



Playing to the crowd: Using *Drosophila* to dissect mechanisms underlying plastic male strategies in sperm competition games

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1. Introduction

Social environments can have profound effects on an individual's fitness, regardless of whether the environment is stable or rapidly changeable, and can be important even in organisms perceived to live in relatively simple societies (Bailey & Moore, 2018; Bilas, Bretman, & Bennett, 2021). The social environment can be defined as any interaction between individuals of the same species. The outcomes of the interactions may depend on the sex, age, relatedness of interacting parties, or simply number or density of other individuals. In this review we will focus on one aspect of the social environment, namely how males use the presence of rival males prior to

mating to predict competition and alter their investment in mating. The idea that males should respond to their competitive environment to gain significant fitness benefits was proposed by Geoff Parker over 50 years ago (Parker, 1970) and shown experimentally by Matt Gage (Gage, 1991), supporting the key components of ejaculate economics theory (Parker, Ball, Stockley, & Gage, 1996, 1997). Over the last couple of decades there has been a rapid increase in studies documenting adaptive plasticity (e.g., reviews by Bretman, Gage, & Chapman, 2011; Magris, 2021; Ramm, 2020; Weir, Grant, & Hutchings, 2011). Together such studies are highlighting the sophisticated plasticity of males that occurs in response to social context.

The strength of intra-sexual competition is determined by the social environment, both population density and Operational Sex Ratio (OSR, ratio of receptive males to females) altering encounter rates with potential mates and rival males (Emlen & Oring, 1977; Kokko & Rankin, 2006). However, the level of competition within a species is unlikely to be static over a male's reproductive lifetime, as population densities and OSRs can be highly variable over short temporal and spatial scales (Kasumovic, Bruce, Andrade, & Herberstein, 2008). It has long been suggested that males could use social information to invest strategically in reproductive efforts (Parker, 1970; Wedell, Gage, & Parker, 2002). Males of many species have multiple mating opportunities but limited resources. This generates a potential trade-off between current and future mating events, thus selecting for strategies that maximize a male's reproductive output through plastic allocation of reproductive effort (Parker, 1982). Factors that could affect the fitness value of any particular mating include the availability, quality and mating status of females, and the amount of competition from other males. For example, in many species female size is positively related to fecundity, so the opportunity to mate with a larger female might induce the male to invest more in that mating (Bonduriansky, 2001). Indeed, if a male has recently mated (i.e., is resource-depleted) this preference can become stronger (Byrne & Rice, 2006). However, if larger females are generally preferred then a male might encounter greater competition for that mate. Therefore, the optimal strategy of an individual male will depend on the strategy rival males employ (Parker & Pizzari, 2010).

Competition between males can occur before copulation, affecting the probability of obtaining matings, and can involve direct aggressive encounters or indirect competition mediated through courtship displays (Emlen & Oring, 1977; Kokko & Rankin, 2006). Taking a meta-analysis

approach, [Weir et al. \(2011\)](#) found that as OSR increased, aggression between males increased and then declined. This suggests that as the number of rivals increases, males fight more for access to females up to the point where the number of rivals makes aggression too costly ([Knell, 2009](#)). Conversely, courtship effort declines with increasing male bias, perhaps because there are simply fewer females to prompt displays, or because males might switch strategies, e.g., to sneak matings ([Weir et al., 2011](#)). So while the presence of rival males obviously implies competition, it can also create selective opportunities for alternative reproductive tactics. An intriguing recent example comes from black widow spiders. Here, in a situation where mate searching is extremely costly and the local OSR is high, males can parasitize rival males' mate searching efforts by following their silk, using social information to more quickly find females despite this resulting in higher competition ([Scott, McCann, & Andrade, 2019](#)).

In polyandrous species, competition between males may continue post-copulation, i.e., through sperm competition and cryptic female choice. In much of the early theory developed to predict optimal male sperm competition strategies, a male's investment relates to the number of sperm produced or allocated to a particular mating ([Parker et al., 1996, 1997](#)). However, this can be thought of more broadly as "mating investment," and so be applied to non-sperm ejaculate components and behavior ([Cameron, Day, & Rowe, 2007](#); [Wedell et al., 2002](#)), and to pre-copulatory behaviors ([Parker, Lessells, & Simmons, 2013](#)). As the "risk" of competition (the likelihood a female will mate more than once) increases, a male's investment in sperm production is predicted to increase ([Parker et al., 1996, 1997](#)). In contrast, when sperm competition "intensity" (the number of competitors) increases, the amount of sperm allocated is predicted to peak when males encounter one rival and decrease thereafter as returns diminish with more competitors ([Parker et al., 1996, 1997](#)). These predictions can be modified by many factors within different mating systems. For example, the pattern of investment is predicted to change depending on whether there is a fair raffle between sperm such that the number of offspring reflects the number of sperm, or whether there is precedence for either the first or subsequent males. The predictions will also differ if males obtain imperfect information about competition levels. Males may trade-off investment in ejaculate components and behavior with other reproductive traits, such as weaponry. Predictions for the sperm and non-sperm portions of the ejaculate may also vary ([Cameron et al., 2007](#)). Male investment and the trade-off between current and future

matings will likely depend on a male's condition and age and are highly likely to depend upon female condition and behavior. Sperm competition relies on females being polyandrous within a mating bout, so the amount of competition males face will depend on female mating rate (Fromhage, McNamara, & Houston, 2008; Tazzyman, Pizzari, Seymour, & Pomiankowski, 2009). Together, the rich theory base on expected competitive outcomes clearly shows the significant fitness gains for males that adjust their mating investment, whether behavior or ejaculate components, in response to cues of sperm competition (Fromhage et al., 2008; Parker & Pizzari, 2010; Tazzyman et al., 2009; Williams, Day, & Cameron, 2005).

There are numerous studies in which the potentially adaptive responses of males to cues of sperm competition have been documented, and a number of excellent reviews show the broad taxonomic occurrence of this phenomenon. Some reviews have focused on strategic ejaculation (Kelly & Jennions, 2011; Magris, 2021; Perry, Sirot, & Wigby, 2013; Ramm, 2020; Wedell et al., 2002), and others on behavioral responses such as courtship effort, mating duration and mate guarding (Bretman, Gage, et al., 2011; Dore et al., 2018; Kasumovic & Brooks, 2011; Weir et al., 2011). However, a male's plastic responses may be achieved through a diverse combination of ejaculate and behavioral components. Our objective in this article is not to provide broad taxonomic coverage, but instead to focus around a single system, to deepen our understanding of the totality of physiological processes and responses that result in adaptive male plasticity. However, we emphasize that such plasticity is widespread (Bretman, Gage, et al., 2011) and hence also include here some additional, recent examples just to illustrate its diversity. Perhaps unsurprisingly given their amenability to experimental studies, many examples of male plasticity come from invertebrates. Examples in which increased exposure to males alters male investment include: the production of more but lower quality nuptial gifts in male-biased populations of *Pisaura mirabilis* spiders (Heimerl et al., 2021), increased mating duration following exposure of males to chemical cues of rival males in the ladybird *Menochilus sexmaculatus* (Chaudhary, Mishra, & Omkar, 2017), increased copulation duration and fertilization success in male soldier flies *Merosargus cingulatus* exposed to rivals (Barbosa, 2012), and increased sperm transfer rate after experiencing the presence of a rival in lesser wax moths (*Achroia grisella*) (Jarrige, Riemann, Goubault, & Schmoll, 2015). Examples from vertebrates include; male green treefrogs *Hyla cinerea* (Neelon & Höbel, 2019) and gladiator frogs *Hypsiboas rosenbergi* (Höbel, 2015) alter courtship calling in response to rivals, likewise

three-spined stickleback *Gasterosteus aculeatus* exposed to a dummy rival male increased courtship effort and increased in redness (attractive to females) (Kim & Velando, 2014), and in greater sac-winged bats *Saccopteryx bilineata*, territorial song production is observed to increase as the number of rival males in the colony becomes greater (Eckenweber & Knörnschild, 2013). Finally, notoriously lacking in sex drive in captivity, male giant pandas (*Ailuropoda melanoleuca*) can be induced to show more interest in a female in oestrous after exposure to scents from rival males (Bian et al., 2013). While not comprehensive, this list of recent findings shows the variety of responses of males to conspecific rivals. They document behavioral changes, and in some cases alterations to ejaculate components. However, there has tended to be less focus on dissecting the drivers, mechanisms and consequences of this plasticity, on which we focus here within a single system, by presenting a detailed discussion of research performed using *Drosophila* fruit flies, mainly *Drosophila melanogaster*. This valuable and tractable research model is providing an exemplar for understanding the proximate and ultimate causes of the behavioral and ejaculate responses to the presence of rival males.

Drosophila of both sexes are highly sensitive to their social environment (Sokolowski, 2010) and have been used extensively to probe the mechanisms of plasticity in response to social cues (Chen & Sokolowski, 2022). Both sexes display changes in social behavior in response to change in group size and density (Roode, Rasool, Schneider, & Levine, 2020), and their position in a group has a genetic basis and is heritable (Wice & Saltz, 2021). Moreover, individuals can exhibit social learning. For example females can alter their oviposition behavior if they learn indirectly of the presence of parasitoids from social partners (Kacsoh, Bozler, Ramaswami, & Bosco, 2015). Social interactions also impact the responses of individual flies to infection (Leech, Evison, Armitage, Sait, & Bretman, 2019) as well as to non-infectious disease such as cancer (Dawson et al., 2018). The social environment can also influence microbiomes (Leech et al., 2021), sleep and feeding behavior (Li et al., 2021). Mating behavior, and in particular the robust, plastic responses made by males to the presence of rival males before mating upon which we focus in this review, is highly sensitive to the social environment. The experimental power available in *D. melanogaster* in particular, has enabled researchers to conduct detailed studies to examine these key plastic responses, and to reveal the underpinning molecular mechanisms involved. We describe below the research that has been conducted to measure the benefits and costs of male plastic responses, to dissect the behavioral

and ejaculate component traits that actually respond, and to examine the mechanisms involved. This covers the processes of initial environmental information gathering and processing, through to the production of the male responses themselves.



2. Combining mating behaviors and ejaculate economics—Is plasticity adaptive?

Behavioral traits can be extremely variable both between, and importantly within, individuals across different social and sexual contexts. A key step in understanding whether variation in behavior is adaptive behavioral plasticity is to test its impact on fitness. Male *D. melanogaster* respond to the presence of a rival male prior to mating by increasing their mating duration (Bretman, Fricke, & Chapman, 2009). In contrast, exposure to multiple males in the mating arena itself results in the opposite effect, i.e., a decrease in mating duration (Bretman et al., 2009). Consistent with this, individual males can accurately track their social environment, increasing or decreasing mating duration depending on their recent experience of exposure to potential rivals (Fig. 1) (Bretman, Westmancoat, Gage, & Chapman, 2012). Males are largely in control of determining the extension of mating duration, as demonstrated in a study where the capacity of females to respond was reduced (Bretman, Westmancoat, & Chapman, 2013). To do this, anesthetized females were decapitated and immobilized. The result was that, while mating duration was overall shorter with such females, the extension of mating duration by males that had been exposed to rivals remained. Supporting the idea that mating duration may be largely influenced by males is the finding that males mutant for the circadian rhythm genes *period* or *timeless* show altered copulation durations, while female flies carrying the same mutations do not (Beaver & Giebultowicz, 2004). A substantial body of work shows that the mating duration response to a rival is a robust and repeatable example of behavioral plasticity. However, the key step in understanding whether variation in behavior is adaptive is to measure its impact on fitness (Bretman et al., 2009). As shown below, the actual association of plastic responses with fitness can be complex.

Similar responses by males to rivals seems widespread among *Drosophila*. An increase in mating duration after exposure to rivals has been observed in *D. pseudoobscura*, *D. subobscura*, *D. montana*, *D. acanthoptera* and *D. nanoptera* (Lizé, Doff, Smaller, Lewis, & Hurst, 2012; Mazzi, Kesaniemi, Hoikkala, & Klappert, 2009; Price, Lizé, Marcello, & Bretman, 2012). These species

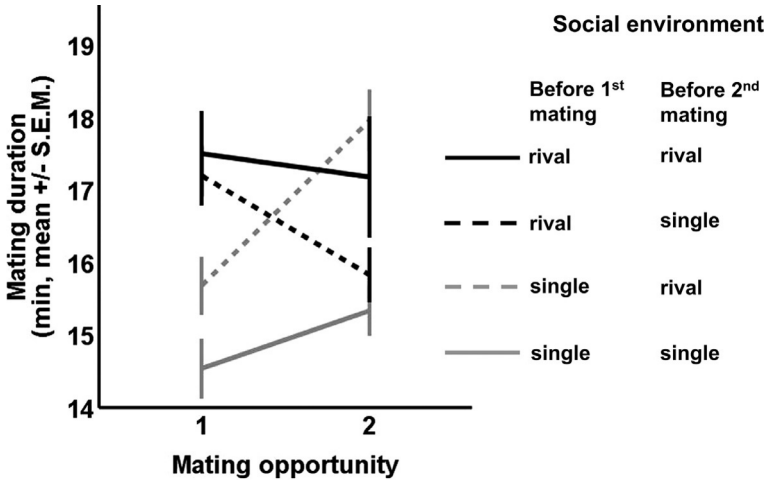


Fig. 1 *D. melanogaster* mating duration in response to exposure to rivals prior to mating, across two mating opportunities. Having documented in single mating events that males previously exposed to rivals increased mating duration (Bretman et al., 2009), this experiment explored whether males could vary their behavior if the environment changed. Initially, males were held alone (gray) or with a rival male (black) for 72 h, then allowed to mate. They were then given the same social condition (solid lines) or switched conditions (dashed lines) for 72 h before mating again. Males mated for longer if they had been exposed to a rival, and followed their most recent experience, showing the response to be fully flexible. Data from Bretman, A., Westmancoat, J. D., Gage, M. J. G., & Chapman, T. (2012). Individual plastic responses by males to rivals reveal mismatches between behaviour and fitness outcomes. *Proceedings of the Royal Society B: Biological Sciences*, 279, 2868-2876.

represent clades of fruit flies that diverged at least 60MYA (Tamura, Subramanian, & Kumar, 2004). The presence of plastic responses to rivals within *D. subobscura* and *D. acanthoptera* is puzzling, as females of these species are believed to mate only once, and have never been observed remating under laboratory conditions. Hence if males are unlikely to experience sperm competition, the reasons for extending mating and potentially investing more in such matings when potential rivals are present are unclear and call into doubt adaptive explanations (Lizé, Doff, et al., 2012). One possibility is that extended mating following exposure to rival males may not always be adaptive and might instead sometimes arise from sources such as fatigue from intense pre-copulatory competition (Lizé, Price, Heys, Lewis, & Hurst, 2014). These observations emphasize the importance of testing for fitness effects of plastic responses and ejaculate composition effects in all of these different *Drosophila* species. So far, the only *Drosophila* species

tested which does not exhibit plasticity in mating duration in response to conspecific males is *D. bifasciata* (Lizé, Price, et al., 2012). The authors suggested that although this species is polyandrous, it forms a more substantial mating/sperm plug than do other *Drosophila* species, perhaps preventing the female from mating for long enough that plasticity in mating duration would not accrue benefits (Lizé, Price, et al., 2012). An alternative explanation is that because this species mates on open sap runnels (rather than in complex rotting fruit as do many other *Drosophila* species) it only ever faces high competition, rendering plasticity ineffective (Lizé, Price, et al., 2012). This potential ecological driver could be investigated by assessing other sap dwelling species.

If plasticity in mating duration is adaptive, then males should accrue fitness benefits by responding to rivals. Consistent with this, the extended mating duration triggered by pre-mating exposure to rivals has been shown in *D. melanogaster* to increase a male's reproductive success by increasing the number of eggs laid by the female he mates with, increasing survival of those eggs to adulthood, increasing the time before remating by the female and thus increasing paternity share, both when such "responding" males are the first or second male to mate (Bretman et al., 2009). Therefore, in these single matings, males appear to gain significantly increased fitness from responding to the threat of sperm competition, as predicted. However, these fitness benefits may decrease over time. For instance, over successive matings, the extended mating duration response is maintained without discernible associated fitness benefits (Bretman et al., 2012). Indeed, over their lifetime, males held with rivals continue to mate for longer than those held singly up until very old age (Bretman, Westmancoat, Gage, & Chapman, 2013). Responding males only achieve a higher offspring output in the first few matings, and overall, both treatments are observed to achieve equivalent lifetime reproductive success. Similarly, Douglas, Anderson, and Saltz (2020) found the initial increase in offspring fathered by males after exposure to rivals declines of successive matings. This reinforces the idea that it is the ability to exhibit plasticity itself that is critical for fitness, as neither "fixed" strategy of always short or always extended mating is better in fitness terms. Moreover, while fitness gains by responding males are often evident (Bretman et al., 2009, 2012; Bretman, Westmancoat, Gage, & Chapman, 2011; Bretman, Westmancoat, Gage, et al., 2013; Hopkins et al., 2019; Mohorianu, Fowler, Dalmay, & Chapman, 2018), this is not the case in all studies (Dore, Bretman, & Chapman, 2020; Hopkins et al., 2019). This suggests that fitness benefits do not occur as a direct result of longer

matings per se, or that any benefits are life-stage or context-dependent. This prompts the question how exactly fitness benefits of extended matings are achieved, which we discuss below.

A potential benefit to extended matings is that males can guard females to prevent immediate remating (Burdfield-Steel & Shuker, 2014). However, in *D. melanogaster* the extension resulting from exposure of males to rivals is a couple of minutes in a mating that typically lasts 10–20 min. Moreover, when males mate in the presence of rivals, mating duration decreases, perhaps due to interference or harassment from the rival (Bretman et al., 2009). Therefore, it seems unlikely that the extended mating could be a direct form of mate guarding. In some species, mating duration has a linear relationship with the transfer of sperm (Simmons, 2001), and so there would be an obvious benefit to males in mating for longer and successfully transferring more sperm. However, in *D. melanogaster* at least, this relationship does not hold, with sperm seemingly delivered in a discrete package mid-way through copulation (Gilchrist & Partridge, 2000; Linklater, Wertheim, Wigby, & Chapman, 2007). Nevertheless, the magnitude of a male's response to rivals is correlated with changes in his ejaculate. For example, Moatt and colleagues (Moatt, Dytham, & Thom, 2014) found that males exposed to rivals had a greater proportion of live sperm in their seminal vesicles, while Garbaczewska, Billeter, and Levine (2013) found that these males transferred more sperm to females. In line with the predictions from models of sperm competition intensity (Parker et al., 1996), there is an increase in sperm transfer following exposure to single rival, and a decrease after exposure to multiple rivals (Hopkins et al., 2019). Similar phenomena have been observed in other fruit flies. For example, in *D. pseudoobscura*, a species with polymorphic sperm, it is observed that males exposed to rivals increase the proportion of fertilizing eusperm transferred to females and thus gain more offspring (Price et al., 2012). A more recent study discovered that there are in fact two morphs of non-fertilizing parasperm, one of which is increased and the other decreased in response to rival male exposure (Alpern, Asselin, & Moehring, 2019). Although not explicitly testing the response to rivals, longer mating duration in *D. montana* is linked to increased offspring output and reduced female remating (Mazzi et al., 2009).

Sperm are not the only critical part of the ejaculate; seminal fluid proteins (Sfps, which include the Accessory Gland proteins, or Acps) also represent a substantial reproductive investment by males (Perry et al., 2013). Indeed, males may become Sfp-depleted before sperm becomes limiting (Hihara, 1981; Linklater et al., 2007). Recently it was estimated that the

D. melanogaster seminal proteome contains about 300 proteins (Wigby et al., 2020). The Sfps have myriad effects, including ensuring sperm reaches storage and influencing female egg laying rate, remating rate, lifespan, sleep, feeding and immune responses (Avila, Sirot, LaFlamme, Rubinstein, & Wolfner, 2011). The observations of increased egg laying and reduced remating by females mated to males previously exposed to rivals (Bretman et al., 2009), and shortened lifespans exhibited by these females (Filice, Bhargava, & Dukas, 2020) are therefore known effects of receipt of Sfps. A direct quantification of two important Sfps, sex peptide and ovulin, transferred to females, showed these increased after males were exposed to rivals (Wigby et al., 2009). A further study combined observations of mating duration, counts of sperm transferred and analysis of the whole ejaculate proteome (Hopkins et al., 2019). Both mating duration and sperm number transferred were increased by the presence of just one rival, as in previous studies (Fig. 2). However, the majority of Sfps showed no response to a single rival, but were transferred in increased amounts in response to multiple rivals (Hopkins et al., 2019). This variation in the

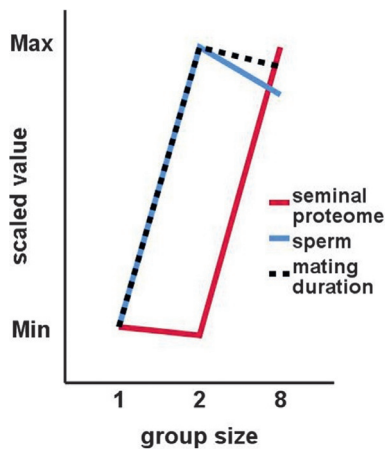


Fig. 2 Relative responses of mating duration, sperm and seminal fluid to varying numbers of rivals. Male *D. melanogaster* were kept singly (1), in a pair (2) or a group of 8 prior to mating. Mating duration and number of sperm transferred to females were assayed. An estimate of seminal proteome transfer was calculated from quantitative proteomics of virgin vs mated males in each group. Here for direct comparison between these three reproductive components, we show each data type relative to a standardized value for treatment 1. While sperm and mating duration showed increases from males held singly to males held in pairs, and a slight decrease in the group of 8, the seminal proteome in contrast showed no response between 1 and 2, and maximal response at 8. Values are calculated from Hopkins et al. (2019).

sensitivity or response of different reproductive components (i.e., mating duration, sperm and seminal fluid) to the number of rivals is intriguing. As discussed in the introduction, theoretical models of sperm competition intensity (i.e., number of competitors) predict that males should invest maximally when experiencing one competitor and decrease investment thereafter. Therefore, males should not just be sensitive to the presence of others but to the number of competitors. Some evidence of this is found in *D. montana*, as the peak extension of mating duration is observed with one competitor but reduced with four rivals (Mazzi et al., 2009). However, we were unable to find such an effect on duration over a range of one to 36 males held together, no further extension was observed beyond one rival, and neither did the density of flies have an effect (Bretman, Fricke, Hetherington, Stone, & Chapman, 2010). This appears to be a common finding. Kelly and Jennions (2011) undertook a meta-analysis of ejaculate size (sperm quantity) plasticity, finding a lack of sensitivity to the number of rivals across a range of studies. The generally poor concordance in *D. melanogaster* between mating duration and precise changes to ejaculate composition, and the difference in sensitivity to the number of rivals, again shows that plastic mating behavior and ejaculate transfer and composition are related but distinct elements of a male's response to rivals.

Given the potential benefits of increased investment in a mating event discussed above, the maintenance of plasticity, rather than all males continually investing more, implies that responding to rivals is costly. Attempts to measure such costs have been through measurements of the effects of male responses to rivals on lifespan and lifetime reproductive success. As noted above, over successive matings, the duration extension and fitness benefits become uncoupled (Bretman et al., 2012). This is exacerbated when the number of rivals is increased, with males exposed to a greater number of rivals showing a sharper decline in their number of offspring produced (Hopkins et al., 2019). Housing males in isolation or with a rival for their entire adult lifetime and allowing weekly mating opportunities, shows that rivals maintain the extended mating response until old age (Bretman, Westmancoat, Gage, et al., 2013). However, responding males only gained fitness benefits in the first few matings, became progressively less successful at mating with age and ultimately died earlier. Therefore, given the response appears costly, one might predict that males in poor condition, for example fed a poor diet, would be unable to mount a response. Consistent with this, the ability to respond was diminished in males that were starved or fed an imbalanced diet, and while males exposed to rivals continued to extend

mating duration, this did not translate into a greater number of offspring (Mason, Rostant, & Chapman, 2016).

Similar patterns of increased aging and reduced lifespan in responding males of monogamous species without any observable benefits has led to the suggestion that the extended mating duration in response to rivals is an outcome of the high cost of pre-copulatory male-male interactions, and is not an adaptive behavior (Lizé, Price, et al., 2014). However, though males are aggressive toward each other, there is no data to support the suggestion that aggression plays a significant role in the patterns observed in lifespan and aging (Bretman, Westmancoat, Gage, et al., 2013; Flintham et al., 2018; Leech, Sait, & Bretman, 2017). Females held in single sex groups do not incur shorter lifespans. Flintham et al. (2018) used a quirk of *D. melanogaster* development to investigate the source of this sex difference in social impacts on lifespan. *D. melanogaster* sex determination is cell-specific, meaning that genetic manipulation can be used to feminize or masculinize cell types within an animal of the opposite sex. When this was employed to masculinize the nervous system of females the resulting flies showed male-like lifespan costs (Flintham et al., 2018). This finding suggests that costs are not directly related to increased investment in ejaculate production itself, as the flies that showed male-like costs were otherwise female and sterile. The presence of other males is not always costly to male lifespan, and can even increase lifespan if rival males are exchanged so they remain young as the focal male ages (Ruan & Wu, 2008). These data show that while interacting with other males can be costly, it is not clear that these are costs of the sperm competition response of extended matings per se. The decrease in lifespan observed may be a consequence of social interactions unrelated to sperm competition, such as alteration of the microbiome (Leech et al., 2021). Moreover, the cost of being plastic has not yet been measured, as the experimental design described above essentially forces males to take one or other strategy (Bretman, Westmancoat, Gage, et al., 2013). To measure direct costs of mating duration plasticity, it is necessary to integrate these experiments with manipulations of the mechanistic basis of the response that we discuss below.

Laboratory studies are an excellent way to interrogate behaviors in controlled manipulations. However, it is unclear how well what is observed in these experiments reflects the natural context. Unfortunately, it is rare to find species that are tractable in both settings (Rodríguez-Muñoz, Bretman, Slate, Walling, & Tregenza, 2010). In *Drosophila*, local densities and sex ratios vary considerably over space and time (Markow, 1988; Soto-Yéber, Soto-Ortiz, Godoy, & Godoy-Herrera, 2019). Understanding an individual fruit fly's

natural social environment is challenging. However they are known to cluster on patchy food resources and to travel distances of up to 2 km, so it is feasible that fruit flies in nature experience variation in the presence of rival males (Markow, 1988; Soto-Yéber et al., 2019). The response to rivals certainly seems robust to specific experimental conditions, being observed by many independent groups across different laboratories. Nevertheless, the possibility remains that this behavior is shaped at least to some extent by laboratory culturing regimes. The wild type strain used in many of these studies, Dahomey, was collected in what is now Benin in the 1970s, hence is highly lab-adapted. The populations are cultured in large, panmictic cage populations, to minimize the effects of genetic drift or selective sweeps. Though the population cages are generally held at a high density, the regular influx of new food and emergence of new cohorts of flies is expected to promote variation in the social environment. If the cages were insufficiently variant in this respect, we would also expect the loss of plasticity because males are constantly under competition. However, plasticity is maintained, suggesting it is either critical to fitness, not costly enough to lose and/or maintained because there is sufficient variation (Dore, Rostant, Bretman, & Chapman, 2021). A recent, elegant study from Churchill, Bridle, and Thom (2020) showed that, in the laboratory, the patchiness of food can also drive male plastic mating duration responses. Groups of three males were allowed to interact on food patches that were clustered or dispersed. On clustered food, individuals spent more time in close proximity and subsequently mated for longer, strongly suggesting the plastic response of males to rivals is ecologically meaningful and that the physical environment also impacts upon the social environment (Churchill et al., 2020). This is consistent with wild observations of *D. melanogaster* and *D. simulans* which suggest that microhabitat heterogeneity alters behaviors such as cluster formation, courtship and aggression (Soto-Yéber et al., 2019).

Adaptive plasticity should only be observed when there is sufficient environmental variation to make responding worthwhile, e.g., when amount of sperm competition varies across mating opportunities. As noted above, plasticity in male mating responses has been maintained over hundreds of generations in laboratory populations kept at relatively constantly high density, and is observed in most *Drosophila* species so far tested, including in monogamous species (Lizé, Doff, et al., 2012). Therefore, responses being observed in situations in which plasticity should not be beneficial because competition is always very high or very low, suggests that it is not particularly evolutionarily labile. In support of this idea, there is little evidence that the degree of plasticity in mating duration is heritable (Bretman, Lizé, Walling, & Price, 2014;

Taylor, Evans, & Garcia-Gonzalez, 2013). Recent evidence shows that the response can be altered by experimental evolution under different sex ratios, with males from male-biased or females-biased selection lines showing differences in their sensitivity to the number of rivals to which they were exposed (Maggu, Ahlawat, Arun, Meena, & Prasad, 2021). However, in neither selection regime was the plasticity of the response lost. Similarly, Dore et al. (2021) found mating duration per se to lengthen over generations under male biased selection, but no change in the plastic response to rivals.

Taken together, the work described above gives a picture of the behavioral component of a male's plastic response to rivals being "hard wired" and generally observed, while the ejaculate response is less consistent. Perhaps this reflects that it is more costly or complex to alter ejaculate components. As we unpick the mechanistic basis of these behavioral and ejaculate components of the response to rivals, we will be able to assess whether they are indeed separate or whether they require cross-talk between tissues such as the brain, testis and the accessory glands. Nevertheless, mating duration itself is a convenient and easily measured proxy that indicates when males are sensing and responding to the presence of rival males.



3. How are males able to be plastic?

In order to show plasticity, individuals must have the capacity to assess environmental information and translate it into their behavioral strategy, and this may occur through a variety of mechanisms (Cardoso, Teles, & Oliveira, 2015). To respond to rivals, males need the sensory ability to identify potential rivals, to process this information via a central hub (usually the brain or nervous system), and change output behavior accordingly. Below, we assess the potential mechanisms at various stages of this process.

3.1 A complex set of sensory information

Work in other animals such as newts, meadow voles, mealworm beetles, bushcrickets, field crickets and flour beetles has shown that rivals can be detected via single cues either in chemosensory (Aragon, 2009; Carazo, Font, & Alfthan, 2007; delBarco-Trillo & Ferkin, 2004; Lane et al., 2015; Thomas & Simmons, 2009) or acoustic modalities (Bailey, Gray, & Zuk, 2010; Gray & Simmons, 2013; Rebar & Greenfield, 2017). However, work in *D. melanogaster* using genetic and physical manipulations of different sensory modalities found a more complex picture (Bretman, Westmancoat, et al., 2011). No manipulation of tactile, auditory, olfactory or visual cues on their

own prevented a male's plastic mating duration response. The presence of a rival can be simulated by providing single males with either pure cVA, a pheromone associated with aggression (Wang & Anderson, 2010), or the entire cuticular hydrocarbon repertoire of another male. This cue on its own is also unable to trigger a male's response to rivals. However, when combinations of sensory cues were tested, it was found that males required a paired combination of sound, smell and touch to respond to rivals, but that any two of those three sensory inputs was sufficient. Interestingly, vision was not involved in a male's plasticity response (Bretman, Westmancoat, et al., 2011). Other researchers have found that separating males from rivals with a permeable plastic divider maintains the mating duration plasticity response and increase in sperm production seen following exposure to rivals but removes any effects on lifespan (Moatt et al., 2014). This suggests that the reduction in lifespan caused by exposed to a rival only occurs when males are in physical contact (Moatt, Dytham, & Thom, 2013). Some results are also mixed—with one study reporting that a male's plastic response does utilize visual cues, as tested using mirrors to simulate the presence of a rival (Kim, Jan, & Jan, 2012), but with a subsequent study failing to replicate this effect (Rouse, Watkinson, & Bretman, 2018). Interestingly, *D. pseudoobscura* also utilize multiple, but contrasting sensory cues to respond to rivals, deploying tactile and olfactory cues and not vision, with the role of auditory cues being unclear (Maguire, Lizé, & Price, 2015).

The complexity of sensory modalities used to respond to the presence of a rival suggests that it may be costly if males make incorrect reproductive investment decisions and/or that a male requires a range of information to assess whether another individual should be considered a rival. *Drosophila* live in mixed species groups, though within these they can form clusters of conspecifics (Soto-Yéber et al., 2019). Although hybridization can occur, there are strong pre-copulatory mechanisms promoting reproductive isolation (Coyne & Orr, 1989). If species can effectively avoid hybridization, then heterospecifics should not be recognized as potential sperm competitors and trigger male plastic responses to rivals. Accordingly, *D. pseudoobscura* males increase mating duration in response to conspecifics but not to *D. persimilis* (Price et al., 2012) or *D. subobscura* males. Likewise, *D. subobscura* males do not respond to *D. pseudoobscura* males as potential rivals (Lizé, Price, et al., 2014). It may be that species identity is an important component of a male's decision to extend mating duration and that at least part of the sensory system used is to detect this information. *D. melanogaster* males with a full sensory repertoire do not respond by extending mating to *D. yakuba*

or *D. virilis*. However, they mount a partial plastic response to *D. simulans* and *D. pseudoobscura* males, which is an unexpected pattern based on their phylogenetic distance from one another (Bretman, Rouse, Westmancoat, & Chapman, 2017). *D. melanogaster* are not known to hybridize with *D. pseudoobscura* and only rarely with *D. simulans*, so it was not expected that they would be perceived as rivals by *D. melanogaster* males. When single sensory modalities of smell, hearing or touch were manipulated, *D. melanogaster* males were more likely to make a mistake, i.e., to respond by extending mating duration upon exposure to species with which they never hybrid mate. In the majority of manipulations that altered the response, it caused *D. melanogaster* to respond to heterospecifics to an equal or greater degree as to conspecifics, rather than reducing their responses. However, no one sensory modality could be identified as critical, and no one manipulation caused a change in the same direction across all four rival species. Likewise, multiple manipulations of the same modality did not cause identical responses. For example, interfering with olfaction by removing antennae caused a fivefold increase in the mating duration response to *D. simulans*, but the use of the olfactory co-receptor defective *Orcy* mutant had little effect. This shows that these sensory manipulations are not interchangeable, and indeed removing parts of the antennae may interfere with both olfaction and hearing (Göpfert & Robert, 2002; van Naters & Carlson, 2007). Nevertheless, the findings suggest that species identity is a key part of the information males require to make accurate responses to rivals (Bretman et al., 2017).

The question of whether relatedness within species determines plasticity responses has also been investigated, to test the prediction of whether males would be less competitive toward close kin. Competition between males can cause collateral damage to females, an idea central to sexual conflict. As previously mentioned, female *D. melanogaster* mated to males that have been exposed to rivals have more offspring earlier in life, but have fewer later in life, and have shorter lifespans (Filice et al., 2020). If males increase their investment under sexual competition, this could have negative consequences for females, so reduce the males' indirect fitness if these females are likely to mate with the males' brothers. Evidence for males adjusting levels of harm when competing with relatives has been found in seed beetles (Lymbery, Tomkins, & Simmons, 2019) and red junglefowl (Tan et al., 2017). However, for a species with likely unstructured populations, such as *D. melanogaster* that lives in genetically homogeneous populations at least as adults, kin selection is not thought to be an important factor in driving the evolution of plasticity (Chippindale, Berggren, Alpern, & Montgomerie, 2015). Nevertheless, flies appear to be able to discriminate

kin as adults, with males being reported to mate for a shorter time with (Lizé, McKay, & Lewis, 2014), and transfer less sperm to (Heys et al., 2018), their sisters. The evidence that relatedness is an important modulator of male–male competition in *D. melanogaster* is mixed. Taylor (2014) found that males responded equally to brothers or non-kin in terms of extending their mating duration. Carazo, Tan, Allen, Wigby, and Pizzari (2014) showed that females are harmed less by matings with males kept with familiar/related rivals, implying that males alter their level of investment in mating competition accordingly. Further work found that the extent of harm varied as a function of male relatedness and familiarity (Le Page et al., 2017). Subsequently, a study using flies recently caught from the wild, under naturalistic levels of sexual competition, found no evidence of relatedness altering male–male aggression or harassment of females (Marquez-Rosado, Garcia-Co, Londoño-Nieto, & Carazo, 2022). Overall, the role of relatedness and familiarity in male–male competition in *D. melanogaster* remains unclear.

3.2 Assessing the amount of competition: Time of exposure to rivals

Key to the extension of mating duration by *D. melanogaster* males exposed to rivals is the length of time males experienced rivals before mating. There is a linear relationship with exposure time and subsequent mating duration, with approximately 24h exposure being required for males to significantly increase duration (Bretman et al., 2010). Conversely, males continue to respond for about 12h after a rival is removed, but this is only the case if the initial exposure time to a rival was at least 36h (Kim et al., 2012; Rouse & Bretman, 2016). Exposure to rivals over many hours is not necessarily required for males to be able to express behavioral plasticity. The mealworm *Tenebrio molitor* alters mate guarding duration after only 20 min of rival exposure (Carazo, Fernández-Perea, & Font, 2012). Indeed, if *D. melanogaster* males were exposed to a rival for 24h, they continued to respond only for 1h after the rival was removed, suggesting that they have the capacity to alter their behavior faster than they actually normally do (Rouse & Bretman, 2016). There may be two reasons as to why plastic changes to male mating duration in *D. melanogaster* take time to develop. Firstly, the mating duration behavior may be linked in some way to ejaculate investment responses and hence males may need time to produce more sperm/Acps. This is supported by research that has described ejaculate proteomics, with the majority of seminal proteins being altered following exposure of a male to multiple rivals (Hopkins et al., 2019). Secondly, in a social environment that varies rapidly, males may require information that a rival is

likely to be resident and not transient, hence worth the investment of a response. Certainly, the amount of social information received alters the speed of a male's mating duration response. Interfering with either olfactory or auditory cues causes males to take longer to significantly increase mating duration, and removing the last antennal segment (manipulating both cues) causes males to continue responding for longer after the rival has been removed (Rouse & Bretman, 2016). These findings suggest that complex cues facilitate the accurate timing of highly flexible reproductive plasticity (Dore et al., 2018).

The amount of time spent with a rival might be integrated with number of rivals to obtain information on whether a threshold "amount" of competition has been reached. This phenomenon is believed to occur in the mealworm *Tenebrio molitor* which "sums" the number of rivals encountered over time. Hence males increase mate guarding duration when exposed sequentially to four rivals over 20 min but do not do so when exposed to one rival for the same amount of time (Carazo et al., 2012). Likewise, *Callosobruchus* seed beetles appear to integrate information on the number of rivals encountered over time, and so respond to the total number of males encountered rather than the number at any one time (Lymbery et al., 2019). Indeed, it has been suggested that the requirement to assess the number of mating rivals was a driver in the evolution of quantity estimation (Shiffman, 2012). As noted earlier, the Sfps component of the response is sensitive to the number of rivals in *D. melanogaster*, whereas the mating duration and sperm elements are insensitive (Bretman et al., 2010; Hopkins et al., 2019). Whether *D. melanogaster* integrates the number of rivals and time has not yet been tested, so it may be that responses occur faster with increased numbers of rivals. However, male *D. melanogaster* from male-biased selection lines do maintain their mating duration responses for longer after rivals are removed than do those from female-biased lines, suggesting there is a genetic basis to perception thresholds (Maggu et al., 2021).

3.3 Bringing the outside in: Genetic and neuronal mechanisms

Once a rival has been sensed, how does this information become integrated with physiological and molecular pathways to produce the mating behavior and associated ejaculate responses? Gene expression seems sensitive to even subtle changes in the social environment. Differences can be seen in a matter of minutes when flies are exposed to the same or opposite sex (Ellis & Carney, 2011) and females show upregulation of immune/stress related

genes upon simply hearing the song of conspecific males (Immonen & Ritchie, 2012). A focused study of expression of seven reproductive genes found mixed evidence of differential expression following variation in social environment (Fedorka, Winterhalter, & Ware, 2011). When exposed to rivals for 72 h prior to mating, two of three seminal fluid protein genes were down regulated, but no differences were seen in expression of four spermatogenesis genes. The number of rivals present during mating caused no alteration in the expression of these genes (Fedorka et al., 2011). Interestingly, downregulation of seminal fluid gene expression was also observed in *Teleogryllus* crickets after exposure to rivals (Sloan, Lovegrove, & Simmons, 2018).

A whole transcriptome approach using mRNA-seq revealed widespread gene expression changes in males following exposure to rivals (Mohorianu et al., 2017). To partition elements of the behavioral and ejaculate components of the response, flies were divided into head/thorax vs abdomen, respectively. Three time points were chosen for the gene expression profiling, 2, 24 and 50 h after a rival had been added, to represent the early build-up, the cusp and the end of the predicted gene expression responses, respectively. The greatest differential regulation was observed at 2 h, with males exposed to rivals increasing expression of sensory genes in the head/thorax and ejaculate-related genes in the abdomen. An investigation of social feeding across 10 *Drosophila* species also found chemosensory-perception gene expression patterns to be particularly variable across species and social conditions (Shultzaberger et al., 2019). Together these findings are consistent with the idea that chemosensory-perception genes are key to facilitating behavioral evolution. As in the study by Fedorka et al. (2011), differentially expressed ejaculate genes observed were mostly those encoding for seminal proteins rather than sperm-related genes. Differential expression was much reduced at 24 h and largely absent at 50 h after the rival was introduced. Differences between replicates functionally mimicked the main treatment effects, which is consistent with the redundancy seen in the sensory cues and suggests that multiple genetic pathways may lead to a similar phenotypic outcome. Along with sets of genes apparently related to detecting or responding to rivals, genes related to immunity, stress responses and longevity were also enriched for expression following exposure to rivals. These functional groups might point to potential mechanisms of costs of exposure to rivals. However, measures of differential gene expression are necessarily correlational. To examine whether the genes identified as socially responsive are necessary for male plastic responses, or are due to

unrelated consequences of social contact, requires functional genetics approaches as outlined below.

The gene expression data suggested that responses of males to rivals requires the coordination of many genes, potentially achieved through Gene Regulatory Networks. Such coordination of gene expression could be achieved through transcriptional regulation, by transcription factors or post-transcriptional mechanisms such as microRNAs that act as gatekeepers of translation to proteins (Bartel, 2004). To test for the presence of such networks, known Sfp sequences that could be targeted by specific miRNAs were assessed, identifying some microRNA seed sequences (5–7 base pair region that matches miRNA to mRNA) that target multiple Sfps and thus represent putative regulatory hubs. The idea that miRNAs are required for the response to rivals was then functionally tested by interfering with miRNA biosynthesis. Males in which miRNA activity was reduced by knocking down a gene critical to their biosynthesis, *droscha*, could not respond to rivals both in terms of mating duration and offspring output (Mohorianu et al., 2018). Therefore, male responses to rivals are at least partly controlled at the post-transcriptional level.

Generally, any behavioral plasticity in response to multiple changing environmental cues would be expected to involve the brain/CNS, as a central processor of input cues and in the coordination of the production of behavior (Mery & Burns, 2010). *D. melanogaster* is a powerful model here, as much work has been performed to investigate their learning and memory abilities, and the genetic and neural basis of associative learning (Dukas, 2008). Reproductive plasticity in males has long been used as a way of interrogating learning and memory, through variations of a “courtship suppression” assay. Female mating status provides information to males about sperm competition risk, as the detection of already mated females necessarily places the male into a competitive situation. Males should therefore be able to assess whether a female has mated and alter their investment accordingly. In terms of changes to mating duration, the evidence is rather mixed as to whether males can respond in this way, with studies finding that they can both increase or decrease duration with a mated female (Friberg, 2006; Sirot, Wolfner, & Wigby, 2011). However, males do consistently reduce courtship effort when exposed to mated or unmated females they are unable to mate with. “Courtship suppression” is a standard assay in fly neurobiology though there are variations in exact procedures (e.g., reviewed by McGuire, Deshazer, & Davis, 2005). In general, males exposed to mated “trainer” females successfully associate olfactory cues with

mating failure and reduce their courtship effort when subsequently exposed to females. Males that successfully learn to tailor courtship effort accrue reduced costs of courtship, and can more successfully target virgin females in later mating tasks (Dukas, 2005). This provides a framework and useful comparison with learning and memory mechanisms underlying the response to rivals.

After a learning period, *Drosophila* memory is characterized by four distinct phases; short term memory (STM) lasting up to an hour after acquisition, medium term memory (MTM) peaking 1–2h after acquisition, and two longer term memory traces (or types) broadly categorized as either anesthesia sensitive memory (ASM) or anesthesia resistant memory (ARM) (Margulies, Tully, & Dubnau, 2005). As the names suggest, ASM and ARM are separated by their sensitivity to anesthesia and are distinctly regulated at the molecular and neuronal level (Dubnau & Chiang, 2013). They can be simply distinguished by anesthetizing flies, for example by placing them on ice for a couple of minutes prior to testing memory recollection. Among males previously exposed to rivals, those that had undergone anesthesia failed to extend mating duration, highlighting ASM as the memory trace associated with this response (Rouse et al., 2018). ASM and ARM can also be distinguished through the genes necessary for their expression, for example ARM requires the gene *radish*. Knock down of the expression of *radish* throughout the nervous system, did not alter a male's ability to respond to rivals, again indicating that ARM is not the relevant pathway used. ASM is reported to be more costly to deploy than ARM (Mery & Kawecki, 2005), implying that correctly responding to rival males is worth the extra cost of ASM.

Some key genes involved in learning and memory pathways, as defined by studies in courtship suppression (McGuire et al., 2005), were found to be differentially expressed when males were exposed to a rival (Mohorianu et al., 2017). Functional genetics has been used to assess whether these differentially expressed genes were necessary for the mating duration extension response. These included genes that are part of the cyclic adenosine monophosphate (cAMP) signaling pathway necessary for ASM, namely *dunce* (*dnc*), *rutabaga* (*rut*), and *amnesiac* (*amn*), and *neurofibromatosis 1* (*NF1*) a GTPase. Broadly, *dnc*, *rut* and *NF1* are required to translate between the acquisition and STM phases, and *amn* between STM and MTM (Margulies et al., 2005) for more in depth reviews of these pathways see Tumkaya, Ott, and Claridge-Chang (2018) and Boto, Stahl, and Tomchik (2020). It is reported that the response to rivals is dependent on

the expression of *rut* and *amn*, but that *dnc* and *NF1* were not necessary (Rouse et al., 2018). The requirement for different genes can indicate the type of learning involved in responding to rivals. Both *rut* and *dnc* are used in the learning phase, but in associative learning assays, it appears *dnc* is used when associating negative reinforcement (a punishment or cost) whereas *rut* forms associations through both negative and positive reinforcement (Xu et al., 2012). For example, *rut* but not *dnc* is required for ethanol preference (Xu et al., 2012), and like alcohol in moderation, the experience of other males may not be entirely negative.

In addition to this genetic component of extended mating duration, it is also possible to identify brain areas responsible for the plastic responses of males to rivals. As olfaction is an important sensory modality by which males recognize competition (Bretman, Westmancoat, et al., 2011), we predicted that the brain area known to control olfactory memory, the mushroom bodies (MBs), are likely to be involved. The MB can be split into separate regions, the γ , α , α' , β and β' lobes, containing distinct bundles of neurons. Unlike much of the invertebrate brain, the MB organization is flexible, potentially enabling greater behavioral plasticity, and leading to the suggestion that it is the part most like vertebrate brains (Aso et al., 2014). We investigated the role of the MB in the response to rivals by both knocking out neural transmission both across the whole MB and within individual lobes, and by restoring *rut* expression to specific lobes after generalized knockout. The results identified that the γ and α'/β' lobes in the MBs were responsible for male behavioral responses to rivals (Rouse et al., 2018). Interestingly, the γ and α'/β' lobes both seem to be required to achieve a behavioral response, as when one, but not the other, were activated with *rut*, males did not respond to rivals. These lobes are associated with short term memory (γ and α'/β') and memory consolidation (α'/β') (Montague & Baker, 2016). The molecular and neural mechanisms highlighted here have parallels with courtship suppression, but are subtly different. For example, courtship suppression seems to require expression through α/β lobes of the MBs to allow for long term plasticity. It may be then that the response to female mating status requires learning and long-term retention, while the response to rival males requires some retention but also the ability to rewrite the memory, thereby quickly tracking changes in the sociosexual environment. This raises the interesting possibility that the type of memory used to enable plasticity is matched to the frequency with which the environment changes.

As with the investigation of the cues males use and gene functional groups that alter in expression, there is some evidence for redundancy in the neuronal mechanisms involved. Kim et al. (2012) report that the MBs were not involved with the extended mating duration and instead what is key is the presence of the ellipsoid body, the center of visual memory. Kim et al. (2012) identified roles for *amn* and *rut*, though they also suggested that ARM was the predominant memory trace needed for male plastic responses. These differences between studies might to some extent be explained by differences in laboratory conditions or between fly lines used. However, this may also indicate that subtle differences in social information can operate through different mechanisms, as recently found in the courtship suppression paradigm. Within courtship suppression assays, the brain region identified as important, is sensitive to the methodology used, specifically whether the female used to train the male and the female used to test his memory were both mated. When a mated female was used in the training phase but a virgin female was used for the memory test, males utilized α/β MB neurons (Montague & Baker, 2016). Whereas, when both trainer and tester females were mated, males utilized MB γ neurons and neurons in the lateral horn (Bates et al., 2020). Therefore, if males use multiple memory pathways to respond to rival males, subtle differences in experimental design could easily lead to seemingly opposing conclusions. This also highlights how complex the basis for assessing and responding to the social environment might be.

Whatever the specific neurogenetic mechanisms involved, it is clear that learning and memory processes are required for full responses to rival males. Social interactions have long been associated with the evolution of cognition (Dunbar, 1998). However, while it has been suggested that the need to count the number of rivals is implicated in the evolution of quantity estimation (Shiffman, 2012), the role of sperm competition is controversial (Lemaitre, Ramm, Barton, & Stockley, 2009). In *D. melanogaster*, an experimental evolution study showed that in lines maintained under monogamy (the absence of sperm competition), males evolved a reduction in learning/memory ability compared to polygamous lines (Hollis & Kawecki, 2014). This cognitive stimulation by sperm competition can also be seen during an individual male's lifetime (Rouse, McDowall, Mitchell, Duncan, & Bretman, 2020). Sperm competition cues were disentangled from general social contact by housing males with rivals of their own species (sperm competition cues) or a species that does not stimulate a mating duration

response (*D. virilis*). Males that had been exposed to potential mating rivals outperformed males only exposed to a social contact in a series of learning and memory tests (e.g., Fig. 3). This suggests sexual competition, not just general social contact, is integral to the development of adult cognition. Moreover, a range of assays was used including one for visual learning, a sensory modality not involved in responding to rivals. This assay also showed males exposed to conspecific rivals outperformed those exposed to heterospecific rivals, suggesting sperm competition cues stimulate cognition generally. Males exposed to conspecifics increased expression of genes associated with neural plasticity. Along with the earlier finding that males use the more costly ASM form of memory, this might point to developmental

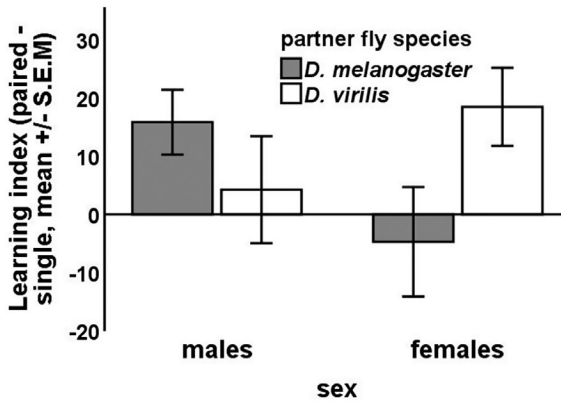


Fig. 3 Social contact alters learning ability in a sex-specific way. Male and female *D. melanogaster* were held alone or exposed to a conspecific or heterospecific (*D. virilis*) same-sex partner for 10 days. Their learning ability was then tested, shown here in a classic associative learning task where they learn to avoid an odor associated with a punishment (bang of the vial). As time of day has a large effect on behavior, and the assay is low throughput, 2–3 flies from the paired treatments were tested alongside the same number of single flies (though all flies were tested alone) within a 30 min period. The learning index was then calculated as a difference between the paired flies and their single fly counterparts (mean \pm standard error S.E.M.). A learning index of 0 means no difference between paired and single flies, a positive value means paired flies were better at learning than single flies, and negative that paired flies were worse. *D. virilis* does not elicit a sperm competition response from *D. melanogaster* males. Males were better at learning after conspecific contact, whereas females performed after heterospecific contact. Data from Rouse, J., McDowall, L., Mitchell, Z., Duncan, E. J., & Bretman, A. (2020). Social competition stimulates cognitive performance in a sex-specific manner. *Proceedings of the Royal Society B: Biological Sciences*, 287, 20201424.

costs associated with plasticity. This could be tested directly by observing whether flies genetically manipulated to be unable to learn and remember rivals do not show the decrease in lifespan usually observed in group housed males.

Interestingly, females do not show the same cognitive responses to social environments. In contrast to findings for males, females from monogamous lines do not show any reduction in cognitive abilities (Hollis & Kawecki, 2014). Indeed, female cognitive performance can be stimulated by the presence of heterospecific females, rather than conspecifics, as found in males (Fig. 3) (Rouse et al., 2020). Females might not identify conspecific females as competitors whereas heterospecifics may be a stronger signal of competition. As previously mentioned, females exposed to conspecific same-sex partners do not show the same reduction in lifespan or the change in their microbiome (Flintham et al., 2018; Leech et al., 2017, 2021). These sex differences in the consequences of exposure to same-sex partners might signify sexual conflict over the ability to mount these responses or between sex variation in the importance of social drivers in the evolution and development of cognition.



4. Future perspectives

The body of work described gives a detailed understanding of plastic responses to sperm competition rivals—from sensory inputs, physiological and cellular processes, to ultimate outcomes (Fig. 4). We identify key questions arising from this work and directions for future study. For example, redundancy has been invoked at the level of sensory cues, differentially expressed genes and neural pathways. Future work could examine whether the pathways are truly redundant, e.g., whether different sensory inputs stimulate different routes through the neurogenetic mechanisms.

In general, the fruit fly is an excellent model to understand how behavioral plasticity is coordinated, and additional probing of the mechanistic basis of the response should be tractable and fruitful. We have noted that the mating duration response and the ejaculate response to rivals (either measured directly through the proteome or indirectly through number of offspring) show some coordination but also crucial differences in sensitivity to the presence of rivals. What is unclear is the extent of separate and shared mechanisms. Candidates for such coordinated regulation include BMP neuropeptide signaling that coordinates ejaculate transfer (Redhai et al., 2016),

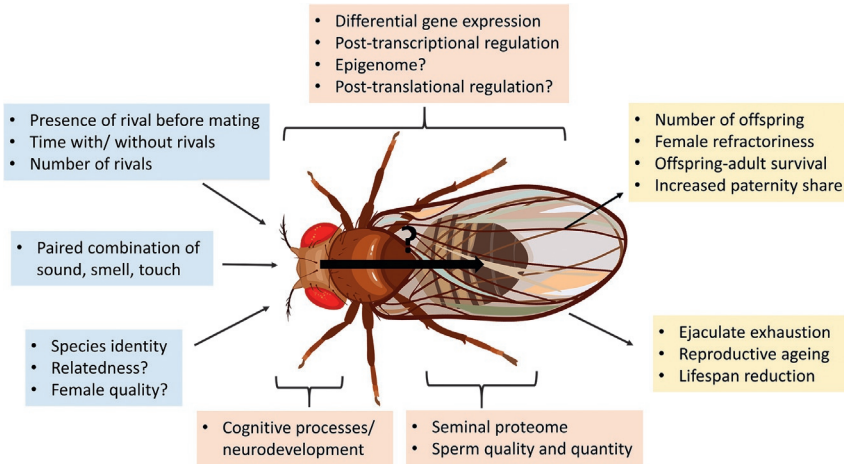


Fig. 4 Summary of male *Drosophila* responses to sperm competition rivals. The diagram partitions the response to rivals from inputs (blue boxes), through internal processes and mechanisms (orange boxes) and to outputs (yellow boxes). So far, the *Drosophila* model of responses to rivals combines understanding the parameters of the social environment to which males are sensitive, the combination of sensory modalities they use and the information that might be conveyed. Inroads have been made into the neural, genetic and physiological processes that enable males to produce a plastic response. The fitness outcomes have been investigated, both the benefits and costs, to evaluate whether investment is really strategic. However, there are some major questions remaining (with question marks), in particular whether there is cross-talk between brain and reproductive tract, or whether the behavioral and ejaculate response are fully separate. This highlights how this response in *D. melanogaster* can be a useful model to interrogate behavioral plasticity incorporating proximate and ultimate causes.

the hormone ecdysone, which is socially-sensitive and mediates accessory gland processes (Leiblich et al., 2019), dopamine which is socially-sensitive and alters mating-drive in males (Zhang, Rogulja, & Crickmore, 2016), and Notch signaling, which mediates queen pheromone-induced reproductive suppression in worker honey bees (Duncan, Hyink, & Dearden, 2016). Exploring these processes will enable us to elucidate how environmental information is integrated at the genomic level, a major question in phenotypic plasticity research (Duncan, Gluckman, & Dearden, 2014). While we have made progress in describing transcriptional, post-transcriptional and proteomic regulation, further functional genetic experiments could identify which genes are key to the sperm competition response and which are unrelated consequences of social contact. This will help us to understand

the true costs of plasticity and perhaps identify gene classes that are central to the evolution of plasticity. We can also probe further levels of control, for example the epigenome has been invoked as key to how plasticity is achieved (Duncan et al., 2014). The role of the epigenome in highly flexible plasticity such as the sperm competition responses described here has been questioned (Cardoso et al., 2015). However, our transcriptome data includes differential expression of histone modifiers (Mohorianu et al., 2017), pinpointing candidate epigenetic mechanisms to interrogate. We hope as these questions are tackled in an increasingly wide range of species that the conservation of the processes can be assessed, enabling a deeper understanding of interplay between behavioral plasticity and evolution. This aim will be facilitated as genetic manipulations in non-model organisms become more widely used, but there are also low-input options. For example, feeding of epigenetic modifier inhibitor chemicals has been successfully employed in honeybees to assess epigenetic control of reproductive plasticity through ovary activation (Duncan, Leask, & Dearden, 2020).

Ultimately it will also be crucial to understand not just male reproductive strategies and mechanisms in response to sperm competition, but also female strategies, mechanisms, and evolutionary interests (Arnqvist, 2014). A fruitful approach may be to consider plasticity in cryptic female choice, though we note that distinguishing female and male effects can be challenging (Firman, Gasparini, Manier, & Pizzari, 2017). Moreover, tests of adaptive plasticity in female reproductive tissues are extremely sparse (Plakke, Deutsch, Meslin, Clark, & Morehouse, 2015). However, we do know that (a) female reproductive tracts of internally fertilizing species—across the full range of animal taxa—undergo dramatic changes during different stages of reproduction (Carmel, Tram, & Heifetz, 2016; Read, Word, Ruschinsky, Timmons, & Mahendroo, 2007), so likely possess the capability for plasticity, (b) females can plastically modify later stages of reproduction, such as provisioning to offspring, or biasing sex allocation (West & Sheldon, 2002), and (c) phenotypic plasticity of sexual traits, in general, appears to be ubiquitous (Cornwallis & Uller, 2010). We therefore expect that like males, trade-offs and constraints will likely influence the female reproductive tract meaning that females will make economic use of resources, and strategically tailor reproductive tracts to boost fitness returns (adaptive plasticity). New theory may be required, along with empirical work spanning genetics and neurobiology, to evolutionary ecology to bring parity with males, and address the question of how male-female interactions shape mating and fertilization outcomes.

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