Perception of male–male competition influences Drosophila copulation behaviour even in species where females rarely remate

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Males in many taxa are known to exhibit behavioural plasticity in response to the perceived intensity of sperm competition, reflected in Drosophila melanogaster by increased copulation duration following prior exposure to a rival. We tested the prediction that males do not adjust their copulation effort in response to the presence of a competitor in Drosophila species where there is little or no sperm competition. Contrary to expectations, male plasticity in copulation duration was found in both Drosophila subobscura and Drosophila acanthatopera, species in which females rarely remate. These results are discussed in relation to the adaptive basis of plasticity in these species.

Keywords: plasticity; monandry; polyandry; copulation duration; female receptivity; social environment

1. INTRODUCTION

Sperm competition—competition between the ejaculates of rival males within the female reproductive tract—has driven the evolution of many traits that enhance male post-copulatory success, for example, prolonged copulation duration, nuptial feeding and sperm partitioning (reviewed in Simmons [1]). Some of these adaptations are fixed within species. For example, species in which sperm competition is intense have evolved disproportionately large testes, so that males can deliver increased sperm numbers per ejaculate (e.g. [2]). Other adaptations are plastic, where behaviour alters in relation to cues that indicate sperm competition risk. Phenotypic plasticity in ejaculate allocation in relation to the level of sperm competition is widespread across diverse animals (reviewed in Wigby et al. [3]).

Drosophila melanogaster is a polyandrous species in which males have recently been observed to prolong copulation in response to cues indicating elevated intensity of sperm competition [4]. Males use olfactory, visual, auditory and touch cues prior to encountering females to assess the presence of competitor males [5], and respond by prolonging copulation and increasing transfer of accessory gland peptides [3,6]. This behaviour enhances male fitness through increasing his success in sperm competition, and by decreasing his mate’s receptiveness to rival males [4].

In this study, we examine the extent to which plasticity in mating behaviour is observed in four species of Drosophila with contrasting mating systems. If plasticity is adaptive solely in relation to sperm competition risk, males would not be expected to adjust their copulation effort and/or ejaculate characteristics in species where female remating is absent or rare, as in these species, sperm competition rarely occurs. While data estimating female remating refractoriness in Drosophila are commonly limited to laboratory assays (reviewed in [7–9]), females have been shown to be least likely to remate in Drosophila subobscura from the obscura group, Drosophila acanthatopera from the nannoptera group, and three species of Hawaiian Drosophila. We therefore compared plasticity in relation to competitor male presence in two species of Drosophila from the obscura clade, the polyandrous Drosophila pseudoobscura and the commonly monandrous D. subobscura [10,11], and two from the nannoptera clade, the highly polyandrous Drosophila nannoptera, and the commonly monandrous D. acanthatopera [12].

2. MATERIAL AND METHODS

(a) Plasticity in copulation duration in D. subobscura and D. pseudoobscura

Multifemale lines of D. subobscura and D. pseudoobscura were collected in 2008 from Vancouver Island (British Columbia) and Show Low (Arizona), respectively. Flies were reared in a humidified room at 18°C, with a 12:12 h photoperiod, on standard corn-sugar–yeast-agar medium. Experimental adult flies were raised at standard densities of 200 larvae per vial, and isolated when virgin using ice anaesthesia. Females were then placed at a standard density of 10 per vial. Males were isolated either individually (treatment ‘S’) or placed with a competitor male (treatment ‘C’) for 8 days.

After 8 days of maturation and conditioning, single female flies were isolated in vials at 20°C. A single male from one of the two treatments was then gently aspirated into each vial. For each male, the times of introduction and the start and the end of copulation were recorded. Vials from the ‘competitor’ treatment that contained dead males were not used, and only one male was taken from each ‘C’ pair to avoid pseudoreplication. Males were removed from the vials once copulation had ended.

Drosophila subobscura females that had mated were retained and their desire to remate tested after 7 and 14 days. To this end, a single 10-day-old male was added to the female’s vial for 2 h on each day and remating scored. Remating was similarly recorded for D. pseudoobscura females at 7 days with a 7-day-old virgin male.

(b) Plasticity in copulation duration in D. nannoptera and D. acanthatopera

Multifemale lines of D. acanthatopera and D. nannoptera collected in 1976 at Oaxaca (Mexico) and in 2002 at Puebla (Mexico) were obtained from the Drosophila species centre (UC San Diego, Stock references: 15090-1693.00 and 15090-1692.11). These species, which naturally inhabit cactus roots in Mexico/Southern US, were reared at 25°C on banana–opuntia medium.

The plasticity of mating behaviour was measured as for obscura flies above, with the following distinctions. At 25°C, males of these species take 7 days to mature and they were thus allowed to mature individually for 8 days. Males were then placed in two treatments (S and C) as before and were maintained in these regimes for 4 days before being placed with a female. Following the initial copulation duration assay, female remating was scored 4 h after copulation for D. nannoptera (which remates daily), and 7 and 14 days after copulation for D. acanthatopera (a species which remates rarely), in each case with a 12-day-old virgin male.
3. RESULTS

In all four species, copulation duration was significantly longer when males were conditioned with a rival before copulation (D. subobscura, t-test (Welch modification), t = 6.39, d.f. = 61.226, p < 0.001; D. pseudoobscura, GLM, t = 4.14, d.f. = 96, p < 0.001; D. acanthoptera, GLM, t = 3.43, d.f. = 163, p < 0.001; D. nannoptera, GLM, t = 2.47, d.f. = 153, p = 0.013; figure 1 and see the electronic supplementary material for full details of the analysis).

In three of the four species studied, copulation latency (time from introduction of a male to mating) was significantly longer when males were conditioned with a rival before copulation (Kruskal–Wallis, D. subobscura, χ² = 3.88, d.f. = 1, p = 0.048; D. pseudoobscura, χ² = 5.51, d.f. = 1, p = 0.018; D. acanthoptera, χ² = 1.90, d.f. = 1, p = 0.167; D. nannoptera, χ² = 10.45, d.f. = 1, p = 0.001; figure 2 and see the electronic supplementary material for full details of the analysis).

Female remating rate was highly variable across the four species, ranging from 0 per cent in the monandrous D. subobscura to 80 per cent in polyandrous species (table 1). The social environment of the first male mated with the female did not affect the female’s propensity to remate in any of the four species (GLM, D. subobscura, z = 0.00, d.f. = 48, p = 1.000; D. pseudoobscura, z = 0.00, d.f. = 93, p = 0.995; D. acanthoptera, z = 0.00, d.f. = 102, p = 0.408; D. nannoptera, z = 1.18, d.f. = 104, p = 0.218).

4. DISCUSSION

Plasticity in mating duration in response to pre-copulatory cues of competition was found in all four Drosophila species in this study, notwithstanding the variety of mating systems displayed by these species. Most notably, copulation duration increased in response to pre-copulatory cues of male–male competition in both D. subobscura and D. acanthoptera, two species in which females remate either rarely or not at all and post-copulatory sexual selection is thus very weak. Indeed, D. subobscura, the species least likely to remate in laboratory assays, demonstrated the largest increase in copulation duration following exposure to a competitor. In this species, mean copulation duration almost doubled following exposure to a competitor (copulation duration increased by 93%), compared with 6–21% increases in copulation duration in other species.

We observed that latency to mate was also affected by social environment prior to mating. Males conditioned with competitors took longer to acquire a mate than males maintained singly prior to mating in three of the four species. Whether this difference is functionally related to the longer copulation obtained by these males is uncertain, and requires further investigation. However, we note that this correlation was not universal in Drosophila (our study and reference [13]).

Previous studies of the moderately polyandrous D. melanogaster demonstrated how plasticity in copulation duration is adaptive in this species [4,13]. Increased copulation duration followed a pre-copulatory cue indicating that post-copulatory sexual selection was likely to be intense. Males experiencing this cue mated for longer, had increased success in sperm competition and induced increased latency to remating in their female partner. This explanation for plasticity does not hold in monandrous species such as D. subobscura and D. acanthoptera, as here there is little or no sperm competition.

Why, therefore, is plasticity observed in the two species where remating is rare? We consider there to be four possible explanations. The first is that plasticity in copulation duration may be maintained by even
weak sperm competition, with very low levels of female remating sufficient to make alteration of male behaviour beneficial. While we observed no remating in \textit{D. subobscura}, \textit{D. acanthoptera} did show low levels of remating after 14 days (similarly noted by Pitnick \\& Markow [12]). Further, while we are confident females of these two species are among the least likely to remate in the genus \textit{Drosophila}, firm attribution of the precise rate of female remating for any particular population or species requires marker-based measurement of paternity in field-collected females (reviewed in Singh et al. [7]). The second explanation relates to evolutionary lag: plasticity is not currently adaptive, but was in the recent past. There is evidence that monandry has evolved recently in \textit{D. subobscura}; all other members of the \textit{obscura} clade exhibit polyandry [10,14,15], and while our Canadian population showed no evidence of female remating, polyandry is observed in some current populations of \textit{D. subobscura} [14,16]. A third explanation is that plasticity is adaptive in monandrous taxa, but has a different driver. As females remate rarely in these species, there is a highly male-biased operational sex ratio, and males suffer strong pre-copulatory sexual selection. The presence of rival males signals that pre-copulatory sexual selection will be particularly intense for males, and future copulations unlikely. In this instance, retaining sperm and accessory fluid for future copulations may represent a waste of effort and males are selected to prolong copulation to ensure that the female partner obtained remains fertile for her entire life. A fourth explanation is that the behaviour is simply non-adaptive in the genus \textit{Drosophila}.

In conclusion, plasticity in mating behaviour is more widespread among mating systems than initially thought, at least within the genus \textit{Drosophila}. Males respond to the presence of a competitor male by prolonging copulation in both polyandrous species and those where females rarely remate. Future research into how plasticity relates to fitness in monandrous mating systems will be valuable.

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