

Heritable variation in the timing of spermatophore removal, a mechanism of post-copulatory female choice in crickets

B. S. MAUTZ¹ & S. K. SAKALUK

Behaviour, Ecology, Evolution and Systematics Section, Department of Biological Sciences, Illinois State University, Normal, IL, USA

Keywords:

Acheta domesticus;
heritability;
post-copulatory female choice;
sexual selection;
spermatophore.

Abstract

Female crickets can exert post-copulatory mating preferences by prematurely removing a male's spermatophore after copulation, which terminates sperm transfer. Although most models of sexual selection assume that female mating preferences are heritable, there has been little work addressing genetic variation underlying post-copulatory mate choice. We used a paternal half-sib design, in which different males were randomly assigned as mates to several females to create half-sib families, to determine the heritability of spermatophore retention time in female house crickets, *Acheta domesticus*. There was significant additive genetic variance in the timing of spermatophore removal by females [$h^2 = 0.50 \pm 0.19$ (\pm SE)], suggesting that the timing of spermatophore removal is determined, in part, by the female's own genotype independent of the quality of her mate. The relatively high heritability of spermatophore retention time may be reflective of the absence of strong selection on this trait, consistent with previous work showing no difference in the fitness of females permitted to freely remove the spermatophore of their mates and those forced to accept complete ejaculates.

Introduction

Female insects can determine which males sire their young even after copulation has occurred by prematurely terminating copulations, failing to store transferred sperm, removing or ejecting stored sperm, or delaying oviposition until after mating with desirable males (Eberhard, 1996). Such mechanisms represent a form of post-copulatory female choice because they often lead to differential fertilization success of males (Thornhill, 1983).

Crickets offer an ideal model with which to examine the evolution of post-copulatory female choice: the ejaculate of a male typically remains attached outside the female's genital opening after mating in the form of an externally attached spermatophore, and females are thus well positioned to influence sperm transfer by removing the spermatophore before complete sperm

transfer has occurred (Sakaluk, 1984; Simmons, 1986; Sakaluk & Eggert, 1996; Ivy & Sakaluk, 2007). Premature removal of the spermatophore has a significant influence on male fertilization success, with those males transferring a greater number of sperm siring a higher proportion of offspring (Sakaluk, 1986; Sakaluk & Eggert, 1996; Calos & Sakaluk, 1998; Eggert *et al.*, 2003; García-González & Simmons, 2005; Bussière *et al.*, 2006).

An underlying assumption of studies of post-copulatory mate choice is that females' preferences vary primarily according to the perceived quality of their prospective mates. However, factors intrinsic to females and unrelated to male phenotype, such as female age, condition or previous mating history, can affect the costs of choice and thereby influence females' preferences (Prosser *et al.*, 1997; Moore & Moore, 2001; Byers *et al.*, 2006; Mautz & Sakaluk, 2008). Another largely unexplored factor is genetic variation in the actual mechanism(s) mediating the female preference. For example, if there was genetic variation in the propensity of females to discard sperm, a common form of post-copulatory choice (Eberhard, 1996), a female's decision to use a male's sperm in fertilizations might be mediated, in part, by her own genotype, independent of the male's phenotype.

Correspondence: Scott K. Sakaluk, Behaviour, Ecology, Evolution and Systematics Section, Department of Biological Sciences, Illinois State University, Normal, IL 61790-4120, USA.
Tel.: +1 309 438 2161; fax: +1 309 438 3722;
e-mail: sksakal@ilstu.edu

¹*Present address:* School of Botany and Zoology, The Australian National University, Canberra ACT 0200, Australia.

Here we report the results of a study designed to determine the heritability of spermatophore retention time in female house crickets, *Acheta domesticus*, using a half-sib design in which different males were randomly assigned as mates to three different females. We predicted that if the timing of spermatophore removal was determined primarily by the quality of a female's mate, there should be little resemblance in the timing of spermatophore removal across half-sib females. However, if the timing of spermatophore removal is determined, in part, by the female's own genotype independent of the quality of her mate, there should be a significant heritability of spermatophore retention time reflected in the resemblance between female half-sibs.

Materials and methods

Experimental design

The parental generation of crickets was obtained as late instar nymphs purchased from Fluker Farms® (Baton Rouge, LA, USA), a commercial cricket supplier whose rearing practices have promoted the maintenance of genetic variation (Fleischman & Sakaluk, 2004). Crickets were housed by sex in 55-L ($59 \times 43 \times 30.5$ cm²) plastic storage containers. Unless otherwise noted, crickets were provisioned *ad libitum* throughout the experiment with Fluker's® cricket chow, water supplied in 40-mL plastic tissue culture flasks plugged with cotton dental rolls, and egg carton to increase rearing surface area, and maintained on a 16 : 8 light : dark cycle at 32° C. Adults were removed the day of their eclosion and held for 5 days until sexual maturity in sex-specific 4.3-L plastic shoebox containers, provisioned as above.

A paternal half-sib breeding design was used to partition phenotypic variation into causal components (Lynch & Walsh, 1998). We chose sires randomly from the population of crickets that had been obtained for any given 'round' of matings. Because space and time limitations necessitated the use of sires from different batches of crickets, it was necessary to include 'round' as a random factor in all subsequent analyses (see below). A total of thirty sires in the two rounds of matings produced the half-sib families. Each sire was paired for 24 h with each of three virgin females (dams), a period of time sufficient to ensure that each female mated with the sire (Kindle *et al.*, 2006). After mating, each female was held for 7 days in a separate plastic shoebox and allowed to oviposit into moistened peat moss. Females were then discarded and the eggs allowed to hatch. Newly hatched nymphs from dam families were divided into two separate plastic shoeboxes of 50 offspring each to control for common environmental effects, and reared to sexual maturity. The positions of the boxes within the environmental chamber were randomized daily to minimize local environmental effects and variance between families.

Experimental observations

Two F1 females and two F1 males were randomly selected from each box within 2 days of adult eclosion, yielding a total of four females and four males per dam, and 12 offspring of each sex per sire. All offspring were weighed using a Mettler® Toledo AG245 electronic balance to the nearest 0.01 mg. After they had been weighed, females were held separately in 0.71-L containers until their use in mating trials. Experimental mating trials were staged in Plexiglas mating chambers ($10.6 \times 3.4 \times 8.0$ cm) illuminated with red light. Each female was paired with a randomly selected, sexually experienced male obtained from the stock colony. Mating in house crickets is initiated when the female mounts a courting male, at which time he attempts to transfer the spermatophore to the female (Khalifa, 1950). We recorded the spermatophore retention time as the time from when the female dismounted the male to the time at which the female removed the spermatophore. If a male failed to court the female within 10 min of his introduction to the mating chamber, he was replaced with a new male because females will not mount males that do not court (Nelson & Nolen, 1997).

Statistical analysis

Data were analyzed using PROC MIXED in SAS version 9.1 (SAS Institute Inc., 2002). All variables were log-transformed to meet the assumption of restricted maximum likelihood (REML). All effects (round, sire, dam, and box) were treated as random effects using the following model: $Y_{ijk} = \mu + R_m + S_j + D_{jk} + B_{jkl} + E_{ijkl}$. Dam was nested under sire in the analysis. Variance component estimates were tested for significance using the χ^2 -restricted likelihood ratio test between the full statistical model and a model where the parameter of interest was constrained to zero (Fry, 2004). The random round effect (R_m) was significant for both male and female mass (though not for spermatophore retention time), and hence, was retained in the analysis. Variance component parameters were generated using REML by expressing the covariance between families as a linear function of the four causal components in the model. Negative estimates of variance were constrained to be zero. Narrow-sense heritabilities were calculated following Lynch & Walsh (1998). To compare sources of phenotypic variation within traits, and sources of variation among different fitness traits, coefficients of variation (CV) for each parameter estimate were calculated according to Houle (1992).

Results

Due to the loss of some offspring, the sample size of the experiment was reduced for each family (Table 1). Variance due to sires did not contribute to mass of

	No. sires	No. dams	No. boxes	No. offspring	Mean	CI (lower, upper)
Female mass (mg)	30	90	175	331	393	(318, 484)
Male mass (mg)	30	90	176	346	351	(297, 415)
Spermatophore retention time (min)	30	90	175	330	22.0	(8.7, 55.3)

Table 1 Mean body mass of male and female half-sib *Acheta domesticus*, and mean spermatophore retention time of mated female half-sibs. Means and confidence intervals (CI) were back-transformed from log-transformed data.

Table 2 Parameter estimates of variance components and coefficient of variation for female and male body mass, and the timing of spermatophore removal by females.

Source	Female mass			Male mass			Spermatophore retention time		
	Estimate	CV	Proportion of variation	Estimate	CV	Proportion of variation	Estimate	CV	Proportion of variation
V_A	4.74×10^{-6}	0.003	0.01	2.21×10^{-5}	0.001	0.03	$8.05 \times 10^{-2**}$	0.90	0.39
V_{Dam}	$1.58 \times 10^{-3*}$	0.007	0.21	$4.09 \times 10^{-4*}$	0.006	0.17	$5.97 \times 10^{-3*}$	0.25	0.11
V_{Round}	$1.56 \times 10^{-3***}$	0.01	0.21	$9.94 \times 10^{-4***}$	0.009	0.26	–	–	–
V_{Box}	$1.22 \times 10^{-3**}$	0.009	0.19	$6.05 \times 10^{-4**}$	0.007	0.20	–	–	–
V_E	5.01×10^{-3}	0.015	0.38	1.83×10^{-3}	0.012	0.34	1.62×10^{-1}	1.17	0.50

Parameter estimate values represent the REML estimate of each variance component from analyses using transformed data to meet test assumptions when necessary (see text).

CV, coefficient of variation based on Houle (1992).

V_A , additive genetic variance; V_{Dam} , variance due to dam; V_{Round} , variance due to round; V_{Box} , variance due to box; V_E , residual environmental variance. Statistically significant values shown in bold. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

offspring of either sex. However, variance due to dams contributed significantly to mass of offspring of both sexes, as did box in which the offspring were reared (Table 2). The confounding box effect should have no effect on interpretation of results given that full sib families were separated into two boxes controlling for the significant box effect in the parameter estimates. Spermatophore retention time showed significant variation due to both sire and dam effects (Table 2). Additionally, there was a significant phenotypic correlation between spermatophore retention time and body mass of female offspring ($r = 0.152$, $P < 0.01$).

Heritability of each trait was calculated from the variance component estimates (Lynch & Walsh, 1998). Spermatophore retention time was significantly heritable [$h^2 = 0.50 \pm 0.19$ (\pm SE)], whereas neither male body mass, nor female body mass, were significantly heritable ($h^2 < 0.01$ in both cases).

Discussion

A growing number of studies across a diverse array of taxa, including various insect species (Gray & Cade, 1999; Jang & Greenfield, 2000; Ritchie, 2000; Iyengar *et al.*, 2002; Reinhold *et al.*, 2002; Mühlhäuser & Blanckenhorn, 2004), fish (Bakker, 1993; Brooks, 2002) and birds (Qvarnström *et al.*, 2006), have revealed significant heritable variation in the mating preferences of females. However, the overwhelming majority of studies have focused on the heritability of females' precopulatory

preferences, and there has been little work addressing the genetic variation underlying post-copulatory mate choice. A notable exception is the study by Clark *et al.* (1999) on sperm competition in *Drosophila*, which revealed that the success of a particular male's sperm in securing fertilizations is contingent, in part, on the genotype of the female. In the present study, we found significant heritable variation in the timing of spermatophore removal by female house crickets, a pervasive mechanism of post-copulatory female choice in crickets (Sakaluk, 1984; Simmons, 1986; Bussière *et al.*, 2006). This result is consistent with a previous study showing that the timing of spermatophore removal is repeatable across successive matings in female *A. domesticus* (Fleischman & Sakaluk, 2004).

Studies of post-copulatory female choice in crickets have shown that the differential spermatophore removal behavior of females can promote sexual selection on males affecting a variety of phenotypic attributes including the size of food gifts synthesized by males (Fedorka & Mousseau, 2002), body size (Sakaluk, 1985; Simmons, 1986; Bateman *et al.*, 2001), and hindwing morphology (Sakaluk, 1997). Models of sexual selection addressing the coevolution of male traits and female mating preferences generally assume that there is heritable variation in both the male trait and the female preference (reviews in Brooks & Endler, 2001; Mühlhäuser & Blanckenhorn, 2004). Although the present results provide some support to this assumption, the extent to which post-copulatory female mating

preferences and target male traits are genetically correlated remains unknown.

Why the heritability of spermatophore removal time should be so high (~ 0.50) remains unclear, especially given that behavioural traits commonly are characterized by low additive genetic variation (Mousseau & Roff, 1987). It may be that the timing of spermatophore removal is not under particularly strong selection. In support of this possibility, Fleischman & Sakaluk (2004) found no difference in the longevity, reproductive output, and offspring quality of female *A. domesticus* permitted to freely remove the spermatophore of their mates and those that were forced to accept complete ejaculates from their mates. These results suggest that differential spermatophore removal does not provide any direct or indirect benefits to females (Fleischman & Sakaluk, 2004). In a related study, Jennions *et al.* (2004) found that female field crickets (*Teleogryllus commodus*), mated to both an unrelated and a sibling male, accepted similar amounts of sperm from both, even though inbreeding leads to decreased fitness; this too suggests that differential spermatophore removal is not necessarily related to female fitness.

An alternative explanation to account for the high heritability of spermatophore retention time is that the scope of post-copulatory female choice was reduced because females in this experiment were mating for the first time. It could be argued that in the absence of any additional mates, females lack a comparative basis upon which to selectively bias the paternity of their offspring through differential spermatophore removal. This, in turn, could eliminate an important residual source of variation in the timing of spermatophore removal, leading to an inflated heritability estimate. However, female crickets typically mate with many different males, and the vast majority of females mating for the first time invariably mate with additional males (review in Sakaluk *et al.*, 2002). Premature removal of the spermatophore by a female mated to an unattractive male would, therefore, reduce his relative fertilization success even if he was the first male to mate with a female (Sakaluk & Eggert, 1996; Bussière *et al.*, 2006). Moreover, Fleischman & Sakaluk (2004) showed that the timing of spermatophore removal was significantly repeatable for female *A. domesticus* mated to five different males, so that the spermatophore retention times of singly mated females appear to be a reasonable proxy for those of multiply-mated females.

We found no measurable additive genetic variation due to sire in mass of offspring of either sex, contrary to a previous study (Ryder & Siva-Jothy, 2001). There was, however, significant variation attributable to dam, which includes additive genetic effects, maternal effects, dominance effects, and shared half-sib environment, among other sources (Lynch & Walsh, 1998). Although we can rule out additive genetic variation as a source of variation in body mass, the half-sib design used in the present

study does not allow us to determine the relative contribution of each of the remaining sources of variation.

A recent study of factors influencing the timing of spermatophore removal in female *A. domesticus* showed that it was unrelated to the mass of the male (Mautz & Sakaluk, 2008). In the present study, however, the time after mating at which the spermatophore was removed was positively correlated with the female's own body mass. Such a relationship could arise because of a functional constraint, a selective constraint or both (Arnold, 1992). First, larger females appear to have greater difficulty reaching around to remove the spermatophore relative to smaller females (B.S. Mautz, pers. obs.), resulting in increased spermatophore retention time. Second, a selective constraint could arise as a consequence of larger females having higher fecundity (Gray, 1997) and a concomitant demand for larger quantities of sperm to avoid sperm limitation (Wedell *et al.*, 2002). Positive selection acting on female body size could result in a preponderance of females who retain spermatophores longer, thus limiting females' options to discriminate among males through premature spermatophore removal.

Acknowledgments

We are indebted to C. Weddle, C. Koenig, J. Leman, and J. Sakaluk for help in rearing and observing crickets. We thank T. Ivy, M. Jennions and two anonymous referees for helpful comments on the manuscript, and D. Byers and R. Bowden for useful suggestions on the design of the study. This research was funded by grants from the National Science Foundation to SKS and C. G. Hamaker, and grants from the Beta Lambda chapter of the Phi Sigma Biological Honours Society to BSM. Additional support to BSM was provided by an NSF GK-12 award to C. J. Moore.

References

- Arnold, S.J. 1992. Constraints on phenotypic evolution. *Am. Nat.* **140**: S85–S107.
- Bakker, T.C.M. 1993. Positive genetic correlation between female preference and preferred ornament in sticklebacks. *Nature* **363**: 255–257.
- Bateman, P.W., Gilson, L.N. & Ferguson, J.W.H. 2001. Male size and sequential mate preference in the cricket *Gryllus bimaculatus*. *Anim. Behav.* **61**: 631–637.
- Brooks, R. 2002. Variation in female mate choice within guppy populations: population divergence, multiple ornaments and the maintenance of polymorphism. *Genetica* **116**: 343–358.
- Brooks, R. & Endler, J.A. 2001. Female guppies agree to differ: phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution* **55**: 1644–1655.
- Bussière, L.F., Hunt, J., Jennions, M.D. & Brooks, R. 2006. Sexual conflict and cryptic female choice in the black field cricket, *Teleogryllus commodus*. *Evolution* **60**: 792–800.

- Byers, J.A., Byers, A.A. & Dunn, S.J. 2006. A dry summer diminishes mate search effort by pronghorn females: evidence for a significant cost of mate search. *Ethology* **112**: 74–80.
- Calos, J.B. & Sakaluk, S.K. 1998. Paternity of offspring in multiply-mated female crickets: the effect of nuptial food gifts and the advantage of mating first. *Proc. R. Soc. Lond. B* **265**: 2191–2195.
- Clark, A.G., Begun, D.J. & Prout, T. 1999. Female x male interactions in *Drosophila* sperm competition. *Science* **283**: 217–220.
- Eberhard, W.G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton, NJ.
- Eggert, A.-K., Reinhardt, K. & Sakaluk, S.K. 2003. Linear models for assessing mechanisms of sperm competition: the trouble with transformations. *Evolution* **57**: 173–176.
- Fedoraka, K.M. & Mousseau, T.A. 2002. Material and genetic benefits of female multiple mating and polyandry. *Anim. Behav.* **64**: 361–367.
- Fleischman, R.R. & Sakaluk, S.K. 2004. No direct or indirect benefits to cryptic female choice in house crickets (*Acheta domesticus*). *Behav. Ecol.* **15**: 793–798.
- Fry, J.D. 2004. Estimation of genetic variances and covariances by restricted maximum likelihood using PROC MIXED. In: *Genetic Analysis of Complex Traits Using SAS* (A.M. Saxton, ed.), pp. 11–34. SAS Publishing, Cary, NC.
- García-González, F. & Simmons, L.W. 2005. Sperm viability matters in insect sperm competition. *Curr. Biol.* **15**: 271–275.
- Gray, D.A. 1997. *Sexual Selection, Phenotypic and Genetic Quality in House Crickets, Acheta Domesticus*. Ph.D. thesis, University of New Mexico, Albuquerque.
- Gray, D.A. & Cade, W.H. 1999. Quantitative genetics of sexual selection in the field cricket, *Gryllus integer*. *Evolution* **53**: 848–854.
- Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* **130**: 195–204.
- Ivy, T.M. & Sakaluk, S.K. 2007. Sequential mate choice in decorated crickets: females use a fixed internal threshold in pre- and postcopulatory choice. *Anim. Behav.* **74**: 1065–1072.
- Iyengar, V.K., Reeve, H.K. & Eisner, T. 2002. Paternal inheritance of a female moth's mating preference. *Nature* **419**: 830–832.
- Jang, E. & Greenfield, M.D. 2000. Quantitative genetics of female choice in an ultrasonic pyralid moth, *Achroia grisella*: variation and evolvability of preference along multiple dimensions of the male advertisement signal. *Heredity* **84**: 73–80.
- Jennions, M.D., Hunt, J., Graham, R. & Brooks, R. 2004. No evidence for inbreeding avoidance through postcopulatory mechanisms in the black field cricket, *Teleogryllus commodus*. *Evolution* **58**: 2472–2477.
- Khalifa, A. 1950. Sexual behavior in *Gryllus domesticus* L. *Behaviour* **2**: 264–274.
- Kindle, T.K., Johnson, K.M., Ivy, T.M., Weddle, C.B. & Sakaluk, S.K. 2006. Female mating frequency increases with temperature in two cricket species, *Gryllobates sigillatus* and *Acheta domesticus* (Orthoptera: Gryllidae). *Can. J. Zool.* **84**: 1345–1350.
- Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.
- Mautz, B.S. & Sakaluk, S.K. 2008. The effects of age and previous mating experience on pre- and post-copulatory mate choice in female house crickets (*Acheta domesticus* L.). *J. Insect Behav.* doi: 10.1007/s10905-008-9120-9.
- Moore, P.J. & Moore, A.J. 2001. Reproductive aging and mating: the ticking of the biological clock in cockroaches. *Proc. Natl Acad. Sci. USA* **98**: 1971–1976.
- Mousseau, T.A. & Roff, D.A. 1987. Natural selection and the heritability of fitness components. *Heredity* **59**: 181–197.
- Mühlhäuser, C. & Blanckenhorn, W.U. 2004. The quantitative genetics of sexual selection in the dung fly *Sepsis cynipsea*. *Behaviour* **141**: 327–341.
- Nelson, C.M. & Nolen, T.G. 1997. Courtship song, male agonistic encounters, and female mate choice in the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae). *J. Insect Behav.* **10**: 557–570.
- Prosser, M.R., Murray, A.M. & Cade, W.H. 1997. The influence of female age on phonotaxis during single and multiple song presentations in the field cricket, *Gryllus integer* (Orthoptera: Gryllidae). *J. Insect Behav.* **10**: 437–449.
- Qvarnström, A., Brommer, J.E. & Gustafsson, L. 2006. Testing the genetics underlying the co-evolution of mate choice and ornament in the wild. *Nature* **441**: 84–86.
- Reinhold, K., Reinhold, K. & Jacoby, K.J. 2002. Dissecting the repeatability of female choice in the grasshopper *Chorthippus biguttulus*. *Anim. Behav.* **64**: 245–250.
- Ritchie, M.G. 2000. The inheritance of female preference functions in a mate recognition system. *Proc. R. Soc. Lond. B* **267**: 327–332.
- Ryder, J.J. & Siva-Jothy, M.T. 2001. Quantitative genetics of immune function and body size in the house cricket, *Acheta domesticus*. *J. Evol. Biol.* **14**: 646–653.
- Sakaluk, S.K. 1984. Male crickets feed females to ensure complete sperm transfer. *Science* **223**: 609–610.
- Sakaluk, S.K. 1985. Spermatophore size and its role in the reproductive behaviour of the cricket, *Gryllobates supplicans* (Orthoptera: Gryllidae). *Can. J. Zool.* **63**: 1652–1656.
- Sakaluk, S.K. 1986. Sperm competition and the evolution of nuptial feeding behavior in the cricket, *Gryllobates supplicans* (Walker). *Evolution* **40**: 584–593.
- Sakaluk, S.K. 1997. Cryptic female choice predicated on wing dimorphism in decorated crickets. *Behav. Ecol.* **8**: 326–331.
- Sakaluk, S.K. & Eggert, A.K. 1996. Female control of sperm transfer and intraspecific variation in sperm precedence: antecedents to the evolution of a courtship food gift. *Evolution* **50**: 694–703.
- Sakaluk, S.K., Schaus, J.M., Eggert, A.-K., Snedden, W.A. & Brady, P.L. 2002. Polyandry and fitness of offspring reared under varying nutritional stress in decorated crickets. *Evolution* **56**: 1999–2007.
- SAS Institute Inc., 2002. *SAS Version 9.1*. SAS Institute Inc., Cary, NC.
- Simmons, L.W. 1986. Female choice in the field cricket *Gryllus bimaculatus*. *Anim. Behav.* **34**: 1463–1470.
- Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *Am. Nat.* **122**: 765–788.
- Wedell, N., Gage, M.J.G. & Parker, G.A. 2002. Sperm competition, male prudence and sperm-limited females. *Trends Ecol. Evol.* **17**: 313–320.

Received 29 March 2008; revised 5 May 2008; accepted 7 May 2008