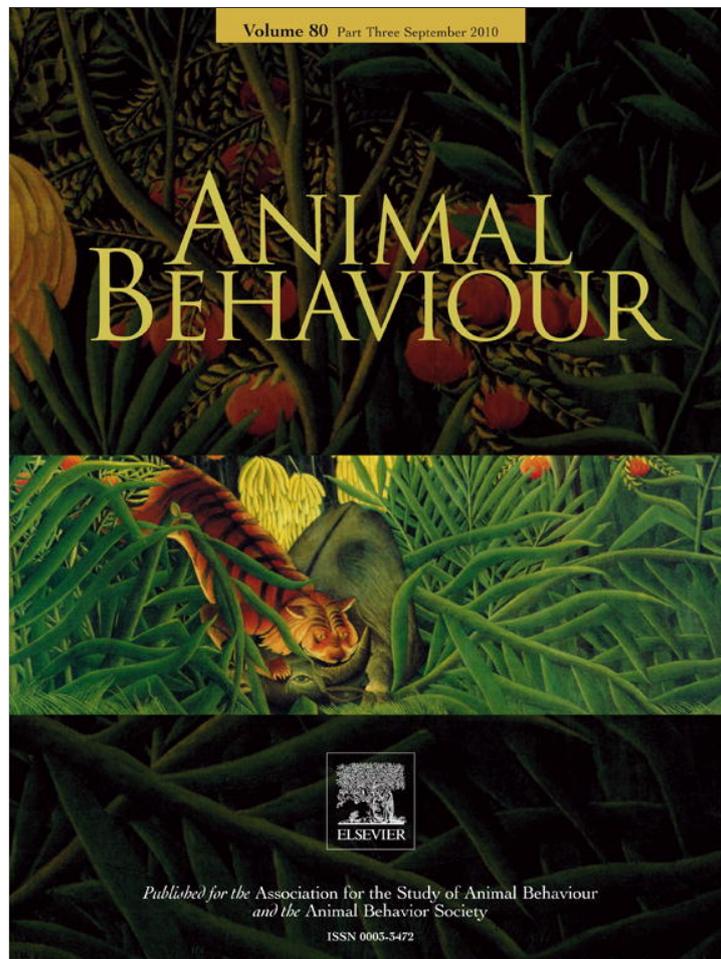


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No preference for novel mating partners in the polyandrous nuptial-feeding spider *Pisaura mirabilis* (Araneae: Pisauridae)

Cristina Tuni*, Trine Bilde

Department of Biological Sciences, Aarhus University

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Polyandrous females may gain genetic benefits for their offspring through postmating sexual selection. To facilitate postcopulatory choice for males of superior genetic quality females are expected to bias precopulatory mate choice towards novel males (i.e. genetically novel sources). Preference for novel partners is also expected to maximize male lifetime reproductive success by allowing males to increase the number of mates. We investigated male and female preference for novel or former mating partners in the spider *Pisaura mirabilis* by offering females novel males (polyandry) or the same male (monogamy). Precopulatory (mate acceptance) and prefertilization (latency to copulation, mating interruption and copulation duration) behaviours were compared between the two treatments. Males provide females with a nuptial prey gift during courtship. Because of the direct benefit associated with nuptial feeding, females should accept males indiscriminately and exert preference only at the prefertilization level. We found that monogamous females remated more readily than polyandrous females, suggesting less resistance to remating with the same male than with novel mates. No differences in female prefertilization responses were found. Lack of preference for novel mates may suggest that direct selection exerted by the nuptial gift rather than indirect selection for genetic benefits is a more likely driver of female remating propensity. Females were nevertheless resistant to remating, suggesting a trade-off between direct benefits and costs of remating. We found no effect of mate novelty on male mating behaviour, indicating either lack of discriminatory ability or that risk of sperm competition creates paternity benefits from remating with the same female.

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In polyandrous species (in which females mate with more than one male) mate choice is typically a sequential process in which a female must assess the quality of each male encountered and decide whether to remate. Direct selection models of mate choice predict that females exert preference for fitness-enhancing male traits, favouring males that provide resources such as food, parental care or high-quality territories (Thornhill & Alcock 1983; Andersson 1994; Kirkpatrick 1996). Material benefits may have a large impact on female fecundity or offspring survival (Thornhill & Alcock 1983; Vahed 1998; Arnqvist & Nilsson 2000), to an extent where they overcome costs associated with additional matings, which include exposure to predation, disease or even physical injury (Daly 1978; Chapman et al. 1995; Knell & Webberley 2004; Arnqvist & Rowe 2005). Therefore, female choice for direct benefits is considered one of the major ultimate forces driving the evolution and maintenance of polyandry (Arnqvist & Nilsson 2000; Arnqvist & Kirkpatrick 2005).

Polyandrous females may also derive fitness benefits via indirect selection for genetic benefits that enhance offspring viability (Jennions & Petrie 2000; Simmons 2005). Indirect genetic benefits can be obtained by promoting sperm competition or cryptic female choice, if postcopulatory sexual selection results in paternity bias towards males that provide their offspring with 'good genes' or 'compatible genes' (Keller & Reeve 1995; Eberhard 1996; Zeh & Zeh 1996, 1997; Bilde et al. 2008). Polyandrous females exerting control over paternity may hence acquire both direct and indirect benefits especially if the costs of postcopulatory sexual selection are small (Jennions & Petrie 2000).

Material benefits accrue whether females remate repeatedly with the same or a novel male, while the potential for acquiring indirect genetic benefits requires mating with novel partners representing different genotypes (i.e. ejaculates of different genetic composition) to facilitate postcopulatory sexual selection. The ability to discriminate against previous partners by means of precopulatory choice, and favour novel mates, can potentially allow for indirect selection to operate. Precopulatory choice for novel partners has been shown in females of crickets, beetles, flies and pseudoscorpions (Bateman 1998; Zeh et al. 1998; Archer & Elgar 1999; Hosken et al. 2003; Ivy et al. 2005). However, in mating

* Correspondence: C. Tuni, Department of Biological Sciences, Ecology and Genetics, Aarhus University, Ny Munkegade 1540, DK-8000 Aarhus C, Denmark.
 E-mail address: cristina.tuni@biology.au.dk (C. Tuni).

systems in which females receive material benefits from males, such as nuptial gifts, selection on female preference for novel partners is more ambiguous. Provided males are able to offer the required quality and quantity of material resource repeatedly, females would not benefit from seeking novel mates. Females of species in which males provide nuptial gifts may exert precopulatory choice to maximize material donations (i.e. preference for males offering larger gifts) rather than intrinsic male quality (Thornhill 1976; Thornhill & Alcock 1983; Boggs 1995; Vahed 1998). However, material and genetic benefits may operate in concert: females that acquire direct benefits from accepting a gift-giving mate may nevertheless exert postcopulatory selection for males of higher genetic quality or superior genetic compatibility (Tregenza & Wedell 2002; Simmons et al. 2006; Bilde et al. 2008). Females that exert cryptic choice will thus have the potential to gain material benefits from repeated copulations and genetic benefits from multiple sires.

From the male's perspective, providing females with material resources can impose considerable costs from energy expenditure, predation risks or food deprivation involved in nuptial donation. Costs of mating are predicted to lead to male mate choice and strategic sperm allocation (Dewsbury 1982; Wedell et al. 2002; Pizzari et al. 2003). Males might invest differentially in mating with females on the basis of female quality (i.e. females producing more and/or better offspring), according to the intensity of sperm competition (i.e. degree of polyandry) or sperm limitation that requires decisions on sperm allocation for future copulations (Pilastro et al. 2002; Reinhold et al. 2002; Wedell et al. 2002; Pizzari et al. 2003). The behavioural phenomenon known as the Coolidge effect (Wilson et al. 1963; Dewsbury 1981; Pizzari 2002) mediates differential sperm allocation, through a decline in remating propensity with a particular female as she becomes sexually familiar (i.e. the more a male copulates with her) and an increase in sexual interest for a novel female (not previously inseminated). Therefore, discriminating against previous partners should lead to a selective advantage in males, allowing those showing preference for novel females to maximize their lifetime reproductive success by increasing the number of mating partners (Bateman 1948; Dewsbury 1981; Pizzari 2002; Wedell et al. 2002).

Investigating remating behaviour towards novel or former mating partners in species in which females receive direct material benefits from males is particularly valuable for understanding the relative strength of direct (material) and indirect (genetic) benefits for the evolution and maintenance of polyandry. The nursery web spider, *Pisaura mirabilis* is one of the very few spider species exhibiting nuptial feeding (Austad & Thornhill 1986; Nitzsche 1988; Itakura 1993, 1998; Costa-Schmidt et al. 2008). Males of *P. mirabilis* court females by offering a nuptial gift consisting of an insect prey wrapped in silk; once the female accepts the gift and starts feeding from it males enter the mating position and initiate sperm transfer (Bristowe 1958; Austad & Thornhill 1986). Therefore, polyandrous females have the potential to base their mating decisions on both direct and indirect benefits. Experimental evidence shows that females mate several times (Austad & Thornhill 1986; Nitzsche 1988; Drengsgaard & Toft 1999; Bilde et al. 2007), although at present there is no evidence for direct fitness benefits of polyandry derived by the nutrient contents of a single nuptial gift (Stålhandske 2001). However, an increase in feeding rate was shown to enhance fecundity in *P. mirabilis* females (Austad & Thornhill 1986) suggesting the potential for polyandrous females to acquire material benefits. Females require a nuptial gift to accept copulation, and copulation duration depends on gift consumption time which is positively correlated with gift size (Bilde et al. 2006, 2007; Prokop & Maxwell 2009; C. Tunı, personal observation). Sperm transfer is positively correlated with copulation time

(Drengsgaard & Toft 1999) and therefore covaries with nuptial gift size and consumption time. Hence, there is strong sexual selection on the male gift-giving trait (Stålhandske 2001; Bilde et al. 2007). Gift construction and donation is probably associated with costs to males from time expenditure and predation risks involved in prey capture, silk investment for gift wrapping (Lang 1996) and food deficiency.

We investigated male and female mate choice towards novel (polyandrous) or former (monogamous) mating partners through controlled laboratory experiments to assess whether: (1) females bias precopulatory mate choice towards novel males (i.e. genetically dissimilar mates) which could facilitate postcopulatory sexual selection; and (2) males allocate more courtship to novel females and less to repeated copulations with former mates to maximize their reproductive success. The effect of encounters with former and novel mates was analysed by means of precopulatory (mate acceptance) and prefertilization (latency to copulation, mating interruption, copulation duration) behaviours as proxies for remating propensity. If females are under direct selection, they are not expected to show precopulatory choice based on male novelty since preference is determined by the presence of the nuptial gift. If direct and indirect benefits operate in concert, we predicted that remating females would accept the nuptial gift (precopulatory choice) and subsequently favour novel mates of a different genotype through shorter latency to copulation and longer copulations (prefertilization choice). Longer copulations would provide novel males with an advantage in sperm competition (Drengsgaard & Toft 1999). Because of the costs of providing a nuptial gift to females and of insemination, we predicted that males should direct their mating effort towards novel partners to maximize reproductive success.

METHODS

Study Organism, Collecting and Rearing Conditions

Pisaura mirabilis is a common and widespread hunting spider belonging to the Palaeartic Pisauridae family. The life cycle in southern Scandinavia is biennial: adults appear in April and females lay eggs in June. Spiderlings hatch in July–August and reach adulthood 2 years later.

Juveniles and subadult spiders were collected in early October 2005 from a grass meadow in Mols peninsula, Eastern Jutland, Denmark. Approximately 200 spiders were placed individually in vials (3 cm in diameter, 7 cm in height) covered by sponge lids and supplied with a substrate of fresh wet moss (*Sphagnum* sp.) to retain high humidity. Spiders collected at this time can be raised to maturity in the laboratory in winter (December–February) while in nature they would have overwintered for the second time to mature the following spring. Spiders were kept at room temperature (approximately 20 °C) and a natural photoperiod. Initially, individuals were fed twice a week with five to seven fruit flies, *Drosophila melanogaster*, from a laboratory culture. Upon maturity, spiders were offered a diet of two or three house flies, *Musca domestica*, and small crickets, *Acheta domesticus*, twice a week. Each individual was checked every third day until the final moult to maturity. Approximately 15 spiders died during moulting. Spiders were assigned to experiments 6–7 days after the final moult when they became sexually mature.

Effect of Novelty on Precopulatory Behaviours

We conducted controlled laboratory experiments from January to April 2006. Spiders were randomly assigned to two treatments, consisting of a monogamous (M) and a polyandrous (P) group. Monogamous females were presented four times to the same male

($N = 22$), while polyandrous females were presented once each to four different males ($N = 27$). To control for mating history, males within the P group were rotated so that on each trial, a focal female was exposed to a male with similar previous experience with other females (i.e. zero, one, two and three previous encounters) as the focal female (Tregenza & Wedell 1998). For a given female in the P group, her first mate would be a virgin, her second mate would have previously mated once, her third mate had previously mated twice, and the fourth mate would have mated three times. Mating experiments were performed in transparent plastic terraria (17×17 cm and 10 cm high) in which the inside bottom was covered with a layer of adsorbent paper. Females were transferred into the terrarium 10 min before the mating experiment, allowing them to leave draglines on the substrate, which elicits male sexual excitement upon contact (Nitzsche 1988). The flies offered to males as nuptial gifts were of similar size (M. J. Albo, unpublished data) across experimental treatments and replicates. Once a male holding a house fly as a nuptial gift was introduced into the same terrarium as a female ($t = 0$ s), we recorded the following male and female precopulatory behaviours (see Table 1). Male-controlled behaviours were: the proportion of males presenting the gift to females; time to gift presentation (from start of trial until the male offered the gift); gift wrapping (the proportion of males that invested in adding more silk to the gift); the female-controlled response was copulation success (Table 1). Individuals were used only once per day, and after each trial spiders were returned to their housing vials. The nutritional state of spiders was equalized by feeding males with a house fly immediately after mating trials in which females were observed consuming the gift received from the male. The trial was terminated after 120 min with no interaction between the sexes, when females took the gift away from males preventing courtship, or if the male was cannibalized.

Effect of Novelty on Prefertilization Behaviours

In the precopulatory assay (see above) not all females copulated with each of the four males with which they were presented. If a female rejected a male, we repeatedly presented the female with the same (M) or novel (P) males until four copulations were accomplished (M group, $N = 16$ females; P group, $N = 19$ females; Fig. 1). This design was aimed at investigating remating propensity towards a particular partner as he/she becomes sexually familiar (i.e. after repeated copulations with the same mate). Male and female prefertilization behaviours within each completed copulation were recorded (see Table 2: male-controlled responses: gift presentation; time to gift presentation; gift wrapping; female-

controlled responses: latency to copulation (the time from the start of the trial to copulation initiation); mating interruption (how many times a female interrupted sperm transfer); copulation duration). Mating trials were conducted following the procedure described above. The number of trials required to accomplish four matings was recorded for comparison between treatments as a proxy of female remating resistance.

Fitness Effects

With the intention of distinguishing the relative contributions of material and genetic benefits we investigated the effect of four accomplished matings (M versus P) on components of female reproductive fitness (fecundity and egg hatching success; Tregenza & Wedell 1998). A control group (C) consisting of a virgin female mated once to a virgin male ($N = 30$) was added to the experimental design to assess the effect of material benefits on singly mated and multiply mated females. If polyandry provided females with genetic benefits, females of the P group should experience higher fitness than the M and C groups since only females mated to novel mates have the potential for postcopulatory choice. Therefore, P females should show increased egg hatching success. Once mating trials from all treatments were completed, spiders were fed throughout their life span and we recorded the number of eggsacs produced by females and the longevity of a subset of individuals. Unfortunately the eggs deposited inside the eggsacs (up to 150 eggs per eggsac, Stålhandske 2001) failed to hatch, because of unknown environmental factors; hence we were unable to assess fitness effects of the C, M and P groups, and could compare only the numbers of eggsacs.

Sexual Cannibalism

We recorded incidents of precopulatory sexual cannibalism and replaced dead males with spiders of the same mating status (virgin, mated once, twice, three times) in order not to alter mating experience. Occurrence of male death-feigning behaviour (thanatosis; Bilde et al. 2006, 2007; Hansen et al. 2008) was also recorded.

Statistical Analysis

Data were analysed with generalized linear models (GLM) with an appropriate error and link function. We ran models on the effect of treatment group (M or P) on male and female behavioural responses, and included trial number and the interaction between treatment group and trial number in the model. Continuous data

Table 1

Precopulatory behaviours: female and male responses during subsequent encounters (second, third, fourth encounters) with formerly encountered (M) and novel (P) individuals

	M encounter			P encounter			Prob>P, χ^2_{df}		
	2	3	4	2	3	4	Group	Trial	Interaction
Male-controlled responses									
Gift presentation % (proportion)	59 (13/22)	68 (15/22)	68 (15/22)	70 (19/27)	63 (17/27)	59 (16/27)	0.6	0.9	0.3
Time to gift presentation (min), median (range)	13 (8–62)	12 (3–36)	17 (2–56)	12 (0.2–62)	12 (0.2–59)	14.5 (2–71)	$\chi^2_{1,147}=0.1$ 0.7	$\chi^2_{2,147}=0.4$ 0.7	$\chi^2_{2,147}=2.4$ 0.8
Gift wrapping % (proportion)	59 (13/22)	48 (10/21)	62 (13/21)	50 (13/26)	41 (11/27)	33 (9/27)	$\chi^2_{2,80}=0.1$ 0.09	$\chi^2_{2,80}=0.5$ 0.5	$\chi^2_{2,80}=0.4$ 0.4
							$\chi^2_{1,147}=3.4$	$\chi^2_{2,147}=1.1$	$\chi^2_{2,147}=1.6$
Female-controlled responses									
Copulation success after first male attempt % (proportion)	76.5 (13/17)	78.5 (11/14)	100 (15/15)	55 (10/18)	70.5 (12/17)	81 (13/16)	0.016*	0.015*	0.3
Copulation success overall % (proportion)	73 (16/22)	64 (14/22)	73 (16/22)	66 (18/27)	63 (17/27)	59 (16/27)	$\chi^2_{1,147}=4.5$ 0.2	$\chi^2_{2,147}=8.9$ 0.6	$\chi^2_{2,147}=2.3$ 0.6
							$\chi^2_{1,147}=1.7$	$\chi^2_{2,147}=0.9$	$\chi^2_{2,147}=0.9$

* Denotes significance.

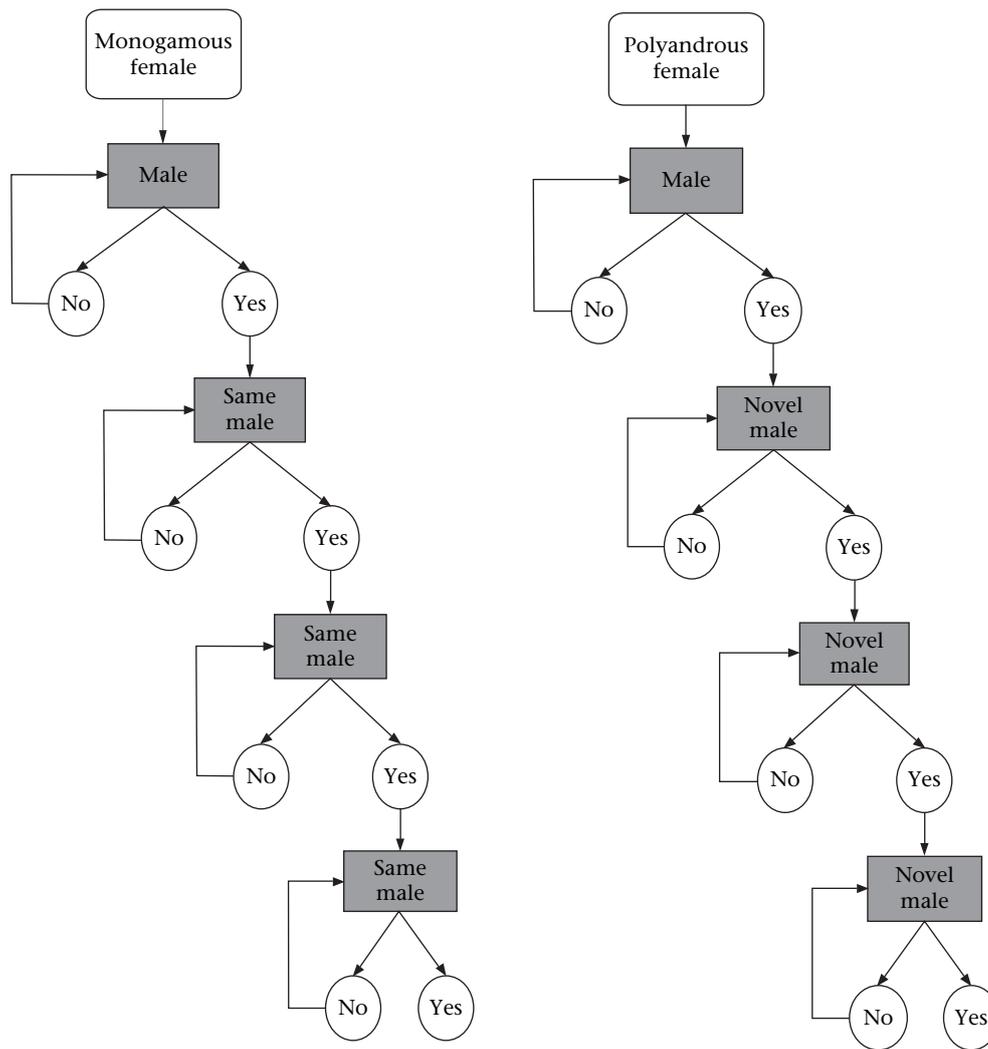


Figure 1. Outline of the experimental design: monogamous females were offered the same male (M group) and polyandrous females novel males (P group) until four completed copulations were accomplished.

were analysed using GLM with Gamma errors (GLM-g); for counts we used the Poisson family (GLM-p) and for proportions the binomial family (GLM-b). GLM-b models were checked for

overdispersion. We present results from the full models. All statistical analyses were performed using JMP 7.0 software (SAS Institute, Cary, NC, U.S.A.).

Table 2
Prefertilization behaviours: female and male behaviours during subsequent accomplished copulations (first, second, third and fourth matings) with former mating partners (M) and sexually novel mates (P)

	M mating				P mating				Prob>P, χ^2_{df}		
	1	2	3	4	1	2	3	4	Group	Trial	Interaction
Male-controlled responses											
Gift presentation % (proportion)	94 (15/16)	81 (13/16)	75 (12/16)	94 (15/16)	68 (13/19)	79 (15/19)	89 (17/19)	84 (16/19)	0.3	0.7	0.15
Time to gift presentation (min), median (range)	8 (0.2–120)	12 (3–62)	14 (3–56)	18.5 (2–60)	8 (1–21)	13 (1–48)	20 (0.2–59)	17 (4–71)	$\chi^2_{1,132}=1.0$	$\chi^2_{3,132}=1.3$	$\chi^2_{3,132}=5.2$
Gift wrapping % (proportion)	53 (8/15)	56 (9/16)	53 (8/15)	73 (11/15)	47 (9/19)	58 (11/19)	58 (11/19)	63 (12/19)	$\chi^2_{1,99}=1.1$	$\chi^2_{3,99}=4.7$	$\chi^2_{3,99}=1.1$
									$\chi^2_{1,132}=0.6$	$\chi^2_{3,132}=2.3$	$\chi^2_{3,132}=0.4$
Female-controlled responses											
Latency to copulation (min), median (range)	15 (2–116)	25.5 (1–67)	21 (5–59)	25.5 (5–110)	16 (3–39)	25 (2–120)	33 (10–86)	25 (6–89)	0.7	0.2	0.4
Copulation duration (min), median (range)	22 (6–56)	24 (6–71)	21 (2–54)	20.5 (5–63)	20 (1–57)	26 (3–78)	23 (1–81)	19 (3–56)	$\chi^2_{1,131}=0.1$	$\chi^2_{3,131}=4.9$	$\chi^2_{3,131}=2.7$
Number of mating interruptions, median (range)	1 (1–3)	1 (1–3)	1 (1–4)	1 (1–6)	1 (1–4)	1 (1–3)	1 (1–4)	1 (1–3)	$\chi^2_{1,132}=0.2$	$\chi^2_{3,132}=2.5$	$\chi^2_{3,132}=1.3$
									$\chi^2_{1,101}=0.1$	$\chi^2_{3,101}=0.2$	$\chi^2_{3,101}=0.7$

RESULTS

Mating Trials

Males performed their courtship display (described by Bristowe 1958) when placed in the terrarium with the female. Successful courtship resulted in females grasping the gift in their chelicerae and initiating gift consumption. At this point, males entered the mating position and initiated sperm transfer by reaching the female epigyne (genital opening) with their pedipalps. Males that were rejected on their first mating attempt commonly added more silk threads to the gift and made renewed attempts (i.e. presented the gift in repeated attempts). Of 270 mating trials performed during the experiment, copulations occurred in 69% ($N = 186$). Precopulatory sexual cannibalism occurred in 7% (19/270) of the trials. The incidence of cannibalistic events was comparable among all treatments (C, M, P; GLM-b model effect of treatment group (C, M, P): $\chi^2_{2,217} = 5.0$, $P = 0.08$) and did not covary significantly with experience (i.e. the sequence of trials 1–4; trial nested within treatment group: $\chi^2_{6,217} = 5.7$, $P = 0.45$). A similar analysis of male thanatosis behaviour showed no significant effect of treatment group on thanatosis (GLM-b: $\chi^2_{2,217} = 0.14$, $P = 0.9$), while sequence of encounters was significant (trials 1–4 nested within treatment group: $\chi^2_{2,217} = 13.8$, $P = 0.03$) with a higher proportion of males death feigning on the fourth encounter. Overall, male death-feigning behaviour occurred in 37% (100/270) of all trials.

Effect of Novelty on Precopulatory Behaviours

Male identity (former-M versus novel-P) was not a significant predictor of mating success (Fig. 2). However, a significantly higher proportion of females in the M group than the P group mated after a male's first mating attempt (a male would present the same gift repeatedly if he was initially rejected by a female, Table 1). The sequence of encounters (trial) with males had a significant effect on mating success in both treatment groups as the proportion of females that mated after a male's first mating attempt increased as the number of encounters with males increased (Table 1). No difference in components of male courtship behaviour between treatments was found; the proportion of males that courted novel females was comparable to that courting former mates, regardless of the sequence of encounters (Table 1). Similarly, gift-wrapping

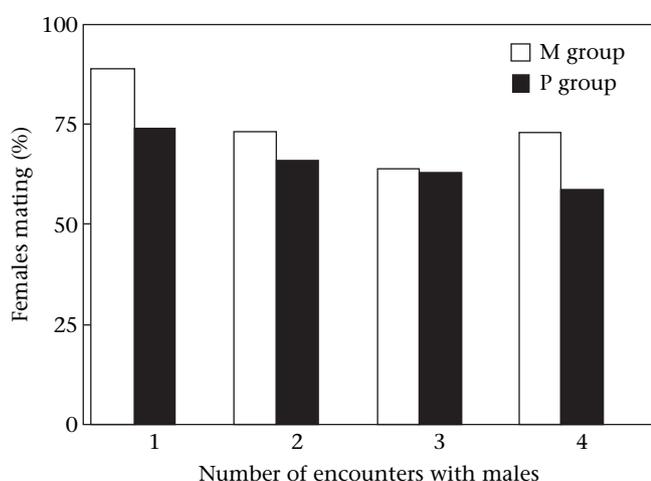


Figure 2. Mating success among females during subsequent encounters (second, third and fourth encounters) with the same males (M group; $N = 22$) and novel males (P group; $N = 27$). The first encounter with a male is given for comparison.

behaviour and latency to gift presentation did not differ between treatment groups and sequence of encounters (Table 1).

Effect of Novelty on Prefertilization Behaviours

Females required one to five encounters with a male to accomplish copulation, indicating resistance to remating. Consequently, females were presented with up to eight males to accomplish four copulations. The mean number of trials required to accomplish first, second, third and fourth matings differed significantly between treatment groups and the trial in question; females from the P group required more trials to accomplish third and fourth matings than females from the M group (GLM-g: group effect: $P < 0.001$; trial effect: $P < 0.001$; interaction: $P < 0.001$; Fig. 3). Data on male and female prefertilization behaviours during each accomplished mating (first, second, third and fourth) are given in Table 2. No significant difference in the female behaviours latency to copulation, copulation duration and frequency of interruptions during copulation was found between different groups during each accomplished mating (Table 2). Similarly, male gift presentation, gift-wrapping behaviour and latency to gift presentation did not differ significantly between either treatment group or sequence of mating (Table 2).

Fitness Effects

Mated females produced one to three eggsacs, with no significant difference between control, M and P treatment groups (Table 3). Among the females that produced an eggsac ($N = 57$), 21 females (59%) produced a second eggsac, 13 (36%) of which produced a third. None of the eggsacs hatched or contained developed embryos, and it was not possible to count the eggs within eggsacs. Female longevity, measured as number of days from development to adulthood until death was similar across groups (Table 3).

DISCUSSION

Precopulatory behaviours revealed that females of the monogamous group accepted more males on their first gift presentation than females of the polyandrous group, suggesting less resistance to mating with a previous male than a novel male. This result was

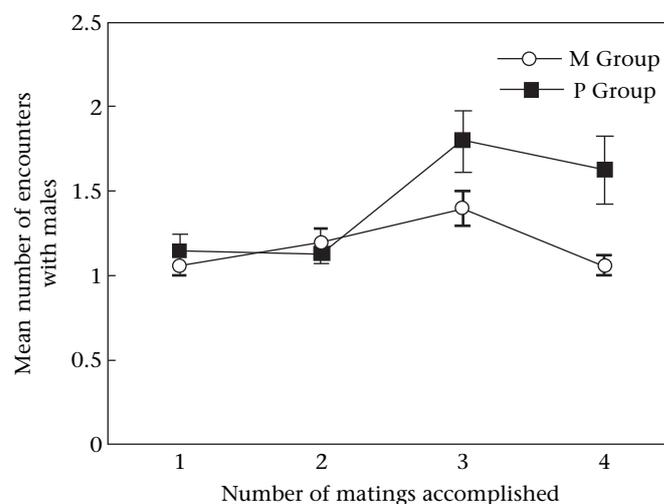


Figure 3. Mean number of encounters with previously encountered males (M group) and novel males (P group) required to accomplish each of the four matings. Error bars indicate SE.

Table 3
Relative fitness variables: treatment effect (M, P, C) on number of eggsacs produced and female longevity (number of days from adulthood to death)

Fitness variables	M treatment	P treatment	C treatment	P
Number of eggsacs				
Mean±SE	2±0.2	2±0.18	1.66±0.18	0.6
Range	1–3	1–3	1–3	
N	16	19	18	
Female longevity				
Median	72	86	106	0.9
Range	48–139	20–157	28–158	
N	11	15	10	

corroborated by the finding that monogamous females required fewer encounters with males to accomplish a total of four copulations than polyandrous females. However, once females engaged in matings no differences in prefertilization responses such as latency to copulation, copulation duration and frequency of mating interruptions were found, suggesting lack of prefertilization discrimination of males. Courtship performance from males was comparable across previous and novel partners suggesting lack of male mate discrimination.

An important factor not always accounted for in studies on female choice for novel males is the possible influence of male behaviour (Bateman 1998; Archer & Elgar 1999; Ivy et al. 2005). In our study, controlling for male and female behaviours allowed us to control for male influence on female responses. Males appeared to court previous and novel females in the same way. Male copulation effort was determined by female acceptance, and male courtship effort prior to copulation was similar across treatments. Therefore, the finding that a higher proportion of monogamous females accepted their mate indicates that females exposed repeatedly to the same male lowered their resistance to remating. In contrast, polyandrous females rejected males in higher proportions forcing these males to re-offer the gift several times until female acceptance and copulation. This explains why the overall outcome of male–female interactions (copulation success) did not differ between polyandrous and monogamous groups.

Remating with a male that a female has previously encountered may represent a safe interaction, for example by reducing the risk of harm (Arnqvist & Rowe 2005). Males may possess harmful adaptations such as genital spines and the transfer of seminal toxins that function to reduce female remating propensity (Johnstone & Keller 2000). It is possible that females reduce the costs of harmful matings by reducing the number of different mates, while retaining direct nutrient benefits from matings with a known mate. However, the observed pattern of female remating behaviour assumes the presence of mate discrimination mechanisms. Discrimination mechanisms have been described in the form of individual recognition in social insects (D'Etorre & Heinze 2005) or self-referencing (female marking males with an individual-specific chemical signature) in crickets (Ivy et al. 2005). Spiders, like other arthropods, rely mainly on chemical signals to mediate different types of interactions such as foraging, predator avoidance and courtship (reviewed in Tietjen & Rovner 1982; Huber 2005). In *P. mirabilis* the silk used by males to wrap the nuptial gift may mediate chemical (i.e. pheromone) communication informing females about male identity (novel or former male). Although pheromone use in male spiders has been largely understudied, evidence suggests that females are capable of chemically assessing male identity (Huber 2005).

Our study revealed no evidence for differences in female prefertilization behaviours, and hence no evidence for indirect selection. Previous studies show that *P. mirabilis* females are capable of stealing the gift from males either during gift display (courtship) or

during copulation, preventing or terminating copulation (Drengsgaard & Toft 1999; Bilde et al. 2006, 2007; Andersen et al. 2008). The lack of behavioural discrimination of males in copulation initiation, frequency of mating interruptions or copulation duration suggests an overall lack of female prefertilization preference. However, the absence of difference in copulation duration with former and novel males does not exclude the potential for female cryptic postcopulatory choice. Preferential sperm use for fertilization of the eggs, differential storage of sperm in the spermatheca or sperm displacement by females are postmating processes in the female reproductive tract that may lead to fertilization bias (Eberhard 1996). If *P. mirabilis* females are capable of postcopulatory mate choice they may acquire indirect genetic benefits. We found no evidence for indirect selection and hence that postmating sexual selection favours polyandry in this system. However, to evaluate fully the effect of multiple sires on fitness, egg hatching success and offspring fitness assays are needed. It is likely that direct selection exerted by the presence of the gift is sufficient to entice females into mating, as they, like other arthropods exhibiting nuptial feeding, are generally receptive to most of the male donations (Thornhill 1976; Thornhill & Alcock 1983; Boggs 1995; Vahed 1998). Indeed, previous studies in this species showed that female hunger level predicted remating propensity (Bilde et al. 2007).

Our study revealed that a proportion of females rejected remating opportunities and required several additional encounters with males for each accomplished copulation. Female resistance to subsequent matings suggests that remating is costly, and indicates a trade-off between direct benefits from the nuptial gifts and costs of remating.

We were not able to detect effects of mate novelty on male courtship intensity or mating behaviour. This result should, however, be taken with caution, as we are not able to exclude the existence of subtle differences in male responses that require high power to detect. Also, we did not control for differential sperm transfer by males among previous and novel mating partners. The behavioural results suggest either lack of precopulatory discrimination of females or lack of preference for novel females and hence no evidence for the Coolidge effect. Similarly, no evidence for the Coolidge effect was found in male decorated crickets, *Gryllobates sigillatus* (Gershman & Sakaluk 2009). Gershman & Sakaluk (2009) suggested this was due to relaxed selection on males for mate recognition if female prefer novel mates. However, given the costs of mating repeatedly with the same female in terms of ejaculate investment, nuptial gift donation and lost mating opportunities, males are expected to balance the costs and reproductive benefits. Males may, for example, adjust ejaculate expenditure based on previous investment in a mate (Dewsbury 1982; Simmons 2001; Wedell et al. 2002; Pizzari et al. 2003). Males are able to alter sperm numbers or spermatophore size according to the reproductive value of their mates (i.e. female age, size, reproductive status or promiscuity; Simmons 2001; Pilastro et al. 2002; Reinhold et al. 2002; Wedell et al. 2002; Pizzari et al. 2003). Therefore, it is possible that *P. mirabilis* males allocate sperm preferentially to sexually novel females (Dewsbury 1981; Pizzari 2002; Koene & Ter Maat 2007; Ódeen & Moray 2008; Steiger et al. 2008).

Alternatively, males may derive net benefits from remating with the same females by maximizing paternity success under sperm competition (e.g. Smith 1979; Ridley 1988; Otronen 1994; Birkhead & Møller 1998). Mating more than once with the same female increases the amount of sperm the male transfers, and thus the number of offspring sired (Drengsgaard & Toft 1999). Furthermore, if females are likely to be polyandrous, males may limit the paternity success of future mates, by loading the female spermatheca (sperm storage organ) and preventing sperm storage from later mates, or by transferring accessory gland products that inhibit

female remating (Eberhard 1996; Simmons 2001). Sperm competition and patterns of sperm priority may be critical in shaping male mating decisions in relation to mated females. In *P. mirabilis*, the last male to copulate was shown to achieve higher fertilization success when females copulated with more than five males, turning first-male advantage into a last-male advantage (Drengsgaard & Toft 1999). If females are highly polyandrous, males protect their paternity by remating with previous partners.

In conclusion, the overall lack of precopulatory preference for novel partners representing novel genetic sources suggests that polyandry in *P. mirabilis* is most likely to be maintained by direct selection for nuptial gifts. However, female resistance to remating suggests a trade-off between direct benefits and costs of mating. Determining the net effects of polyandry on fitness is necessary to assess the role of indirect selection on female mate choice. The level of sperm competition may dictate the high male motivation in remating regardless of female novelty. Estimates of female natural mating rate, sperm allocation patterns and paternity patterns are needed to acquire a better understanding of male mating decisions.

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References

- Andersen, T., Bollerup, K., Toft, S. & Bilde, T. 2008. Why do males of the spider *Pisaura mirabilis* wrap their nuptial gifts in silk: female preference or male control? *Ethology*, **114**, 775–781.
- Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.
- Archer, M. S. & Elgar, M. A. 1999. Female preference for multiple partners: sperm competition in the hide beetle, *Dermestes maculatus* (DeGeer). *Animal Behaviour*, **58**, 669–675.
- Arnqvist, G. & Kirkpatrick, M. 2005. The evolution of infidelity in social monogamous passerines: the strength of direct and indirect selection on extrapair copulation in females. *American Naturalist*, **165**, S26–S37.
- Arnqvist, G. & Nilsson, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour*, **60**, 145–164.
- Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict*. Princeton, New Jersey: Princeton University Press.
- Austad, S. N. & Thornhill, R. 1986. Female reproductive variation in a nuptial-feeding spider *Pisaura mirabilis*. *Bulletin of the British Arachnological Society*, **7**, 48–52.
- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity*, **2**, 349–368.
- Bateman, P. W. 1998. Mate preference for novel partners in the cricket *Gryllus bimaculatus*. *Ecological Entomology*, **23**, 473–475.
- Bilde, T., Tunı, C., Elsayed, R., Pekar, S. & Toft, S. 2006. Death feigning in the face of sexual cannibalism. *Biology Letters*, **2**, 23–25.
- Bilde, T., Tunı, C., Elsayed, R., Pekar, S. & Toft, S. 2007. Nuptial gifts of male spiders: sensory exploitation of the female's maternal care instinct or foraging motivation? *Animal Behaviour*, **73**, 267–273.
- Bilde, T., Friberg, U., Maklakov, A. A., Fry, J. D. & Arnqvist, G. 2008. The genetic architecture of fitness in a seed beetle: assessing the potential for indirect genetic benefits of female choice. *BMC Evolutionary Biology*, **8**, 295.
- Birkhead, T. R. & Møller, A. P. 1998. *Sperm Competition and Sexual Selection*. London: Academic Press.
- Boggs, C. L. 1995. Male nuptial gifts: phenotypic consequences and evolutionary implications. In: *Insect Reproduction* (Ed. by S. R. Leather & J. Hardie), pp. 215–242. New York: CRC Press.
- Bristowe, W. S. 1958. *The World of Spiders*. London: Collins.
- Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. 1995. Cost of mating in *Drosophila melanogaster* female is mediated by male accessory gland products. *Nature*, **373**, 241–244.
- Costa-Schmidt, L. E., Carico, J. E. & Araújo, A. M. 2008. Nuptial gifts and sexual behaviour in two species of spider (Araneae, Trechaleidae, Paratrechalea). *Naturwissenschaften*, **95**, 731–739.
- Daly, M. 1978. Cost of mating. *American Naturalist*, **112**, 771–774.
- D'Ettoire, P. & Heinze, J. 2005. Individual recognition in ant queens. *Current Biology*, **15**, 2170–2174.
- Dewsbury, D. A. 1981. Effects of novelty on copulatory behavior: the Coolidge effect and related phenomena. *Psychological Bulletin*, **89**, 464–482.
- Dewsbury, D. A. 1982. Ejaculate cost and male choice. *American Naturalist*, **119**, 601–610.
- Drengsgaard, I. L. & Toft, S. 1999. Sperm competition in a nuptial feeding spider, *Pisaura mirabilis*. *Behaviour*, **136**, 877–897.
- Eberhard, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton: Princeton University Press.
- Gershman, S. N. & Sakaluk, S. K. 2009. No Coolidge effect in decorated crickets. *Ethology*, **115**, 774–780.
- Hansen, L. S., Gonzales, S. F., Toft, S. & Bilde, T. 2008. Thanatosis as an adaptive male mating strategy in the nuptial gift-giving spider *Pisaura mirabilis*. *Behavioral Ecology*, **19**, 546–551.
- Hosken, D. J., Martin, O. Y., Born, J. & Huber, F. 2003. Sexual conflict in *Sepsis cynipsea*: female reluctance, fertility and mate choice. *Journal of Evolutionary Biology*, **16**, 485–490.
- Huber, B. A. 2005. Sexual selection research on spiders. *Biological Reviews*, **80**, 363–385.
- Itakura, Y. 1993. The life history and nuptial feeding of a nursery web spider, *Pisaura lama*. *Insectarium*, **30**, 88–93.
- Itakura, Y. 1998. Discovery of nuptial feeding in the spider, *Perenethis fascigera* (Araneae: Pisauridae). *Acta Arachnologica*, **47**, 173–175.
- Ivy, T. M., Weddle, C. B. & Sakaluk, S. K. 2005. Females use self-referent cues to avoid mating with previous mates. *Proceedings of the Royal Society B*, **272**, 2475–2478.
- Jennions, M. D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*, **75**, 21–64.
- Johnstone, R. A. & Keller, L. 2000. How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *American Naturalist*, **156**, 368–377.
- Keller, L. & Reeve, H. K. 1995. Why do females mate with multiple males? The sexually selected sperm hypothesis. *Advances in the Study of Behavior*, **24**, 291–315.
- Kirkpatrick, M. 1996. Good genes and direct selection in evolution of mating preferences. *Evolution*, **50**, 2125–2140.
- Knell, R. J. & Webberley, K. M. 2004. Sexually transmitted disease of insects: distribution, evolution, ecology and host behaviour. *Biological Reviews of the Cambridge Philosophical Society*, **79**, 557–581.
- Koene, J. M. & Ter Maat, A. 2007. Coolidge effect in pond snails: male motivation in a simultaneous hermaphrodite. *BMC Evolutionary Biology*, **7**, 212.
- Lang, A. 1996. Silk investments in gifts by males of the nuptial feeding spider *Pisaura mirabilis* (Araneae: Pisauridae). *Behaviour*, **133**, 697–716.
- Nitzsche, R. O. M. 1988. 'Brautgeschenk' und Umspinnen der Beute bei *Pisaura mirabilis*, *Dolomedes fimbriatus* und *Thaumasia uncatata* (Arachnida, Araneida, Pisauridae). *Verhandlungen des Naturwissenschaftlichen Vereins Hamburg*, **30**, 353–393.
- Otronen, M. 1994. Repeated copulations as a strategy to maximize fertilization in the fly *Dryomyza anilis* (Dryomyzidae). *Behavioral Ecology*, **5**, 51–56.
- Ödeen, A. & Moray, C. M. 2008. *Drosophila melanogaster* virgins are more likely to mate with strangers than familiar flies. *Naturwissenschaften*, **95**, 253–256.
- Pilastro, A., Scaggiante, M. & Rasotto, M. B. 2002. Individual adjustment of sperm expenditure accords with sperm competition theory. *Proceedings of the National Academy of Sciences, U.S.A.*, **99**, 9913–9915.
- Pizzari, T. 2002. Sperm allocation, the Coolidge effect and female polyandry. *Trends in Ecology & Evolution*, **17**, 456.
- Pizzari, T., Cornwallis, C. K., Løvlie, H., Jakobsson, S. & Birkhead, T. R. 2003. Sophisticated sperm allocation in male fowl. *Nature*, **426**, 70–74.
- Prokop, P. & Maxwell, M. R. 2009. Female feeding regime and polyandry in the nuptially feeding nursery web spider, *Pisaura mirabilis*. *Naturwissenschaften*, **96**, 259–265.
- Reinhold, K., Kurtz, J. & Engqvist, L. 2002. Cryptic male choice: sperm allocation strategies when female quality varies. *Journal of Evolutionary Biology*, **15**, 201–209.
- Ridley, M. 1988. Mating frequency and fecundity in insects. *Biological Reviews of the Cambridge Philosophical Society*, **63**, 509–549.
- Simmons, L. W. 2001. *Sperm Competition and its Evolutionary Consequences in the Insects*. Princeton, New Jersey: Princeton University Press.
- Simmons, L. W. 2005. The evolution of polyandry: sperm competition, sperm selection and offspring viability. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 125–146.
- Simmons, L. W., Beveridge, M., Wedell, N. & Tregenza, T. 2006. Postcopulatory inbreeding avoidance by female crickets only revealed by molecular markers. *Molecular Ecology*, **15**, 3817–3824.
- Smith, R. L. 1979. Repeated copulation and sperm precedence: paternity assurance for a male brooding water bug. *Science*, **205**, 1029–1031.
- Stålhandske, P. 2001. Nuptial gift in the spider *Pisaura mirabilis* maintained by sexual selection. *Behavioral Ecology*, **12**, 691–697.
- Steiger, S., Franz, R., Eggert, A. K. & Müller, J. K. 2008. The Coolidge effect, individual recognition and selection for distinctive cuticular signatures in a burying beetle. *Proceedings of the Royal Society B*, **275**, 1831–1838.
- Thornhill, R. 1976. Sexual selection and nuptial feeding behaviour in *Bittacus apicalis*. *American Naturalist*, **110**, 529–548.
- Thornhill, R. & Alcock, J. 1983. *The Evolution of Insect Mating Systems*. Cambridge, Massachusetts: Harvard University Press.
- Tietjen, W. J. & Rovner, J. S. 1982. Chemical communication in lycosids and other spiders. In: *Spider Communication: Mechanisms and Ecological Significance*

- (Ed. by P. N. Witt & J. S. Rovner), pp. 249–279. Princeton, New Jersey: Princeton University Press.
- Tregenza, T. & Wedell, N.** 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution*, **52**, 1726–1730.
- Tregenza, T. & Wedell, N.** 2002. Polyandrous females avoid costs of inbreeding. *Nature*, **415**, 71–73.
- Vahed, K.** 1998. The function of nuptial feeding in insects: a review of empirical studies. *Biological Reviews*, **73**, 43–78.
- Wedell, N., Gage, M. J. G. & Parker, G. A.** 2002. Sperm competition, male prudence and sperm limited females. *Trends in Ecology & Evolution*, **17**, 313–320.
- Wilson, J. R., Kuehn, R. E. & Beach, F. A.** 1963. Modification in the sexual behavior of male rats produced by changing the stimulus female. *Journal of Comparative and Physiological Psychology*, **56**, 636–644.
- Zeh, J. A. & Zeh, D. W.** 1996. The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society B*, **263**, 1711–1717.
- Zeh, J. A. & Zeh, D. W.** 1997. The evolution of polyandry II: post copulatory defences against genetic incompatibility. *Proceedings of the Royal Society B*, **264**, 69–75.
- Zeh, J. A., Newcomer, S. D. & Zeh, D. W.** 1998. Polyandrous females discriminate against previous mates. *Proceedings of the National Academy of Sciences, U.S.A.*, **95**, 13732–13736.