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THE EVOLUTION OF REPEATED MATING IN THE BURYING BEETLE, *NICROPHORUS VESPILLOIDES*

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Animals of many species accept or solicit recurring copulations with the same partner; i.e., show repeated mating. An evolutionary explanation for this excess requires that the advantages of repeated mating outweigh the costs, and that behavioral components of repeated mating are genetically influenced. There can be benefits of repeated mating for males when there is competition for fertilizations or where the opportunities for inseminating additional mates are rare or unpredictable. The benefits to females are less obvious and, depending on underlying genetic architecture, repeated mating may have evolved as a correlated response to selection on males. We investigated the evolution of repeated mating with the same partner in the burying beetle *Nicrophorus vespilloides* by estimating the direct and indirect fitness benefits for females and the genetics of behavior underlying repeated mating. The number of times a female mated had minimal direct and no indirect fitness benefits for females. The behavioral components of repeated mating (mating frequency and mating speed) were moderately negatively genetically correlated in males and uncorrelated in females. However, mating frequency and mating speed were strongly positively genetically correlated between males and females. Our data suggest that repeated mating by female *N. vespilloides* may have evolved as a correlated response to selection on male behavior rather than in response to benefits of repeated mating for females.

KEY WORDS: **Behavior genetics, correlated selection, direct benefits, indirect benefits, mating behavior, quantitative genetics, repeated mating, sexual selection.**

Repeated mating occurs in many animal species (Hunter et al. 1993); that is they accept or solicit sequential copulations with the same partner (we adopt the terminology of Hunter et al. (1993) and Drapeau et al. (2001), where repeated mating refers specifically to recurring mating between the same male and female pair. The related phenomenon of multiple mating refers to mating between an individual and several different partners.) There are potential costs to repeated mating such as increased risk of predation (Rowe 1994) infection (Hurst et al. 1995; Thrall et al. 2000), exposure to inter- and intrasexual competition (Andersson 1994) and time and energy costs (Thornhill and Alcock 1993). In males, benefits of and therefore selection for repeated mating occur when the opportunities for attracting an additional mate are rare or unpredictable and when the potential for sperm competition exists (Parker 1970; Thornhill and Alcock 1983). Under such conditions, males may use multiple copulations with their partner as a means to increase their assurance of paternity (Alcock 1994). From the female perspective, selection for repeated mating is more difficult to explain (Hunter et al. 1993). Female egg production is limited compared with male sperm production, and each additional mating is unlikely to provide increasing fitness benefits to females if the male passes sufficient sperm in a single copulation (Parker 1970). Thus, the default expectation is that there should be selection against repeated mating in females.

Although the conditions for fitness benefits of repeated mating for females are more restrictive they do exist. In some species, male-derived nutrients or chemicals that are transferred during mating may elevate female fitness (Arnqvist and Nilsson 2000). Specifically, repeated mating may increase the fecundity, fertility, and survival of females (Kamimura 2003; Dunn et al. 2005; Klemme et al. 2007), and may also increase the offspring's survival (Eady et al. 2000; Tregenza et al. 2003; Jennions et al. 2007). Repeated mating can also protect against infertility by ensuring the use of young sperm (Siva-Jothy 2000; Reinhardt and Siva-Jothy 2005), avoiding unsuccessful sperm transfer (Garcia-Gonzalez 2004), or by compensating for mating with sperm-depleted males (Wedell et al. 2002).

Repeated mating may evolve in the absence of direct benefits to females if females copulate repeatedly to comply with male behavior. This may occur in species in which there is no cost of repeated mating (Hunter et al. 1993) or the costs of engaging in repeated mating are lower than the benefits of resisting male sexual advances (Clutton-Brock and Parker 1995). A related but more controversial idea is that mating propensity in females has evolved due to a genetic correlation between the sexes (Halliday and Arnold 1987; Arnold and Halliday 1988, 1992). Halliday and Arnold (1987) suggest that selection on male mating propensity can lead to a correlated response in females, as long as selection for increased mating propensity in males is stronger than selection against it in females.

Discriminating among the various hypotheses for the evolution of repeated mating therefore requires measurement of the effects of variation in repeated mating on direct and indirect components of female fitness combined with estimation of the genetic basis of behavior underlying repeated mating. In this study we use the burying beetle *Nicrophorus vespilloides* to investigate both selection on and inheritance of repeated mating, as such integrated studies involving repeated mating are lacking. Repeated mating is typical in burying beetles. A selective advantage of repeated mating for males has already been demonstrated as repeated mating acts in mate guarding and as a defense against sperm competition (Eggert and Müller 1989a; Müller and Eggert 1989; Eggert 1992; Müller et al. 2007). In this study we begin to examine the fitness consequences of repeated mating for females by examining potential benefits, and examine the quantitative genetics of behaviors involved in repeated mating. First, we examine the effects of the number of times a female mates with the same partner on female fecundity and fertility (Experiment 1). Second, we test if male fertility is inversely related to the number of times the male has mated, therefore selecting for repeated mating by females (Experiment 2). Third, we consider the effects of varying the number of copulations of each pair on the development, performance, and survival of the females' offspring (Experiment 3). Fourth, we examine variation in levels of repeated mating (i.e., the number of times a female mates with the same male) under conditions in which females would benefit from staying with a male compared to conditions in which females would not benefit from staying with a male (Experiment 4). Finally, we examine the genetic architecture of behavioral components of repeated mating in males and females (Experiment 5) using a half-sibling breeding design. Our approach allows us to integrate our knowledge of components of selection on female repeated mating and the genetics of behavior underlying repeated mating to thereby improve our understanding of the evolution of repeated mating in this species. We are specifically interested in understanding why repeated mating may have evolved, rather than the limits to repeated mating, and therefore focus on potential benefits to females. For the aspects of female fitness that we measured, we report limited direct benefits to females that engage in repeated mating and no indirect benefits at all for their offspring. A significant positive genetic correlation between male and female behavior underlying repeated mating coupled with the previously demonstrated benefits to repeated mating in males suggests that selection for repeated mating in females may have evolved as a correlated response.

THE MATING SYSTEM OF BURYING BEETLES

Male *N. vespilloides* exhibit alternative mate finding tactics in which there are clear benefits to repeated mating (Müller and Eggert 1989; Eggert 1992). Some males search for small vertebrate carcasses, which are essential for reproduction, and fight to obtain or protect a carcass from other conspecifics. If no female is present at a carcass, the winning male occupies a central position on the carcass and emits pheromone to attract a female (Eggert and Müller 1989a,b; Eggert 1992). When a female arrives at a carcass, the male engages in contact and noncontact mate guarding, and in repeated mating with the female during carcass burial and oviposition (Eggert 1992). Females rarely leave the carcass unless displaced by other females. In addition, some males release pheromone to attract a female in the absence of a carcass (Eggert and Müller 1989a,b) and mate with any female that is attracted.

The reproductive benefits of emitting pheromone without a carcass depend on the number of females that a given male attracts and the level of female promiscuity (Eggert 1992; House et al. 2007). In contrast, repeated mating by males that defend a carcass helps assure a high level of paternity (Müller and Eggert 1989; House et al. 2007), although the sperm of rival males is displaced very slowly (Müller and Eggert 1989) and most broods are of mixed paternity (Müller et al. 2007). Slow displacement may occur if males transfer small sperm volumes or no sperm due to sperm depletion (Müller and Eggert 1989; Garcia-Gonzalez 2004). From the female's perspective, copulating with males that emit pheromone without a carcass ensures fertility under situations in which females discover and use resources for breeding alone (Eggert 1992). Nothing is known about the potential benefits for females that engage in repeated mating with guarding males. Sperm are viable for roughly 3 weeks (Eggert 1992) however, there is currently no information as to whether there are dose-dependent effects of repeated mating on components of female or offspring fitness, or whether behavioral components of repeated mating are genetically correlated in males and females.

Methods

We collected over 700 *N. vespilloides* from the wild (Kennel Vale, Cornwall, England) in August 2006 and maintained an outbred laboratory stock population derived from these individuals. In the laboratory, each female was placed in an individual breeding chamber; that is, a transparent plastic container (17 × 12 × 6 cm) filled with 2 cm of moist soil and a 20–25 g mouse carcass (Livefoods Direct Ltd, Sheffield, UK). Females that successfully reared a brood were removed from the breeding chamber and frozen when the larvae dispersed from the carcass, thereby contributing no more than one brood to the population. The dispersed larvae were removed from the breeding chamber and housed in individual rearing containers; clear plastic containers (8 × 8 × 3.5 cm) filled with 2 cm of soil. After eclosion, each virgin offspring was fed two decapitated mealworms (*Tenebrio*) twice a week. Some of these virgin adults were used as parents in the genetic breeding design and the remaining adults were randomly mated at sexual maturity to form an F_2 population with no inbreeding. Subsequent generations of stock and experimental animals were reared and maintained under identical conditions. All experiments and rearing of offspring were undertaken in a constant temperature room at 20 ± 1°C with a 16L:8D light regime.

GENERAL EXPERIMENTAL PROCEDURES

All trials were conducted in individual transparent, plastic mating chambers (11 × 11 × 3 cm). The female was always introduced into the mating chamber before the male. During premating interactions, males and females exhibited a variety of behaviors that range from passive to aggressive. Males tend to initiate physical contact by walking or running at a female and touching her with their mouthparts and antennae. Some females respond by mating whereas others run away or interact aggressively by biting, kicking and grappling with their partner. These females will mate after a period of combat. Once the female is receptive to mating, the male climbs on the female's back, curls his abdomen downward, and inserts his adeagus (intromittent organ) into the female vagina. Following mating, the male retracts his adeagus and remains on the female's back, mate guarding, or climbs off the female's back and walks away.

In experiments 1, 2 and 3 we experimentally manipulated the number of times a pair mated to investigate the effects of variation in levels of repeated mating. Males were more sexually ardent if they were rested following mating so pairs were separated at the end of a mating and returned to their individual containers until the next mating. This ensured that the last mating in the series was completed within 8 h of the first copulation. In experiments 4 and 5 we examined behavioral components of repeated mating. During an observation period (50 min) we recorded the number of copulations (= mating frequency). Copulation was defined as having occurred whenever the male's adeagus was visibly inserted into the female's vagina. In experiment 5 we also recorded (1) the mating speed as the time in seconds from the entry of the male in the mating chamber to the commencement of mating and (2) the mating duration in seconds, defined from the successful insertion of the adeagus to its removal.

DIRECT BENEFITS OF REPEATED MATING

Experiment 1: Fertility and fecundity benefits for females

In the first experiment we examined the effects of varying levels of repeated mating on female fecundity and fertility by experimentally manipulating the number of times females were mated to the same male (i.e., 1, 2, 3, 6, 9, 12, or 15 times). Virgin males and females were randomly paired and assigned a mating treatment at random a priori. This random assignment of females to the different levels of repeated mating was used to ensure that an effect of the repeated mating treatment was not obscured by any effect of female body size. Each female completed her entire quota of matings (i.e., $n = 34$ for one mating, $n = 36$ for two matings, $n = 46$ for three matings, $n = 26$ for six matings, $n = 27$ for nine matings, $n = 27$ for 12 matings, $n = 28$ for 15 matings) on the same day that the first mating was commenced.

Females were transferred to individual breeding chambers and allowed access to a carcass 14 h after mating. Exposure to a carcass stimulates egg laying. Eggs were removed from the soil and were counted at 48 and 96 h after the female was provided with a carcass. Eggs were subsequently incubated on moist cotton pads at 20 ± 1°C with a 16:8 L:D regime. We recorded the number of eggs that hatched twice daily until all eggs hatched or had turned black (a sign of unfertilized eggs).

We restricted our analyses to eggs that were laid during 96 h to avoid including infertile eggs that were a result of aging sperm. Although females may mate repeatedly to counter the negative effects of sperm aging (Siva-Jothy 2000), our experiment was not designed to test this hypothesis as all matings were conducted on the same day. Furthermore, preliminary analysis suggested that the total number of eggs that hatch in the first 96 h is a good predictor of a female's total reproductive output following repeated mating (proportion of hatching eggs; $F_{1,116} = 195.128$, $P \leq 0.0001$, $r^2 = 0.63$).

Experiment 2: Repeated mating for infertility avoidance

In a second experiment we examined the effects of repeated mating on sperm production to test the idea that males may become sperm limited, and repeated mating may avoid reduced fertility and fecundity in females. We mated each male ($n = 26$) with each of five different females. The male was allowed to copulate three times with each female, totaling 15 matings for each male. The fertility and fecundity of the five females was used as a proximate measure of male sperm depletion using the same procedure as in experiment 1. Males were randomly paired to five female partners. Random assignment of the partners was used to ensure that any evidence of sperm depletion was not obscured by effects associated with female body size on fecundity or fertility. We also ensured that each male completed his last mating within 8 h of the first mating to increase the likelihood of detecting evidence of sperm depletion.

INDIRECT BENEFITS OF REPEATED MATING

Experiment 3: Fitness benefits for offspring

In our third experiment, we examined the indirect benefits of varying levels of repeated mating by manipulating the number of times females were allowed to copulate (i.e., one, two, or three times) with the same male. Males and females were paired at random ($n = 33$ one mating; $n = 36$ two matings; $n = 32$ three matings) and each female completed the quota of matings within 2 h of the first mating. No pair refused to mate.

We estimated offspring performance and fitness (Lock et al. 2004) by measuring components of development, size, and survival. Females from all treatments were provided with 14–19 g mouse carcasses and allowed to breed. The effect of variation in carcass mass on offspring fitness was examined statistically in our analyses. We checked the breeding chambers twice daily and recorded when larvae first appeared on the carcass and when all larvae dispersed from the carcass. At the time of dispersal each larva was individually weighed and then housed in individual rearing containers. We checked the rearing chambers daily and recorded when larvae pupated and pupae eclosed. At eclosion the length of the pronotum was measured with digital callipers. After dispersal, we recorded whether individuals survived to eclosion. Thus we recorded offspring development times as the duration of the larval period on the carcass (time between arrival on the carcass and dispersal), the duration of the prepupal wandering phase (time between dispersal and pupation), and the duration of the pupal phase (time between pupation and ecolsion as an adult). Offspring size was measured as both the mass of individual larvae at dispersal and their pronotum length at adult eclosion. Larvae do not feed after dispersal before becoming adult. Survival to adulthood was measured as the proportion of dispersing larvae that eclosed as adults.

CONTEXT-DEPENDENT MATING BEHAVIOR

Experiment 4: Mating in the presence or absence of a resource

In our fourth experiment, we examined the mating frequency of males and females in the presence ($n = 15$) or absence ($n = 15$) of a carcass. These two conditions approximate the circumstances under which mating occurs in nature (Eggert 1992). Each pair was observed for five consecutive 50-min observation periods with a 10-min break between successive observation periods. The number of times a pair mated within each 50-min period was recorded to give the mating frequency.

GENETIC ARCHITECTURE OF REPEATED MATING

Breeding design

In experiment 5 we used a conventional paternal half-sibling breeding design (Lynch and Walsh 1998) to estimate the genetic basis of mating behavior. Families from 30 sires each mated to three dams were established from unrelated male and female virgin stock beetles. Sexually mature pairs were placed in individual breeding chambers. The sires remained with each dam for 24 h and were then placed with a new dam. The dams were subsequently allowed to breed on a freshly thawed mouse. Dams that successfully reared a brood were removed after the offspring dispersed, and the dispersed larvae were housed in individual rearing containers. When these beetles reached sexual maturity, behavioral observations were made of these virgin sons and daughters.

Measures of mating behavior

We recorded the mating behavior of males and female offspring derived from this half-sibling design to quantify the genetic components of repeated mating. Six to seven adult males (sons) and six to seven adult females (daughters) were observed from each family. Focal males and females were paired with randomly selected virgin stock animals and placed in individual mating chambers. In this way the mating behavior was assigned to a single individual of the pair. Pairs were observed continuously for 50 min and mating frequency, mating duration, and mating speed were recorded. We analyzed behavior of the first interaction because not all pairs mated more than once.

Statistical analysis

In experiment 2, where we examined the effects of repeated mating on male sperm production, there were two males for which all five females failed to produce fertile eggs, two males for which two females failed to produce fertile eggs, and two males for which one female failed to produce fertile eggs. These missing data values posed a problem because repeated measures analysis does not accommodate missing values. We therefore excluded these males from the analysis. In experiment 3, females that produced broods with less than five offspring were excluded because estimates based on few offspring may be unreliable. Eight females were excluded from each mating treatment due to this criterion ($n = 1$ single mating, $n = 5$ two matings, $n = 2$; three matings).

Statistical analyses were performed using the JMP (SAS, Cary, NC) or Stat View (Abacus Concepts Inc, Berkeley, CA) statistical packages. All means are presented ± SE. Quantitative genetics data were also analyzed with ASREML (VSNi Limited, Hemel Hempstead, United Kingdom). Because our design follows a standard half-sibling breeding design, and is balanced for numbers of dams per sire, we also fitted a standard least-squares analysis of variance (ANOVA) to estimate mean squares associated with sires and dams nested within sires. JMP gave the same results as ASREML, and restriction-fragment-length ploymorphism(REML) analyses never differed by more than a few percent from the standard least-squares ANOVA. We therefore used the latter because calculating statistical significance is more straightforward with the least-squares estimates (Lynch and Walsh 1998). All data were examined for departure from normality before being used in analyses. Proportional data, (i.e., egg hatching success and offspring survival) were normalized by transforming to logits (Snedecor and Cochran 1978).

Genetic analysis

In total, data on the mating behavior of 603 daughters and 582 sons from 30 sires with three dams per sire were analyzed. Narrow sense heritabilities, additive genetic correlations, and standard errors in females and males were calculated from these estimates as described by Becker (1984). Significance was determined by F-tests associated with sire estimates, with Satterthwaite's corrections for imbalance in the number of offspring per sire (Lynch and Walsh 1998). Degrees of freedom associated with the denominator are therefore not whole numbers. Genetic correlations within a sex were determined by multivariate analysis of variance (MANOVA). Genetic correlations were only estimated if both trait measures had nonzero additive genetic variance (Lynch and Walsh 1998). We estimated the additive genetic correlations between the sexes using the variance due to the overall sire effects (V_{SIRE}) and the variance component of the interaction between sire and sex ($V_{\mathrm{SIRE} \times \mathrm{SEX}}$). These estimates were obtained by a single MANOVA with sire, dam nested within sire, sex, sire by sex, dam nested within sire by sex as factors. The genetic correlation was estimated as $r_A = \frac{V_{SIRE}}{V_{SIRE} + V_{SIRE \times SEX}}$ (Astles et al. 2006).

Results

DIRECT BENEFITS OF REPEATED MATING

Experiment 1: Fecundity and fertility benefits for females

Levels of repeated mating had no effect on female fecundity measured as the total number of eggs laid by a female ($F_{6,217} = 0.48$, $P = 0.82$; Fig. 1A) but had a significant influence on female fertility measured as the proportion of eggs that hatched ($F_{6,217} = 4.52$, $P = 0.0002$, $r^2 = 0.11$; Fig. 1B). Tukey's test of post-hoc differences suggested that the fertility of females that mated repeatedly with the same male quickly reached an asymptote after two or more copulations (Fig. 1B).

Experiment 2: Repeated mating for infertility avoidance

The high average fertility of females mated to the same male demonstrated that males transfer sperm effectively during repeated copulations (average hatching success; first female = 0.76 ± 0.20, second female = 0.76 ± 0.21, third female = 0.82 ± 0.17, fourth female = 0.92 ± 0.06, fifth female = 0.80 ± 0.22). If males were sperm depleted, we would expect to see a decline in the proportion of eggs that hatched as a function of the order in which females were mated to a male. We found just the opposite, with the proportion of eggs that hatched increasing with later matings (MANOVA; $F_{4,16} = 5.12$, $P = 0.007$). This appeared to be unrelated to female investment as the number of eggs that were laid by successive females did not differ as a consequence of being the first, second, third, fourth, or fifth female to mate with the same male ($F_{4,16} = 1.92$, $P = 0.155$).

INDIRECT BENEFITS OF REPEATED MATING

Experiment 3: Fitness benefits for offspring

Different levels of repeated mating, carcass mass, and brood size did not significantly influence offspring development time (MANOVA, Wilks' $\lambda = 0.86$, $F_{8,174} = 1.66$, $P = 0.11$). However, there was an overall significant effect on size of offspring (MANOVA; $F_{4,88} = 0.25$, $P = 0.0006$). Univariate ANOVA showed that carcass mass and brood size both had significant effects on offspring mass at the time of dispersal from the carcass (carcass mass: $F_{1,92} = 16.41$, $P < 0.0001$; brood size: $F_{1,92} = 44.55$, $P < 0.0001$), whereas there was no significant effect of

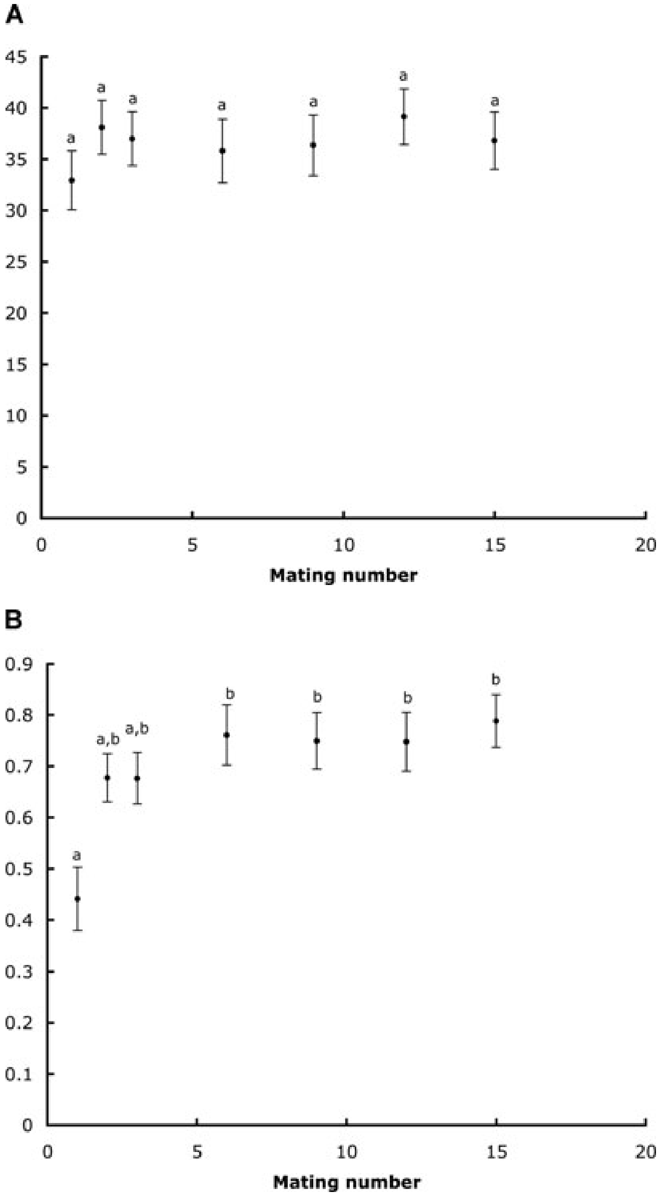

Figure 1. Effects of different levels of repeated mating on (A) total egg production and (B) the proportion of egg hatching. Mean ± SE for females that mated 1, 2, 3, 6, 9, 12, or 15 times with the same male. Tukey's test was used to determine significant differences between pairs. Different lower case letters indicate significant pairwise comparisons in both figures.

different levels of repeated mating ($F_{1,92} = 0.47$, $P = 0.62$). Similarly, there was a highly significant effect of carcass mass and brood size on offspring adult size (carcass mass: $F_{1,92} = 10.14$, $P = 0.002$; brood size: $F_{1,92} = 18.62$, $P \leq 0.0001$), but again level of repeated mating was not significant ($F_{1,92} = 1.03$, $P = 0.36$). Thus, offspring that have fewer siblings or are reared on heavier carcasses achieve a greater body mass at the time of dispersal and ultimately attain a bigger adult body size.

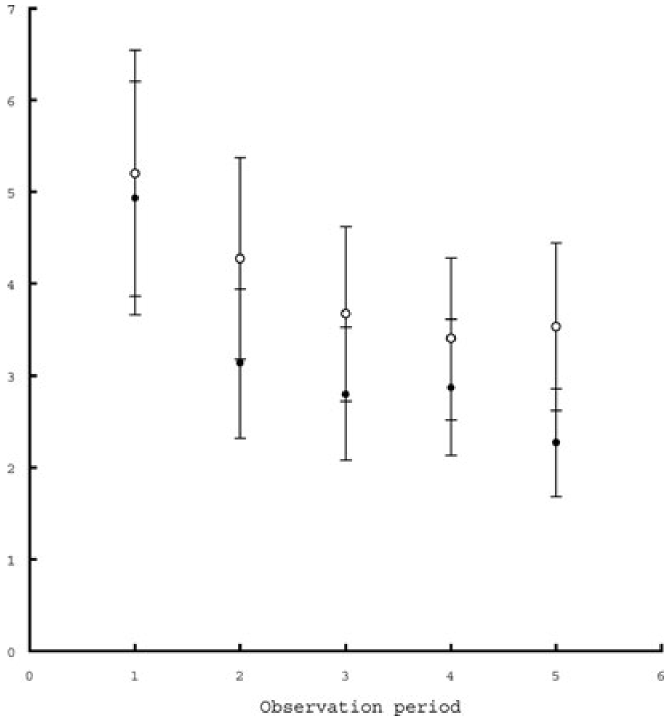

Figure 2. The mating frequency of females housed with a single male for five consecutive observation periods in the presence (filled circles, $n = 15$) or absence of a carcass (open circles, $n = 15$).

We found no significant effects of our measured variables on offspring survival (carcass mass: $F_{1,92} = 0.37$, $P = 0.54$; brood size: $F_{1,92} = 3.64$, $P = 0.06$; level of repeated mating: $F_{2,92} = 0.48$, $P = 0.62$).

CONTEXT-DEPENDENT MATING BEHAVIOR

Experiment 4: Mating in the presence or absence of a resource

Pairs engaged in repeated mating both in the absence or presence of a carcass, although the frequency of mating under both conditions declined during the observation periods (MANOVA; $F_{4,25} =$, $P = 0.0002$; Fig. 2). The mating frequency was higher when pairs copulated in the absence of a carcass compared to when pairs copulated in the presence of a carcass (MANOVA; $F_{1,28} = 0.013$, $P = 0.013$). There was no effect of the interaction between observation period and mating frequency in the presence or absence of a carcass (MANOVA; $F_{4,25} = 0.82$, $P = 0.728$).

GENETIC ARCHITECTURE OF REPEATED MATING

Experiment 5: Genetics of repeated mating

The behavioral components of repeated mating (i.e., mating frequency, mating speed, and mating duration) were variable for both males and females. However, the heritabilities of these traits were moderate for males and low for females (Table 1). There were significant sire effects for female mating speed ($F_{29,61.32} = 1.8007$,

Table 1. **Male and female phenotypic and genetic values for behavioral components of repeated mating in the burying beetle *N. vespilloides*. Mating frequency is measured in counts; mating duration and mating speed measured in seconds.**

Variable	Mean±SE	V_P	V_A	h^2±SE	*n*
Male mating frequency	3.68±0.07	2.66	0.70	**0.27±0.15**	582
Male mating duration	92.90±1.85	1966.78	79.78	**0.24±0.13**	581
Male mating speed	180.58±8.62	45088.35	18017.09	**0.40±0.19**	582
Female mating frequency	3.65±0.06	2.21	0.26	0.12±0.10	603
Female mating duration	89.15±2.09	2599.54	−63.96	−0.02±0.05	600
Female mating speed	190.06±10.22	63880.32	10422.78	**0.16±0.10**	603

Variables with significant (P<0.05) sire effects are in bold.

$P = 0.027$), but not for female mating frequency ($F_{29,60.978} = 1.464$, $P = 0.106$) or female mating duration ($F_{29,61.556} = 0.8684$, $P = 0.655$). In males, there were significant sire effects for mating frequency ($F_{29,62.385} = 2.4366$, $P = 0.0017$), mating duration ($F_{29,61.82}, = 2.0745$, $P = 0.0083$), and mating speed ($F_{29,61.105} = 2.2734$, $P = 0.0035$). The magnitude of additive genetic variation (V_A) was smaller in females than in males whereas phenotypic variation (V_P) was similar in the two sexes (Table 1). Thus, the low h^2 for females reflects a smaller contribution of additive genetic effects and a larger contribution of environmental effects in females compared to males.

Phenotypic correlations among traits associated with mating were of similar magnitude for male and female samples (Table 2). In particular, phenotypic correlations were low between mating duration and the other traits (Table 2). The only strong phenotypic correlation was between mating frequency and mating speed, which was negative. Genetic correlations show a similar pattern in males, with a strong negative genetic correlation between mating frequency and mating speed (Table 3). Genetic correlations between mating frequency and mating duration, and between mating duration and mating speed, were also negative in males but weaker. In contrast, in females the genetic correlation between mating speed and mating frequency was positive but very low (Table 3). The genetic correlation between mating frequency and mating speed in females was close to zero (Table 3). Other genetic correlations could not be calculated for females due to the zero h^2 for mating duration. Finally, there were strong genetic correlations between the sexes, approaching a value of 1.0 for mating frequency and 0.5 for mating speed (Table 3).

Table 2. **Phenotypic correlations for behavioral components of repeated mating in female (*n*=600; above diagonal) and male (*n*=581; below diagonal) *N. vespilloides*.**

	Mating frequency	Mating duration	Mating speed
Mating frequency		−0.09 (P=0.037)	−0.24 (P<0.0001)
Mating duration	−0.05 (P=0.268)		−0.04 (P=0.293)
Mating speed	−0.20 (P<0.0001)	−0.01 (P=0.893)	

Discussion

Previous research on the burying beetle *N. vespilloides* has shown that repeated mating is common (Eggert and Müller 1989a; Müller and Eggert 1989; Eggert 1992) and beneficial for males (Müller and Eggert 1989; Müller et al. 2007). We do not find similar benefits of repeated mating for females as long as they mate more than once. There is no evidence that females mate repeatedly either to obtain male substances that elevate female fecundity or compensate for infertile matings. Furthermore, there is no evidence of fitness benefits to offspring that are raised by mothers that mate repeatedly. Fitness of offspring was primarily influenced by sibling competition and quantity of resources available, which is

Table 3. **Estimates of additive genetic correlations (r_A) in *N. vespilloides* among behavioral components influencing repeated mating in females (above diagonal) and males (below diagonal), and for correlations between the sexes (along the diagonal). Genetic correlations between mating duration in females and other traits were not calculated given the estimate of zero heritability for mating duration in females. Significance determined by likelihood-ratio test comparing a full model with one in which the genetic covariance is specified as zero, or, in the case of intersexual genetic correlations, testing the hypothesis of no significant sire effect in the model (Fry 1992).**

	Mating frequency	Mating duration	Mating speed
Mating frequency	0.91 (P=0.005)	—	0.05 (P=0.640)
Mating duration	−0.21 (P=0.803)	—	—
Mating speed	−0.67 (P=0.004)	−0.13 (P=0.949)	0.46 (P<0.0001)

consistent with previous research on *N. vespilloides* where repeated mating was uncontrolled (Bartlett 1987; Smiseth and Moore 2002; Lock et al. 2004; Smiseth et al. 2007a,b). At best, there might be weak selection for repeated mating for fertility assurance, as at least two copulations are required before females have a high level of fertility. However, we found evidence for a genetic correlation between male and female mating frequency and mating speed. This may explain why repeated mating has evolved in females, even though the fitness payoffs in the traits that we measured are low.

SELECTION ON FEMALE REPEATED MATING

Nicrophorus vespilloides engaged in extensive repeated mating regardless of the presence or absence of a carcass. This finding is comparable with our genetic analysis showing that there is lower environmental variance influencing components of mating behavior in males compared to females, suggesting that males control mating (Table 1). In our quantitative genetic design, males and females were analyzed separately (i.e., only the male or a female of a pair were analyzed), and the individual that was not from a family in the breeding design was treated as an environmental influence on mating rate. If males were able to determine remating by females, direct genetic influences on female behavior are expected be low whereas environmental influences are expected to be high. Likewise, if females determine repeated mating, direct genetic influences on male behavior are expected to be low whereas environmental influences are expected to be high. We found that environmental variance was high for females, which suggests that males tend to control repeated mating. However, there are still additive genetic influences on female behavior, so the pattern of mating is not entirely dependent on males. Thus, there is potential for evolution of female as well as male mating behavior.

We find no support for the hypothesis that females gain dose-dependent, fecundity benefits through repeated mating. In contrast to our prediction that repeated mating would increase fecundity, females that mated just once were as fecund as those that mated repeatedly. This finding differs from studies that have demonstrated that male-derived nutrients or chemicals that are transferred during mating elevate female fecundity (Gwynne 1984; Simmons 1990; Wagner et al. 2001; Fedorka and Mousseau 2002). Unlike systems in which males transfer products that influence female reproduction, *N. vespilloides* mates for less than 90 sec on average, suggesting that few, if any, nutrients or other stimulatory substances are transferred. Furthermore, the absence of fecundity benefits may reflect the greater role of the presence of carrion for the stimulation of egg production rather than repeated mating. For example, in *N. tomentosus*, the burial and preparation of a carcass by a female is the major cue that triggers oocyte development, ovulation and oviposition (Scott and Traniello 1987). It seems possible that the same behavioral cues are required for female *N. vespilloides* also.

We found no evidence that females that mated repeatedly invested more in their offspring. The lack of indirect effects of repeated mating on offspring fitness is consistent with Tregenza and Wedell (1998) and Ivy and Sakaluk (2005) who also found no effects of repeated mating on offspring life history. In contrast, Eady et al. (2000), Tregenza et al. (2003), and Jennions et al. (2007) found that the life histories of offspring are less variable when females mate repeatedly with the same male compared to the offspring of females that mate to different males. Thus, indirect effects on offspring fitness appear to be important in some species but irrelevant in others. In the case of *N. vespilloides*, the influence of repeated mating was insignificant relative to the mass of the carcass and the number of siblings that compete for limited resources. The importance of the carrion resource for offspring fitness is further reinforced by the behavior of parents, who can reduce the size of the brood by filial cannibalism in response to limited food available from small carcasses (Bartlett 1987).

Females of many species require numerous matings to protect against infertility (Wedell et al. 2002; Garcia-Gonzalez 2004). Our results suggest that this does not apply to *N. vespilloides* given that virgin females, mated to extensively mated males, did not suffer a reduction in fertility. In this species, infertile matings appear to be rare as a female that mated with a virgin male was as fertile as a female that was inseminated by the same male that had previously engaged in extensive repeated mating. Thus, it appears that males are capable of rapidly replenishing their sperm supply and of transferring additional sperm during repeated mating. Nonetheless, females that copulated twice or more produced more viable eggs than females that copulated only once. These findings are consistent with those of Müller and Eggert (1989), who suggest that males transfer small sperm volumes during copulation, as males can only achieve a high level of paternity by mating very frequently. However, it does not appear that fertility benefits exert appreciable selection on female repeated mating behavior under all conditions. When a female breeds with a male on a carcass, the results of this study suggest that the mating frequency is well beyond the level required to ensure fertility. However, females also rear offspring without the assistance of a male 14–35% of the time (Müller et al. 2007). In this context, infertility should select for repeated mating behavior in females for fertility assurance but the optimum for females is likely to be lower than for males.

We did not examine costs of repeated mating for females, as we focused on the reasons why repeated mating might have evolved in *N. vespilloides*. It is certainly possible that costs exist. However, this would suggest a constraint on repeated mating, which simply exasperates our need to understand how females might overcome the costs of mating repeatedly. Specifically, we have focused on (1) fecundity and fertility benefits for females and (2) development, performance, and survival benefits for their offspring as empirical studies have found the strongest evidence that mating frequency positively influences these components of fitness (Arnqvist and Nilsson 2000; Eady et al. 2000; Tregenza et al. 2003; Jennions et al. 2007). Nonetheless several studies that found that mating frequency also positively influenced other components of female fitness (e.g., egg mass: Fox 1993; longevity: Arnqvist and Nilsson 2000). There are also a number of possible scenarios in which repeated mating may increase female fitness in systems in which reproduction is dependent on a limited resource or is influenced by paternal care (Hunter et al. 1993). For example, females may trade repeated mating for consumption of the carcass, increased paternal care, or a reduction in infanticide of a female's brood. Therefore, for a holistic test of the adaptive value of repeated mating for females, we need to measure a wider range of fitness components to gain a more accurate estimate of total female fitness. Despite these limitations, our measures of the direct and indirect benefits of repeated mating for females suggest that the benefits are low.

NONADAPTIVE EXPLANATIONS FOR FEMALE REPEATED MATING

Components of mating behavior are genetically influenced in *N. vespilloides*. Our data on genetic correlations suggest that there is a common genetic basis for variation in mating frequency and mating speed within males and between the sexes. In contrast, the duration of copulation is genetically influenced in males but not in females. In males, mating duration was negatively genetically correlated with the other components of mating behavior. However, the magnitude of the correlation, relative to those between the other components of mating, suggests that this genetic correlation would not be a strong constraint to the evolution of repeated mating in males. In contrast, the evolution of repeated mating in females is likely to be constrained by selection for repeated mating in males due to the low levels of genetic variance in components of mating and the covariance between males and females for those traits that are genetically influenced.

If repeated mating in females where a simple byproduct of selection for repeated mating in males, why is selection on males favored? Theoretically, the limited opportunities for reproduction in *N. vespilloides* (Scott 1998) and the risk of sperm competition (Bartlett 1988; House et al. 2007), should favor mechanisms such as repeated mating that ensure a high confidence of paternity for males (Alcock 1994). For example, male water bugs, *Abedus herberti* (Smith 1979) and male dung beetles, *Onthaphagus taurus* (Hunt and Simmons 2002), achieve a high level of paternity assurance by repeated mating. The results of Müller and Eggert (1989), suggest that paternity assurance would select for repeated mating in males. Males can achieve a high level of paternity when they defend a carcass and copulate very frequently with the same female during oviposition (Müller and Eggert 1989). In addition, it seems likely that the negative genetic correlation between mating speed and mating frequency reflects selection for frequent matings with short durations. At present it is unclear how mating duration affects male fitness. The phenotypic and genetic correlation between mating duration and mating frequency suggests that there is a trade-off between mating duration and mating frequency. However, there is no relationship between mating duration and the fertilization success of two males that each mated once to the same female (C. M. House, unpubl. data).

CONCLUSION

Overall, our study suggests that repeated mating in female *N. vespilloides* evolves primarily as a correlated response to selection for repeated mating in males. Studies in *Drosophilia melanogaster* also show correlated responses in males and females to selection on mating speed (Manning 1963; Stamenkovic-Radak et al. 1992 but see Butlin 1993). However, the evidence is inconsistent, as other selection experiments in *D. melanogaster* have failed to show significant correlated responses to selection for mating speed and/or frequency (Gromoko and Newport 1988; Veuille and Mazeau 1988; Sgro et al. 1998). In other systems, such as domestic chickens, *Gallus domesticus* (Cheng and Siegel 1990), stalk-eyed flies, *Cyrtodiopsis dalmanni* (Grant et al. 2005), and bean beetles, *Callosobruchus chinensis* (Harano and Miyatake 2007), there is no evidence for genetic correlations between males and females for mating speed and/or frequency. Thus our study provides unique empirical support for the theory of Halliday and Arnold (1987).

A major difference between the quantitative genetic aspect of our study and previous studies is the direct measurement of mating frequency rather than the use of "mating speed" as a proxy (but see Grant et al. 2005). While selection is predicted to favor an association between mating speed and mating frequency (Cade 1984), and we have argued above that mating speed and mating frequency are both components of repeated mating, the extent to which these traits overlap is unclear. For example, Casares et al. (1993) investigated the determinants of mating speed in *D. melanogaster* and found an important role for the female genotype. This result suggests that it may be erroneous to assume that male genotypes with faster mating speeds also fertilize more females (Casares et al. 1993). Thus, it is prudent to quantify mating frequency directly.

Arguments against nonadaptive hypotheses for the evolution of repeated mating in females have included selection against the genetic correlation due to the different physiological basis for repeated mating in males and females (Pyle and Gromko 1981; Sherman and Westneat 1988; Gromko 1992) and the potential adaptive advantages to females that mate with different males (Jennions and Petrie 2000; Simmons 2005). Our experiments suggest that there are no physiological constraints and minimal benefits to females from engaging in repeated mating. Both of these characteristics of repeated mating in female *N. vespilloides* would contribute to the maintenance of the genetic correlation between male and female repeated mating. However, many of the tests of genetic correlations in mating focused on multiple mating rather than repeated mating. Repeated mating in burying beetles is the most stereotyped female sexual behavior but there is also evidence of polyandrous mating in this species (Müller et al. 2007). This raises two interesting questions that warrant further attention (Hunter et al. 1993; Drapeau et al. 2001): Does the genetic basis to repeated mating also influence the propensity to mate polyandrously, and, does selection differ when females mate polyandrously rather than repeatedly with the same male?

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LITERATURE CITED

Alcock, J. 1994. Postinsemination associations between males and females in insects: the mate-guarding hypothesis. Annu. Rev. Enotomol. 39:1–21.

Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton NJ.

Arnold, S. J., and T. Halliday. 1988. Multiple mating: natural selection is not evolution. Anim. Behav. 36:1547–1548.

———. 1992. Multiple mating by females: the design and interpretation of selection experiments. Anim. Behav. 43:178–179.

Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. Anim. Behav. 60:145–164.

Astles, P. A., A. J. Moore, and R. F. Preziosi. 2006. A comparison of methods to estimate cross-environment genetic correlations. J. Evol. Biol. 19:114–122.

Bartlett, J. 1987. Filial cannibalism in burying beetles. Behav. Ecol. Sociobiol. 21:179–183.

———. 1988. Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera: Silphidae). Behav. Ecol. Sociobiol. 23:297–303.

Becker, W. A. 1984. Manual of quantitative genetics. Academic Enterprises, Pullman.

Butlin, R. 1993. A comment on the evidence for a genetic correlation between the sexes in *Drosophila melanogaster*. Anim. Behav. 45:403–404.

Cade, W. H. 1984. Genetic variation underlying sexual behavior and reproduction. Am. Zool. 24:355–366.

Casares, P., M. C. Carracedo, E. S. Miguel, R. Pineiro, and L. Garcia-Florez. 1993. Male mating speed in *Drosophila melanogaster*: differences in genetic architecture and in relative performance according to female genotype. Behav. Genet. 23:349–357.

Cheng, K. M., and P. B. Siegel. 1990. Quantitative genetics of multiple mating. Anim. Behav. 40:406–407.

Clutton-Brock, T. H. and G. A. Parker. 1995. Sexual coercion in animal societies. Anim. Behav. 49:1345–1365.

Drapeau, M. D., B. F. Fuller, C. L. Rauser, and A. D. Long. 2001. Repeated mating in a lek-mating insect, *Drosophila melanogaster*. Dros. Inf. Serv. 84:136–140.

Dunn, D. W., J. P. Sumner, and D. Goulson. 2005. The benefits of multiple mating to female seaweed flies, *Coelopa frigida* (Diptera: Coelpidae). Behav. Ecol. Sociobiol. 58:128–135.

Eady, P. E., N. Wilson, and M. Jackson. 2000. Copulating with multiple mates enhances female fecundity but not egg-to-adult survival in the bruchid beetle *Callosobruchus maculatus*. Evolution 54:2161–2165.

Eggert, A.-K. 1992. Alternative male mate-finding tactics in burying beetles. Behav. Ecol. 3:243–254.

Eggert, A.-K., and J. K. Müller. 1989a. Mating success of pheromone-emitting *Necrophorus* males: do attracted females discriminate against resource owners? Behavior 110:1–4.

———. 1989b. Pheromone-mediated attraction in burying beetles. Ecol. Entomol. 14: 235–237.

Fedorka, K. M., and T. A. Mousseau. 2002. Material and genetic benefits of female multiple mating and polyandry. Anim. Behav. 64:361–367.

Fox, C. W. 1993. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera, Bruchidae). Oecologia 96:139–146.

Fry, J. D. 1992. The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. Evolution 46:540–550.

Garcia-Gonzalez, F. 2004. Infertile matings and sperm competition: the effect of "nonsperm representation" on intraspecific variation in sperm precedence patterns. Am. Nat. 164:457–472.

Grant, C. A., T. Chapman, A. Pomiankowski, and K. Fowler. 2005. No detectable genetic correlation between male and female mating frequency in the stalk-eyed fly *Cyrtodiopsis dalmannii*. Heredity 95:444–448.

Gromko, M. H. 1992. Genetic correlation of male and female mating frequency: evidence from *Drosophila melanogaster*. Anim. Behav. 43:176–177.

Gromko, M. H., and M. E. A. Newport. 1988. Genetic basis for remating in *Drosophila melanogaster*. II. Response to selection based on the behavior of one sex. Behav. Genet. 18:621–632.

Gwynne, D. T. 1984. Courtship feeding increases female reproductive success in bushcrickets. Nature 307:361–363.

Halliday, T., and S. J. Arnold. 1987. Multiple mating by females: a perspective from quantitative genetics. Anim. Behav. 35:939–941.

Harano, T., and T. Miyatake. 2007. No genetic correlation between the sexes in mating frequency in the bean beetle, *Callosobruchus chinensis*. Heredity 99:295–300.

House, C. M., J. Hunt, and A. J. Moore. 2007. Sperm competition, alternative mating tactics, and context-dependent fertilisation success in the burying beetle, *Nicrophorus vespilloides*. Proc. R. Soc. Lond. B 274:1309–1315.

Hunt, J., and L. W. Simmons. 2002. Confidence of paternity and paternal care: covariation revealed through the experimental manipulation of the mating system in the beetle *Onthophagus taurus*. J. Evol. Biol. 15:784–795.

Hunter, F. M., M. Petrie, M. Otronen, T. Birkhead, and A. P. Moller. 1993. Why do females copulate repeatedly with one male? Trends Ecol. Evol. 8:21–26.

Hurst, G. D. D., R. G. Sharpe, A. H. Broomfield, L. E. Walker, T. M. O. Majerus, I. A. Zakharov, and M. E. N. Majerus. 1995. Sexually transmitted disease in a promiscuous insect, *Adalia bipunctata*. Ecol. Entomol. 20:230–236.

Ivy, T. M., and S. K. Sakaluk. 2005. Polyandry promotes enhanced offspring survival in decorated crickets. Evolution 59:152–159.

Jennions, M. D., and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biol. Rev. 75:21–64.

Jennions, M. D., J. M. Drayton, R. Brooks, and J. Hunt. 2007. Do female black field crickets *Telleogryllus commodus* benefit from polyandry? J. Evol. Biol. 20:1469–1477.

Kamimura, Y. 2003. Effects of repeated mating and polyandry on the fecundity, fertility and maternal behaviour of female earwigs, *Euborellia plebeja*. Anim. Behav. 65:205–214.

Klemme, I., J. A. Eccard, and H. Ylonen. 2007. Why do female bank voles, *Clethrionomys glareolus*, mate multiply? Anim. Behav. 73:623–628.

Lock, J. E., P. T. Smiseth, and A. J. Moore. 2004. Selection, inheritance, and the evolution of parent-offspring interactions. Am. Nat. 164:13–24.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer Associates Inc, MA.

Manning, A. 1963. Selection for mating speed in *Drosophila melanogaster* on the behaviour of one sex. Anim. Behav. 11:116–120.

Müller, J. K., and A.-K. Eggert. 1989. Paternity assurance by "helpful" males: adaptations to sperm competition in burying beetles. Behav. Ecol. Sociobiol. 24:245–249.

Müller, J. K., V. Braunisch, W. Hwang, and A.-K. Eggert. 2007. Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*. Behav. Ecol. 18:196–203.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45:525–567.

Pyle, D. W., and M. H. Gromko. 1981. Genetic basis for repeated mating in *Drosophila melanogaster*. Am. Nat. 117:133–146.

Reinhardt, K., and M. T. Siva-Jothy. 2005. An advantage for young sperm in the house cricket *Acheta domesticus*. Am. Nat. 165:718–723.

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

Scott, M. P. 1998. The ecology and behavior of burying beetles. Annu. Rev. Entomol. 43:595–618.

Scott, M. P., and J. F. A. Traniello. 1987. Behavioural cues trigger ovarian development in the burying beetle, *Nicrophorus tomentosus*. J. Insect Physiol. 33:693–696.

Sgro, C. M., T. Chapman, and L. Partridge. 1998. Sex-specific selection on time to remate in *Drosophila melanogaster*. Anim. Behav. 56:1267–1278.

Sherman, P. W., and D. F. Westneat. 1988. Multiple mating and quantitative genetics. Anim. Behav. 36:1545–1547.

Simmons, L. W. 1990. Nuptial feeding in tettigoniids: male costs and the rates of fecundity increase. Behav. Ecol. Sociobiol. 27:43–47.

———. 2005. The evolution of polyandry: sperm competition, sperm selection, and offspring viability. Annu. Rev. Ecol. Evol. Syst. 36:125–146.

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

Smiseth, P. T., and A. J. Moore. 2002. Does resource availability affect offspring begging and parental provisioning in a partially begging species? Anim. Behav. 63:577–585.

Smiseth, P. T., L. Lennox, and A. J. Moore. 2007a. Interaction between parental care and sibling competition: parents enhance offspring growth and exacerbate sibling competition. Evolution 61: 2331–2339.

Smiseth, P. T., R. S. J. Ward, and A. J. Moore. 2007b. How do parents influence asymmetric sibling competition? Experimental evidence from a species with partially dependent young. Ecology 88: 3174–3182.

Smith, R. L. 1979. Repeated copulation and sperm precedence: paternity assurance for a male brooding water bug. Science 205:1029–1031.

Snedecor, G. W., and W. G. Cochran. 1978. Statistical methods, 6th edn. Iowa State Univ. Press, IA.

Stamenkovic-Radak, M., L. Partridge, and M. Andjelkovic. 1992. A genetic correlation between the sexes for mating speed in *Drosophila melanogaster*. Anim. Behav. 43:389–396.

Thornhill, R., and J. Alcock. 1983. The evolution of insect of mating systems. Harvard Univ. Press, London.

Thrall, P. H., J. Antonovics, and A. P. Dobson. 2000. Sexually transmitted diseases in polygynous mating systems: prevalence and impact in reproductive success. Proc. R. Soc. Lond. B 267:1555–1563.

Tregenza, T., and N. Wedell. 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. Evolution 52:1726–1730.

Tregenza, T., N. Wedell, D. J. Hosken, and P. I. Ward. 2003. Maternal effects on offspring depend on female mating pattern and offspring environment in yellow dung flies. Evolution 57:297–304.

Veuille, M., and S. Mazeau. 1988. Genetic variability of sexual behavior in a natural population of *Drosophila melanogaster*. Behav. Genet. 18:389–403.

Wagner, W. E., R. J. Kelley, K. R. Tucker, and C. J. Harper. 2001. Females receive a life-span benefit from male ejaculates in a field cricket. Evolution 55:994–1001.

Wedell, N., M. J. G. Gage, and G. A. Parker. 2002. Sperm competition, male prudence and sperm-limited females. Trends Ecol. Evol. 17:313–320.

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