Current Biology *17*, 32–36, January 9, 2007 ©2007 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2006.10.054

Report

Paternal Indirect Genetic Effects on Offspring Viability and the Benefits of Polyandry

Francisco García-González[1,*] and Leigh W. Simmons[1]
[1] Centre for Evolutionary Biology
School of Animal Biology, M092
The University of Western Australia
Nedlands, WA 6009
Australia

Summary

Although females are expected to maximize their reproductive success with only one or a few matings [1], the females of many species mate with multiple partners [2]. Experimental studies have found evidence for an increase in egg or embryo viability when females mate polyandrously [3]. These studies have been interpreted in the context of genetic-benefit models that propose that multiple mating increases offspring viability because it allows females to select male genotypes that influence viability directly or because it allows females to avoid genetic incompatibility [2, 4–8]. However, no studies have examined directly the precise mechanisms by which parents influence embryo viability. Using a morphological marker that enabled us to determine paternity and survival of embryos sired by individual male crickets in both sperm-competitive and -noncompetitive situations, we show that males inducing high embryo viability enhance the viability of embryos sired by inferior males. These results indicate that paternal effects and interacting phenotypes determine embryo viability. They show that a male's reproductive success is modified by the interaction between indirect genetic effects of sperm competitors. Importantly, our findings show that the benefits accruing to offspring of multiply mated females need not be transmitted genetically.

Results and Discussion

Parents contribute to offspring phenotype both genetically and environmentally. When the environment provided by the parents is determined genetically, then the environment is heritable, and its effects on offspring phenotype are known collectively as indirect genetic effects [9, 10]. Indirect genetic effects can result from the expression of maternal or paternal genes, in which case they are known as maternal and paternal effects, respectively [9, 10]. They can also occur when the individual in which the genetic effect is expressed and the individual whose phenotype is affected are unrelated, in which case they are known as interacting phenotypes [11]. Indirect genetic effects can have far-reaching implications for evolutionary processes because they allow responses to selection in traits for which there is no additive genetic variation [9–13]. Maternal effects on offspring performance are thought to be widespread because mothers usually provide nutritional or other environmental contributions to eggs, embryos, newborn offspring, or infants [9, 14, 15]. With notable exceptions [9, 16], paternal effects have been largely dismissed when males are not involved in extended parental care.

Previous studies of the Australian field cricket *Teleogryllus oceanicus* have shown that polyandrous females have a higher prehatching embryo viability than females mated the same number of times to single males [17] and that differences between males in their ability to induce embryo viability are heritable [18]. Additive genetic variation in embryo viability due to sires could arise because of genes that control development of the embryo per se. Interestingly, however, a role for male accessory-gland products on embryo viability was implicated by a genetic correlation between the hatching success induced by males and heritable variation in accessory-gland weight [18]. Thus, variation in embryo viability may represent a paternal indirect genetic effect.

In this study, we distinguish between paternal environment and paternal genetic effects on embryo viability. For this purpose we used crickets with a homozygous recessive morphological marker (white eye, *we*) that can be identified midway through embryo development. This enabled us to determine paternity and subsequent embryo viability of the offspring of two males, a *we* and a wild-type black eye (*be*) male mated to the same *we* female. We put this tool to the task of revealing, for the first time, changes in the survival of the embryos sired by individual males in both sperm-competitive and -noncompetitive contexts. The predictions arising from the existence of paternal genetic effects or paternal environmental effects can be tested with these data. If embryo survival is determined by the direct action of a sire's genes, a male inducing low or high embryo viability in his offspring with monandrous females should induce the same low or high embryo viability in his offspring when he mates in competition with another male, irrespective of his competitor's ability to sire viable offspring. In contrast, if paternal environment effects influence embryo viability, the survival of the embryos of a male inducing low embryo viability in his offspring with monandrous females should be increased in sperm-competitive situations involving a male with a greater ability to produce viable embryos. Conversely, the survival of embryos sired by a male capable of inducing high embryo viability might be reduced in sperm-competitive situations involving a male with inferior ability to produce viable embryos.

Mean (±1SE) fertilization success (proportion of eggs laid that absorbed water and began development; see Figure 1) across singly mated females (n = 49) was 88.24 ± 1.63% (range 60–100). The proportion of fertilized eggs that successfully hatched across monandrous females was also high (89.9 ± 1.35%, range 59–100). Across their monandrous females, fertilization success was repeatable for males (repeatability ± 1SE as in

*Correspondence: pgarcia@cyllene.uwa.edu.au

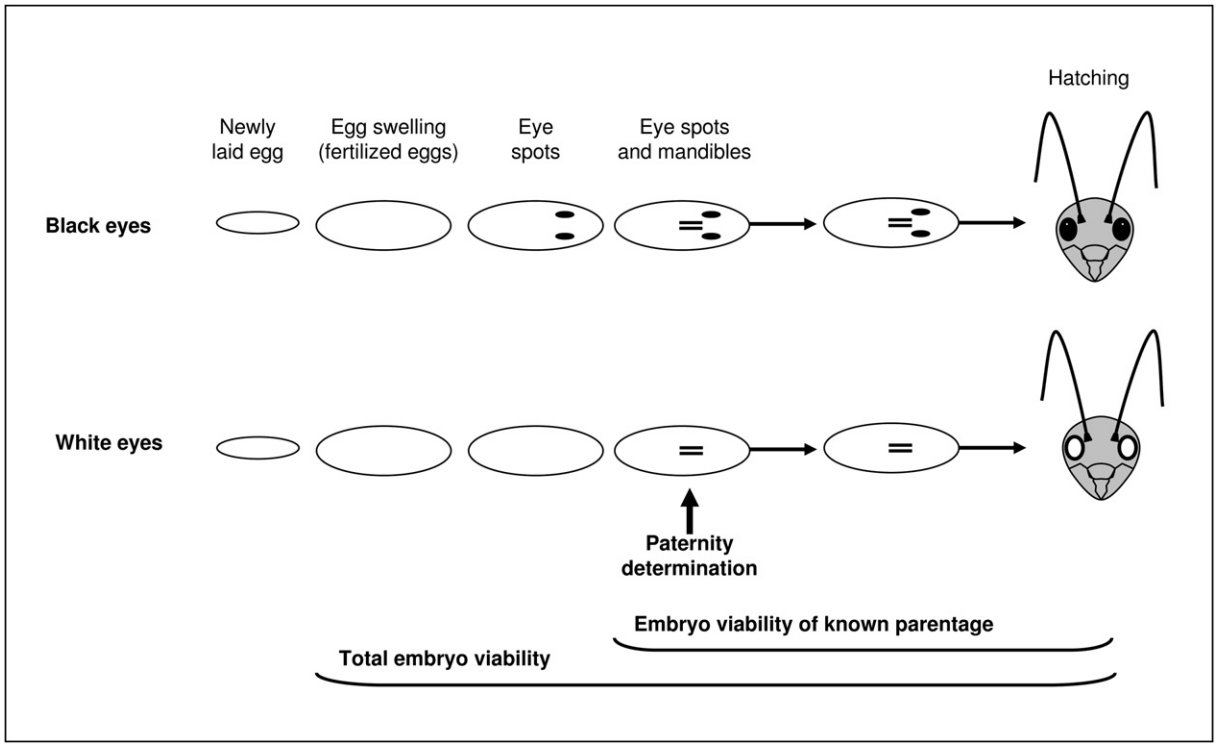

Figure 1. Outline of the Developmental Stages from which Embryo Viability and Paternity Were Determined

Fertilized eggs absorb water and swell within 2–3 days of being laid. Fertilization success was thus calculated as the proportion of eggs that swelled. After fertilization, we monitored the development of the embryos' head capsules, clearly visible through the chorion. Paternity could be assigned midway through embryo development (7–10 days after eggs were laid), when the mandibles began to sclerotize (as evidenced by two black marks visible through the chorion) and the developing eyes could be scored for color. We noted the number of eggs that reached the paternity-assignment stage and the number that successfully hatched (14–19 days after being laid). Embryo viability was not related to cricket morph (total embryo viability $F_{1,22} = 0.006$, $p = 0.93$; embryo viability from eye spots and mandibles to hatching $F_{1,22} = 0.9$, $p = 0.35$).

Becker [19], $R = 0.56 \pm 0.14$, $p = 0.001$). Consistent with previous evidence of additive genetic variation in male ability to sire viable embryos, the males in this study induced repeatable embryo viability across their two monandrously mated females (R of proportion of fertilized eggs that successfully hatched = 0.36 ± 0.18, $p = 0.035$). ANOVA with female family (block; see Experimental Procedures) as a random factor, and male identity as a random factor nested within the block, rendered weak and nonsignificant female-family effects on fertilization success (arcsine transformed) ($F_{11,25} = 2.11$, $p = 0.11$) and on the proportion of fertilized eggs that hatched (arcsine transformed) ($F_{11,25} = 2.52$, $p = 0.063$).

For each pair of males in each block, we determined the male with relatively higher embryo viability (HV) and the male with relatively lower embryo viability (LV) according to the average embryo viability across their two monandrous mates. The hypothesis of paternal genetic effects on embryo viability predicts no change in the survival of the embryos of LV males from monandrous to polyandrous situations, regardless of their competitors' ability to induce embryo viability. Alternatively, the hypothesis that paternal effects influence embryo viability predicts that when a female mates polyandrously, the survival of the embryos of LV males should be modified according to the sperm competitors' ability to induce embryo viability. We found the latter to be true: The greater the difference in embryo viability between the two males when mating singly, the greater the increase in embryo survival for the LV male when both males mated with the same female (Figure 2A). We bootstrapped regression coefficients by using 1000 simulations to gain 95% confidence limits for the relationship depicted in Figure 2A. The 95% confidence intervals on the regression coefficient did not overlap with zero (0.26, 1.81), indicating that the relationship is robust. HV-male embryo viability was also influenced by LV males. When both males were mated to the same female, the viability of embryos sired by HV males was decreased to a greater extent when the difference between the HV and LV males when mating singly was greater (Figure 2B). However, this effect seems weaker than that observed for the changes in LV-male embryo viability and may be driven by a single interacting pair (95% confidence intervals on the regression coefficient after bootstrapping: −3.48, 0.29).

We found no evidence to suggest that paternity was biased toward males able to induce high embryo viability. The proportion of offspring sired by the second male to mate (P_2) was independent of his ability to induce high viability: When paternity was assessed at the eye-spot stage of embryo development, the mean ± SE P_2 was 0.32 ± 0.10 for HV-LV matings and 0.14 ± 0.07 for LV-HV matings (t test for dependent samples, $t = 1.36$, d.f. 10, $p = 0.204$). Analysis of paternity at hatching yielded quantitatively similar results.

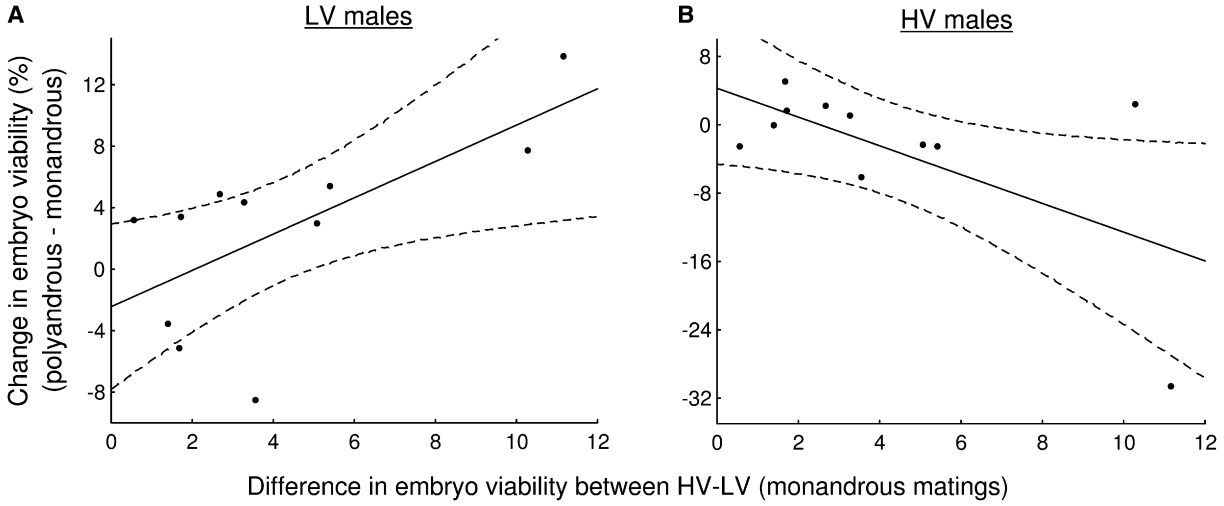

Figure 2. Interacting Paternal Effects on Offspring Viability

(A) The change in viability of an individual LV male's embryos from monandrous to polyandrous situations is plotted against the relative abilities of male pairs to induce embryo viability under monandry (LV: male of a pair with relatively low embryo viability. HV: male of a pair with relatively high embryo viability). This relationship is positive and significant ($r = 0.67$, $p = 0.025$, $n = 11$). Each data point belongs to one statistically independent block of full-sibling sisters.

(B) The change in viability of an individual HV male's embryos from monandrous to polyandrous situations is plotted against the relative abilities of male pairs to induce embryo viability under monandry ($r = -0.61$, $p = 0.045$, $n = 11$).

Discontinuous lines indicate the 95% confidence intervals for the fitted line.

Previously, we documented significant additive genetic variance among male crickets in their ability to produce viable embryos (heritability of embryo viability is 0.46 [18]). The data reported here suggest that these differences are unlikely to be due to genes that determine embryo viability per se but rather that they are due to heritable variation in paternal environments or to paternal indirect genetic effects [9, 10]. Our data indicate that environmental influences on embryos can originate as paternal effects in species in which males are not involved in parental care.

Given the genetic correlation between embryo viability and accessory-gland investment in this species [18], accessory-gland products (Acps) transferred with sperm at copulation seem to be the factors most likely to mediate the paternal effects we have observed. Not only do Acps influence aspects of female reproductive biology such as remating, oviposition, and sperm storage [20–22], but they can also play a critical role in egg development [22]. In some species, Acps stimulate both the production and uptake of yolk into oocytes [22], and a number of studies have shown that seminal-fluid products are incorporated in the eggs of mated females [23]. In the seed beetle *Stator limbatus*, offspring development time is determined by both the rearing host of the mother and that of the father [16], and it has been suggested that the paternal rearing host may affect the composition of a male's ejaculate, which would in turn affect the composition of the eggs laid by his mate [24]. In *Teleogryllus* it is known that accessory-gland products stimulate vitellogenesis and oviposition (reviewed in [25]). Genetic variation in the expression of these seminal-fluid proteins represents a mechanism by which differences in embryo viability between males can arise, and it accounts for indirect genetic effects of the kind we have documented in this study. Although our results show that environmental influences on embryos originate as paternal effects, we are unable to determine whether these effects impact developing embryos directly or whether they do so indirectly by generating changes in maternal provisioning.

Our data suggest that the effect of HV males on the viability of embryos sired by LV males may be stronger than the effect of LV males on the embryos sired by HV males. If embryo viability is determined by a threshold response to the quantitiy and/or quality of Acps received, we would expect to see increases in LV males' embryo viability but little or no change in the viability of HV males' offspring. Studies of seminal-product effects on oviposition in *T. commodus* suggest that females can exhibit either dose-dependent or threshold responses, depending on the chemical structure of injected proteins [26]. Further research into the precise mechanism of Acp action on embryo viability is required before firm conclusions can be drawn.

Our study holds important implications for postcopulatory sexual selection. Sperm-competition studies generally use paternity data collected at hatching or birth to calculate fertilization success. However, paternity assessed in this way can be confounded by differences in embryo mortality [27]. Our study highlights the fact that even though males might exhibit repeatability in competitive fertilization success, ultimately a male's paternity success depends also on his ability to induce embryo viability and on the abilities of his sperm competitors. This effect of interacting phenotypes on embryo viability will hold important implications for evolutionary responses to selection under sperm competition.

In a recent study of a viviparous pseudoscorpion, Zeh and Zeh [28] showed that a male that mates with his sister sires about twice as many inbred surviving offspring if the female also mates with an unrelated male. The authors concluded that outbred embryos exert a rescuing effect on inbred half-siblings and suggested two possible mechanisms: Outbred embryos could exhibit a better

ability to draw nutrients into the communal brood sac; alternatively, outbred embryos could establish a non-self presence in mixed inbred/outbred broods, and this presence could activate the normal cascade of feto-maternal interactions. Both mechanisms represent differential maternal allocation based on interactions between maternal and offspring genotypes. Zeh and Zeh's [28] findings could also be mediated by paternal effects if, for example, females responded differently to the Acps of related and unrelated males. Population crosses of bruchids, *Callosobruchus maculatus,* have shown how male genetic background can influence a female's response to his ejaculate [29]. Our findings differ from those of Zeh and Zeh, however, because their effects seem to depend on interactions between parental genotypes (genetic incompatibility), whereas ours depend on intrinsic sire effects (good genes). Moreover, our results show that offspring viability benefits arising from mixed paternity extend to egg-laying animals, and they do so more widely than in consanguineous matings.

There are a growing number of experimental studies that report an increased survival of embryos produced by polyandrous females [3]. Current genetic-benefit models for the evolution of polyandry propose that increased embryo viability in multipy mated females is determined by direct transmission of paternal genes [2, 3]. Our results suggest that the benefits accrued for the offspring of polyandrous females need not be transmitted directly from fathers if the fathers can provide environments that promote embryo viability and if these environmental effects are themselves genetically determined. Our findings thereby offer a potential indirect genetic benefit that could promote the evolution of polyandry.

Experimental Procedures

Animals

We used crickets exhibiting a morphological homozygous recessive marker, white eye (*we*), to examine paternal genetic and environmental effects on developing embryos [30]. *We* is a neutral marker because the mutation does not affect the fertilization capacity of sperm, nor does it affect embryo viability [31, 32]. White-eyed crickets from a stock population maintained in the lab for more than 30 generations and F2 wild-type black-eyed (*be*) males derived from mated females collected in Carnarvon (North Western Australia) were used in this study. All the animals were bred in plastic containers in a constant-temperature room (25°C), maintained on a 12:12 hr light:dark cycle, fed with cat chow ad libitum, and supplied with a Petri dish containing a pad of moist cotton wool for oviposition. Crickets were older than 1 week but younger than 1 month when used in our mating trials.

We generated 20 full-sibling homozygous *we* families by mating unrelated pairs of males and females from the stock population. Nymphs were kept in 5 liter containers, and the sexes were separated before the penultimate instar. These families provided the 12 families, or blocks, used in our experimental design.

Experimental Design

The mating protocol employed was analogous to previous designs used for looking at the genetic benefits of polyandry [33] but possessed important modifications. We used twelve blocks (families) of six sisters, each sister being assigned at random to either a monandrous or polyandrous mating group. For each family, a *be* and a *we* male, unrelated to the females, were mated either singly or in competition. In the monandrous group, the *be* male was mated twice to each of two sisters, and the *we* male was mated twice to each of another two sisters. In the polyandrous group, both males were mated once each to each of the remaining two sisters. In this way, all females received two copulations, either with the same male (*be-be* or *we-we*) or with different males (*be-we* or *we-be*). Although a male's ability to induce high viability in his offspring was not related to the phenotypic marker (see Figure 1), by alternating the mating order of *we* and *be* males for the two polyandrous females within every block, we could be sure that mating order was also alternated with respect to the ability of each pair of males (HV-LV or LV-HV) to induce embryo viability.

Of particular interest to the predictions tested was knowing the viability of embryos sired by each of the competing males under polyandrous matings. For the embryos of polyandrously mated females, sire identity could be determined once the developing mandibles had begun to sclerotise and the eye spots took on their color (Figure 1). Thus, we were able to analyze the viability of embryos with known parentage from this stage to hatching for each of the two males when they mated in competition and compare this same measure of embryo viability determined from their monandrous mates. The experimental protocol therefore allowed us to determine the survival of embryos sired by each male in both noncompetitive and competitive contexts and to assess maternal effects by comparing among female family variances. The use of sisters within each block to some degree controlled for any female-driven variance in embryo viability. Although this control may not be perfect, we found no significant female effects on fertilization success or embryo viability (see Results and Discussion), either because female effects were relatively weak compared with male effects or because our design was effective in controlling for female effects.

Within monandrous females, embryo viability across the later part of the embryonic period, from paternity assignment to hatching, is significantly correlated with viability assessed from egg swelling to hatching (arcsine transformed data, $r = 0.56$, $p = 0.00003$, $n = 49$), although it does underestimate total viability because some embryos die before paternity can be assigned (mean ± SE percentage of embryo mortality from fertilization to eye spot/mandible marks stage across monandrous females, 6.9 ± 1.1%). Our estimate of offspring viability is therefore conservative because it could not include the total variance in embryo mortality.

Matings were carried out in small plastic boxes (7 cm × 7 cm × 5 cm). After mating, the males were left to guard the females for 40 min, thus preventing females from removing the spermatophore. Spermatophores were experimentally removed after this period to standardize the volume of ejaculate and numbers of sperm received by each female [31]. In crickets, sperm and seminal fluid are transferred simultaneously. After their first copulation, males were allowed to recover for 1 hr before pairs were established again for a second copulation. To ensure that males had young, viable sperm [32, 34], they were mated to nonexperimental females before being exposed to experimental females. After their matings, females were provided with a Petri dish containing damp sand and allowed to oviposit for 1–2 days (until females had laid at least 50 eggs). Eggs were rinsed daily from the sand, and a random sample of 50 eggs was placed onto a double layer of moist filter paper and incubated at 25°C. Eggs were placed in a grid of five rows of ten eggs so that individual egg development could be tracked.

Each block (group of sisters mated to two males) involved two sisters mated monandrously to one of the males, two other sisters mated to the other male (except for one block in which one of the males was mated with three females instead of two), plus two sisters mated polyandrously to the two males (except for one block in which the two males were mated to three females instead of two). Therefore, across blocks, we used 49 females mated monandrously, 25 females mated polyandrously, and 24 males. Blocks were statistically independent because each comprised full-sibling sisters mated to two males. Although we completed 12 blocks, the sample size for analyses involving the ranking of males according to their embryo viability was reduced to 11 because males in one block showed no difference in embryo viability and thus could not be ranked.

Means are presented with ± 1SE.

Acknowledgments

This work was funded by Australian Research Council grants to F.G.-G. and L.W.S. and the West Australian Centres of Excellence in Science and Innovation Program. We thank Stephen Robinson

for conducting the matings, Jason Kennington for the bootstrapping, and Jon Evans for comments.

Received: August 7, 2006
Revised: October 16, 2006
Accepted: October 25, 2006
Published: January 8, 2007

References

1. Bateman, A.J. (1948). Intrasexual selection in *Drosophila*. Heredity *2*, 349–368.
2. Jennions, M.D., and Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. Biol. Rev. *75*, 21–64.
3. Simmons, L.W. (2005). The evolution of polyandry: Sperm competition, sperm selection, and offspring viability. Ann. Rev. Ecol. Evol. Syst. *36*, 125–146.
4. Curtsinger, J.W. (1991). Sperm competition and the evolution of multiple mating. Am. Nat. *138*, 93–102.
5. Keller, L., and Reeve, H.K. (1995). Why do females mate with multiple males? The sexually selected sperm hypothesis. Adv. Stud. Behav. *24*, 291–315.
6. Yasui, Y. (1997). A "good sperm" model can explain the evolution of costly multiple mating by females. Am. Nat. *149*, 573–584.
7. Yasui, Y. (1998). The "genetic benefits" of female multiple mating reconsidered. Trends Ecol. Evol. *13*, 246–250.
8. Zeh, J.A., and Zeh, D.W. (2003). Toward a new sexual selection paradigm: Polyandry, conflict and incompatibility. Ethology *109*, 929–950.
9. T.A. Mousseau and C.W. Fox, eds. (1998). Maternal Effects as Adaptations (Oxford: Oxford University Press).
10. Wolf, J.B., Brodie, E.D., III, Cheverud, J.M., Moore, A.J., and Wade, M.J. (1998). Evolutionary consequences of indirect genetic effects. Trends Ecol. Evol. *13*, 64–69.
11. Moore, A.J., Brodie, E.D., and Wolf, J.B. (1997). Interacting phenotypes and the evolutionary process.1. Direct and indirect genetic effects of social interactions. Evolution Int. J. Org. Evolution *51*, 1352–1362.
12. Wolf, J.B., Brodie, E.D., and Moore, A.J. (1999). Interacting phenotypes and the evolutionary process. II. Selection resulting from social interactions. Am. Nat. *153*, 254–266.
13. Qvarnstrom, A., and Price, T.D. (2001). Maternal effects, paternal effects and sexual selection. Trends Ecol. Evol. *16*, 95–100.
14. Reinhold, K. (2002). Maternal effects and the evolution of behavioral and morphological characters: A literature review indicates the importance of extended maternal care. J. Hered. *93*, 400–405.
15. Wilson, A.J., Coltman, D.W., Pemberton, J.M., Overall, A.D.J., Byrne, K.A., and Kruuk, L.E.B. (2005). Maternal genetic effects set the potential for evolution in a free-living vertebrate population. J. Evol. Biol. *18*, 405–414.
16. Fox, C.W., Waddell, K.J., and Mousseau, T.A. (1995). Parental host-plant affects offspring life-histories in a seed beetle. Ecology *76*, 402–411.
17. Simmons, L.W. (2001). The evolution of polyandry: An examination of the genetic incompatibility and good-sperm hypotheses. J. Evol. Biol. *14*, 585–594.
18. García-González, F., and Simmons, L.W. (2005). The evolution of polyandry: intrinsic sire effects contribute to embryo viability. J. Evol. Biol. *18*, 1097–1103.
19. Becker, W.A. (1984). Manual of Quantitative Genetics (Washington, D.C.: Pullman).
20. Wolfner, M.F. (1997). Tokens of love: Functions and regulation of *Drosophila* male accessory gland products. Insect Biochem. Mol. Biol. *27*, 179–192.
21. Simmons, L.W. (2001). Sperm Competition and Its Evolutionary Consequences in the Insects (Princeton, NJ: Princeton University Press).
22. Gillott, C. (2003). Male accessory gland secretions: Modulators of female reproductive physiology and behavior. Annu. Rev. Entomol. *48*, 163–184.
23. Sirot, L.K., Lapointe, S.L., Shatters, R., and Bausher, M. (2006). Transfer and fate of seminal fluid molecules in the beetle, *Diaprepes abbreviatus*: Implications for the reproductive biology of a pest species. J. Insect Physiol. *52*, 300–308.
24. Fox, C.W., and Mousseau, T.A. (1998). Maternal effects as adaptations for transgenerational phenotypic plasticity in insects. In Maternal Effects as Adaptations, T.A. Mousseau and C.W. Fox, eds. (Oxford: Oxford University Press), pp. 159–177.
25. Simmons, L.W. (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.
26. Stanley-Samuelson, D.W., Peloquin, J.J., and Loher, W. (1986). Egg-laying in response to prostaglandin injections in the Australian field cricket, *Teleogryllus commodus*. Physiol. Entomol. *11*, 213–219.
27. Gilchrist, A.S., and Partridge, L. (1997). Heritability of pre-adult viability differences can explain apparent heritability of sperm displacement ability in *Drosophila melanogaster*. Proc. R. Soc. Lond. B. Biol. Sci. *264*, 1271–1275.
28. Zeh, J.A., and Zeh, D.W. (2006). Outbred embryos rescue inbred half-siblings in mixed-paternity broods of live-bearing females. Nature *439*, 201–203.
29. Brown, D.V., and Eady, P.E. (2001). Functional incompatibility between the fertilization systems of two allopatric populations of *Callosobruchus maculatus* (Coleoptera: Bruchidae). Evolution Int. J. Org. Evolution *55*, 2257–2262.
30. Weigensberg, I., Carrière, Y., and Roff, D.A. (1998). Effects of male genetic contribution and paternal investment to egg and hatchling size in the cricket, *Gryllus firmus*. J. Evol. Biol. *11*, 135–146.
31. Simmons, L.W., Wernham, J., García-González, F., and Kamien, D. (2003). Variation in paternity in the field cricket *Teleogryllus oceanicus*: No detectable influence of sperm numbers or sperm length. Behav. Ecol. *14*, 539–545.
32. García-González, F., and Simmons, L.W. (2005). Sperm viability matters in insect sperm competition. Curr. Biol. *15*, 271–275.
33. Tregenza, T., and Wedell, N. (1998). Benefits of multiple mates in the cricket *Gryllus bimaculatus*. Evolution Int. J. Org. Evolution *52*, 1726–1730.
34. Reinhardt, K., and Siva-Jothy, M.T. (2005). An advantage for young sperm in the house cricket *Acheta domesticus*. Am. Nat. *165*, 718–723.