Female genotype affects male success in sperm competition

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SUMMARY

The central question addressed by most studies of sperm competition is: 'what determines which male's sperm are used at fertilization?' Empirical and theoretical studies that address this question have traditionally focused on adaptations which enhance male fertilization success while treating the female as a receptacle in which sperm competition is played out. Here we provide evidence which suggests that female genotype strongly influences the outcome of sperm competition. When the sperm of two males are in competition the proportion of offspring fathered by the second male to mate (P_2) was found to be highly repeatable only if the male pair were mated to three different, but genetically similar females (full-sisters to each other; unrelated to either of the males). In contrast, if a male pair were mated to three females that were unrelated then P_2 was either non-repeatable or marginally repeatable. We also show that male success in sperm competition is determined, to a large extent, by gamete and/or male–female compatibility. This conclusion is derived from the observation that P_2 was repeatable among full-sisters mated to different, yet genetically similar male pairings, whilst P_2 was non-repeatable among full-sisters mated to different, genetically distinct male pairings.

1. INTRODUCTION

When a female mates with two or more males within a single reproductive cycle, there is competition among the sperm for the fertilization of the ovum or ova (Parker 1970). In most insect species that have been studied the last male to mate fertilizes the majority of subsequent eggs (Ridley 1989). This is referred to as last-male sperm precedence and is typically measured as a species-specific sperm precedence value or P_2 value (Boorman & Parker 1976). However, in virtually all species studied there is considerable intraspecific variability around these mean P_2 values (Lewis & Austad 1990). Those studies that have addressed intraspecific variation in P_2 often, but not always (Eady 1994a), reveal subtle components of male reproductive behaviour in relation to fertilization success (Siva-Jothy & Tsubaki 1989; Simmons & Parker 1992; Lewis & Austad 1994; Radwan 1996). However, even in these studies there is considerable residual P_2 variation. Some of this unexplained variation may be attributable to variability in female reproductive biology. Because most post-copulatory competition among males for paternity is played out within the bodies of females it seems intuitively likely that variation in female behaviour, morphology and physiology is likely to influence the outcome of sperm competition

(Eberhard 1996). In *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) the last male to mate fertilizes on average 80% of the eggs subsequently laid, although there is considerable variation about this mean P_2 value (range 0–1; Eady 1991, 1994a). Previous studies of sperm competition on this beetle have found such variation to be unexplained by either male size, female size, copulatory behaviour or the number of sperm transferred during copulation (Eady 1994a, 1995). Lack of a clear correlation between paternity and male phenotypic characteristics may be due to the measurement of inappropriate male characteristics or it could result from an overwhelming effect of variation in female reproductive behaviour, morphology and physiology on male fertilization success. Studying the effect of female variability on last-male sperm precedence is difficult because only one P_2 value per female can be generated under standardized conditions. However, if the extent of P_2 was largely female-determined then one would predict that the pattern of sperm use would be more similar amongst closely related females than it would amongst distantly related females. Therefore, we compared the repeatability of P_2 values obtained from male pairs mated in succession to: (i) females that were genetically similar (full-sisters); and (ii) females that were genetically less-similar (non-sibs). If genotype-related variation in female reproductive biology does influence male success in sperm competition

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then we predict that P_2 values obtained from each male pair will be more repeatable when the male pairs are mated to sisters, rather than unrelated females.

2. MATERIALS AND METHODS

(a) Culture methods

Stock cultures of *C. maculatus* beetles were maintained on black-eyed beans, *Vigna unguiculata* (L.) Walp in a constant temperature environment at 26 °C and 35% relative humidity with a 16L:8D photoperiod. Two different strains of beetle were used in this study: one, an outbred stock derived from Niamey, Niger, and the other a laboratory stock originally derived from Brazil approximately 14 years ago. The beetles from both the Niamey and Brazilian stocks were cultured on a weekly basis with approximately 10 000, and 500 beetles, respectively, being transferred to 6000, and 2000 fresh black-eyed beans, respectively. Because the life-cycle of these beetles takes four weeks, four cultures of each strain were maintained, each generation separated by one week. Because this method creates four genetically isolated populations approximately 5000 beetles from the outbred Niamey population were transferred between adjacent cultures each week. To obtain families, pairs consisting of a single virgin male and female, from the same culture box, were placed in separate containers containing approximately 150 beans and allowed to mate and oviposit until their natural deaths. Virgin adult siblings were obtained by isolating individual beans containing pre-emergent adults in polystyrene petri dishes subdivided into 25 compartments.

(b) Experiments

Experiments were carried out separately on the two strains of *C. maculatus* beetle which differed with respect to the inter-mating intervals of females. Females of the Niamey strain remated readily 24 h following the initial copulation, whilst females of the Brazilian strain remated readily 48 h following copulation. The basic experimental design was to compare the repeatability of P_2 when pairs of males were mated to: (i) three genetically similar females (full-sibs) or, (ii) three genetically less-similar females (non-sibs). Male pairs (all initially virgin) were mated to three separate females (all initially virgin). In approximately half of the trials the three females were full-sibs, whilst in the other half of the trials the three females were non-relatives. Each female triplicate was considered a replicate and within each replicate the original inter-mating interval and mating order (i.e. of the pair of males one was always the first male to mate) were maintained. The experiment was performed separately on both the Niamey and the Brazilian strain beetles. When Niamey strain beetles were used, inter-mating intervals were 24 h, and when Brazilian strain beetles were used, the inter-mating intervals were 48 h. Within both the Niamey and Brazilian experiments some full-sister trios were also full-sibs to other female trios (i.e. full-sibs between replicates). In the Niamey experiment the males of each pair were not related either within or between replicates. However, within the Brazilian experiment males were not related within replicates, but on some occasions they were related (full-sibs) between replicates. Thus, within the Niamey experiment groups of full-sib females (between replicates) were mated to different male pairs that were genetically dissimilar between replicates and, within the Brazilian experiment groups of full-sib females (between replicates) were mated to different, but genetically similar, male pairs (between, not within, replicates) (figure 1).

Figure 1. The diagram represents the two treatments used to examine the effect of female variability on last-male sperm precedence in the Brazilian strain experiment. In treatment 1, male pairs were mated to three females, each from a genetically distinct family. In treatment 2, male pairs were mated to three females, all full-sibs. Enclosed boxes represent female families. Within treatment 2, females were full-sibs within each replicate and on some occasions females were full-sibs between replicates (i.e. females 1, 2 and 3 of replicate 1 were full-sibs of females 1, 2 and 3 of replicate 2). The males of each pair were not related within replicates but on some occasions were full-sibs between replicates (i.e. male A1 was a full-sib of male A2, and male B1 was a full-sib of male B2; male A was not related to male B). This produced nine groups of genetically similar females being mated to male pairs that were genetically similar between, but not within pairs. For the Niamey experiment, treatments 1 and 2 were exactly the same except all male pairs were randomly chosen and unrelated within and between replicates.

During the inter-mating intervals females were allowed to oviposit on 30 black-eyed beans (*Vigna unguiculata*). Following the second mating, females oviposited on 50 black-eyed beans until their natural deaths. The eggs laid were incubated at 26 °C, 35% relative humidity, with a 16L:8D photoperiod and the extent of last-male sperm precedence estimated using the genetic marker technique (Eady 1991). In the analyses, each female triplicate (or duo, as some male pairs failed to mate with all three females) was considered a replicate and the intra-class correlation coefficients r_1 , and their standard errors, were calculated according to Lessells & Boag (1987) and Becker (1984). Because the P_2 data obtained from this study were nonparametric, probabilities based on estimates derived from randomization tests associated with the statistical analyses are presented (Sokal & Rohlf 1995).

3. RESULTS

When the same two males were mated to three separate females which were not full-sibs P_2 was repeatable in the Niamey but not the Brazilian strain; $F_{13,22} = 4.17$, $p < 0.05$, intra-class correlation coefficient $r_1 = 0.55$ (s.e. ± 0.05) and $F_{31,62} = 1.2$, $p = 0.27$, $r_1 = 0.07$ (s.e. ± 0.12), respectively (figure 2a,b). However, if the

three females were full-sisters then P_2 was highly repeatable in both the Niamey and Brazilian strains; $F_{12,20} = 22.68$, $p < 0.001$, $r_1 = 0.9$ (s.e. ± 0.16) and $F_{34,64} = 14.2$, $p < 0.001$, $r_1 = 0.82$ (s.e. ± 0.06), respectively (figure 2c,d). Comparison of the intra-class correlation coefficients between the two treatments revealed that r_1 was indeed higher in the full-sister treatments as compared to the non-sib treatments in both the Niamey and Brazilian strains: $t = 2.06$, d.f. = 69, $p < 0.05$ and $t = 5.98$, d.f. = 191, $p < 0.001$, respectively. Randomization tests (Sokal & Rohlf 1995), in which P_2 values were randomly assigned between female trios within treatments, revealed that for the Niamey and Brazilian strains, the probability of obtaining $r_1 \geq 0.9$ and $r_1 \geq 0.82$, respectively, was $p < 0.001$, and the probability of obtaining $t \geq 2.06$ and $t \geq 5.98$, was $p < 0.001$. These probabilities are based on 1000 randomizations of the data. Within the Niamey experiment there was no difference in the extent of P_2 between groups of full-sisters (i.e. across replicates) mated to different, unrelated male pairs; $F_{7,41} = 0.62$, $p = 0.7$. However, within the Brazilian experiment there was a difference in the extent of P_2 between groups of full-sisters (i.e. across replicates) mated to different, related male pairs; $F_{8,90} = 13.8$, $p < 0.001$.

If individual P_2 values were artefacts of differential pre-adult mortality associated with genetic incompatibility (Zeh & Zeh 1996) then P_2 would correlate positively with the pre-adult mortality of offspring from the female's first mate (measured as per cent of eggs failing to produce adult beetles). No such correlation existed; $r_s = 0.12$, $n = 60$, $p > 0.2$. In addition P_2 was not related to the difference in pre-adult mortality before and after the second matings of females; $r_s = 0.09$, $n = 60$, $p > 0.2$. Again, if P_2 was an artefact of genetic compatibility then it would correlate positively with the difference in offspring viability. Finally, if P_2 was an artefact of genetic incompatibility then the pre-adult mortality of offspring derived from full sisters mated to male pairs would be repeatable. This was not the case; $r_1 = -0.01$, $F_{10,18} = 0.97$, $p = 0.5$.

4. DISCUSSION

Our results indicate that the female plays an important role in determining male fertilization success under conditions of sperm competition. In two separate experiments, the extent of P_2 of individual male pairs was found to be more repeatable when variability between females was reduced by mating males to three separate full-sibling females. When male pairs were mated to three unrelated females, P_2 values were considerably more variable.

Why the sperm of certain male phenotypes do well or badly within the reproductive tracts of certain female genotypes is unclear. In *Tribolium castaneum*, the interaction between male and female genotypes explains a significant amount of variation in P_2 (Lewis & Austad 1990). However, in the study of Lewis & Austad (1990), genotypic variation was between strains rather than within strains as in the present study. Thus, between strain interactions may have resulted from males (and their ejaculates) being adapted to prevailing

Figure 2. Repeated measures of P_2 (the proportion of eggs fertilized by the second male to mate). (a) In the Brazilian strain, when the same male pair were mated to unrelated females, P_2 was not repeatable; e.g. Spearman rank correlation coefficient for P_2 (measure 1) versus P_2 (measure 2), $r_s = 0.129$, $n = 32$, $p > 0.05$. (b) In the Niamey strain, when the same male pair were mated to unrelated females, P_2 was non-repeatable after two measures; e.g. Spearman rank correlation coefficient for P_2 (measure 1) versus P_2 (measure 2), $r_s = 0.022$, $n = 14$, $p > 0.05$. However, P_2 was repeatable after three measures (see text for details). (c) In the Brazilian strain, when the same male pair were mated to full-sisters, P_2 was repeatable; e.g. Spearman rank correlation coefficient for P_2 (measure 1) versus P_2 (measure 2), $r_s = 0.677$, $n = 35$, $p < 0.001$. (d) In the Niamey strain, when the same male pair were mated to full-sisters, P_2 was repeatable; e.g. Spearman rank correlation coefficient for P_2 (measure 1) versus P_2 (measure 2), $r_s = 0.634$, $n = 13$, $p < 0.05$.

female phenotypes (Eberhard 1996; Rice 1996; Gomendio & Roldan 1993). Somatic–gametic incompatibility mechanisms appear to regulate male fertilization success in the ascidian *Diplosoma listerianum* (Bishop *et al.* 1996) and, in the sand lizard *Lacerta agilis* (Olsson *et al.* 1996), where the success of a male's sperm, in competition with the sperm of other males, depends on his relatedness to the female. Such sperm selection could occur within the female reproductive tract and/or at the surface of the ova. The results of our study also hint towards a form of male–female and/or somatic–gametic compatibility. This is because the fertilization success of a male is more variable when female genotypic variability is increased. This could be due to related females having similar properties of sperm displacement, storage and utilization. However, this seems unlikely because P_2 values were not repeatable among families of females mated to separate, genetically different male pairings. If, however, families of females were mated to separate male pairs that had similar genotypes (i.e. the males of one pair were related to the males of a second pair as in the Brazilian experiment) then P_2 was repeatable, suggesting that certain male genotypes do well or badly within the reproductive environments of certain female genotypes. Such a result could come about by somatic–gametic compatibility or be the product of male–female compatibility. For example, Eady (1994b, 1995) hints towards a mechanism of sperm flushing in *C. maculatus*. A male's ability to displace sperm may depend on his ability to remain *in copula* (females kick at males during copulation, Eady 1994a) or his ability to stimulate sperm uptake (or ejection) into (or from) the spermathecae. Such abilities may depend critically on the genotypes of both the male and female rather than the interaction between the soma of the female and the genotypes of the sperm.

The results of this study have important implications for the notion of sexual selection by cryptic female choice (Eberhard 1996), vindicating those who have argued that females should not be considered as passive during male–female interactions (Eberhard 1996; Eberhard & Cordero 1995; Batten 1992). The results do, however, present some difficulties for the empirical detection of cryptic female choice. If variation in paternity is not linked to any male character, it will not result in sexual selection by cryptic female choice (Eberhard 1996). Because paternity in *C. maculatus* appears to be largely determined by male and female compatibility (phenotypic or genotypic), it will be more difficult to find male and/or female characteristics that correlate with paternity. However, this does not rule out the possibility of cryptic female choice operating. It simply makes it more difficult to detect. Males are known to vary in their ability to obtain fertilizations (Lewis & Austad 1990, 1994; Radwan 1996). For example, where the genetic marker technique has been employed in sperm competition experiments, some genotypes are consistently better at obtaining fertilizations than others (Lanier *et al.* 1979). However, to detect subtle differences between males of a single strain in their sperm competitive abilities it may now be necessary to perform such experiments controlling for female variability. In

conclusion, a combination of male and female characters determine the outcome of sperm competition in *C. maculatus* with the latter exerting a much stronger influence than previously thought.

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