

NO EVIDENCE FOR INBREEDING AVOIDANCE THROUGH POSTCOPULATORY MECHANISMS IN THE BLACK FIELD CRICKET, *TELEOGRYLLUS COMMODUS*

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Abstract.—Several studies suggest that females mate multiply so that they can preferentially fertilize eggs with the sperm of genetically more compatible males. Unrelated males are expected to be genetically more compatible with a female than her close relatives. We tested whether black field crickets, *Teleogryllus commodus*, can bias sperm usage toward unrelated males by comparing egg hatching success of females mated to two of their siblings (SS), two sibling males unrelated to the female (NN) or to one unrelated male and a sibling male (NS or SN). Egg hatching success was highly repeatable. Hatching success varied significantly among females of the three mating types ($P = 0.011$, $n = 245$ females). The estimated mean hatching success of 36.8% for SS females was significantly less than the 43.4% of NN females, indicating an effect of inbreeding on hatching success. If females preferentially use the sperm of a less closely related male, the hatching success of NS/SN females should be closer to 43.4% than 36.8%. It was, in fact, only 34.9%. This does not differ significantly from the value expected if the two males contributed an equal amount of sperm that was then used randomly. Although polyandry may confer indirect genetic benefits, our results provide no evidence that female *T. commodus* gain these benefits by biasing paternity toward genetically more compatible males through postcopulatory mechanisms.

Key words.—Cryptic female choice, genetic incompatibility, inbreeding, mate choice, multiple mating, polyandry, sexual selection.

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Molecular paternity analysis has unequivocally demonstrated that in many species females mate with more than one male during a single reproductive bout (polyandry; Birkhead and Møller 1998; Zeh and Zeh 2003). Polyandry has historically been considered of little benefit to females (Bateman 1948). There is, however, now widespread evidence that polyandrous females have higher lifetime offspring production than monogamous females (Hosken and Stockley 2003). In insects, the two most widely documented direct benefits for females that mate multiply are: (1) ensuring sufficient sperm to fertilize all their eggs (Ridley 1988); and (2) increased egg production through the transfer of nutrients, essential trace elements or oviposition stimulants by males (Arnqvist and Nilsson 2000). It is therefore tempting to consider the matter settled: females mate multiply because it directly increases lifetime offspring production.

Intriguingly, however, there are also many species in which it seems improbable that multiple mating increases lifetime fecundity. The best-known example is extrapair copulation by birds. Females appear to receive only sperm from these extrapair matings. At the same time they risk decreased parental investment by their social mate if he concludes that his share of paternity in a brood is reduced (Whittingham and Dunn 2001). Researchers have therefore suggested a plethora of possible genetic (indirect) benefits that may accrue to polyandrous females (Jennions and Petrie 2000; Tregenza and Wedell 2000; Zeh and Zeh 2001, 2003). In support of these claims, a positive correlation between a female's number of mates and offspring viability has been reported for several species (e.g., Madsen et al. 1992; Keil and Sachser 1998; Tregenza and Wedell 1998).

The main potential genetic benefits of polyandry fall into two categories. First, polyandry may generate fitter offspring if there is a genetic correlation between male sperm competitiveness/fertilization ability and net offspring fitness. To date, studies have measured components of offspring fitness such as growth rate (Hosken et al. 2003), sons' attractiveness (Pai and Yan 2002) or sperm competitiveness (Bernasconi and Keller 2001), and daughters' fecundity (e.g., Konior et al. 2001; Kozielska et al. 2004). Second, polyandry may be beneficial even if there is no genetic correlation between sperm competitiveness and net fitness (e.g., Simmons 2003). Postcopulatory mechanisms may still result in eggs being preferentially fertilized by genetically more compatible sperm (Zeh and Zeh 1996, 1997, 2003). Several studies suggest that the sperm of genetically more compatible males preferentially fertilize the eggs of polyandrous females. This claim is based primarily on greater egg hatching success or fewer stillborn offspring for females experimentally assigned to a polygamous rather than monogamous treatment while controlling the total number of mating (e.g., Tregenza and Wedell 1998; Newcomer et al. 1999; Worden and Parker 2001; Fedorka and Mousseau 2002; Kamimura 2003). Ultimately, however, to confirm that polyandry is adaptive, regardless of the types of genetic and/or nongenetic benefits conferred, it will be necessary to estimate the long-term, asymptotic fitness of females experimentally assigned to polyandrous and monogamous treatments (Kokko et al. 2003; Hunt et al. 2004).

Inbreeding is the most clearly understood process leading to the combination of genetically incompatible genomes

(Keller and Waller 2002). Consequently, several studies have investigated whether polyandry allows for inbreeding avoidance via postcopulatory mechanisms. There is circumstantial or direct evidence that females preferentially use genetically dissimilar sperm in at least seven species (an ascidian, *Diplosoma listerianum*, Bishop 1996; Bishop et al. 1996; lizard, *Lacerta agilis*, Olsson et al. 1996; fruitfly, *Drosophila melanogaster*, Mack et al. 2002; newt, *Triturus alpestris*, Garner and Schmidt 2003; cricket, *Gryllus bimaculatus*, Tregenza and Wedell 2002; Bretman et al. 2004; marsupial, *Antechinus agilis*, Kraaijeveld-Smit et al. 2002; and domestic fowl, *Gallus gallus*, T. Pizzari cited in Harris 2003). In five species, however, preferential fertilization dependent on sperm genotype was not detected (shrew, *Sorex araneus*, Stockley 1997; field cricket, *Teleogryllus oceanicus*, Simmons 2001; decorated cricket, *Gryllodes supplicans*, Stockley 1999; mallard, *Anas platyrhynchos*, Cunningham and Cheng 1999; dung fly, *Scathophaga stercoraria*, Hosken et al. 2003). Unfortunately, most of these studies had fairly small sample sizes and may have failed to detect preferential sperm use due to low statistical power (see Mack et al. 2002).

The generality of the claim that polyandrous females preferentially use genetically more compatible sperm to fertilize their eggs is largely untested. To date, there are only two published studies where a highly controlled experimental mating design has directly demonstrated a benefit to polyandry due to reduced fertilization success by more closely related males. In *Gryllus bimaculatus*, a female mated to two of her brothers had significantly lower egg hatching success than a female mated to two brothers to whom she was not related. Thus, there is a cost to inbreeding. The hatching success of a female mated to a brother and an unrelated male was, however, almost identical to that of a female mated to two unrelated males. This strongly implies that the unrelated male preferentially fertilized her eggs (Tregenza and Wedell 2002). Subsequent paternity tests have confirmed this explanation, although the unrelated male's share of paternity was not positively correlated with the proportion of eggs that hatched, which may reflect the compounding influence of variation in male or female effects on absolute hatching success (Bretman et al. 2004). In *D. melanogaster*, when a female was housed with an unrelated stock male and either a focal brother, half-brother, or cousin of the female the focal male's share of paternity increased if he was less closely related to the female (Mack et al. 2002). This study did not, however, control for possible differences in the number of copulations by males that vary in their relatedness to the focal female.

We used a controlled mating experiment to further test the claim that female Australian black field cricket, *Teleogryllus commodus*, use polyandry as a mechanism of inbreeding avoidance.

MATERIALS AND METHODS

Study Species

We collected approximately 120 gravid, naturally mated female *T. commodus* in Canberra, Australia, in February–March 2002 to establish a breeding stock. The population was maintained in six to eight large tanks per generation. For the current experiment we paired virgin F_2 adults and reared

their offspring separately to generate 68 families of full-siblings. As soon as their ovipositors were visible, female nymphs were transferred into a female-only container with their sisters to ensure their virginity.

In *T. commodus* there is no compelling evidence that females gain a direct benefit from mating repeatedly. Males do not produce a nutritious spermatophylax, and the spermatophore is small, weighing, on average, about 0.66% of body mass (Evans 1988). Although Wagner et al. (2001) found that repeated mating increased fecundity in another field cricket with small spermatophores, comparison of female *T. commodus* experimentally assigned either one or three matings with a single male revealed no difference in lifetime fecundity or egg hatching success (M. D. Jennions, J. Hunt, and R. C. Brooks, unpubl. data).

Mating Protocol

Experimental females were all virgins at the start of the mating trials and at least 10 days old to ensure sexual maturity. Experimental males were allowed to mate with a general stock female the day before being used in the experiment. This ensured that they had a fresh spermatophore available and were reproductively active. Males were also at least 10 days old.

We created 36 blocks of eight crickets, four full-sibling females (sisters) and four males following the protocol of Tregenza and Wedell (2002). Each block requires six crickets from one full-sibling family (four females, two males) and two crickets from another full-sibling family (two males). In each block two males (S1, S2) were full-siblings (brothers) of the four females and two males (N1, N2) were not related to the females, but were brothers to one another. Females were assigned to one of four mating regimes that mated them with: (1) S1 then S2; (2) S2 then N1; (3) N2 then S1; or (4) N1 then N2. We also created 49 blocks of three sibling females (sisters) and four males. In each block two males (S1, S2) were full-siblings of the females and two males (N1, N2) were unrelated siblings from another family. Females were assigned to one of three double mating regimes that mated them with: (1) S1 then S2; (2) N1 then N2; or (3) S2 then N1. Males and females were placed together in pairs in small plastic containers (9 × 9 × 9 cm) and allowed to mate. The exact age of individuals was unknown, however, within each block individuals of the same sex reached adulthood in the same week.

In the first round of mating, if a male did not mate within the first hour he was replaced with one of his brothers. Most males mated within the first 15 min. After a successful mating, the pair was allowed to remain together for 60 min. to prevent the female from prematurely removing the spermatophore (Evans 1988; see also Simmons et al. 2003). The male was then removed. We then waited 2 h before pairing the female with her second male. Following the second mating, the pair was again allowed to remain together for 60 min before the male was removed.

Blocks were established over several weeks in seven sets so the random factor set was included in the analysis. In total 84.2% of the available females ($N = 245$ of 291 females) produced sufficient eggs (>10) in the first week to estimate

hatching success. For 51 families, females were only used in one block. For the other 17 families, females were used in two blocks in different sets, with the family of origin of the unrelated males differing between the blocks (cf. Tregenza and Wedell [2002], who used each family in two blocks).

Egg Hatching Success

After a female had successfully mated twice, the second male was removed. The female was then provided with cat food, a water vial, and a small petri dish of moist sand for oviposition. After seven days the dish was removed. The dish was immersed in a beaker of water and the sand gently swirled to bring the eggs to the surface. Eggs were then transferred to a pad of moist cotton wool in a large covered petri dish, which was sealed in an individual plastic container. We counted out 100 eggs from each of 210 females. Another 35 females produced between 11 and 97 eggs (mean \pm SE: 60.3 ± 4.6 eggs). Although total lifetime fecundity was not recorded, there was no trend for females that mated to two unrelated males to be more likely to produce sufficient eggs (> 10 eggs) after seven days than females mated to two of their brothers ($N = 71$ vs. 68 females; expected ratio 1:1).

For 205 females that produced more than 110 eggs, we placed a second batch of eggs in another container. The second batch of eggs consisted of 100 eggs from 163 females and 14–98 eggs (mean \pm SE: 58.6 ± 3.6) from 42 females. This allowed us to test whether the hatching success of individual females was significantly repeatable. We checked containers for emerging nymphs every 2–3 days for a 95 day period. It was necessary to monitor egg hatching for a prolonged time because it took 53.7 ± 1.4 days (mean \pm SE) between the first and last nymph hatching ($N = 181$ females that laid 100 eggs of which 10 or more hatched).

Statistical Analysis

Data on egg hatching success (the ratio of nymphs hatching to eggs laid) represent proportions. We therefore analyzed hatching success in a generalized linear mixed model (GLMM) with binomial error. Analyses were run in S-Plus 6.4 using the Mass library of Venables and Ripley (2002) and the function `glmmPQL`. Model parameters are estimated using a maximum-likelihood approach and are therefore capable of dealing with unbalanced data. We first tested whether there was any effect on mating order by comparing hatching success in SN and NS matings. There was no difference (see Results), so we pooled the two mating types. This is consistent with work on the congener *T. oceanicus* in which mating order has no effect on paternity in the clutches of doubly mated females (Simmons et al. 2003).

We then used a GLMM to test whether female identity explained variation in hatching success using both sets of eggs from the 205 females that produced more than 110 eggs. Thus, there were two datapoints per female. The three random factors in the model were set ($N = 7$), block nested within set ($N = 85$), and female identity ($N = 205$). Female hatching success was highly repeatable among females. To examine the effect of mating type on hatching success, we used the first set of eggs from the largest available sample of 245 females. The initial full model included the random factors

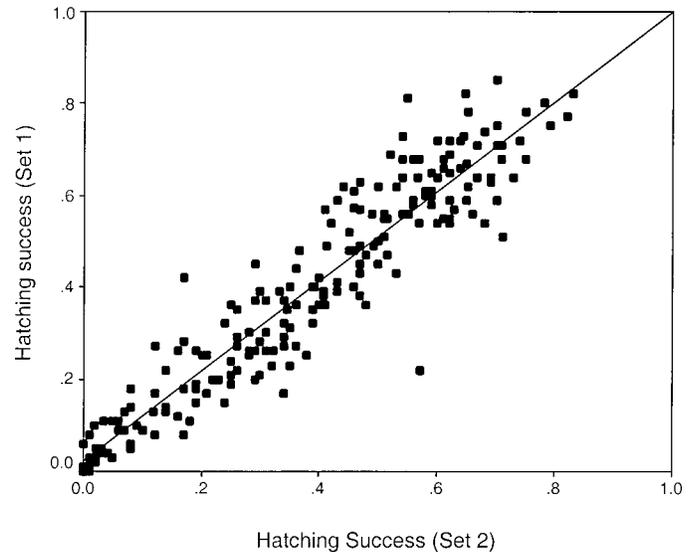


FIG. 1. The proportion of eggs that hatched from the first and second batch of eggs ($N = 205$ females). The line of perfect equality is included.

set ($N = 7$) and block nested within set ($N = 85$), and the fixed factor mating type (SS, SN/NS, and NN). We then compared the full model to a reduced model that excluded the factor set using a log-likelihood ratio (LLR) test to determine whether its inclusion increased the fit of the model to the data.

Model output in a GLMM presents P -values using the standard error of the difference between a focal level of a categorical fixed factor and each other level (Crawley 2002). We treated SS as the focal treatment and compared it to SN/NS and NN. We used one-tailed tests because there is a clear prior prediction that inbreeding is detrimental. The hatching success of a female mated to two of her brothers should therefore be lower than that of a female mated to two unrelated males. Likewise, a female mated to a brother and an unrelated male should have higher hatching success than one mated to two brothers, regardless of whether females preferentially use the sperm of the unrelated male. To compare the NN and SN/NS treatments we made NN the focal treatment. Again we used a one-tailed test because of the clear prediction that NN will have higher hatching success than SN/NS due to inbreeding effects.

RESULTS

Repeatability of Hatching Success

Hatching success was highly repeatable between two batches of eggs from the same female (Fig. 1). A comparison of a fully nested model with and without the inclusion of the term female identity showed that it explained a significant amount of the variance in hatching success, even when the effects of set and block were taken into consideration (LLR = 155.39, $df = 1$, $P < 0.0001$). The correlation between the proportion of eggs hatching in the first and second batch was similar for SS, SN/NS, and NN females ($r_s = 0.90$, $N = 54$; $r_s = 0.95$, $N = 93$; $r_s = 0.92$, $N = 58$, all one-tailed tests P

TABLE 1. Parameter estimates for the differences between means for fixed effects from a generalized linear mixed model (with binomial error) of the logit transformation of the proportion of eggs hatching for the three mating types (see text). Back-transformed means are presented in the text. The standard errors for the two mating types are for the difference between each treatment and the SS treatment. (SS, sibling-sibling mating; SN/NS, sibling/nonsibling mating; NN, nonsibling/nonsibling mating).

Fixed effects (<i>N</i>)	Value	SE	df	<i>t</i>	<i>P</i> _{2-tailed}
Intercept	-0.5413	0.268	158	2.019	0.045
SS (68)	0	—	—	—	—
SN/NS (106)	-0.0841	0.124	158	0.678	0.499
NN (71)	0.2775	0.136	158	2.037	0.043

< 0.0005). There was a very small (1.4%) but significant decrease in hatching success between the first and second batch of eggs (40.0 vs. 38.6%; Wilcoxon matched-pairs test, $P = 0.014$, $N = 205$). This suggests that eggs more likely to hatch were preferentially transferred to the first container, probably because they were slightly larger. Due to this minor decrease in hatching success, we limited our analysis of the effect of mating type on hatching success to the first batch of eggs collected from each female.

Effect of Mating Treatment on Hatching Success

The order in which a female mated with her brother and an unrelated male had no effect on hatching success. There was no difference in hatching success between SN and NS females, whether we used all available data (35.7% vs. 33.3%; $F_{1,29} = 0.426$, $P = 0.519$, $N = 110$ females) or fully balanced data from the 30 blocks with data for both females (37.2% vs. 34.0%; $F_{1,29} = 0.382$, $P = 0.542$, $N = 60$ females). Therefore, we consider the treatments SN and NS as synonymous in subsequent analyses.

There was a significant effect of female mating type on egg hatching success ($F_{2,158} = 4.62$, $P = 0.011$, $N = 245$ females). The estimated mean hatching success was 36.8% for SS females, 34.9% for SN/NS, females and 43.4% for NN females (Table 1). Hatching success was significantly greater for NN than SS females (one-tailed test, $t_{158} = 2.04$, $P = 0.022$) or SN/NS females ($t_{158} = 2.99$, $P = 0.0016$). There was no significant difference between SS and SN/NS females ($t_{158} = 0.68$; no *P*-value is presented as the one-tailed hypothesis was falsified because the mean hatching success of SS females was greater than that of SN/NS females; see Rice and Gaines 1994). The estimated hatching success of 34.9% for SN/NS females did not differ significantly from the 40.1% expected if N and S males contributed an equal amount of sperm that was then used randomly by the female (i.e., the mean of 36.8% + 43.4%; two-tailed test $t_{158} = 0.89$, $P = 0.811$).

The random term set significantly improved the fit of the model (LLR = 54.08, df = 1, $P < 0.001$) and was therefore included in the final model. The term block did not significantly improve the fit of the model (LLR = 0.36, df = 1, $P = 0.55$). It was, however, retained in the final model because the design of the experiment meant that some males mated to more than one female per block. A model that excluded block still showed a significant effect of female mating type

on egg hatching success ($F_{2,236} = 4.50$, $P = 0.012$, $N = 245$ females). Hatching success for NN females was significantly greater than that for SS females (one-tailed test, $t_{236} = 2.03$, $P = 0.022$) or SN/NS females ($t_{236} = 2.94$, $P = 0.0018$). There was no significant difference between SS and SN/NS females ($t_{236} = 0.65$).

DISCUSSION

Effect of Mating Treatment on Hatching Success

We used males and females of known relatedness to test directly whether polyandry is beneficial because it reduced maternal-paternal genetic incompatibility. Inbreeding is the most commonly reported source of genetic incompatibility between genomes (Keller and Waller 2002). Inbreeding depression is due largely to dominance interactions between the alleles inherited from each parent (Charlesworth and Charlesworth 1987; Falconer and MacKay 1996) and it is likely that genetic incompatibility effects that reduce hatching success in insects are due to reduced zygote and embryo viability. Our study provides the first evidence for inbreeding depression in hatching success in black field crickets, *T. commodus*. Inbreeding between full-siblings caused a 6.6% decrease in hatching success. Although there is limited data on inbreeding depression in insects (Roff 1997; Saccheri et al. 1998), studies of other gryllid crickets have also reported that inbreeding reduces hatching success (Tregenza and Wedell 2002) and has negative effects on other life-history and morphological traits (Roff and DeRose 2001).

Given inbreeding depression, selection should favor mate choice and/or postcopulatory mechanisms that reduce the likelihood of insemination or fertilization by close relatives (Pusey and Wolf 1996). The strength of selection and whether such inbreeding avoidance mechanisms will evolve depends on the relative frequency with which wild females encounter related and unrelated males. It seems unlikely that females in field crickets such as *T. commodus*, which occur in large, eruptive populations, will encounter siblings in the wild. However, in the field cricket *Gryllus bimaculatus*, which appears to have a similar breeding biology to *T. commodus*, females are less likely to mate with closely related males (Simmons 1989, 1991) and, following multiple mating, there is preferential fertilization by the sperm of less closely related males (Bretman et al. 2004). This suggests that females encounter their brothers in the field and that this has been a strong enough force to select for both mechanisms of inbreeding avoidance in *G. bimaculatus*. In our study, however, we found no evidence that female *T. commodus* bias fertilization toward less closely related, and thus genetically more compatible, mates. This may indicate that in *T. commodus* adult females rarely encounter their brothers so that there is little opportunity for selection for inbreeding avoidance, or that despite being advantageous selection has not acted to reduce inbreeding via postcopulatory mechanisms. However, our failure to detect a benefit is unlikely to be due to low statistical power. First, our study used data on the hatching success of 245 females, which is more than double the sample size used in the comparable study of Tregenza and Wedell (2002), who reported a medium to large effect size of $r = 0.45$ for the influence of female mating type on hatching

success in *G. bimaculatus*. Second, hatching success was not subject to substantial measurement error as our two estimates per female were highly correlated.

There is direct evidence based on sperm movement that female animals can bias paternity through active sperm choice (sensu Birkhead 2000). In ascidians, for example, interactions between sperm and the female soma prevent certain sperm from reaching the site of fertilization in a manner similar to that in the well-studied self-incompatibility system of pollen-stigma interactions in plants (Bishop 1996; Bishop et al. 1996). Likewise, direct interactions between eggs and sperm lead to fertilization success that depends on male and female genotype in mice (Rulicke et al. 1998) and sea urchins (Palumbi 1999). To date, however, only two studies have conclusively demonstrated reduced fertilization success for closely related males. *Gryllus bimaculatus* females mated to two siblings had an egg hatching success of 34%, which is significantly lower than the 53% when mated to two unrelated males. Thus, there is a cost to inbreeding. However, the hatching success of females mated to a sibling and an unrelated male was 55%. This strongly implies that the unrelated male preferentially fertilized the eggs (Tregenza and Wedell 2002), a conclusion subsequently confirmed using paternity testing (Bretman et al. 2004). In *D. melanogaster* the share of paternity (relative to an unrelated stock male) of a focal male that was either a brother, half-brother, cousin, or unrelated to the female was such that brothers gained significantly less paternity than focal unrelated males (Mack et al. 2002). However, no other comparison between male types was significant (i.e., half-brothers did as well as unrelated males). This suggests that it may be difficult to extrapolate from results showing biased fertilization when females mate with a close relative to claims that females can generally bias fertilization toward genetically more compatible sperm from the population at large. Finally, when sand lizards (*Lacerta agilis*) females were experimentally assigned two mates in the laboratory, the less closely related male sired a greater proportion of the offspring (Olsson et al. 1996).

We found no evidence that *T. commodus* females biased fertilization toward less closely related males. Thus, there is no evidence that avoidance of genetic incompatibility due to inbreeding drives polyandry. In another study we also found no evidence that females bias fertilization toward males that increase offspring hatching success or several other measures of offspring performance (M. D. Jennions, J. Hunt, and R. C. Brooks, unpubl. ms.). Whether our finding will prove to be the general rule in other animal species is unknown.

In our view, too many authors are now invoking biased sperm use to avoid genetic incompatibility as the force driving polyandry without possessing the requisite data. As Tregenza and Wedell (2000) have noted, variation in genetic compatibility has become a default explanation for observed benefits of polyandry. We think this explanation should be directly tested. Although there are a few species providing sufficient evidence to conclude that polyandry reduces genetic incompatibilities in offspring (e.g., *Cordylochernes scorpioides* and *G. bimaculatus*; Zeh 1997; Tregenza and Wedell 1998; Newcomer et al. 1999; Bretman et al. 2004), there are simply too few studies available to conclude that this phenomenon is widespread.

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LITERATURE CITED

- Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* 60: 145–164.
- Bateman, A. J. 1948. Inter-sexual selection in *Drosophila*. *Heredity* 2:349–368.
- Bernasconi, G., and L. Keller. 2001. Female polyandry affects their sons' reproductive success in the red flour beetle *Tribolium castaneum*. *J. Evol. Biol.* 14:186–193.
- Birkhead, T. R. 2000. Defining and demonstrating postcopulatory female choice—again. *Evolution* 54:1057–1060.
- Birkhead, T. R., and A. P. Møller, eds. 1998. Sperm competition and sexual selection. Oxford Univ. Press, Oxford, U.K.
- Bishop, J. D. D. 1996. Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum*. 1. Autoradiographic investigation of sperm movements in the female reproductive tract. *Proc. R. Soc. Lond. B.* 263:369–376.
- Bishop, J. D. D., C. S. Jones, and L. R. Noble. 1996. Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum*. 2. Investigation of male mating success using RAPD markers. *Proc. R. Soc. Lond. B.* 263:401–407.
- Bretman, A., N. Wedell, and T. Tregenza. 2004. Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. *Proc. R. Soc. Lond. B.* 271:159–164.
- Charlesworth, B., and D. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* 18:237–268.
- Crawley, M. J. 2002. Statistical computing: an introduction to data analysis using S-Plus. John Wiley and Sons, Chichester, U.K.
- Cunningham, E. J. A., and K. M. Cheng. 1999. Biases in sperm use in the mallard: no evidence for selection by females based on sperm genotype. *Proc. R. Soc. Lond. B.* 266:905–910.
- Evans, A. R. 1988. Mating systems and reproductive strategies in three Australian gryllid crickets: *Bobilla victoriae* Otte, *Balamara gida* Otte and *Teleogryllus commodus* (Walker) (Orthoptera: Gryllidae; Nemobiinae; Trigonidiinae; Gryllinae). *Ethology* 78:21–52.
- Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman, New York.
- Fedorka, K. M., and T. A. Mousseau. 2002. Material and genetic benefits of female multiple mating and polyandry. *Anim. Behav.* 64:361–367.
- Garner, T. W. J., and B. R. Schmidt. 2003. Relatedness, body size and paternity in the alpine newt, *Triturus alpestris*. *Proc. R. Soc. Lond. B.* 270:619–624.
- Harris, W. E. 2003. Sperm tales. *ISBE Newsletter* 15:14–18.
- Hosken, D. J., and P. Stockley. 2003. Benefits of polyandry: a life history perspective. *Evol. Biol.* 33:173–194.
- Hosken, D. J., T. W. J. Garner, T. Tregenza, N. Wedell, and P. I. Ward. 2003. Superior sperm competitors sire higher-quality young. *Proc. R. Soc. Lond. B.* 270:1933–1938.
- Hunt, J., L. Bussière, M. D. Jennions, and R. C. Brooks. 2004. What is genetic quality? *Trends Ecol. Evol.* 19:329–333.
- Jennions, M. D., and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol. Rev. Cambr. Philos. Soc.* 75:21–64.
- 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.
- Keil, A., and N. Sachser. 1998. Reproductive benefits from female promiscuous mating in a small mammal. *Ethology* 104:897–903.
- Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.* 17:230–241.

- Kokko, H., R. Brooks, M. D. Jennions, and J. Morley. 2003. The evolution of mate choice and mating biases. *Proc. R. Soc. Lond. B.* 270:653–664.
- Konior, M., J. Radwan, and M. Kolodziejczyk. 2001. Polyandry increases offspring fecundity in the bulb mite. *Evolution* 55: 1893–1896.
- Kozielska, M., A. Krzeminska, and J. Radwan. 2004. Good genes and the maternal effects of polyandry on offspring reproductive success in the bulb mite. *Proc. R. Soc. Lond. B.* 271:165–170.
- Kraaijeveld-Smit, F. J. L., S. J. Ward, P. D. Temple-Smith, and D. Paetkau. 2002. Factors influencing paternity success in *Antechinus agilis*: last-male sperm precedence, timing of mating and genetic compatibility. *J. Evol. Biol.* 15:100–107.
- Mack, P. D., B. A. Hammock, and D. E. L. Promislow. 2002. Sperm competitive ability and genetic relatedness in *Drosophila melanogaster*: similarity breeds contempt. *Evolution* 56: 1789–1795.
- Madsen, T., R. Shine, J. Loman, and T. Hakansson. 1992. Why do female adders copulate so frequently? *Nature* 355:440–441.
- Newcomer, S. D., J. A. Zeh, and D. W. Zeh. 1999. Genetic benefits enhance the reproductive success of polyandrous females. *Proc. Natl. Acad. Sci. USA* 96:10236–10241.
- Olsson, M., R. Shine, T. Madsen, A. Gullberg, and H. Tegelstrom. 1996. Sperm selection by females. *Nature* 383:585.
- Pai, A. T., and G. Y. Yan. 2002. Polyandry produces sexy sons at the cost of daughters in red flour beetles. *Proc. R. Soc. Lond. B.* 269:361–368.
- Palumbi, S. R. 1999. All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. *Proc. Natl. Acad. Sci. USA* 96:12632–12637.
- Pusey, A., and M. Wolf. 1996. Inbreeding avoidance in animals. *Trends Ecol. Evol.* 11:201–206.
- Rice, W. R., and S. D. Gaines. 1994. Heads I win, tails you lose: testing directional alternative hypotheses in ecological and evolutionary research. *Trends Ecol. Evol.* 9:235–237.
- Ridley, M. 1988. Mating frequency and fecundity in insects. *Biol. Rev. Cambr. Philos. Soc.* 63:509–549.
- Roff, D. A. 1997. *Evolutionary quantitative genetics*. Chapman and Hall, New York.
- Roff, D. A., and M. A. DeRose. 2001. The evolution of trade-offs: effects of inbreeding on fecundity relationships in the cricket *Gryllus firmus*. *Evolution* 55:111–121.
- Rulicke, T., M. Chapuisat, F. R. Homberger, E. Macas, and C. Wedekind. 1998. MHC-genotype of progeny influenced by parental infection. *Proc. R. Soc. Lond. B.* 265:711–716.
- Saccheri, I., M. Kuussaari, M. Kankare, M. Vikman, P. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392:491–494.
- Simmons, L. W. 1989. Kin recognition and its influence on mating preferences of the field cricket, *Gryllus bimaculatus* (Degeer). *Anim. Behav.* 38:68–77.
- . 1991. Female choice and the relatedness of mates in the field cricket, *Gryllus bimaculatus*. *Anim. Behav.* 41:493–501.
- . 2001. The evolution of polyandry: an examination of the genetic incompatibility and good-sperm hypotheses. *J. Evol. Biol.* 14:585–594.
- . 2003. The evolution of polyandry: patterns of genotypic variation in female mating frequency, male fertilization success and a test of the sexy-sperm hypothesis. *J. Evol. Biol.* 16: 624–634.
- Simmons, L. W., J. Wernham, F. Garcia-Gonzalez, and D. Kamien. 2003. Variation in paternity in the field cricket *Teleogryllus oceanicus*: no detectable influence of sperm numbers or sperm length. *Behav. Ecol.* 14:539–545.
- Stockley, P. 1997. No evidence of sperm selection by female common shrews. *Proc. R. Soc. Lond. B.* 264:1497–1500.
- . 1999. Sperm selection and genetic incompatibility: Does relatedness of mates affect male success in sperm competition? *Proc. R. Soc. Lond. B.* 266:1663–1669.
- Tregenza, T., and N. Wedell. 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution* 52:1726–1730.
- . 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. *Mol. Ecol.* 9:1013–1027.
- . 2002. Polyandrous females avoid costs of inbreeding. *Nature* 415:71–73.
- Venables, W. N., and B. D. Ripley. 2002. *Modern applied statistics with S*. 4th ed. Springer-Verlag, New York.
- 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.
- Whittingham, L. A., and P. O. Dunn. 2001. Male parental care and paternity in birds. *Curr. Ornithol.* 16:257–298.
- Worden, B. D., and P. G. Parker. 2001. Polyandry in grain beetles, *Tenebrio molitor*, leads to greater reproductive success: material or genetic benefits? *Behav. Ecol.* 12:761–767.
- Zeh, J. A. 1997. Polyandry and enhanced reproductive success in the harlequin-beetle-riding pseudoscorpion. *Behav. Ecol. Sociobiol.* 40:111–118.
- Zeh, J. A., and D. W. Zeh. 1996. The evolution of polyandry. I. intragenomic conflict and genetic incompatibility. *Proc. R. Soc. Lond. B.* 263:1711–1717.
- . 1997. The evolution of polyandry. 2. Post-copulatory defences against genetic incompatibility. *Proc. R. Soc. Lond. B.* 264:69–75.
- . 2001. Reproductive mode and the genetic benefits of polyandry. *Anim. Behav.* 61:1051–1063.
- . 2003. Toward a new sexual selection paradigm: polyandry, conflict and incompatibility. *Ethology* 109:929–950.

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