Ethology

RESEARCH PAPERS

The Effects of Experimentally Induced Polyandry on Female Reproduction in a Monandrous Mating System

Göran Arnqvist* & José A. Andrés†

* Animal Ecology, Department of Ecology and Evolution, Evolutionary Biology Centre, University of Uppsala, Uppsala, Sweden
† Department of Ecology and Evolutionary Biology, Corson Hall, Cornell University, Ithaca, NY, USA

Correspondence
Göran Arnqvist, Animal Ecology, Department of Ecology and Evolution, Evolutionary Biology Centre, University of Uppsala, Norbyvägen 18D, SE – 752 36 Uppsala, Sweden.
E-mail: goran.arnqvist@ebc.uu.se

Received: September 8, 2005
Initial acceptance: November 2, 2005
Final acceptance: November 24, 2005
(J. Schneider)

doi: 10.1111/j.1439-0310.2006.01211.x

Abstract

Females of most insect species maximize their fitness by mating more than once. Yet, some taxa are monandrous and there are two distinct scenarios for the maintenance of monandry. While males should always benefit from inducing permanent non-receptivity to further mating in their mate, this is not necessarily true for females. Since females benefit from remating in many species, cases of monandry may reflect successful male manipulation of female remating (i.e. sexual conflict). Alternatively, monandry may favor both mates, if females maximize their fitness by mating only once in their life. These two hypotheses for the maintenance of monandry make contrasting predictions with regards to the effects of remating on female fitness. Here, we present an experimental test of the above hypotheses, using the monandrous housefly (*Musca domestica*) as a model system. Our results showed that accessory seminal fluid substances that males transfer to females during copulation have a dual effect: they trigger female non-receptivity but also seem to have a nutritional effect that could potentially enhance female fitness. These results suggest that monandry is maintained in house flies despite potential benefits that females would gain by mating multiply.

Introduction

For females, benefits of polyandry might arise through different pathways. First, females may gain direct benefits from remating. Such direct benefits include replenishment of depleted or unviable sperm supplies (e.g. Thornhill & Alcock 1983; Arnqvist 1989; Siva-Jothy 2000), the transfer of nuptial gifts and nutrients (e.g. Wedell 1997; Wiklund et al. 2001), access to resources (e.g. Martens & Rehfelt 1989), and protection from male harassment (e.g. Rowe 1992). Secondly, females may benefit from indirect genetic benefits from polyandry, such that their offspring exhibit increased viability and/or reproductive success (see Jennions & Petrie 2000 for a review). However, females also suffer several potential ecologic, physiologic and/or energetic costs from remating (e.g. Arnqvist 1989; Rowe 1994; Chapman et al. 1995; Rice 1996; Crudgington & Siva-Jothy 2000; Arnqvist & Rowe 2005). Despite such potential costs, however, a meta-analysis showed that insect females mated once only generally gain from a second mating (Arnqvist & Nilsson 2000).

In the light of the different benefits described above, monandry is a female strategy that is poorly understood (Arnqvist & Nilsson 2000; Wiklund et al. 2001; Zeh & Zeh 2003). Independently of how monandry originally evolved, it may in theory be maintained by two different routes. First, as males invariably benefit if their mate do not remate with other males while females usually benefit from remating (see above), monandry may reflect a sexual conflict over female mating rate and successful male manipulation of females (Parker 1979; Arnqvist & Rowe 2005). In other words, males might impose

Ethology **112** (2006) 748–756 © 2006 The Authors
Journal compilation © 2006 Blackwell Verlag, Berlin

monandry upon their mates at the expense of female fitness. There is some empirical support for this possibility. For example, females of many monandrous species willingly remate if the stimulus provided by males (i.e. the amount of seminal substances transferred) is experimentally reduced (Riemann et al. 1967; Riemann & Thorson 1969; Klowden 1999; Andrés & Arnqvist 2001; but see Klowden 2001) and, more importantly, females of monandrous insect species have been shown to actually or potentially benefit from polyandry (Baer & Schmid-Hempel 1999, 2001; Jones 2001). Secondly, monandry may not be associated with sexual conflict if females maximize their fitness by mating just once. Selection in both sexes would then favor monandry. This would be the case if, for example, the ecologic costs of mating are unusually high or in some cases where males exhibit paternal care of offspring (Arnqvist & Kirkpatrick 2005).

These two hypotheses for the maintenance of monandry make contrasting predictions with regards to the potential effects of remating on female fitness in monandrous insects. If monandry indeed reflects a sexual conflict over female mating rate, females should benefit from remating. On the other hand, if monandry is favored by selection in both sexes there should be a net negative effect of remating on female fitness. This negative effect might arise from ecologic costs, such as time or energy waste, or from direct negative effects of the ejaculate on female fitness (Chapman et al. 1995, 1998; Holland & Rice 1999; Civetta & Clark 2000; Johnstone & Keller 2000). An obvious dilemma in this context is the fact that the biology of particular model systems may disallow assessments of these predictions, simply because it may be difficult or even impossible to induce female remating in monandrous taxa.

In this paper, we strive to test these contrasting predictions in order to provide insights into the mechanisms involved in the evolutionary maintenance of monandry in the naturally monandrous house fly, *Musca domestica*, in which monandry can be artificially manipulated while ensuring full transfer of sperm (Murvosh et al. 1964; Andrés & Arnqvist 2001). In particular, we test if experimentally induced polyandry enhances female fitness via direct benefits (i.e. sperm supplies and seminal products). Furthermore, our experimental design allowed us to disentangle the independent effects of sperm and accessory seminal substances on female fitness. Our results suggest that monandry in this species is maintained by sexual conflict in which males are able to enforce monandry upon their mates despite of the beneficial effects that multiple mating would have for females.

Methods

Study Organism

Houseflies (*M. domestica*; Diptera; Muscidae) are monandrous, such that the great majority of females mate only once (Riemann et al. 1967; Riemann & Thorson 1969; Leopold et al. 1971a). Males show pre-copulatory courtship (Meffert & Bryant 1991; Meffert & Regan 2002; Meffert & Hagenbuch 2005) but there is no evidence for copulatory courtship. Loss of receptivity to further matings, as well as stimulation of oviposition, is mainly caused by accessory seminal substances transferred to females with the ejaculate (Riemann et al. 1967; Adams & Nelson 1968; Riemann & Thorson 1969; Leopold et al. 1971a,b). These proteins and peptides (Terranova et al. 1972) have a dose-dependent effect on female physiology and reproductive behavior (Riemann & Thorson 1969). Reduced amounts of accessory seminal substances induce oviposition without loss of receptivity to further mating (Riemann & Thorson 1969; Andrés & Arnqvist 2001) and the ability of male houseflies to induce oviposition and loss of receptivity in females varies across populations (Andrés & Arnqvist 2001; Hicks et al. 2004). Thus, geographic populations differ in their reproductive behavior, presumably due to divergence of the seminal signal–receptor system. As a result, there might be significant differences in the way the different populations respond to seminal accessory substances (Andrés & Arnqvist 2001; Hicks et al. 2004).

Stocks and Rearing Methods

Two different wild-type populations were selected because of their contrasting reproductive behavior. The first population (S) derives from a natural farm population in Umeå, northern Sweden, and previous experiments have shown that the oviposition rate of females of this population depends on the population identity of their mate (Andrés & Arnqvist 2001). The second population (M), is a laboratory wild-type stock derived from several wild populations in Minnesota, USA. In this population, the oviposition rate of the females is independent of the population identity of their mate (Andrés & Arnqvist 2001).

Flies were reared at 25–27°C, at a relative humidity of 60–70% and under a 12L:12D light cycle. Adults were provided with dry food and water ad libitum. Larvae were reared in an artificial medium and each new generation was founded by approx. 400 randomly chosen pupae (for a full description of the rearing protocol; see Andrés & Arnqvist 2001).

Experimental Design

In houseflies, as in many other insects (e.g. Eberhard 1996), transfer of sperm to females during copulation precedes transfer of accessory ejaculate components. Full sperm transfer is achieved after approx. 10 min of copulation (Murvosh et al. 1964) after which accessory seminal substances are transferred, and full transfer of such substances requires at least 40 min of copulation (Leopold et al. 1971a,b). Thus, the amount of accessory substances transferred to the female can be manipulated by interrupting matings (Riemann et al. 1967; Riemann & Thorson 1969; Andrés & Arnqvist 2001). In this paper, we exploited this fact to examine the distinct effects of different components of the ejaculate (i.e. sperm vs. accessory gland substances) on three different components of female fitness: lifespan, fecundity, and fertility.

For each strain, a random sample of approx. 400 pupae was introduced into a virgin chamber for hatching. Shortly after emergence, the sexes were separated under light and brief CO_2 anesthesia. Males were isolated individually in experimental chambers consisting of a net cylinder (7.5-cm high, 9-cm diameter) provided with water and dry food. Females where kept together in small insectaries. Six days after emergence, each female was paired with a single male in an experimental chamber. Females from the M population were randomly assigned to one of four different treatments (see Table 1) (n = 31–36 per treatment). In treatments 1 (control) and 2 (induced polyandry) females were mated for 20 min after which copulation was terminated by aspirating the mating pair out of the chamber and gently separating them by hand. In treatments 3 (enforced monandry) and 4 (optional monandry), mating was not interrupted. If no mating occurred during the first 3–4 h after introducing the female, the male was removed and the female was offered a second male. No female was exposed to more than two putative first mates. Following the first matings, males were removed, oviposition substrates were introduced and females were kept isolated in the chambers for the next 48 h. After this first mating, oviposition substrates were replaced every 24 h (see Andrés & Arnqvist 2001 for details).

Three days after the first mating, all females underwent a second stage of each treatment. Females from treatments 1 and 3 were placed with males with inoperative genitalia, while females from treatments 2 and 4 were paired with intact males. The genitalia of the former group of males was made inoperative by covering the genitalia with a thin coating of paraffin wax the day prior to exposure to the experimental females. Behavioral assays showed that this treatment did not affect male courtship behavior: there were no significant differences in the number of mating attempts per minute between manipulated and intact males ($F_{1,16} = 0.004$, $p = 0.96$). However, the treatment completely impeded copulation. All males were kept with females in the mating chambers for 6 h and then removed. A male, intact (induced polyandry and optional monandry treatments) or with inoperative genitalia (control and enforced monandry treatments), was thereafter introduced to each female for 6 h every third day until her death and all matings were recorded. Therefore, all females experience the same exposure to courting males throughout the experiment. For all females, new oviposition substrates were introduced every 24 h, starting immediately after the first copulation. Food was replaced every second week and deaths were scored daily.

Table 1: Experimental design

Treatment	First mating interrupted	Potential mating rate	Realized mating rate	Realized sperm dose	Realized AS dose
1. Control	Yes	1	1	Standard	Much reduced
2. Induced polyandry	Yes	>1	2	Double	Standard
3. Enforced monandry	No	1	1	Standard	Standard
4. Optional monandry	No	>1	1	Standard	Standard

For each treatment, we present the potential and the realized number of matings that females engaged in during the course of the experiment. Realized sperm and accessory seminal substances (AS) doses are given relative to a standard normal mating.

Ethology **112** (2006) 748–756 © 2006 The Authors
Journal compilation © 2006 Blackwell Verlag, Berlin

In terms of receipt of ejaculate substances, thus, females in treatment 1 (control) received one full dose of sperm but a much reduced dose of accessory seminal substances. Females in treatment 3 (enforced monandry) experienced a single full copulation only. Females in treatment 2 (induced polyandry) remated once (see below) as a result of their first copulation being interrupted, and thus received two doses of sperm but only a single full dose of accessory seminal substances. Females in treatment 4 (optional monandry) mated once only (see below) and thus received the same amount of ejaculate substances as females in treatment 3 (see Table 1 for a summary). Ideally, a treatment in which females received two full doses of both sperm and accessory seminal substances would have completed the design. However, this is not possible in truly monandrous species like *M. domestica* in which a single dose of seminal substances induces lifelong female refractiveness. However, the potential direct benefits of remating can still be inferred by a set of planned comparisons of the treatments described above.

Oviposition substrates containing eggs were incubated for 36 h at 25°C and 70% relative humidity. At that time, all eggs laid were counted and scored as either hatched or unhatched under a dissecting microscope. Female body size was measured as their wing length, using a digitizing tablet placed under a dissecting microscope provided with a side-mounted camera lucida. This experiment allowed us to analyze the effects of mating treatment on three different female fitness components: lifespan, lifetime fecundity, and fertility.

Under our experimental conditions, females lived for up to 65 d. This situation is unlikely to closely reflect the natural conditions under which monandry is maintained in this species (Ragland & Sohal 1973). Further, populations may differ in the effects of mating. The experiments described above were therefore replicated with both the M and the S population (n = 15 females per treatment and population). In this second experiment, however, the experiments were terminated after only 12 d of oviposition, and we measured two female fitness components: fecundity and fertility.

Statistical Analyses

Our experimental design allowed us to test not only for the effect of remating on different female fitness components, but also to disentangle the independent effects of sperm and accessory seminal substances. This was achieved by performing the following planned comparisons. First, we tested for the effect of sperm dose by contrasting enforced monandrous females with induced polyandrous ones. As females of both treatments received the same amount of seminal substances (see Table 1), this contrast specifically test for direct effects of sperm dose on female fitness. Secondly, we tested for the effect of accessory seminal substances by contrasting control females that received an insignificant amount of these substances, with all females that received a full dose (see Table 1).

Females accidentally killed or lost during the experiments (n = 9) and those which laid no eggs (n = 3) were excluded from analyses. Statistical analyses involving lifespan or the number of eggs laid were analyzed using conventional statistical models. The assumptions made by these models (i.e. normality of residuals and homogeneity of variances) were assessed, and data were transformed if necessary to meet these assumptions. Data on fertility rate were analyzed in generalized linear models of the number of hatched eggs, using binomial errors and a logit link function with the total number of eggs laid per female as the binomial denominator (McCullagh & Nelder 1989; Crawley 1993). To compensate for overdispersion, we used the method of Williams (1982) prior to statistical inference (Crawley 1993). These analyses were carried out using GLIM 3.77® (Royal Statistical Society, London, UK).

Results

Mating Rates and the Effect of Female Size

In two of our experimental treatments, induced polyandry and optional monandry, females were given the opportunity to remate after their first mating. All but one in the former group, but none in the latter actually remated. Body size was not significantly correlated with lifespan ($r = -0.043$, $p = 0.701$, $n = 86$), fecundity ($r = 0.126$, $p = 0.200$, $n = 86$) or fertility ($r = 0.144$, $p = 0.263$, $n = 86$) among M females, or with fecundity ($r = 0.223$, $p = 0.301$, $n = 28$) or fertility ($r = 0.124$, $p = 0.512$, $n = 28$) among S females (data for treatments 3 and 4). There was no difference in the $\bar{x}$ body size of females assigned to the different treatments (M females: ANOVA, $F_{3,111} = 0.525$, $p = 0.660$; S females: ANOVA, $F_{3,57} = 0.998$, $p = 0.400$).

Effects on Female Lifespan

Overall, our experimental treatments significantly affected lifespan among M females (Fig. 1; $F_{3,111} =$

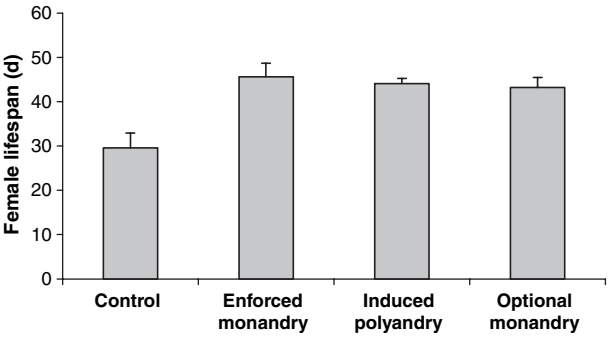

Fig. 1: Mean (± SE) female lifespan across the experimental mating treatments

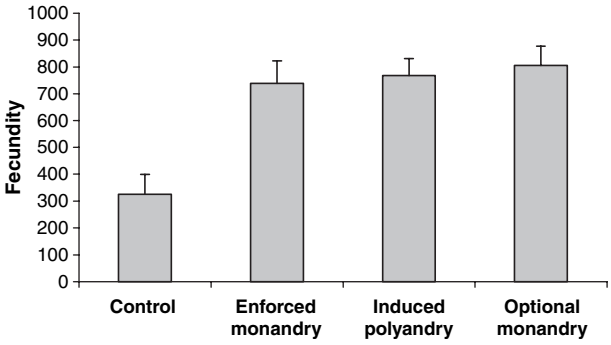

Fig. 2: Effects of the experimental mating treatment on female lifetime fecundity, measured as $\bar{x}$ (± SE) number of eggs laid

4.062, p = 0.009). To assess the potential role of the two main components of the ejaculate (i.e. sperm and accessory seminal substances) on female lifespan, we first compared the $\bar{x}$ lifespan of enforced monandrous and induced polyandrous females. As is evident from Fig. 1, the lifespan of females from these two treatments were statistically inseparable ($F_{1,64} = 2.056$, p = 0.156). A comparison between the $\bar{x}$ lifespan of control females with that of the females that received a full dose of seminal substances showed that the latter females lived for longer than did control females ($F_{1,113} = 6.494$, p = 0.012). These results strongly suggest that accessory seminal substances increase female lifespan.

Effects on Female Fecundity Rate

Fecundity showed no apparent decline over time among M females, and fecundity rate was thus estimated as the $\bar{x}$ number of eggs laid per day from a female's first mating until her death. Overall, our experimental treatments had a significant effect on daily fecundity ($F_{3,111} = 2.855$, p = 0.040). Sperm dose had no detectable effect on female fecundity rate, as there was no significant difference in $\bar{x}$ egg production rate between enforced monandrous (16.47 ± 4.5) and induced polyandrous females (17.41 ± 1.1; $F_{1,64} = 0.151$, p = 0.902). However, control females laid on average fewer eggs per day (10.96 ± 2.8) than females that received a full dose of accessory seminal substances (17.42 ± 0.8; $F_{1,113} = 7.536$, p = 0.007).

Effects on Female Lifetime Fecundity

The $\bar{x}$ number of eggs laid during the entire lifetime of females differed across treatments (Fig. 2; $F_{3,113} = 4.14$, p = 0.008). Control females laid fewer eggs than did females that received a full dose of accessory seminal substances ($F_{1,113} = 10.32$, p = 0.002). In contrast, there were no differences in the total number of eggs laid between females that received the same quantity of accessory substances but a different amount of sperm ($F_{1,64} = 0.368$, p = 0.546).

Effects on Fertility

To test if remating affects female lifetime fertility, we analyzed the proportion of fertile eggs in a generalized linear model. Mean fertility ranged from 75% to 84% across treatments, but there was no significant differences between treatments ($\chi^2_3 = 5.29$, p = 0.133), suggesting that a single mating is enough to fertilize all the eggs a female lays in her lifetime. We note that there was no apparent changes in $\bar{x}$ fertility as female aged.

Effects on Fecundity and Fertility Early in Life

Assessment of treatments effects early in life may aid in detecting more subtle effects of remating on female fecundity and fertility, especially considering the fact that females under our experimental conditions showed elevated lifespan compared with natural conditions (Ragland & Sohal 1973). Therefore, we analyzed effects on fecundity and fertility in our second expt, restricted to the total number and hatching rate of eggs laid during the first 12 d after a female's first mating. Our treatment had a significant effect on fecundity early in life, although populations differed in the number of eggs laid (see Table 2). We note that females from the two populations responded in a very similar way to our experimental treatment, as revealed by the non-significant treatment × population interaction (cf. Hicks et al.

Ethology **112** (2006) 748–756 © 2006 The Authors
Journal compilation © 2006 Blackwell Verlag, Berlin

Table 2: ANOVA of female fecundity during the first 2 wk of oviposition

	F	df	p-value
Treatment	7.648	2,106	<0.001
Population	4.999	1,106	0.027
Treatment × Population	0.110	2,106	0.895

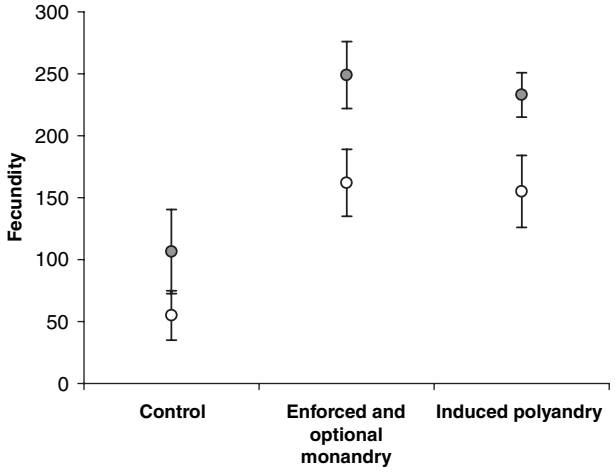

Fig. 3: The $\bar{x}$ (±SE) number of eggs laid during the first 2 wk of oviposition across treatments by females from the M (filled circles) and the S (open circles) population (treatments 3 and 4 pooled)

2004). The treatment effect stems from the fact that control females laid about half as many eggs as females receiving a full dose accessory seminal substances (Fig. 3; $F_{1,54} = 19.519$, $p < 0.001$). In contrast, sperm dose had no significant effect on fecundity as enforced monandrous and induced polyandrous females laid a similar number of eggs ($F_{1,86} = 0.934$, $p = 0.436$).

To analyze the effects of strain and mating treatment on the early fertility of the females we employed a generalized linear model (see Methods) including both factors. However, neither population nor treatment had a significant effect on the rate of fertile eggs laid during the first 2 wk of oviposition (test of full model; $\chi^2_3 = 1.305$, $p = 0.728$).

Discussion

Our results showed that the amount of sperm that female house flies received had no detectable effect on their reproductive performance, as females which received two doses of sperm did not differ from those that received one. Apparently, the sperm transferred during a single mating is sufficient to maintain full fertility during a female's entire life in this monandrous species. In contrast, a reduction in the amount of accessory seminal substances transferred had dramatic effects: females that received only a small amount of such substances suffered approx. 50% fitness loss. Thus, our results strongly suggest that monandrous females would indeed benefit from multiple matings, through the receipt of larger amounts of accessory seminal substances. This conclusion rests on the assumption that female benefits from accessory seminal substances are positively dose dependent, but we note that there is strong empirical support for this assumption in insects (see Arnqvist & Nilsson 2000).

In theory, positive effects on female reproduction of seminal substances may be nutritional or hormonal (Karlsson 1998; Stjernholm & Karlsson 2000). Although the distinction between these two effects is non-trivial (Eberhard 1996; Vahed 1998), the fact that female house flies that received a reduced amount of seminal substances exhibited a reduced daily fecundity as well as a shorter lifespan suggests that some of the products of the chemically complex ejaculate of this species (Terranova et al. 1972) indeed have a nutritional effect in females. Hormonal effects would instead be characterized by a depressed daily fecundity coupled with a prolonged lifespan (Karlsson 1998; Stjernholm & Karlsson 2000). This general conclusions is also supported by Hicks et al. (2004) who showed that mated females tended to live longer than virgin females, although they did not control for female egg production.

Overall, our results support the hypothesis that house fly females could potentially increase their fitness by mating multiply. In contrast to other monandrous insects, this increase in fitness does not seem to be due to indirect genetic benefits associate with sperm variability (Baer & Schmid-Hempel 1999, 2001) or to the replenishment of depleted or old sperm supplies (Siva-Jothy 2000; Jones 2001) but to a direct effect of the seminal substances of the ejaculate (Kalb et al. 1993; Chapman et al. 1995; Herndon & Wolfner 1995; Dickinson & Klowden 1997; Wedell 1997; Klowden 1999; Wiklund et al. 2001). If true, then the house fly ejaculate is truly a double-edged sword: while some accessory seminal substances have a net positive effect on female fitness, other seminal components are obviously responsible for the actual induction of female monandry (Riemann et al. 1967; Riemann & Thorson 1969; Leopold et al. 1971a,b). Monandry would then reflect sexual conflict, as males effectively render females incapable of enjoying the direct benefits of

polyandry (see also Baer & Schmid-Hempel 1999; Arnqvist & Nilsson 2000; Jones 2001; Wiklund et al. 2001).

The evolutionary origin and maintenance of true monandry in insects is somewhat of a paradox still unresolved (e.g. Arnqvist & Nilsson 2000; Wiklund et al. 2001; Andersson et al. 2004). We suggest that studies of the effects of seminal substances on female reproductive behavior and physiology could shed some light on the evolution of monandry. One particular scenario assumes that monandry evolved from a pre-existing polyandrous mating system. Under this scenario, seminal substances evolved via post-mating sexual selection (see Eberhard 1996) as male adaptations to reduce sperm competition by lowering receptivity to further mating in their mates (Parker 1984; Eberhard 1996; Simmons & Siva-Jothy 1998; Simmons 2001). Selection in males could then have increased the efficiency of this induction of refractiveness in females, until female fitness was compromised as a direct result of depressed mating rates. At this point, females could obviously counter adapt (i.e. evolve resistance) by metabolizing the accessory substances and using them for somatic maintenance and/or production of eggs (Arnqvist & Nilsson 2000; Wiklund et al. 2001; Arnqvist & Rowe 2005). This scenario could explain why females of monandrous species, like the house fly, benefit from accessory seminal substances. However, it is not clear why females, once having evolved the ability to use accessory seminal substances to their own benefit, have not also evolved the ability to better neutralize the substances that induce female non-receptivity to remating (Andersson et al. 2004). Further, if monandry is maintained entirely by selection for reduced sperm competition among males, it is very difficult to see how monandry in its strictest sense could ever be evolutionarily stable. The reason is simply that the level of sperm competition is relaxed, and eventually ceases to exist, as monandrous females increase in frequency in a population. Thus, when male adaptations that cause monandry in females become efficient they will simultaneously eliminate the selection that caused their evolution in the first place. Given that the production of proteinaceous accessory seminal substances is costly (see Vahed 1998), a certain frequency of polyandry among females thus seems necessary to maintain male investment in substances that induce female non-receptivity. This may contribute the rarity of true monandry among non-social insects (Thornhill & Alcock 1983; Arnqvist & Nilsson 2000).

Acknowledgements

The research reported here was supported by grants from The Swedish Research Council to GA and an EU Marie Curie Fellowship to JAA.

Literature Cited

Adams, T. S. & Nelson, D. R. 1968: Bioassay of crude extracts for the factor that prevents second matings in female *Musca domestica*. Ann. Entomol. Soc. Am. **61**, 112—116.

Andersson, J., Borg-Karlson, A. K. & Wiklund, C. 2004. Sexual conflict and anti-aphrodisiac titre in a polyandrous butterfly: male ejaculate tailoring and absence of female control. Proc. R. Soc. Lond. B **271**, 1765—1770.

Andrés, J. A. & Arnqvist, G. 2001: Genetic divergence of the seminal signal-receptor system in houseflies: the footprints of sexually antagonistic coevolution? Proc. R. Soc. Lond. B **268**, 399—405.

Arnqvist, G. 1989: Multiple mating in a water strider: mutual benefits or intersexual conflict? Anim. Behav. **38**, 749—756.

Arnqvist, G. & Kirkpatrick, M. 2005: The evolution of infidelity in socially monogamous passerines – the strength of direct and indirect selection on extrapair copulation behavior in females. Am. Nat. **165**, S26—S37.

Arnqvist, G. & Nilsson, T. 2000: The evolution of polyandry: multiple mating and female fitness in insects. Anim. Behav. **60**, 145—264.

Arnqvist, G. & Rowe, L. 2005: Sexual Conflict. Princeton Univ. Press, Princeton, NJ.

Baer, B. & Schmid-Hempel, P. 1999: Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. Nature **397**, 151—154.

Baer, B. & Schmid-Hempel, P. 2001: Unexpected consequences of polyandry for parasitism and fitness in the bumblebee, *Bombus terrestris*. Evolution **55**, 1639—1643.

Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. & Partridge, L. 1995: Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature **373**, 211—217.

Chapman, T., Miyatake, T., Smith, H. K. & Partridge, L. 1998: Interactions of mating, egg production and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata*. Proc. R. Soc. Lond. B **265**, 1879—1894.

Civetta, A. & Clark, A. G. 2000: Correlated effects of sperm competition and postmating female mortality. Proc. Natl Acad. Sci. USA **97**, 13162—13165.

Crawley, M. J. 1993: GLIM for Ecologists. Blackwell, Oxford, UK.

Ethology **112** (2006) 748–756 © 2006 The Authors
Journal compilation © 2006 Blackwell Verlag, Berlin

Crudgington, H. & Siva-Jothy, M. T. 2000: Genital damage, kicking and early death. Nature **407**, 855—856.

Dickinson, J. M. & Klowden, M. J. 1997: Reduced transfer of male accessory gland proteins and monandry in *Aedes aegypti* mosquitoes. J. Vector Ecol. **22**, 95—98.

Eberhard, W. G. 1996: Female Control: Sexual Selection by Cryptic Female Choice. Princeton Univ. Press, Princeton, NJ.

Herndon, L. A. & Wolfner, M. F. 1995: A *Drosophila* seminal fluid protein, Acp26AA, stimulates egg-laying in females for 1 day after mating. Proc. Natl Acad. Sci. USA **92**, 10114—10118.

Hicks, S. K., Hagenbuch, K. L. & Meffert, L. M. 2004: Variable costs of mating, longevity, and starvation resistance in the housefly *Musca domestica* (Diptera: Muscidae). Environ. Entomol. **33**, 779—786.

Holland, B. & Rice, W. R. 1999: Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes reproductive load. Proc. Natl Acad. Sci. USA **96**, 5083—5088.

Jennions, M. D. & Petrie, M. 2000: Why do females mate multiply? A review of the genetic benefits. Biol. Rev. **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. Am. Nat. **156**, 368—377.

Jones, T. M. 2001: A potential cost of monandry in the lekking sandfly *Lutzomyia longipalpis*. J. Insect Behav. **14**, 385—399.

Kalb, J. M., Dibenedetto, A. J. & Wolfner, M. F. 1993: Probing the function of *Drosophila melanogaster* accessory glands by direct cell ablation. Proc. Natl Acad. Sci. USA **90**, 8093—8097.

Karlsson, B. 1998: Nuptial gifts, resource budgets, and reproductive output in a polyandrous butterfly. Ecology **79**, 2931—2940.

Klowden, M. J. 1999: The check is in the male: male mosquitoes affect female physiology and behavior. J. Am. Mosq. Control **15**, 213—220.

Klowden, M. J. 2001: Sexual receptivity in *Anopheles gambiae* mosquitoes: absence of control by male accessory gland substances. J. Insect Physiol. **47**, 661—666.

Leopold, R. A., Terranova, A. C. & Swilley, E. M. 1971a: Mating refusal in *Musca domestica*: effects of repeated mating and decerebration upon frequency and duration of copulation. J. Exp. Zool. **176**, 353—360.

Leopold, R. A., Terranova, A. C., Thorson, B. J. & Degrugillier, M. E. 1971b: The biosynthesis of the male housefly secretion and its fate in the mated female. J. Insect Physiol. **17**, 987—1003.

Martens, A. & Rehfelt, G. 1989: Female aggregation in *Platycypha caligata* (Odonata: Chlorocipidae): a tactic to evade male interference during oviposition. Anim. Behav. **38**, 369—374.

McCullagh, P. & Nelder, P. A. 1989: Generalized Linear Models. Chapman and Hall, London.

Meffert, L. M. & Bryant, E. H. 1991: Mating propensity and courtship behavior in serially bottlenecked lines of the housefly. Evolution **45**, 293—306.

Meffert, L. M. & Hagenbuch, K. L. 2005: The genetic architecture of house fly mating behavior. Curr. Top. Dev. Biol. **66**, 189—213.

Meffert, L. M. & Regan, J. L. 2002: A test of speciation via sexual selection on female preferences. Anim. Behav. **64**, 955—965.

Murvosh, C. M., Fye, R. L. & Labrecque, G. C. 1964: Studies on the mating behavior of the house fly, *Musca domestica* L. Ohio J. Sci. **64**, 264—271.

Parker, G. A. 1979: Sexual selection and sexual conflict. In: Sexual Selection and Reproductive Competition in Insects (Blum, M. S. & Blum, N. A., eds). Academic Press, New York, pp. 123—166.

Parker, G. A. 1984: Sperm competition and the evolution of animal mating strategies. In: Sperm Competition and the Evolution of Animal Mating Systems (Smith, R. L., ed.). Academic Press, Orlando, FL, pp. 1—60.

Ragland, S. S. & Sohal, R. S. 1973: Mating behavior, physical activity, and aging in the housefly, *Musca domestica*. Exp. Gerontol. **8**, 135—145.

Rice, W. R. 1996: Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature **381**, 232—234.

Riemann, J. G. & Thorson, B. J. 1969: Effect of accessory material on oviposition and mating by female houseflies. Ann. Entomol. Soc. Am. **62**, 828—834.

Riemann, J. G., Moen, J. M. & Thorson, B. J. 1967: Female monogamy and its control in houseflies. Insect Physiol. **13**, 407—418.

Rowe, L. 1992: Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. Anim. Behav. **44**, 189—202.

Rowe, L. 1994: The costs of mating and mate choice in water striders. Anim. Behav. **48**, 1049—1056.

Simmons, L. W. 2001: Sperm Competition and its Evolutionary Consequences in Insects. Princeton Univ. Press, Princeton, NJ.

Simmons, L. W. & Siva-Jothy, M. T. 1998: Sperm competition in insects: mechanisms and the potential for selection. In: Sperm Competition and Sexual Selection (Birkhead, T. R. & Møller, A. P., eds). Academic Press, San Diego, CA, pp. 341—434.

Siva-Jothy, M. T. 2000: The young sperm gambit. Ecol. Lett. **3**, 172—174.

Stjernholm, F. & Karlsson, B. 2000: Nuptial gifts and the use of body resources for reproduction in the green-veined white butterfly *Pieris napi*. Proc. R. Soc. Lond. B **267**, 807—811.

Terranova, A. C., Leopold, R. A., Degrugillier, M. E. & Johnson, J. R. 1972. Electrophoresis of the male accessory secretion and its fate in the mated female. J. Insect Physiol. **18**, 1573—1591.

Thornhill, R. & Alcock, J. 1983: The Evolution of Insect Mating Systems. Harvard Univ. Press, Cambridge, MA.

Vahed, K. 1998: The function of nuptial feeding in insects: review of empirical studies. Biol. Rev. **73**, 43—78.

Wedell, N. 1997: Ejaculate size in bushcrickets: the importance of being large. J. Evol. Biol. **10**, 315—325.

Wiklund, C., Karlsson, B. & Leimar, O. 2001: Sexual conflict and cooperation in butterfly reproduction: a comparative study of polyandry and female fitness. Proc. R. Soc. Lond. **268**, 1661—1667.

Williams, D. A. 1982: Extra-binomial variation in logistic linear models. Appl. Statist. **31**, 144—148.

Zeh, J. A. & Zeh, D. W. 2003: Toward a new sexual selection paradigm: polyandry, conflict and incompatibility. Ethology **109**, 929—950.

Ethology **112** (2006) 748–756 © 2006 The Authors
Journal compilation © 2006 Blackwell Verlag, Berlin