doi:10.1111/j.1558-5646.2011.01317.x

NO EVIDENCE FOR POSTCOPULATORY INBREEDING AVOIDANCE IN *DROSOPHILA MELANOGASTER*

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Received December 7, 2010
Accepted March 20, 2011

Selection to avoid inbreeding is predicted to vary across species due to differences in population structure and reproductive biology. Over the past decade, there have been numerous investigations of postcopulatory inbreeding avoidance, a phenomenon that first requires discrimination of mate (or sperm) relatedness and then requires mechanisms of male ejaculate tailoring and/or cryptic female choice to avoid kin. The number of studies that have found a negative association between male–female genetic relatedness and competitive fertilization success is roughly equal to the number of studies that have not found such a relationship. In the former case, the underlying mechanisms are largely unknown. The present study was undertaken to verify and expand upon a previous report of postcopulatory inbreeding avoidance in *D. melanogaster*, as well as to resolve underlying mechanisms of inbreeding avoidance using transgenic flies that express a sperm head-specific fluorescent tag. However, siblings did not have a lower fertilization success as compared to unrelated males in either the first (P_1) or second (P_2) mate role in sperm competition with a standard unrelated competitor male in our study population of *D. melanogaster*. Analyses of mating latency, copulation duration, egg production rate, and remating interval further revealed no evidence for inbreeding avoidance.

KEY WORDS: **Cryptic female choice, inbreeding depression, mate choice, postcopulatory sexual selection, sperm competition.**

Mating between close relatives often leads to a decline in fitness known as inbreeding depression (Charlesworth and Charlesworth 1999). The magnitude of inbreeding depression varies among traits, environments, and species (Keller and Waller 2002), and it can be strong enough to drive small populations to extinction (Saccheri et al. 1998). It is thus not surprising that a variety of adaptations to avoid inbreeding have evolved, including sex-specific dispersal and premating and postcopulatory discrimination against kin (Pusey and Wolf 1996; Tregenza and Wedell 2000). For example, female sticklebacks (*Gasterosteus aculeatus*; Frommen and Bakker 2006) and cockroaches (*Blattella germanica*; Lihoreau et al. 2007) prefer to mate with unrelated partners. The evolution of postcopulatory mechanisms of inbreeding avoidance is predicted especially when female mate choice is limited or when mate relatedness cues are absent or inaccurate (Birkhead 1998). Such mechanisms have been described in the fowl (*Gallus gallus*; Pizzari et al. 2004) and the least killifish (*Heterandria formosa*; Ala-Honkola et al. 2010), for which most copulations are male-coerced. In the fowl, females appear to regulate the amount of sperm that reach the perivitelline layer of the eggs based on male relatedness (Pizzari et al. 2004), and in the least killifish, fewer sperm were collected from the reproductive tracts of females mated to full-siblings compared to females mated to unrelated males (Ala-Honkola et al. 2010).

Due to the inherent kin-selected fitness benefits of inbreeding, theory predicts that inbreeding should only be avoided if the associated costs are very high (Kokko and Ots 2006). This may explain why inbreeding is by no means universally avoided, although inbreeding depression is very common (Keller and Waller 2002). Experiments quantifying competitive fertilization success using two males that differ in their relatedness to the female have revealed that unrelated males sire a greater proportion of progeny in a small marsupial (*Antechinus agilis*; Kraaijeveld-Smit et al. 2002), the house mouse (*Mus musculus*; Firman and Simmons 2008), the alpine newt (*Triturus alpestris*; Garner and Schmidt 2003), two species of crickets (*Gryllus bimaculatus*; Bretman et al. 2004, 2009 and *Teleogryllus oceanicus*; Simmons et al. 2006), the fruit fly (*Drosophila melanogaster*; Mack et al. 2002) and the white campion (*Silene latifolia*; Teixeira et al. 2009). In contrast, unrelated males exhibited no sperm competition advantage over related males in the mallard duck (*Anas platyrhynchos*; Denk et al. 2005), the migratory locust (*Locusta migratoria*; Teng and Kang 2007), Peron's treefrog (*Litoria peronii*; Sherman et al. 2008), or the guppy (*Poecilia reticulata*; Evans et al. 2008). Similarly, correlational studies of wild populations have revealed a negative relationship between relatedness and paternity success in the sand lizard (*Lacerta agilis*; Olsson et al. 1996a) and the ruff (*Philomachus pugnax*; Thuman and Griffith 2005), but not in the common shrew (*Sorex araneus*; Stockley 1997) or the red squirrel (*Tamiasciurus hudsonicus*; Lane et al. 2007).

Mack et al. (2002) report that female *D. melanogaster* may adaptively bias sperm use against related males. Specifically, a first male's fertilization success ("sperm defense" or P_1), when competing against a standard unrelated male, was found to be lower when the female was his full-sibling than when she was unrelated. Half-sibling males and those of lesser degrees of relatedness suffered no reduction in sperm defense (Mack et al. 2002). Neither the effects of inbreeding on sperm offense (i.e., the proportion of progeny sired by the second male to mate or P_2) nor the mechanisms underlying the association between relatedness and P_1 were examined by Mack et al. (2002).

The recent development of *D. melanogaster* transgenic lines whose sperm heads fluoresce green or red (Manier et al. 2010) present an opportunity to discern the fate of sperm within the female reproductive tract while discriminating between the sperm of different males, and hence to investigate the mechanisms of inbreeding avoidance. The present study aimed to (1) replicate the P_1 experiment of Mack et al. (2002), (2) quantify the relationship between mate relatedness and P_2 and (3), resolve the mechanisms of ejaculate-female interactions underlying postcopulatory inbreeding avoidance in this species. As we did not find postcopulatory inbreeding avoidance, we present data from only the first two aims.

Methods

EXPERIMENTAL POPULATIONS AND METHODS

P_1 and P_2 were quantified in two separate experiments. In each experiment, a focal pair of males (a "test" male and a "standard competitor" male) was mated to the test male's full-sibling and to an unrelated female. For the P_1 experiment, the test male mated to both females first, and for the P_2 experiment, the test male mated to both females second. Both experiments were balanced for mating order (i.e., whether the test male's first mating was with a sibling or a nonsibling female) by randomly assigning half of the males from each family to each mating order. The use of a standard competitor male removes the influence of male $\times$ male interactions on P_1 and P_2, thus enhancing our ability to detect any male $\times$ female interactions (see Bjork et al. 2007) through pairwise comparisons of paternity success in related and unrelated females. Similarly, to remove variation in P_1 and P_2 attributable to the male's mating history (Bjork et al. 2007), all test and competitor males were initially mated to nonexperimental virgin females one day before their first experimental mating.

Test males and females were from a line genetically engineered to produce sperm that are tagged with a red fluorescent protein (RFP; DsRed-Monomer) that had been backcrossed for six generations to the LH_M wild-type strain (for details on the fly strains and the genetic transformation methods, see Manier et al. 2010). The standard competitor males were from LH_M-bw^D line (Chippindale et al. 2001) with a fixed brown dominant eye color that allows unambiguous paternity assignment of all offspring. Both LH_M and a LH_M-bw^D lines were maintained in population cages of approximately 1000 individuals at 24°C and 12L:12D on standard cornmeal-molasses-agar-yeast medium (5.4% cornmeal, 7% molasses, 0.5% agar, 2% yeast, 1.2% ethanol, 0.4% propionic acid, 0.06% methylparaben added to water). Families for this experiment were created by placing pairs of randomly selected virgin females and virgin males from the RFP-line into plastic 8-dram vials containing cornmeal-molasses-agar-yeast medium and a few grains of live yeast. Each pair was transferred to a new vial three times a week to avoid larval crowding. Virgin females and males for the experiment were collected under CO_2 anesthetization. Standard competitor males were bottle reared and collected as virgins.

In both the P1 and the P2 experiment, we used four randomly selected males and females from each of 20 different families and assigned the females randomly to "sibling" and "unrelated" mating roles. Thus the initial sample size was $N = 80$ for both the $P_1$and P_2experiments. All males were 4-d old and all females 3- to 5-d old at their own first experimental mating day.

On the first experimental mating day, the first females and the first males were individually paired in vials to mate. For all pairs, we recorded the time when flies were paired in a vial, the

start of copulation, and the end of copulation. Following copulation, each male was moved to an individual vial until his second mating (see below). Females were provided the opportunity to remate with the second male 2, 3, 4, and 5 days after their first mating by aspirating the second male into the female's vial in the morning and providing a 4-h opportunity to interact. Again, after copulation, the male was moved to his own vial until his second test mating.

Four days after their first copulation, the first males were mated to their second test female. After copulating, the male was removed from the vial and frozen. Second females were provided a 4-h opportunity to remate with the second male on days 2–5 after their first mating. After copulation, second males were removed from the second female's vial and frozen. Although the remating interval for second males was variable due to variation in remating latency among females, this experimental schedule enabled at least a one-day remating interval for all second males.

We quantified inbreeding depression in egg-to-adult viability in the P_1 experiment for all eggs laid during the two-day interval between the first mating and the initial exposure to the second male. P_1 and P_2 were estimated from offspring produced in the first three days after remating, with females transferred to fresh vials daily. In summary, collected data permitted quantification of (1) egg-to-adult viability, (2) P_1, (3) P_2 and, as measures that might have revealed possible inbreeding avoidance mechanisms, (4) time to first copulation, (5) copulation duration with the first male and the second male, (6) the number of eggs produced prior to remating, (7) female remating interval, and (8) the number of eggs produced during 3 days after the second mating.

DATA ANALYSES

Only those test males that successfully mated with both females were included in the analyses. Males were also excluded from the P_1 and the P_2 analyses (1) if either of their mates did not lay any eggs, (2) if their mate from the P_1 experiment produced no progeny prior to remating and had a P_1 value of "0," (3) if the female had a P_1 value "1," (4) if the female in the P_2 experiment produced no progeny prior to remating and had a P_2 value of "1," or (5) if the female had a P_2 value of "0," because any of these occurrences are symptomatic of an unsuccessful copulation or of infertility. In *D. melanogaster*, P_2 values are typically around 0.8 (e.g., Bjork et al. 2007). Final sample size for P_2 analyses was $N = 59$ (males originated from 20 families) and $N = 35$ for P_1 analyses (males originated from 18 families). The lower N for the P_1 experiment resulted from the lower female remating rate in this experiment compared to the P_2 experiment.

To test the effect of relatedness on P_1, P_2, time to the first copulation (P_1 exp.), copulation duration with the first male (P_1 exp.) and the second male (P_1 exp. and P_2 exp.), number of eggs produced after the first mating (P_1 exp.) and after the second mating (P_2 exp.), and female remating day (P_1 exp. and P_2 exp.), we ran separate general linear mixed models estimated using restricted maximum likelihood (REML). Due to heteroscedasticity in residuals, time to the first copulation (P_1) and the first copulation duration (P_1) were $\log_e$-transformed. P_1 and P_2 were arcsine square root transformed as they are proportions. "Male nested within male family" was treated as a random factor, as we had two observations for each male (one when he mated with a sibling; one when he mated with a nonsibling) and males originated from 20 families. The significance of the random term was assessed using likelihood ratio tests (REML; Zuur et al. 2009). The random term was significant only in the analysis of P_2 and the female's second copulation duration (both in P_1 and P_2 exp.) but, to be conservative, we did not remove the random term from any of the models as it was part of the design. Relatedness (sibling or nonsibling), mating order of the male ("sibling female first, nonsibling second" or "nonsibling female first, sibling second") and female order (first or second mate of a given male) were entered as fixed factors into the full models. In the analysis of both P_1 and P_2, we also entered the number of eggs produced before remating as a covariate (as it estimates the number of first male sperm used). In addition, in the analysis of the number of eggs produced after the first mating (P_1 exp.), we included remating day as a fixed factor, because the longer it took a female to remate, the more eggs she produced before remating.

Female order was used as a variance covariate in the analysis of both the time to the first copulation (P_1 exp.) and the number of eggs produced after the first mating (P_1 exp.; different variances allowed for the first and the second female, varIdent function in R) because it improved the models based on likelihood ratio test (REML) and decreased heteroscedasticity in residuals. The optimal fixed structure of the models was determined by comparing nested models using likelihood ratio tests (maximum likelihood, ML) and the final model was refitted with REML as suggested by Zuur et al. (2009). We performed model validations by examining the homogeneity and independence of errors.

We were not able to find a transformation (even with the Box–Cox method) to normalize residuals in the offspring viability data after the first mating. Therefore these data were analyzed with a nonparametric paired Wilcoxon signed rank test. All statistical analyses were performed with R 2.10.1 (R Development Core Team 2009).

Results

There was no difference in the egg-to-adult viability of offspring sired by brothers (mean $\pm$ SD, 0.83 ± 0.21) or unrelated males (0.86 ± 0.20) measured over two days after the first mating in P_1 experiment (Wilcoxon $V = 277.5$, $P = 0.54$, $N = 35$). Therefore,

we did not adjust P_1 and P_2 values for offspring viability. Viabilities were estimated on average from 134 ± 69 eggs produced per female. P_1 and P_2 values were estimated on average from 118 ± 49 and 127 ± 47 offspring produced per female, respectively.

We found no evidence for postcopulatory adaptations for inbreeding avoidance in *D. melanogaster*. Full-siblings of females exhibited levels of competitive fertilization success, in both first and second male roles, that were not statistically different from males that were genetically unrelated to females (Tables 1 and 2). Parameter estimates from the full models for the difference between full-sibling and unrelated treatment are 0.0017 (SE of the estimate 0.0028) for the P1 experiment and 0.0016 (SE 0.0007) for the P2 experiment, with full-sibling treatments having the lower values. Corresponding results were obtained for all of the quantified reproductive traits that individually could represent adaptations contributing to any patterns in sperm precedence. Specifically, neither the time until the first copulation nor copulation duration was affected by whether the male was a sibling or a nonsibling to each virgin female in the P_1 experiment. Also, females did not alter the rate of egg production, the remating interval or the duration of copulation with the standard competitor male after being inseminated by a full-sibling as compared to an unrelated male (Tables 1 and 2). Similarly in the P_2 experiment, nonvirgin females did not delay remating or alter copulation duration when presented with full-siblings as compared to unrelated males, nor did they alter their egg laying behavior based on the relatedness of their second mate (Tables 1 and 2).

Discussion

The existence of inbreeding avoidance mechanisms is intuitive for species in which matings between relatives are likely to occur and inbreeding depression is severe, such as the sand lizard (Olsson et al. 1996a,b), the plant *S. latifolia* (Teixeira et al. 2009), or the least killifish (Ala-Honkola et al. 2009, 2010). Population sizes of the least killifish have been shown to collapse during droughts and to recover slowly (Ruetz et al. 2005), with subsequent population growth primarily from reproduction by surviving individuals rather than by migrants (Ruetz et al. 2005). Consequently, mating between relatives is quite likely, with matings between siblings resulting in severe inbreeding depression in numerous traits (Ala-Honkola et al. 2009).

Even though inbreeding depression may occur in *D. melanogaster* (e.g., Charlesworth and Charlesworth 1999), the population and reproductive biology of this species does not suggest that adaptations to avoid inbreeding should be expected. Natural populations of *D. melanogaster* tend to be very large, consisting of thousands of individuals (e.g., Kusakabe et al. 2000; Shapiro et al. 2007) with high dispersal capability (Coyne and Milstead 1987). Hence, matings between relatives should be uncommon. Moreover, because females remate frequently in nature (i.e., every few days; e.g., Harshman and Clark 1998; Imhoff et al. 1998; Kuijper and Morrow 2009), with sperm displacement resulting in high last-male sperm precedence (Bjork et al. 2007; Manier et al. 2010), the fitness costs associated with insemination by a rare, closely related individual would be low.

Table 1. Means, standard deviations, and the significance of the factor relatedness in full (linear mixed effects) models comparing P_1, P_2, and other reproductive behaviors between full-sibling and unrelated pair treatments.

TRAIT	Mean (SD)		Significance of relatedness in the full model		
	Sibling	Unrelated	df	*t*	*P*-value
P1 EXPERIMENT					
P1	0.15 (0.14)	0.18 (0.18)	32	−0.79	0.43
Time to first copulation (min)	28.4 (27.1)	24.9 (19.9)	34	0.8	0.43
Copulation duration with the first male (test male) (min)	26.4 (9.2)	24.9 (6.7)	34	0.84	0.4
Number of eggs produced before remating	135 (78)	131 (61)	35	0.42	0.68
Remating day	3.2 (1.0)	3.2 (1.2)	36	0	1
Copulation duration with the second male (min)	*23.5 (5.7)	*24.5 (4.9)	35	−1.41	0.17
P2 EXPERIMENT					
P2	0.80 (0.17)	0.83 (0.15)	56	−1.52	0.13
Copulation duration with the second male (test male) (min)	29.7 (6.5)	29.8 (6.6)	56	−0.10	0.92
Remating day	2.9 (1.0)	3.0 (1.0)	58	−0.87	0.39
Number of eggs laid after remating	151 (43)	138 (45)	58	1.65	0.10

*Refers to whether the first male was a sibling or unrelated.

Table 2. Fixed effects of final (linear mixed effects) models for P_1, P_2, and other reproductive behaviors measured.

Measured trait	Fixed effect	Parameter estimate	SE	df	*t*	*P*-value
P1 EXPERIMENT						
P1	Intercept (arc sine sqrt transformed)	0.39	0.03	35	15.2	0.000
Time to first copulation (min)	Intercept (log-transformed)	2.99	0.1	36	29.6	0.000
Copulation duration with the first male (test male) (min)	Intercept (log-transformed)	3.2	0.04	36	89.7	0.000
Number of eggs produced before remating	Intercept	−8.47	14.1	36	−0.6	0.552
	Female order (second)	−23.95	10.0	36	−2.39	0.022
	Remating day	48.2	4.1	36	11.8	0.000
Remating day	Intercept	3.2	0.13	38	23.8	0.000
Copulation duration with the second male (min)	Intercept	24.9	0.87	36	28.6	0.000
	Female order (second)	−1.9	0.77	36	−2.45	0.019
P2 EXPERIMENT						
P2	Intercept (arc sine sqrt transformed)	1.06	0.05	57	20.4	0.000
	Male mating order (sib first)	−0.10	0.05	38	−2.17	0.037
	Female order (second)	0.06	0.03	57	2.03	0.047
	No. of eggs prod. before remating	0.0009	0.0003	57	3.46	0.001
Copulation duration with the second male (test male) (min)	Intercept	30.8	0.96	57	32.1	0.000
	Female order (second)	−2.3	0.86	57	−2.65	0.010
Remating day	Intercept	3.0	1.0	60	30.5	0.000
Number of eggs laid after remating	Intercept	145	4.1	60	35.7	0.000

Consistent with this contention, we found no evidence that male–female relatedness influences competitive fertilization success in *D. melanogaster*. In other words, males of our study population do not appear to strategically ejaculate (Wedell et al. 2002), and females do not strategically select sperm (Birkhead 1998), based on the relatedness of their mates. An independent set of experiments executed contemporaneously with the present study and using the same experimental population (RFP-LH_M) revealed that males of this population are capable of extraordinarily sophisticated tailoring of the number of sperm ejaculated based on female mating status, fecundity, and age (Lüpold et al. 2011), indicating that such facultative adjustments are generally possible. Some of our results further indicate no premating inbreeding avoidance by *D. melanogaster*. Specifically, females mated just as quickly with sibling first or second males as with unrelated first or second males. Mating speed is commonly used as an index of male attractiveness (e.g., Taylor et al. 2007; Hosken et al. 2008). These results complement an extensive study by Wei et al. (unpubl. ms.) demonstrating very little premating inbreeding avoidance in a different experimental population of *D. melanogaster*.

Our findings contrast with an earlier report by Mack et al. (2002) showing that full-sibling males had significantly lower P_1 scores relative to unrelated males, although lesser degrees of relatedness (e.g., half-siblings; $R_{1/4}$) did not influence P_1. In the Mack et al. (2002) study, the effect of relatedness was about 0.06 with siblings and unrelated males having P_1 values of 0.23 and 0.29, respectively. Our parameter estimates for relatedness effects (from the full statistical models) were much lower (0.0017 for P_1 and 0.0016 for P_2) and the differences between full-sibling and unrelated means were 0.03 both in the P_1 and P_2 experiments (Table 1). Thus, in both studies the differences between treatments were in the same direction and relatively small. Unfortunately, neither P_2 nor other possible mechanisms of discrimination were examined by Mack et al. (2002), thus precluding more extensive comparisons with our study.

One obvious potential explanation for the differing results is divergent adaptation between the respective experimental populations of *D. melanogaster.* The population used by Mack et al. (2002) was collected from nature (Watkinsville, Georgia) in 2000, whereas we used an RFP-sperm line that had been backcrossed to an LH_M-population for six generations. LH_M is a large outbred wild-type population founded in 1991 from central California (Chippindale et al. 2001). It has adapted to the laboratory for over 200 generations and has been maintained in population cages supporting over 1000 individuals. Even though DNA microsatellite data indicate that population subdivision in North

American *D. melanogaster* is low (likely due the commensal habits and the high dispersal capability of these flies; Coyne and Milstead 1987), there are differences between populations from the east and the west coast of the United States (Caracristi and Schlötterer 2003).

It is noteworthy that we did not find inbreeding depression in egg-to-adult viability in our study, also in contrast to Mack et al. (2002). It could thus be argued that the two populations differ in the intensity of selection for inbreeding avoidance. However, subsequent experiments in our laboratory on the same study population as reported on here have revealed significant inbreeding depression in P_2 and in mating latency of both sexes (O. Ala-Honkola, unpubl. data). Inbreeding depression in P_2 is likely to decrease fitness as P_2 is linked with lifetime reproductive success (Fricke et al. 2010). Thus, inbreeding is not totally cost free in our study population, although it is likely to be less harmful than in the study population of Mack et al. (2002). Populations may vary in the severity of inbreeding depression depending on the level of ancestral inbreeding, with higher levels of ancestral inbreeding having purged deleterious alleles and thus resulting in lower inbreeding depression (Swindell and Bouzat 2006a,b). As investigations with diverse taxa accumulate, a more nuanced understanding of the relationships between inbreeding risk, inbreeding depression, and the evolution of mechanisms to both discriminate relatedness of mates and/or gametes and to respond adaptively to such recognition should emerge.

ACKNOWLEDGMENTS

We are grateful to K. Berben and L. Paish for technical assistance. We thank C. Wei, H. Løvlie, S. Wigby, T. Pizzari, and two anonymous referees for helpful comments on earlier drafts of this manuscript. This research was supported by grants from the Academy of Finland to OA-H. (135684), the Swiss National Science Foundation to SL (PBSKP3_130878), and from the U.S. National Science Foundation to SP (DEB-0814732).

LITERATURE CITED

Ala-Honkola, O., A. Uddström, B. Diaz Pauli, and K. Lindström. 2009. Strong inbreeding depression in male mating behaviour in a poeciliid fish. J. Evol. Biol. 22:1396–1406.

Ala-Honkola, O., L. Tuominen, and K. Lindström. 2010. Inbreeding avoidance in a poeciliid fish (*Heterandria formosa*). Behav. Ecol. Sociobiol. 64:1403–1414.

Birkhead, T. R. 1998. Cryptic female choice: criteria for establishing female sperm choice. Evolution 52:1212–1218.

Bjork, A., W. T. Starmer, D. M. Higginson, C. J. Rhodes, and S. Pitnick. 2007. Complex interactions with females and rival males limit the evolution of sperm offence and defence. Proc. R. Soc. Lond. B. 274:1779–1788.

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.

Bretman, A., D. Newcombe, and T. Tregenza. 2009. Promiscuous females avoid inbreeding by controlling sperm storage. Mol. Ecol. 18:3340–3345.

Caracristi, G., and C. Schlötterer. 2003. Genetic differentiation between American and European *Drosophila melanogaster* populations could be attributed to admixture of African alleles. Mol. Biol. Evol. 20:792–799.

Charlesworth, B., and D. Charlesworth. 1999. The genetic basis of inbreeding depression. Genet. Res. 74:329–340.

Chippindale, A. K., J. R. Gibson, and W. R. Rice. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. Proc. Natl. Acad. Sci. USA 98:1671–1675.

Coyne, J. A., and B. Milstead. 1987. Long-distance migration of *Drosophila*. 3. Dispersal of *Drosophila melanogaster* alleles from a Maryland orchard. Am. Nat. 130:70–82.

Denk, A. G., A. Holzmann, A. Peters, E. L. M. Vermeissen, and B. Kempenaers. 2005. Paternity in mallards: effects of sperm quality and female sperm selection for inbreeding avoidance. Behav. Ecol. 16:825–833.

Evans J. P., R. C. Brooks, S. R. K. Zajitschek, and S. C. Griffith. 2008. Does genetic relatedness of mates influence competitive fertilization success in guppies? Evolution 62:2929–2935.

Firman, R. C., and L. W. Simmons. 2008. Polyandry facilitates postcopulatory inbreeding avoidance in house mice. Evolution 62:603–611.

Fricke, C., O.Y. Martin, A. Bretman, L. F. Bussière, and T. Chapman. 2010. Sperm competitive ability and indices of lifetime reproductive success. Evolution 64:2746–2757.

Frommen, J. G., and T. C. M. Bakker. 2006. Inbreeding avoidance through nonrandom mating in sticklebacks. Biol. Lett. 2:232–235.

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.

Harshman, L. G., and A. G. Clark. 1998. Inference of sperm competition from broods of field-caught *Drosophila*. Evolution 52:1334–1341.

Hosken, D. J., M. L. Taylor, K. Hoyle, S. Higgins, and N. Wedell. 2008. Attractive males have greater success in sperm competition. Curr. Biol. 18:R553–R554.

Imhoff, M., B. Harr, G. Brem, and C. Schlötterer. 1998. Multiple mating in wild *Drosophila melanogaster* revisited by microsatellite analysis. Mol. Ecol. 7:915–917.

Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends Ecol. Evol. 17:230–241.

Kokko, H., and I. Ots. 2006. When not to avoid inbreeding. Evolution 60:467–475.

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.

Kuijper, B. and Morrow E. H. 2009. Direct observation of female mating frequency using time-lapse photography. Fly 3:118–120.

Kusakabe, S., Y. Yamaguchi, H. Baba, and T. Mukai. 2000. The genetic structure of the Raleigh natural population of *Drosophila melanogaster* revisited. Genetics 154:679–685.

Lane, J. E., S. Boutin, M. R. Gunn, J. Slate, and D. W. Coltman. 2007. Genetic relatedness of mates does not predict patterns of parentage in North American red squirrels. Anim. Behav. 74:611–619.

Lihoreau, M., C. Zimmer, and C. Rivault. 2007. Kin recognition and incest avoidance in a group-living insect. Behav. Ecol. 18:880–887.

Lüpold, S., M. K. Manier, O. Ala-Honkola, J. M. Belote, and S. Pitnick. 2011. Male *Drosophila melanogaster* adjust ejaculate size based on female mating status, fecundity and age. Behav. Ecol. 22:184–191.

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.

Manier, M. K., J. M. Belote, K. S. Berben, D. Novikov, W. T. Stuart, and S. Pitnick. 2010. Resolving mechanisms of competitive fertilization success in *Drosophila melanogaster*. Science 328:354–357.

Olsson, M., A. Gullberg, and H. Tegelstrom. 1996b. Malformed offspring, sibling matings, and selection against inbreeding in the sand lizard (*Lacerta agilis*). J. Evol. Biol. 9:229–242.

Olsson, M., R. Shine, and T. Madsen. 1996a. Sperm selection by females. Nature 383:585.

Pizzari, T., H. Løvlie, and C. K. Cornwallis. 2004. Sex-specific, counteracting responses to inbreeding in a bird. Proc. R. Soc. Lond. B. 271:2115–2121.

Pusey, A., and M. Wolf. 1996. Inbreeding avoidance in animals. Trends Ecol. Evol. 11:201–206.

R Development Core Team. 2009. R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. ISBN 3–900051-07–0, URL http://www.R-project.org (accessed December 1, 2010).

Ruetz III, C. R., J. C. Trexler, F. Jordan, W. F. Loftus, and S. A. Perry. 2005. Population dynamics of wetland fishes: spatio-temporal patterns synchronized by hydrological disturbance? J. Anim. Ecol. 74:322–332.

Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491–494.

Shapiro, J. A., W. Huang, C. H. Zhang, M. J. Hubisz, J. Lu, D. A. Turissini, S. Fang, H. Y. Wang, R. R. Hudson, R. Nielsen et al. 2007. Adaptive genic evolution in the *Drosophila* genomes. Proc. Natl. Acad. Sci. USA 104:2271–2276.

Sherman, C. D. H., E. Wapstra, T. Uller, and M. Olsson. 2008. Males with high genetic similarity to females sire more offspring in sperm competition in Peron's tree frog *Litoria peronii*. Proc. R. Soc. Lond. B 275:971–978.

Simmons, L. W., M. Beveridge, N. Wedell, and T. Tregenza. 2006. Postcopulatory inbreeding avoidance by female crickets only revealed by molecular markers. Mol. Ecol. 15:3817–3824.

Stockley, P. 1997. No evidence of sperm selection by female common shrews. Proc. R. Soc. Lond. B. 264:1497–1500.

Swindell, W. R., and J. L. Bouzat. 2006a. Reduced inbreeding depression due to historical inbreeding in *Drosophila melanogaster*: evidence for purging. J. Evol. Biol. 19:1257–1264.

———. 2006b. Ancestral inbreeding reduces the magnitude of inbreeding depression in *Drosophila melanogaster*. Evolution 60:762–767.

Taylor, M. L., N. Wedell, and D. J. Hosken. 2007. The heritability of attractiveness. Curr. Biol. 17:R959–R960.

Teixeira, S., K. Foerster, and G. Bernasconi. 2009. Evidence for inbreeding depression and post-pollination selection against inbreeding in the dioecious plant *Silene latifolia*. Heredity 102:101–112.

Teng, Z., and L. Kang. 2007. Egg-hatching benefits gained by polyandrous female locusts are not due to the fertilization advantage of nonsibling males. Evolution 61:470–476.

Thuman, K. A., and S. C. Griffith. 2005. Genetic similarity and the nonrandom distribution of paternity in a genetically highly polyandrous shorebird. Anim. Behav. 69:765–770.

Tregenza, T., and N. Wedell. 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. Mol. Ecol. 9:1013–1027.

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.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. S. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer, New York, NY.

Associate Editor: T. Chapman