable to identify relatives or avoid fertile matings with them, and the bet-hedging hypothesis explains multiple mating as a means of compensating for occasional failures to identify or bias fertilizations in favour of high-quality mates.

Although I found a trend consistent with the hypothesized bet-hedging mechanism linking non-random multiple paternity and offspring growth (Fig. 3), it was not statistically supported. Polyandrous females with larger secondary mates did not benefit more from multiple mating than females with relatively large first mates or similarly sized mates. Furthermore, having an experimentally assigned non-fighter instead of a natural fight-winning male as first mate was not a greater detriment to singly mated females compared to polyandrous females, again contradicting an expectation of the bet-hedging hypothesis. Further study is needed using larger samples, and more opportunity for the potential benefits of bet-hedging to manifest, in the form of larger fitness differences between potential sires.

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REFERENCES

- Alcock, J., Eickwort, G. C. & Eickwort, K. R. 1977. The reproductive behavior of *Anthidium maculosum* (Hymenoptera: Megachilidae) and the evolutionary significance of multiple copulations by females. *Behav. Ecol. Sociobiol.*, **2**, 385–396.
- Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.
- Andersson, M. & Iwasa, Y. 1996. Sexual selection. *Trends Ecol. Evol.*, **11**, 53–58.
- Arnqvist, G. 1989. Multiple-mating in a water strider: mutual benefits or intersexual conflict? *Anim. Behav.*, **38**, 749–756.

- Boake, C. R. B. 1986. A method for testing adaptive hypotheses of mate choice. *Am. Nat.*, **127**, 654–666.
- Boake, C. R. B. 1994. Evaluation of applications of the theory and methods of quantitative genetics to behavioral evolution. In: *Quantitative Genetic Studies of Behavioral Evolution* (Ed. by C. R. B. Boake), pp. 305–325. Chicago: The University of Chicago Press.
- Brown, J. H., Marquet, P. A. & Taper, M. L. 1993. Evolution of body size: consequences of an energetic definition of fitness. *Am. Nat.*, **142**, 573–584.
- Falconer, D. S. 1989. *Introduction to Quantitative Genetics*. 3rd edn. London: Longman.
- Gibson, R. M. & Bachman, G. C. 1992. The costs of female choice in a lekking bird. *Behav. Ecol.*, **3**, 300–309.
- Godin, J.-G. J. & Briggs, S. E. 1996. Female mate choice under predation risk in the guppy. *Anim. Behav.*, **51**, 117–130.
- Hall, C. A. S., Cleveland, C. J. & Kaufmann, R. 1986. *Energy and Resource Quality*. New York: John Wiley.
- Halliday, T. R. & Verrell, P. A. 1988. Body size and age in amphibians and reptiles. *J. Herpetol.*, **22**, 253–265.
- Hamilton, W. D. & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, **218**, 384–387.
- Howard, R. D., Whiteman, H. H. & Schueller, T. I. 1994. Sexual selection in American toads: a test of a good genes hypothesis. *Evolution*, **48**, 1286–1300.
- Johnstone, R. A. 1994. Honest signalling, perceptual error and the evolution of 'all-or-nothing displays'. *Proc. R. Soc. Lond. Ser. B*, **256**, 169–175.
- Lande, R. 1977. On comparing coefficients of variation. *Syst. Zool.*, **26**, 214–217.
- Lande, R. & Arnold, S. J. 1983. The measurement of selection on correlated characters. *Evolution*, **37**, 1210–1226.
- Lenington, S. 1983. Social preferences for partners carrying 'good genes' in wild house mice. *Anim. Behav.*, **31**, 325–333.
- Kasuya, E. 1992. Female mate preference and offspring fitness in the melon fly. *Ecol. Res.*, **7**, 277–281.
- Kirkpatrick, M. & Ryan, M. J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature, Lond.*, **350**, 33–38.
- Korzeniak, U. & Jasienski, M. 1990. Opportunity for non-random mating and components of offspring fitness in the flour beetle, *Tribolium confusum*. *Anim. Behav.*, **40**, 408–409.
- Møller, A. P. 1992. Frequency of female copulations with multiple males and sexual selection. *Am. Nat.*, **139**, 1089–1101.
- Møller, A. P. 1994. Male ornament size as a reliable cue to enhanced offspring viability in the barn swallow. *Proc. natn. Acad. Sci. U.S.A.*, **91**, 6929–6932.
- Moore, A. J. 1994. Genetic evidence for the good genes process of sexual selection. *Behav. Ecol. Sociobiol.*, **35**, 235–241.
- Nicoletto, P. F. 1995. Offspring quality and female choice in the guppy. *Anim. Behav.*, **49**, 377–387.
- Petrie, M. 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature, Lond.*, **371**, 598–599.
- Petrie, M., Hall, M., Halliday, T., Budgley, H. & Pierpoint, C. 1992. Multiple-mating in a lekking bird: why do peahens mate with more than one male and with the same male more than once. *Behav. Ecol. Sociobiol.*, **31**, 349–358.
- Pomiankowski, A. & Møller, A. P. 1995. A resolution of the lek paradox. *Proc. R. Soc. Lond. Ser. B*, **260**, 21–29.
- Pomiankowski, A., Iwasa, Y. & Nee, S. 1991. The evolution of costly mate preferences. I. Fisher and biased mutation. *Evolution*, **45**, 1422–1430.
- Reynolds, J. D. 1996. Animal breeding systems. *Trends Ecol. Evol.*, **11**, 68–72.
- Reynolds, J. D. & Cote, I. M. 1995. Direct selection on mate choice: female redblip blennies pay more for better mates. *Behav. Ecol.*, **6**, 175–181.
- Reynolds, J. D. & Gross, M. R. 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. *Proc. R. Soc. Lond. Ser. B*, **250**, 57–62.
- Ridley, M. 1988. Mating frequency and fecundity in insects. *Biol. Rev.*, **63**, 509–549.
- Simmons, L. W. 1987. Female choice contributes to offspring fitness in the field cricket, *Gryllus bimaculatus* (De Geer). *Behav. Ecol. Sociobiol.*, **21**, 313–321.
- Statistica 1993. *Statistica for Windows*, Version 4.0E. Tulsa, Oklahoma: Statsoft.
- Stockley, P., Searle, J. B., MacDonald, D. W. & Jones, C. S. 1993. Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. *Proc. R. Soc. Lond. Ser. B*, **254**, 173–179.
- Suter, R. B. & Parkhill, V. S. 1990. Fitness consequences of prolonged copulation in the bowl and doily spider. *Behav. Ecol. Sociobiol.*, **26**, 369–373.
- SYSTAT 1996. *SYSTAT for Windows: Statistics*, Version 6.00. Evanston, Illinois: SYSTAT.
- Walker, W. F. 1980. Sperm utilization strategies in nonsocial insects. *Am. Nat.*, **115**, 780–799.
- 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. 1991a. Multiple paternity and first mate sperm precedence in the sierra dome spider, *Linyphia litigiosa*. *Anim. Behav.*, **41**, 135–148.
- Watson, P. J. 1991b. Multiple paternity as genetic bet-hedging in female sierra dome spiders (*Linyphia litigiosa*: Linyphiidae). *Anim. Behav.*, **41**, 343–360.
- Watson, P. J. 1993. Foraging advantage of polyandry for female sierra dome spiders (*Linyphia litigiosa*: Linyphiidae) and assessment of alternative direct benefit hypotheses. *Am. Nat.*, **141**, 440–465.
- Watson, P. J. & Lighton, J. R. B. 1994. Sexual selection and the energetics of copulatory courtship in the sierra dome spider, *Linyphia litigiosa*. *Anim. Behav.*, **48**, 615–626.
- Zeh, J. A. & Zeh, D. W. 1994. Last-male sperm precedence breaks down when females mate with three males. *Proc. R. Soc. Lond. Ser. B*, **257**, 287–292.
- Zeh, J. A. & Zeh, D. W. 1996. The evolution of polyandry-I: intragenomic conflict and genetic incompatibility. *Proc. R. Soc. Lond. Ser. B*, **263**, 1711–1717.