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Male control of mating duration following exposure to rivals in fruitflies ☆

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ABSTRACT

Males of many species assess the likely level of sperm competition and respond adaptively, for example by increasing the level of courtship they deliver, by transferring more sperm or seminal fluids or by extending matings. In mechanistic terms, it may be easier for males to adjust the level of their investment to the likely level of sperm competition for male-limited traits such as sperm and seminal fluid production over which they have control. However, for shared traits, such as mating duration, that are expressed at a level determined by direct interactions between males and females, adaptive responses by males to competition could be constrained. This need not be the case, however, if males have significant influence over the expression of such traits. Understanding which sex can most influence the expression of shared traits in response to sexual competition is important in order to document the range of strategic, plastic responses that are available to each sex. However, direct tests of these ideas require, as in this study, measurements of the effect on a shared trait of manipulating the ability of one, but not the other, sex to influence it. We studied the responses of male *Drosophila melanogaster* to sexual competition, in which mating duration is increased following exposure to rivals, resulting in significantly increased paternity share. Males were allowed to respond normally to the presence of rivals prior to mating, but female responses to males were reduced via decapitation and immobilisation. We found that matings with both intact and decapitated, immobilised females were significantly longer with males that had been exposed to rivals prior to mating. Hence males could maintain their responses to rivals with intact and decapitated females, suggesting significant male influence over the ability to extend mating duration in this context. However, overall, mating duration was significantly longer with intact in comparison to decapitated females. Whether this is due to a female influence over mating duration in general, or whether males respond differently to immobilised females, is not yet known. Gaining a fuller understanding of sex-specific control of plastic traits will be important in the future for understanding how reproductive traits evolve and function.

1. Introduction

Males of many species can respond to the likely threat of post-mating competition (Parker et al., 1996, 1997) by altering their behaviour prior to mating (Bretman et al., 2011a) and/or the amount of sperm or seminal fluid proteins allocated to each partner (Wedell et al., 2002; Wigby et al., 2009). For males to accurately and adaptively match the expression of a trait to their competitive environment they must be able to significantly influence the expression of that trait. For apparently male-limited traits such as sperm and seminal fluid production, the degree of control of sex-specific expression should be high. However, this may not be the case for 'shared' reproductive traits, such as mating duration, that arise as an emergent property of the interaction between males and females (Arnqvist and Rowe, 2005). Intuitively, the value of shared traits should be influenced by both sexes. However, this need not be true if one sex has evolved predominant control or precise mechanisms for matching the value of the trait to the environment. Determining the relative influence of each sex over shared traits that can exhibit plasticity to the social and sexual environment is important to understand the repertoire of plastic responses that are available to each sex.

In order to test whether there is sex specific control of a plastic shared trait we require a system in which the shared trait can be expressed, but where one sex is rendered incapable of exerting any influence over it. In this study we were able to achieve this by adapting methodology from classic studies of courtship in

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Drosophila melanogaster (Cook and Cook, 1975; Grossfield, 1972; Spieth, 1966). We tested directly whether there was male-specific control over the ability to extend matings following increased levels of sexual competition prior to mating. Our hypothesis was that if extending matings in response to an increased risk of sperm competition is an adaptive strategy employed by males, then they must be able exert significant influence over the expression of that shared trait.

Across several species of *Drosophila*, males exposed to rivals prior to mating subsequently mate for significantly longer than controls not exposed to rivals (Bretman et al., 2009; Lizé et al., 2012a; Mazzi et al., 2009; Price et al., 2012) but see (Lizé et al., 2012b). In *D. melanogaster* this extended mating duration is associated with significant fitness benefits for males (i.e. increased paternity in a competitive and non competitive context) mediated at least in part by the transfer of increased quantities of seminal fluid proteins (Bretman et al., 2009; Wigby et al., 2009). Other mechanisms may also exist, for example in *Drosophila pseudoobscura* responses to rivals are associated with the transfer of increased numbers of sperm (Price et al., 2012). Females gain short-term productivity benefits from mating with males that have previously been exposed to rivals (Bretman et al., 2009). The longer-term fitness consequences for females are not yet known, though there are predicted to be costs. For example, receipt of seminal proteins by females can cause short term benefits in terms of increased egg laying, but longer term costs in terms of reduced lifespan and overall lifetime reproductive success (Wigby and Chapman, 2005). Therefore, matings with males that were previously exposed to rivals, that transfer more Sfps, may be disadvantageous to females. Hence there is the possibility for sexual conflict over mating duration.

We hypothesise that because males can gain significant fitness benefits from extended mating duration following exposure to rivals (Bretman et al., 2009), they should be selected to exert a significant influence over mating duration in this social context. Its important to note that such an effect may or may not be related to sex specific control of mating duration *per se*. Our knowledge of the control of mating duration in *Drosophila* in general comes from (i) crosses between different genetic strains, artificially selected lines or different karyotypes in which mating duration appears to follow the male line of origin (e.g. in *D. melanogaster* (MacBean and Parsons, 1967), *D. pseudoobscura* (Kaul and Parsons, 1965; Parsons and Kaul, 1966) and *Drosophila athabasca* (Patty, 1975)), and (ii) interspecific crosses in which in *D. melanogaster*, *Drosophila simulans*, *Drosophila mauritiana* and *Drosophila sechellia* mating duration follows the pattern of the male rather than the female's species (Jagadeeshan and Singh, 2006). These are indirect methods of assessing sex-specific control, which may explain why in different species of *Drosophila* roles for both sexes in the determination of this shared trait have been identified (*Drosophilamontana* (Mazzi et al., 2009), *Drosophilaelegans* (Hirai et al., 1999) and *Drosophilamojavensis* (Krebs, 1991)). Correlates of sex specific control of mating duration, such as female resistance behaviour in the form of 'shaking' have also been investigated in theory and empirical tests (Blanckenhorn et al., 2007).

Our aim was to use a direct assay for male-specific control of variation in mating duration specifically in response to sexual competition. We tested for male control of mating duration following exposure to rivals by using live decapitated and immobilised females. In this way, the expression of the shared trait could be measured, as males will still vigorously court and mate with immobilised and decapitated females (Cook and Cook, 1975; Grossfield, 1972; Spieth, 1966). However, such females have significantly reduced responses to males, allowing us to detect male and female influences. We predicted that, if males are controlling mating duration in the context of increased sexual competition, then mating duration would be extended after a period of exposure to a rival in both intact and decapitated females. We also predicted that female status (intact versus decapitated) should have a significant effect on female attractiveness manifested, for example, as an effect of female treatment on mating latency.

2. Materials and methods

Fly rearing and all experiments were conducted in a 25 °C humidified room, with a 12:12 h light:dark cycle. Flies were maintained in glass vials (75 × 25 mm) containing 8 ml standard sugar–yeast medium (Bass et al., 2007). Wild type flies were from a large laboratory population originally collected in the 1970s in Dahomey (Benin), as used previously in our related studies (Bretman et al., 2009, 2010, 2011b, 2012). Larvae were raised at a standard density of 100 per vial, supplemented with live yeast liquid. At eclosion, flies were collected and the sexes separated using ice anaesthesia. Males were assigned randomly to two treatments, either maintained singly or exposed to a rival male for three days until the matings occurred. Rival males were identified by using a small wing clip (wing tips were clipped using a scalpel under CO_2 anaesthesia). Virgin females were stored 10 per vial on medium supplemented with live yeast granules, until the day of mating at 4 days post eclosion. Up to 1 h before the introduction of a male, females were either aspirated singly into fresh vials, or, using CO_2 anaesthesia, decapitated and pinned through the thorax onto the surface of the food, using a fine mounting pin (0.20 mm, Austerlitz). Focal males were then introduced to the vials containing intact or decapitated females and mating latency and duration recorded. Pairs were given 2 h to mate. In a pilot study, we optimised the positioning of the pinned females just above the food surface to maximise opportunities. Nevertheless as we predicted that males would mate less frequently with the pinned females, we adjusted the samples sizes to start with 60 for the decapitated treatments and 30 for the intact female treatments. One male exposed to a rival was lost during transfer.

Statistical analyses were performed in R v 2.14.0 (Ihaka and Gentleman, 1996). The effect of female status and male exposure to rivals on the number of successful matings was analysed using a generalised linear model (GLM) with binomial errors. The effect of female status and male exposure to rivals on latency to mate and mating duration was analysed using a GLM with quasi Poisson errors (to account for overdispersion). Factors were subtracted from the maximal model using analysis of deviance.

3. Results

Mating frequency, latency to mating and mating duration were significantly affected by both male exposure to rivals and female status. There were, however, no interactions between female status and male exposure to a rival for any of these traits. Almost all males mated given an intact female mated (28/30 single males and 28/29 males exposed to rivals; Table 1). Just over half of the males given a decapitated female mated successfully (34/60 single males and 36/60 paired males; Table 1). As predicted, males took significantly longer to mate with decapitated females, and, consistent with previous work, males exposed to rivals took marginally longer to mate in comparison to males kept alone prior to mating (Table 1, Fig. 1A). Overall, matings were also significantly shorter in duration with decapitated females (Table 1, Fig. 1B). In line with the main prediction, males exposed to rivals prior to mating mated for significantly longer than males kept alone, regardless of whether their mate was intact or decapitated (Table 1, Fig. 1B).

Table 1
Analysis of the effect on male exposure to rivals and female status (live or decapitated) on the number of successful matings in a 2 h period, on latency to mate and on mating duration. Shown are the results of analysis of deviance based upon generalised linear models.

Variable	Source	χ^2_1	*P*
Number of matings	Male exposure	0.272	0.602
	Female status	30.841	<0.0001
	Male exposure*female status	−0.188	0.664
Latency to mate	Male exposure	53.105	0.042
	Female status	1407.3	<0.0001
	Male exposure*female status	−15.25	0.275
Mating duration	Male exposure	20.949	0.0005
	Female status	28.77	<0.0001
	Male exposure*female status	−0.587	0.561

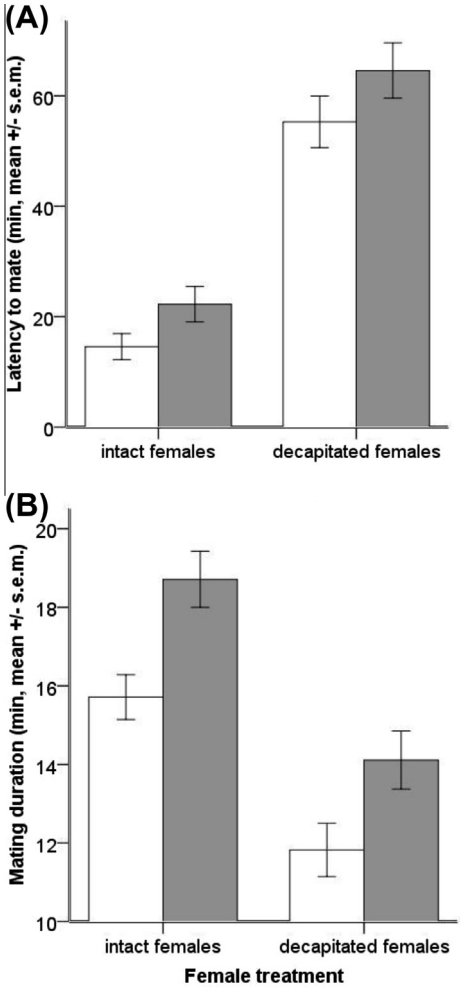

Fig. 1. (A) Latency to mate and (B) mating duration of matings between live or decapitated females and males held singly (white bars) or with a rival (grey bars) for the 3 days prior to mating.

4. Discussion

Taken together, our results suggest that both sexes exert influence over mating duration in this species. We found that mating was always significantly longer in matings between males exposed to rivals prior to mating regardless of female treatment. Female responses to males were presumably reduced in the decapitated females, suggesting that males exert significant influence to extend mating duration in this context. This finding provides support for our hypothesis that males exert control over the duration of extended matings in response to the potential level of sperm competition. However, matings were also significantly slower to start and shorter with decapitated females. This indicates a second important finding, that inputs from females also play an important role in the duration of mating itself.

Previous studies in different *Drosophila* species have reported extended mating duration following exposure of males to rivals (Bretman et al., 2009, 2010, 2011b, 2012, 2013; Lizé et al., 2012a; Price et al., 2012; Wigby et al., 2009). In these studies, it has generally been assumed that the extension of mating duration is under a significant degree of male control, because it results in significant benefits for males (Bretman et al., 2009; Price et al., 2012). By influencing the extent to which mating duration is extended following exposure to rivals males can therefore respond adaptively to the likely level of sperm competition (Parker et al., 1996, 1997). Such responses are therefore predicted to be strongly selected. However, it cannot be discounted that the extension of mating duration could be driven by female responses to the type of male encountered. Our data suggest that the extension of mating duration in this context is indeed under male control, as responses by males to the potential threat of sperm competition were seen in matings with intact and with decapitated females in which female responses to males should be minimised. However, there may be other effects of female decapitation. For example, decapitated females can remain alive for up to 7 days and are reported to respond to physical contact (Spieth, 1966) although in our experiments the females did not exhibit rejection behaviours as previously observed (Spieth, 1966). Females were also immobilised so they could not move away from males. What does seem clear though is that the decapitation treatment minimised the ability of females to exhibit rejection responses towards males and thereby influence the duration of mating through this mechanism. There was an effect, however, of female decapitation on the overall duration of mating. Males took significantly longer to mate, and mated for a significantly shorter time overall, with decapitated females. This is consistent with previous work showing that male *D. melanogaster* will court decapitated females (Cook and Cook, 1975; Grossfield, 1972; Spieth, 1966), but at a reduced rate (Cook and Cook, 1975). This is also in line with evidence that in *Drosophila palustris* and *D. subpalustris* the proportion of inseminated decapitated females was half that of intact females (Grossfield, 1972). However, the findings contrast with a study in *D. montana*, in which males were observed to mate for longer with decapitated females (Mazzi et al., 2009).

Females could influence courtship and mating duration in complex ways. For example, the manner in which the ovipositor is extruded can determine rejection or acceptance behaviour (Lasbleiz et al., 2006). Wild type patterns of courtship in males presumably therefore depend upon elements of female behaviour or other inputs that were not present in our immobilised, decapitated females. If females influenced mating duration through their rejection behaviours, then we might expect males to mate for longer with decapitated females in which such rejection is minimised. However, the opposite was found, as matings were shorter overall when with decapitated females. This suggests that there may be some positive feedback from females to prolong mating duration. Whether this is because females also benefit from extended matings or whether it reflects a reduction in reproductive investment by males who perceive these inactive females to be unattractive and in poor condition, is not yet unclear.

It is possible that our results could represent an outcome of sexual conflict (e.g. see Blanckenhorn et al., 2007). For example, in *D. montana*, in which mating duration is negatively associated with female willingness to remate (Mazzi et al., 2009), it is suggested that longer copulations prevent females from accruing benefits from multiple mating. Likewise, in *D. melanogaster* prolonged mat-

ings also decrease a female's subsequent willingness to remate (Fricke et al., 2009). Furthermore, females mated to males that have been exposed to rivals receive more of at least one seminal fluid protein, sex peptide (Wigby et al., 2009), which can significantly reduce female fitness (Wigby and Chapman, 2005). Prolonged matings in the context of responses to elevated sperm competition risk may therefore be costly to females, whilst simultaneously conferring benefits to males (Bretman et al., 2009). Such potential for conflict would be minimised if both sexes gain productivity from extended matings following exposure of males to rivals (Bretman et al., 2009). More evidence of the fitness outcomes for females of the extended duration of mating in response to socio-sexual context is therefore needed in order to settle this issue.

Breeding experiments suggest that there is a genetic basis for the male influence of mating duration in general. For example, mating duration is reported as significantly heritable in males but not females (father–son $h^2 = 0.46$ (Gromko, 1987), mother–daughter $h^2 \approx 0$ (Gromko, 1989)). As expected, therefore, mating duration is evolutionarily labile, responding significantly to artificial selection within seven generations (Gromko et al., 1991). Other genes, such as the behavioural clock genes *period* and *timeless* that govern circadian rhythms in both males and females are also known to have pleiotropic effects on mating duration (Beaver and Giebultowicz, 2004). Nevertheless, the genetic architecture of mating duration in *D. melanogaster* remains to be resolved.

The evidence for either sex having predominant control over mating duration in *Drosophila* is mixed, with some studies finding evidence for male control (Jagadeeshan and Singh, 2006; Kaul and Parsons, 1965; MacBean and Parsons, 1967; Parsons and Kaul, 1966; Patty, 1975) and others suggesting roles for both sexes (see Hirai et al., 1999; Krebs, 1991; Mazzi et al., 2009). Our data cannot definitively resolve this issue, but do reveal that males maintain their mating duration response according to the likely threat of sperm competition, regardless of female inputs. This then might suggest that complete male control is not necessarily required in order for shared traits to represent adaptive plastic male strategies in response to the competitive environment. Therefore although we predict that in species in which females have significant sex-specific influence over mating duration, males will be less likely to have the capacity to respond precisely to the threat of sexual competition through adjusting mating duration, this may not necessarily always be the case. Wider investigations of these plastic strategies, their fitness outcomes for both sexes, and sex-specific control are therefore required. Given more evidence of the extent of sex-specific control over shared traits in general it may also then be possible to determine whether this occurs due to an attempt to resolve sexual conflict, because of a coincidence of interests, or because of better information gathering by one sex than the other about what the value of the shared trait should be.

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