

Relatedness of Rivals Has No Influence on the Expression of Plastic Mating Behavior in Male *Drosophila Melanogaster*

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Abstract Phenotypic plasticity in mating behavior can be an asset to reproductive success and is observed in a wide variety of species. In particular, the presence of a rival male in the pre-mating arena can elicit behavioral responses from males that directly increase their reproductive success. Although many studies have demonstrated a recurring suite of behavioral responses by males to rivals, they have focused on the sex ratio, number and the density of competitors. However, a key factor not examined so far is how the relatedness of rival males influences the expression of plastic mating behavior, as individuals gain less by competing against their siblings than non-related rivals. I tackle this issue here by exposing full sibling males of *Drosophila melanogaster* to a range of different social environments in the sensitive period between eclosion and first mating. This is a prime period of sexual maturation during which males perceive the level of sexual competition they may face in the mating arena and respond accordingly. I found that the copulation durations of males exposed to a rival male were significantly longer than males kept in isolation. However, the copulation durations of males kept with a sibling or non-related rivals were indistinguishable. In contrast to this, there were no significant differences in mating latencies between any of the males that had matured in different social environments. Together these results confirm that the relatedness of rival males does not affect the plasticity of copulation duration that directly benefits reproductive fitness. The role of relatedness in the plasticity of mating latency or 'attractiveness' of males to females requires further study.

Keywords: rival males, mating behavior, phenotypic plasticity, *Drosophila*

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1. Introduction

Phenotypic plasticity in mating behavior is a key area of interest in sexual selection as it can directly influence reproductive fitness [1]. A recent review of 54 studies across 51 species indicates that male behavioral adjustments to competitors in the mating arena are common within and across a diverse range of species [1]. Recurring behavioral responses to increased numbers of competitors or sexual rivals include increased aggression, increased lengths of copulation duration and decreased mating latencies-the overall time spent engaged in courtship behavior [2]. Plasticity in mating behavior may be related to the sex ratio, number or density of competitors either in the pre-mating arena, in the presence of females or during mating itself [2]. Competitors may be detected by a range of sensory stimuli including olfactory, visual and tactile cues [3]. The ubiquitous nature of plasticity in mating behavior suggests the fitness benefits are substantial.

Fitness benefits of plasticity in mating behavior have been particularly well studied in fruitflies [4]. In

Drosophila melanogaster, the length of copulation duration has been directly linked to the amount of seminal fluid proteins transferred to females, the subsequent number of eggs laid and the refractory period of remating in females, all of which serve to maximise mating and fertilization success [3,4]. However, for plasticity in mating behavior to persist, the fitness benefits must outweigh the costs. One key aspect that can change the relative fitness pay-offs to plasticity in a competitive environment is the relatedness of the rival male to the focal male. For example, when competing against closely related kin in sperm competition, males replace the competitor's sperm with genetically similar sperm, gaining little in terms of overall benefit to the genotype [5]. It follows therefore, that the benefits of plasticity in mating behavior will be diminished when responding to sibling rivals compared to responding to unrelated rivals. Some previous work on olfactory perception in *D. melanogaster* has suggested that exposure to groups of siblings can increase courtship intensity in focal males [6], however, these results were specific to transgenic flies and did not directly compare related and non-related rivals or examine copulation duration. Thus, it remains unknown how the relatedness of rival males affects the plastic

nature of mating behavior in *D. melanogaster* compared to unrelated rivals. This is particularly relevant when large cohorts of males in closed laboratory populations emerge from pupal cases simultaneously or congregate on communal resources that function as a mating arena. In these instances, males have a higher chance of being exposed to full or half-siblings prior to mating than males that disperse away from natal sites upon emergence or compete and copulate with females over a wider area.

In this study, I tackle this issue and examine whether the relatedness of the rival is an important influence over plasticity in mating behavior. To achieve this I bred full sibling families of male *D. melanogaster* and split them simultaneously across three social environments during the period between adult eclosion and first mating. This is a sensitive period of sexual maturation during which males can assess the level of mating competition in their environment. I used triads of full siblings as focal males to standardize genotypes across rival male treatments and isolate the effects of relatedness and social environment. I aimed to distinguish whether being exposed to a sibling during sexual maturation produces the same plastic response in mating behavior as being exposed to an unrelated rival.

2. Materials and Methods

Adult *Drosophila melanogaster* were collected in the Swan Valley region of Western Australia in May 2009 and maintained in mass populations of over 500 individuals with overlapping generations until the start of the experiment in November 2009. Populations were fed on a *Drosophila* medium containing water, oats, sugar, agar and baker's yeast, with a sprinkling of dry yeast. Populations were kept on a 12 hr light: dark cycle at 26°C. To generate the full sibling families for the experiment, I mated 90 pairs of males and females from the stock population. I allowed females three days following mating in which to lay eggs and then removed them from their vials. From each family vial I collected four sons within 12 hours of eclosion and split them into three social environment treatments. Males (hereafter 'focal males') were either housed in isolation, with one of their brothers collected at the same time, or with an unrelated, mature (5-6 days old) male from the stock population. I kept all focal males in their respective social environments for seven days. I then transferred each focal male to a fresh vial with a 3-4 day old, sexually mature virgin female from the stock population. I recorded the behavioral responses of two traits associated with male mating

success-mating latency and copulation duration. I recorded how long each male took, to the nearest minute, to achieve a successful copulation (mating latency) and how long each copulation lasted (copulation duration). Mating trials took place over three hours in the morning from first light, and three hours in the evening prior to dark with triads of focal males from all social environments represented in the same mating windows to reduce variation due to time of day.

2.1. Data Analysis

I tested 238 focal males from 90 families for their behavioral plasticity. Copulation duration was normally distributed but mating latency was log-transformed to improve normality prior to testing. I first examined the correlation between the two mating behaviors assayed. Copulation duration and (transformed) mating latency were significantly, negatively correlated with each other (Pearson correlation: $-.0156$, $p = .016$, $n = 238$). However, since the correlation was weak, I examined each variable separately to improve power [7]. I conducted a univariate analysis of variance (ANOVA) with either copulation duration or (transformed) mating latency as dependent variables and social environment as a fixed factor. Both behavioral traits met assumptions of normality across groups for these analyses (Levene's test of Equality of variances: Copulation duration $F = .19$, $p = .82$; mating latency $F = .92$, $p = .4$). I used SPSS v 20 (IBM) to perform all statistical procedures.

3. Results

3.1. Copulation Duration

Focal males that had been exposed to another male during the period of sexual maturation significantly increased their copulation duration over males that had been kept in isolation until paired with a female (Table 1). Mean and SE of copulation duration of males in three different social environments: males in isolation = mean 18.31 minutes, SE .51, $N = 77$; males exposed to their own brother = mean 20.61, SE .52, $N = 76$; males exposed to a mature, unrelated male = mean 20.91, SE .48, $N = 85$. This effect was evident whether males were exposed to a mature, unrelated male or a sibling of corresponding age and experience (Figure 1), suggesting that relatedness of the rival male does not significantly diminish the benefits of plasticity in mating behavior.

Table 1. Univariate ANOVAs for male mating behavior after maturing in different social environments

Trait	Source of variance	d.f.	F	p	Power
Copulation duration	Social environment	2	7.84	.001	.95
	Error	235			
Mating latency	Social environment	2	2.47	.086	.49
	Error	235			

Results significant at $\alpha < .001$ are in bold. Tukey's HSD posthoc tests for copulation duration indicate that males kept in isolation were significantly different from all other groups-males in isolation vs males exposed to a brother: -2.29 , $p = .005$; males in isolation versus males exposed to a mature, unrelated male: -2.59 , $p = .001$. However, males that were exposed to a brother were not

significantly different from males exposed to a mature, unrelated male: $-.30$, $p = .906$. None of the posthoc tests for mating latency showed any significant differences between males in their responses to different social environments.

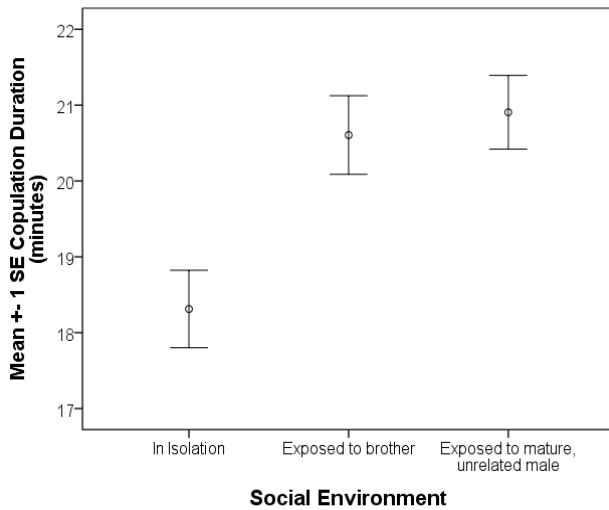


Figure 1. Males increase their copulation duration after being exposed to any rival male

3.2. Mating Latency

Although there appeared to be a trend for males exposed to a rival to decrease mating latency the differences between focal males in all of the social environment groups were small and non-significant. It is possible in this case that the data suffered from low power (Table 1 and Figure 2). Mean and SE of mating latency of males in three different social environments: males in isolation = mean 22.12 minutes, SE 2.53, N = 77; males exposed to their own brother = mean 22.72 minutes, SE 3.28, N = 76; males exposed to a mature, unrelated male = mean 19.05, SE 3.05, N = 85.

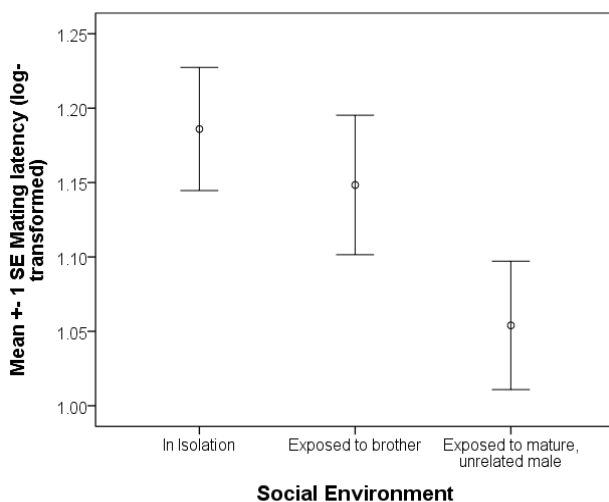


Figure 2. Males decrease their mating latency after being exposed to a rival male

4. Discussion

Phenotypic plasticity in mating behavior has been linked to significant fitness advantages in insects [4]. Specifically, males that increase their copulation duration in response to the presence of rivals encountered prior to or during the time of mating produce more offspring and reduce the remating propensity of their mates [3,4]. However, the fitness pay-offs to plastic behavior can be

modified by the relatedness of rivals as the net fitness benefits gained against a shared genotype are diminished over those gained against an unrelated competitor [5]. This is potentially important when plasticity of behavior is costly to the individual and/or where the likelihood of encountering kin more frequently than unrelated competitors is high. I found that male *D. melanogaster* were equally as likely to increase their copulation duration when they had been exposed to a full sibling rival of corresponding age and experience as when exposed to a mature, unrelated rival. This confirms that the benefits of increasing copulation duration remain positive, even when accounting for the diminished returns from competing against a full sibling. Increased copulation duration has most commonly been interpreted as a form of post-copulatory ‘mate-guarding’ in many species [1,2]. In their review of four closely related *Drosophila* species Lize et al. [8] dismiss sperm competition as a universal explanation for this behavior as it is also seen in closely related species where multiple mating is absent or infrequent. Since males are unlikely to gain a large advantage in competing for fertilizations against a full sibling, this further suggests that mate guarding is the most likely explanation in this species. One possibility is that the relatedness of the rival male was not easily determined by the focal male so that all additional males are simply counted as ‘rivals’. Olfactory signals function as highly developed kin recognition systems in social insects, such as ant colonies, and are generally well acknowledged as effective conspecific and mate recognition systems in solitary insects such as *Drosophila* [9]. However, whether olfactory cues can fine tune recognition between siblings and non-related rivals in *Drosophila* is not known. In any case the effect of any ability to recognise kin on plasticity of copulation duration was negligible. One explanation is that when the perceived operational sex ratio is male biased, either through an abundance of males or a scarcity of female encounters as were represented in this design, it is more costly for males to seek and compete for new females. In this context, extending copulation duration is not driven by the need to out-compete rivals *per se*, but is simply a low cost activity for the individual.

By contrast, I found no significant differences in mating latency between any of the focal males, although there was a weak trend for males to shorten their mating latencies after exposure to a rival and this effect was most pronounced in focal males exposed to a mature, unrelated rival. The non-significant trend is most likely due to the fact that male control over mating latency is diluted by its dependence on female preferences. In *Drosophila* species there is generally no forced copulation [10] and mating latency is predominantly taken as a measure of a female’s preference for the male they are presented with [11,12,13]. To this extent, the variation in female phenotypes will inflate the variation in male plastic responses. However, despite randomly allocating females to focal males to account for this, the trend across rival identity is still evident. Although not conclusive, the emerging pattern remains intriguing and suggests a role for relatedness and/or maturity in the plasticity of mating latency.

Since I cannot partition the effects of relatedness from maturity with this design, the specific roles of each remain purely speculative at this point. However, previous work

into olfactory cues in *Drosophila* does suggest some potential explanations. For example, focal males have been found to increase their courtship intensity more after being exposed to groups of mature, unrelated males [14] than groups of same-age siblings [6]. While this does suggest that rival age could be more influential than relatedness, again, these effects were not specifically isolated from each other. More recent work has shown that cuticular chemical compounds involved in species and mate recognition decrease with age in virgin males and females [15]. This makes it unlikely that an older male would significantly change a focal male's attractiveness to females over a younger male by physically transferring chemicals to him. A plausible explanation is that an unrelated individual may contribute different chemical compounds than a sibling, which enhances the overall attractiveness of a focal male. Flies in mixed social groups have been shown to display both higher total cuticular hydrocarbons levels [16] and have greater mating success [17] than those kept in genotypically homogeneous groups. Further work is needed to specifically establish whether maturity and/or relatedness are significant influences over plasticity in mating latency.

5. Conclusions

Taken together these results suggest that relatedness between rivals has no significant influence over the expression of plasticity of copulation duration but that it may influence the plasticity of mating latency (attractiveness) of males. This suggests that investigations based on mass bred laboratory stocks where large cohorts of males eclose and mature simultaneously with siblings may need to account for relatedness in deducing accurate assays of plasticity of mating behavior and reproductive success in this species.

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Statement of Competing Interests

The author has no competing interests.

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