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# Adventures in Time and Space: What Shapes Behavioural Decisions in Drosophila melanogaster?

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# Adventures in Time and Space:

# What Shapes Behavioural Decisions in *Drosophila melanogaster?*

By

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## Abstract

Variation in behaviour can be observed both between individuals, based on their condition and experience as well as between populations due to sources of heterogeneity in the environment. These behavioural differences have evolved as a result of natural and sexual selection where different strategies may be favoured depending on the costs and benefits associated with those behaviours. In this thesis I examine two sources of heterogeneity within the environment and their behavioural consequences: how spatial complexity mediates sexual selection over time, and how inter and intraspecific signals and individual condition influence social oviposition behaviour. By increasing spatial complexity, we were able to manipulate male-female interaction rate which in turn influenced courtship behaviour and male-induced harm, the consequence of this was an increase in female fecundity especially in the later days of the assay and no change in offspring fitness. These results supported the idea that spatial complexity is able to mediate sexual selection through decreased harm to females. Oviposition decisions are of high consequence to an individual's fitness and can be shaped by many environmental conditions. Instead of expending energy to evaluate all their different costs and benefits of the conditions of potential oviposition sites females can chose to rely on the signals left by others, in this case it would be beneficial for females to identify signals most like themselves. While we found females oviposited with individuals of the same species and diet, when given the option they showed more interest in and laid more eggs on media that previously held virgin males, bringing into question many assumptions of copying behaviour. In *Drosophila melanogaster* the only control females have over their offspring is who they mate with and where they oviposit their eggs, thus, these two factors can have a long-lasting impact on individual fitness for future generations. It is also important to consider how the standard lab environment may be shaping

these behaviours, and the consequences this has for the evolutionary trajectory of lab populations.

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# Chapter 1

## Environmental factors and individual experience influences variation and plasticity in behaviour expression

### Abstract

Natural and sexual selection favour morphological, physiological, and behavioural phenotypes that provide individuals with a fitness advantage over other conspecifics in their environment. Many innate behaviours are a form of genotypic expression, where behavioural strategies can be considered as phenotypes each with associated costs and benefits. Evolutionary game theory models are often used to predict the success of behavioural strategies relative to the frequency of behavioural phenotypes within the population. However, these models seldom consider behavioural plasticity at the individual level. There are many potential sources of variation including heterogeneity in the environment and individual condition, which can lead to behavioural variation within both species and populations. Changes in the environment such as food quality/availability, social interactions, and predation risk can influence behaviours and decision-making. Furthermore, individual variation in condition and experience can lead to the development of behavioural differences. Examining these factors will result in a more comprehensive and realistic understanding of how individuals make decisions that affect their fitness, and ultimately shape evolution in their species.

## Introduction

Individuals often face challenges in their environment, and how they respond frequently contributes to their lifetime fitness. For individuals to succeed they must be able to perceive relevant stimuli in their environment and respond appropriately (McGrath, 2013), and the propensity for animals to produce the desired response to a given stimuli defines their behaviour. While many behaviours are consistent species-wide, populations exhibit behavioural polymorphisms/variation and individuals demonstrate behavioural plasticity in their response to stimuli (Lombal et al. 2010; Pruitt & Riechert, 2010). These behavioural differences can be attributable to different genotypes, learned behaviour and/or they can be a result of environmental plasticity (Berlincourt & Arnould 2015; Pollock et al. 2017; Mets & Brainard, 2018). These behaviours can persist through generations because individuals inherit the genetic information that facilitates the development of these abilities from their parents (Alcock 2001), and/or are taught through parent-offspring interactions during offspring rearing (Kedar et al. 2000). Changes in behaviour can either be considered adaptive or maladaptive depending on how an individual’s fitness is impacted by the outcomes of decisions they make. The best decision to maximize fitness may be determined by several factors within the environment and/or based on the condition and experience of the individual. An adaptive behaviour should be favoured in response to changes in environmental or individual state, where the benefits of the behaviour exceed the costs (Houston & McNamara, 1999; Ha, 2010). In this thesis, I will focus on the causes and consequences of variation in individual behaviour and how it shapes decision making in both environmental and social contexts.

## Behavioural strategies and selective pressure

Behaviours, like other phenotypes, are subject to selective pressures, and variation in behaviours can result in different consequences arising from the decisions made. When phenotypic behaviour reflects genetic variation, there is opportunity for selection to change allele frequency in a population. What kind of behaviours are favoured by natural (and sexual) selection? To answer this question biologists often use an evolutionary stable strategy (ESS) modeling approach, wherein each behavioural strategy is analysed based on its associated costs and benefits. However, we must also take into consideration the interactions of other individuals within a population whose interests do not entirely coincide (Hammerstein, 1998). In this case, game theory is used to assess different strategies, how they might interact, and which results in the best pay-off, where to win an evolutionary game is to have high reproductive success.

When considering the ESS approach, one might expect to observe the same (winning) strategies to be expressed throughout a population. However, individuals may need to adjust their strategies depending on the changing conditions of the environment and the strategies used by others. Some strategies may be beneficial under some conditions but costly in others, which can depend on numerous factors such as the resources individuals are competing for, and the varying strategies employed by others within and between species (Barnard, 2004). Some strategies which appear to have the best cost: benefit ratio for an individual would no longer be optimal if it were employed by the majority. For instance, an aggressive individual might do well when competing against passive individuals; but if the more aggressive strategies becomes common, this strategy will become more costly due to increased challenges and conflicts from others (see “Hawk-Dove game” *sensu*. Smith, 1982). Individuals seldom behave in isolation, thus game theory can be applied to many facets of an individual’s fitness, such as foraging behaviour, physical contests, and reproduction. Solitary foraging decisions can be assessed using patch models, where individuals decide whether to continue or quit exploiting a resource patch as the quantity of resources are continuously depleted (Steven & Krebs, 1986). While this is done alone, individuals will still be influenced by others indirectly, via resources availability, population density, and potential contests resulting from their interaction. Social foraging decisions can sometimes be assessed using the “producer-scrounger game” (Barnard & Sibly, 1981) where producers actively search for resources and share this information with conspecifics, and scroungers never look for food themselves but always join in the foraging. In this case producers incur all the costs (time and energy) of finding food patches, while scroungers only benefit. This leads to a higher fitness when scroungers are greatly out numbered by producers, but as the proportion of scroungers in the population rises, this strategy becomes increasingly less favourable (Giraldeau & Livoreil, 1998). When it comes to physical contests between individuals, how they are assessed depends on whether combatants are evenly matched. For instance, in the “Hawk-Dove” model, noticeably weaker individuals, determined by their resource-holding potential, would do better to avoid contests altogether (Smith, 1982). When the differences between competitors are indiscrete and the outcome is less predictable it can be more costly to engage in overly aggressive contests. In such a case we can look to the “War of Attrition” model where individuals rarely escalate to the point of injury and the winner is determined by how long one is willing to continue the contest (Smith & Price, 1973). Reproduction can be looked at with multiple models depending on the social conditions. The simplest model, the Nash equilibrium, suits many populations where males favour more attractive females, which are courted at a disproportionately higher rate even though they can mate with a limited number of males, thus it is costly to exclusively court the most attractive female (Smith 1982). In species with social hierarchies, models of “optimal skew” are used to describe the distribution of reproduction among group members which vary in social dominance (Reeve, 1998).

Often when considering evolutionary game theory, strategies are considered a product of gene expression where natural selection is the principle decision maker and individuals have less control over their choices (Hammerstein, 1998). However, there are many factors in both the environment and an individual’s past experiences that may influence their decisions and support plasticity in strategies. For animals to maximize their fitness (low costs and high reproductive output) they must make decisions based on the perceived costs and potential benefits of a given action. These costs and benefits may be perceived differently depending on the environment and the state of the individual itself (Houston & McNamara). Potential costs like predation and competition may be weighted differently based on the physical complexity and/or population density of the environment. Where more complex environments provide cover and refuge (Everett & Ruiz, 1993), and higher population reduces an individual’s chances of incurring costs personally (Hager & Helfman, 1991). Likewise, an individual’s condition may change the perceived value of potential benefits, for instance, how hunger influences foraging decisions (Gillette *et al.* 2000), while age and experience may affect mate choosiness (Moore & Moore, 2001). This highlights the importance of a dynamic game theory model (Houston & McNamara), where an individual’s success is not only determined by the behavioural strategy they employ relative to its frequency in the population, but an individual’s ability to adapt its strategy based on changes in environment and individual experience.

## Environmental heterogeneity as a source of behavioural variation

There are numerous ways that the environment can influence an animal's behaviour, such as the availability of food, its spatial ecology, or the physical distribution of conspecifics and/or predators. An individual’s diet is an important factor in decision making, and variation in the quantity, quality, or microbial communities present, can have a profound influence on the expression of behaviours. For instance, in commercial breeds of chicken *Gallus gallus*, hens who have unpredictable access to food modify their foraging behaviour, adopt a more conservative feeding strategy. Their offspring tend to also adopt similar foraging behaviours despite never being exposed directly to food deprived conditions (Nätt *et al*. 2009). While this is an adaptive behaviour for the mother to deal with limited food resources, their offspring’s fitness could be negatively affected if they are unnecessarily restricting their food intake and forgoing resource acquisition. A study by Clark *et al*. (1997) observed that environmental/diet quality affected the quantity and quality of sex pheromone produced in cockroaches *Nauphoeta cinerea*, which resulted in changes in courtship behaviour, such that both male and females from low quality environments were slower to respond to courtship signals and required more courtship effort to achieve copulation than did cockroaches from high quality environments. An environment that offers access to high quality food may promote the growth individuals in relatively good condition, who will exert stronger mate preference in order to maximize their fitness compared to those in worse physiological condition (Cotton *et al*. 2006). This condition dependent mate-choice has been observed in crickets, *Gryllus pennsylvanicus* and *Teleogryllus commodus,* and stalk eyes flies, *Cyrtobiosis dalmanni*, (Hingle *et al*. 2001; Hunt *et al*. 2005; Judge *et al* 2014). In species where males provide nuptial gifts to their mates, the quality of a female’s diet can determine how willing she is to mate. Nutritionally deprived females mate more quickly, resulting in weaker sexual selection on males (Fox & Moya-Laraño, 2009). The content of an individual's diet can also influence their behaviours and decision making.  In Cotton mice, *Peromyscus gossypinus,* offspring inherit food preferences from their parents and mate assortatively based on diet. This continuous influence of diet on reproductive behaviour may be an important facilitator of reproductive isolation and ultimately speciation (Delaney & Hoekstra, 2018). In the fruit fly, *Drosophila melanogaster*, assortative mating can persist even when the offspring are not exposed to the same diets as their parents (Najarro *et al*. 2015). This could be due to the initial transfer of gut micro-organisms from the mother via the egg casing (Bakula 1969). In Chapter 3, I discuss how diets can also influence oviposition decisions in *D. melanogaster*, where females are able to detect difference between the microflora on the egg casings from mothers of differing diets and choose to oviposit assortatively.

There are many social interactions that may influence an individual's decision making, co-operative or aggressive encounters with conspecifics, interactions with potential mates or close calls with would-be predators. The number of individuals sharing the environment can impact both resource availability or quality, and the type or frequency of interactions between conspecifics. In brown trout, *Salmo trutta,* adults encountering an increasing density of younger conspecifics, become more defensive of their resources and begin displaying more aggressive behaviours (Kaspersson *et al.* 2010). Fire salamanders, *Salamandra salamandra,* collected from streams and caves display higher levels of aggression and cannibalism when population densities are high and food resources are low. This pattern is strongest in salamanders collected from caves, where these conditions occur naturally (Manenti *et al.* 2015). As these studies show, individuals in overcrowded populations have higher costs via reduced resource availability and increased aggression. There is an abundance of evidence that suggests increased population density leads to more instances of dispersal in both vertebrate and invertebrate species

(Bengtsson *et al.* 1994; Aars & Ims, 2002; Bowler & Benton, 2004). Dispersal as a strategy can be costly (energy, time, risk) and is often only employed when these costs are outweighed by the benefits of a new environment and costs of remaining in the current environment (Bowler & Benton, 2004; Bonte *et al.* 2011). An individual's decision to disperse is a behavioural adaptation to a changing social environment. In chapter 2, I will be examining the effect of manipulating the frequency of interactions between conspecifics of *D. melanogaster* in a closed system where dispersal is restricted. Specifically, I wanted to investigate how changing interaction frequency influences the male to female harm, as well as reproductive and offspring fitness.

The composition of an individual's social groups may influence the decisions they make in their lifetime. Potential mates can be considered an environmental resource, and an individual's fitness may be limited by availability of the opposite sex and the number of same-sex rivals. For instance, in *D. melanogaster*, males exposed to a population of predominantly low-quality (small-bodied) females, will lower their threshold of mate acceptance and mate with low quality females after failing to copulate with high quality females (Balaban-Feld & Valone, 2017). When males in these populations are experimentally presented with both high and low-quality (decapitated) females, a significant number of those that had previously mated with low quality females retained their preference, the few males that had successfully mated with high quality females previously also kept their preference (Balaban-Feld & Valone, 2017). On the female side, high-quality individuals, who experience greater than average attention from males, subsequently exhibit greater choosiness for mates (Johnstone *et al.* 1995; Monier *et al.* 2018). However, when sex ratios change, individuals may have to adjust their reproductive strategies accordingly. In guppies, when there are male-biased sex ratios, males will reduce their rate of courtship while simultaneously increasing their interference behaviours. In this situation some males may begin to copulate with females by sneaking past other males, and females increase their choosiness resulting in a higher selection pressure on male phenotypes (Jirotkul, 1999). In some species, females have the ability to manipulate the sex ratio of progeny in response to environmental conditions. In order to decrease the competition against their sons, selection favours females who bias their offspring's sex ratio to produce more females, through providing potential mates to unrelated males and less direct competition to male kin (Macke *et al.* 2011). In sheep, *Ovis canadensis*, older females have been shown to bias sex ratios depending on the conditions of the environment, when conditions are favourable they will produce daughters every year, in poor conditions they will produce sons (though they are more costly produce) every other year (Martin & Festa-Bianchet, 2011).

The presence of predators within a natural system can also have a profound impact on the expense of their preys' habitat selection, forging behaviour, reproductive strategies and level of offspring investment. Prey species often trade-off foraging efficiency against safety from predators, either by altering their use of certain resources or through increased anti-predator vigilance, thereby interrupting foraging (Lima & Dill, 1990). Larval tiger salamanders, *Ambystoma tigrinum*, preferentially forage in shallow waters where vegetation is plentiful. However, when predaceous beetles, *Dytiscus dauricus*, are introduced, salamander larvae move to deeper waters where they are safe from predation but as a result suffer from reduced food availability (Holomuzki, 1986). While foraging, the carabid beetle, *Harpalus affinis*, responds to olfactory signals from predators by being less selective in its food preference. This is manifested as a greater consumption of seeds, but of a greater variety of qualities (Charalabidis *et al.* 2017). Several species of birds exhibit decreased handling time when foraging in exposed areas at higher predation risk compared to how the behave in safer areas where they spend more time breaking up food items for easier digestion (Valone & Lima, 1987). In some species the cost of vigilance against predators may be offset by a social group, as the group size increases individuals spend less time being vigilant (Lima & Dill, 1990; Sorato *et al.* 2012). This grouping behaviour is often a trade-off between safety and competition, this can lead to varying sizes in social groups depending on food availability. In the threespine stickleback, hungry fish will form smaller groups than those that are well-fed (Van Havre & FitzGerald, 1988).

The act of reproduction often leaves mating pairs vulnerable to predation as individuals engaging in copulation are less vigilant and less mobile (Lima & Dill, 1990). Increased predation can result from low mobility during reproduction as seen in amphipods, *Gammarus spp.*, and water striders, *Gerris remigis* (Ward, 1986; Sih *et al*. 1990), although the opposite pattern was observed in milkweed beetles, *Tetraopes tetraophthalmus*, which were less visible to predators due to their decreased movement (McCauley & Lawson, 1986; Magnhagen, 1991). In the broadhead skink, *Eumeces laticeps*, females adjust their behavioural response to predators depending on their condition, typically females run to escape predators, gravid females will instead reduce locomotion and rely more on crypsis (Cooper *et al.* 1990). In species where sexual selection has resulted in the evolution of conspicuous traits in males, these bright colours often come at the cost of higher predation risk. When guppies, *Poecilia reticulata*, are exposed to predatory cichlids, *Crenicichla alta*, not only do they reduce the frequency of bright phenotypes through natural selection, but female guppies will change their preference to drab males in response to perceived predators (Godin & Briggs, 1996). Much like foraging, some species may attempt to negate predation risks during reproduction via synchronous breeding in large groups

(Lima & Dill, 1990). However, like any group activity this has the potential to increase competition for both food resources and mates (Caraco & Pulliam, 1984).

In egg-laying species, in particular those where females do not provide additional parent care post-oviposition, the placement of eggs can have a profound influence on the fitness in the subsequent generation. Since offspring are largely undefended from predators during these vulnerable early life stages, gravid females may assess prospective oviposition sites for potential threats to pre-emptively protect their progeny. Spider mites, *Tetranychus urticae,* are able to perceive direct cues from specialist predators *Phytoseiulus persimilis* previously interacting with an egg-laying site as well as indirect predatory signals, such as damaged conspecific eggs and dead adults (Grostal & Dicke, 1999). The present of these signals on a leaf's surface will result in significantly fewer eggs being oviposited on those surfaces compared to the control leaves. Avoidance of signals from a specialized predator may be an evolved trait; recognizing and avoiding injured conspecifics may be an example adaptive behaviour (Grostal & Dicke, 1999). Predation risk has also been shown to influence avian reproductive strategies; Fontaine and Martin (2006) observed over a thousand nest of various community breeding birds over 4 breeding seasons. In half of these sites predators were continuously removed and the others left as control. Parents nesting in the low predation risk sites invested more in their offspring producing larger and more eggs in their nest and feeding their nestlings at a higher rate. When considering how behavioural variation is expressed in nature it is important to consider the environmental conditions that may facilitate behavioural plasticity. As discussed above, resource availability, social groups and predation risk can affect decisions made by individuals regarding habitat, mate choice, and offspring rearing, which can have lasting impacts on individual fitness.

## Sources of behavioural variation between individuals

Individuals within a species can vary greatly in their behaviours as a result of particular conditions and experiences, some of this heterogeneity may be acting on individuals before they've experienced their environment first hand. In frogs, *Rana dalmatina*, if embryos are exposed to chemical cues from a predator this can not only cause slowed development and smaller hatchling size but also behavioural difference in tadpoles' predator response for 10 or more days after hatching (Gazzola *et al*. 2015). In the case of dispersal, life-history has been attributed to an individuals decision to emigrate; where environmental conditions favour dispersal only individuals with genetic and hormonal predispositions will ultimately seek out a new habitat (Bowler & Benton 2004).

Prior experience can shape an individual's future behaviour when foraging, competing against rivals, and choosing a mate. Depending on the environment experienced by animals they may have varying foraging success, for instance, brown trout when raised in a hatchery are slower and less efficient in foraging novel prey than wild-caught trout originating from the same river (Sundström & Johnsson, 2001). In ant colonies, the propensity to forage is determined not only by their condition, where ants with higher fat reserves are less inclined to forage, but also those with low fat reserves who have had recent positive foraging experience will have a higher propensity to forage more (Robinson *et al.* 2012). Physical contests between rivals can be costly for both individuals, using time and energy and possibly increasing their risk of injury or predation (Arnott & Elwood, 2009). Winners of these contests will leave with some rewards, a defended territory, a mate, or food resources, while losers will only incur costs. Thus, it is important for individuals to be able to assess and compare their fighting abilities with rivals to avoid no-win scenarios (Whitehouse, 1997). The fighting ability of animals, also known as their resource-holding potential (RHP), can be influenced and inferred by a number of factors, body size, phenotypic variation, physiological condition (age and resource access), as well as individuals past contest experience (Arnott & Elwood, 2009). Studies have shown that outcomes of previous contest can affect the future behaviours in subsequent contests; where previous winner are more likely to win future contests and past losers are likely to lose, this is known as the winner-loser effect (Otronen, 1990; Whitehouse, 1997; Hsu & Wolf, 1999). This pattern may be due to the animals' actual ability to fight, though there is evidence that suggests previous contests only effect an individual's perception of their own fighting ability (Garcia *et al.*2013). Additionally, the loser-effect will only persist from recent contests, suggesting it's influence on RHP is plastic (Hsu & Wolf, 1999). This change in RHP perception results in a behavioural change, where losers become less aggressive while the opposite it true for winners (Hsu *et al.* 2006). In *D. melanogaster* losing contests also results in decreased courtship behaviour in males (Tesco *et al.* 2016).

As an animal ages or gains further experience it may adjust its reproductive strategies to maximize its fitness, in the two-spotted spider mite, *Tetranychus urticae*, males express plasticity in their mating behaviours depending on their age and the age of their rivals. Young males of this species are more likely to engage in sneaking behaviour to approach females, especially if there are older (predominantly) fighter males present (Sato at a. 2014). While age and sexual experience show no effect on male-male aggressive behaviour in *Drosophila melanogaster*, previously mated males were less aggressive to females than virgin males who were more likely to engage in coercive mating behaviour (Baxter & Dukas 2017). In a study by Wilgers and Hebets (2012) the condition of wolf-spiders, *Rabidosa rabida*, was measured by manipulating both diet and age to observe their effect on mate choice. They found that young females were choosier when selecting a mate, preferring those from high quality diets regardless of their own diet. For the banded demoiselle, *Calopteryx splendens*, previous copulation experience can lead to differences in behaviour; experienced males have higher frequency and lower latency of courtship displays than naïve males. Experienced females of this species remain closer to males, engage in more copulation, and have a greater expression of preference than their naïve counterparts (Verzijden *et al.* 2014). In *D. melanogaster,* past mating experiences can shape future intersexual interactions, where females who previously mated with more harmful males spent less time post-copulation associating with males than females that had mated with low-harm males (Filice & Long, 2017). In chapter 3, I will be examining whether prior sexual experience in *D. melanogaster* influences oviposition decisions. Prior experience can have a multitude of effects on an individual's behaviour regarding foraging, physical contests and reproduction, factors which can be consequential to individual fitness variation.

## Thesis objectives

In this thesis I explored individuals' mating and oviposition decisions in *D. melanogaster*, and how they can be influenced by changing the environmental context. As discussed in this chapter, there are a multitude of factors that may shape individuals' decisions, such as environmental and social influences, as well as the variation expressed by different individuals depending on their condition and experience. However, in nature none of these factors are isolated; for behaviours to be adaptative they may need to be able to respond to a dynamic environment and conditions.

In Chapter 2 I tested how spatial complexity and encounter rates influence remating, reproductive strategies, and offspring fitness, and how this interaction changes over time. Using these data, I discuss whether spatial complexity strengthens selection of males, by increasing search costs, or benefits females, by providing refuge from male harm. This experiment was motivated by the work of Byrne et al. (2008) and more recently by Yun et al. (2017), who looked at refuge availability and environmental complexity/population density, respectively, and how they mediate male induced harm and female fitness. This work was also inspired by other studies by MacLellan *et al.* (2009) and Singh et al. (2017) who suggested environmental complexity lead to stronger selection against deleterious mutations by increasing the search costs for males. Since these studies presented contradicting hypotheses as to how sexual selection is mediated by the complexity of the environment, the goal of the Chapter 2 is to provide further insight into this interaction. Although these studies have shown interest in how spatial complexity may mediate selection, many of the proposed methods cause additional environmental or individual variation which may change behaviours. Our experimental design increases physical complexity/surface area to affect encounter rates while eliminating other sources of variation such as food resources, population density, and humidity.

In Chapter 3, I tested oviposition site choice copying behaviour to determine (i) how specific this behaviour is in response to signals from varying species and diets, (ii) whether this behaviour is in response to relevant signals from other ovipositing females (iii) and if this behaviour differs as a result of female state (virgin or mated). This experiment was inspired by Golden and Dukas' (2014) research as they were able to show that female *D. melanogaster* could detect and interpret microbiotic cues left behind by other females. I was curious to see how well females could discriminate between cues left behind by individuals of different species, raised on different diets, and conspecifics of different sexes / mating experience to test the limitations of this observed copying behaviour. I hypothesized that if females are expressing copying behaviour during oviposition they would associate with (and lay more eggs on) dishes with signals that are most similar to their own in regard to species and diet. Furthermore, females would prioritize signals left by other ovipositing females over males or virgin females, and this ovipositing behaviour should be distinctly different between gravid and virgin females. These assays are important when studying behavioural variation in response to different signals and how this may contribute to speciation due to behavioural isolation.

The information gained from these experiments will help contextualize the behavioural variation observed between populations and individuals of the species *D. melanogaster*. Whether these differences are a result of changes to the physical or social environmental, or individual condition and previous experience. Determining how these factors influence behavioural strategies can provide us with insight into how individuals assess the costs and benefits associated with their decisions and how these values may change throughout their lifetime. As behavioural isolation has been shown to encourage speciation (Boake *et al.* 2002), these studies provide an exciting perspective on how behavioural adaptation can shape the evolutionary trajectory of species.

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# Chapter 2

## Spatial environmental complexity mediates sexual conflict and sexual selection in *Drosophila melanogaster*.

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## Abstract

Sexual selection is an important agent of evolutionary change, but the strength and direction of selection often varies over space and time. One potential source of heterogeneity may lie in the opportunity for male-male and/or male-female interactions imposed by the spatial environment. It has been suggested that increased spatial complexity permits sexual selection to act in a complementary fashion with natural selection (hastening the loss of deleterious alleles and/or promoting the spread of beneficial alleles) via two (not mutually exclusive) pathways. In the first scenario, sexual selection acts more strongly on males in complex environments, allowing males of greater genetic quality a greater chance of outcompeting rivals, with benefits manifested indirectly in offspring. In the second scenario, increased spatial complexity reduces opportunities for males to antagonistically harm females, allowing females (especially those of greater potential fecundities) to achieve greater reproductive success (direct fitness benefits). Here, using *Drosophila melanogaster*, we explore the importance of these mechanisms by measuring direct and indirect fitness of females housed in simple or complex spatial environments. We find strong evidence in favour of the female-conflict-mediated pathway as individuals in complex environments remated less frequently and produced more offspring than those housed in a simpler spatial environment.

*Keywords*: sexual selection, sexual conflict, spatial complexity, mate choice, mating systems

## Introduction

Patterns of non-random mating that arise as a consequence of either intra- or inter-sexual selection can have potentially dramatic consequences for a species' evolutionary trajectory. Understanding the factors that shape the outcome of these two (not necessarily independent) forms of sexual selection (and, by extension, their often-complicated relationship with respect to natural selection) is of great importance to evolutionary biologists. In both intra- and inter-sexual selection, one potentially important mediating factor is the physical characteristics of the environment in which organisms are located. In this study, we set out to examine how changes in spatial complexity potentially alter the outcome of mating dynamics in *Drosophila melanogaster*, a model species for the study of sexual selection and conflict.

It has long been recognized that the degree of complexity in an environment can influence the speed and/or direction of adaptive evolution via natural selection (Łukasik *et al.* 2006). Environmental heterogeneity can generate different and more varied selective pressures than those arising in a simpler, homogenous environment (Miller and Svensson 2014). As adaptations are, by definition "phenotypic variants that result in the highest fitness among a specific set of variants in a given environment" (Reeve and Sherman 1993). If there is environmental uniformity, directional selection will act more efficiently on traits, than when there is spatial and/or temporal heterogeneity resulting in distinct selective regimes. It is also becoming increasingly evident that the operation of sexual selection may also be strongly shaped by the complexity of the local environment, by altering the dynamics of male-male and/or male-female interactions. The spatial ecology of an environment can potentially affect the operation of intra-sexual selection by changing the frequency and types of intraspecific interactions, which can have important consequences for how sexual selection and conflict operate.

Environmental spatial complexity may involve the presence of physical barriers or obstacles, which limit the frequency of encounters between both potential mates and/or rivals. This change in encounter rates can directly shape the type of mating strategies adopted by males. To understand the potential importance of spatial complexity, let us first consider another factor that can influence male-male encounter rates: population density. The density of a population can influence mating decisions made by males, and their investment strategies at the pre- and post-copulatory levels. In high density populations it is likely that males engage in scramble competitions (Parker 2000; Thornhill and Alcock 1983) where access to females depends largely on an individual's ability to outmaneuver rivals, whereas at lower densities success may depend on the ability to exclude access to females through contest competitions (Arak 1983). If the major factor influencing this male behaviour is encounter rate, then spatially complex environments may be functionally equivalent to a low-density environment. In a short-term assay conducted using *Drosophila melanogaster* males placed in two vastly different sized environments, MacLellan *et al.*(2009) found that the strength of sexual selection acting against males possessing visible mutations (with potentially deleterious effects) was greater in larger chambers, presumably due to the increased search effort required to find females. As this mating assay ran for only 24h and most females mated only once, most of this variation was attributed to searching efficiency and pre-copulatory traits, rather than traits involved in pre-or post-copulatory male-male contest competition (or female choice, for that matter). Differences in male-male encounter rates may also favour different phenotypes in males. In many species males have evolved weaponry for battling with same-sex rivals, and selection on these traits can be influenced by male population density. In both dung beetles (*Onthophagus taurus*), and in mites (*Sancassania berlesei*) fighter males are favoured by selection at low population densities, but at high densities males with relatively smaller weapons were more successful at mating (Łukasik *et al.* 2006; Moczek 2004; Radwan *et al.* 2002). An opposite pattern was seen in earwigs, *Forficula auricularia* where, at high population densities, males with relatively larger horns are more successful (Tomkins and Brown 2004). The benefits of aggressive behaviours may also depend on intra-sexual encounter rate. It has been suggested that aggressiveness may be favoured in environments with intermediate encounter rates, as males in low-densities could benefit more from investing in mate-searching behaviours, while in high-densities overly aggressive males incur excessive costs from frequent spars with rivals (Knell 2009). Variation in male-male encounter rates also shape traits involved in post-copulatory sexual selection. For instance, in high-density environments where encounter rates and polyandry are high, males experience a greater risk of sperm competition (Jarrige *et al.* 2015; Long and Montgomerie 2006). Bretman *et al.*(2009) found that male *D. melanogaster* housed with rivals prior to encountering females mated for longer, and sired a greater fraction of offspring in twice-mated females. However, increased investment into sperm and ejaculates is costly and may only be beneficial in crowded environments where there is heightened post-copulatory competition (Parker and Pizzari 2010). Thus, there is great potential for variation in spatial ecology to influence the strength of *intra*-sexual selection operating in a population.

The spatial complexity of an environment can also impact *inter*-sexual selection. The decision whether or not to mate (and with whom) can be influenced by factors including individual condition (which might be associated with age and/or nutrition levels), effectiveness in mate assessment and sampling strategies, as well as access to mates, intensity of male-male competition, presence of rival females and predation risk, all of which can be potentially affected by ecological context (Miller and Svensson 2014). In environments with higher spatial complexity, if encounter rates between males and females are reduced, both sexes may adjust their behavioural strategies to account for increased search costs (Hack, 1998; Parker 1983). Search costs often necessitate a trade-off against mate choosiness, as more energy is expended in finding a potential mate, and there is a greater cost of rejecting that individual (Barry and Kokko 2010; Booksmythe *et al.* 2010; Lindström and Lehtonen 2013). Such a trade-off was observed by Gotthard *el at*. (1999) in a study of two populations of speckled wood butterflies, *Pararge aegeria*, which differed in sex-ratios and the costs of searching for mates due to differences in male eclosion patterns. Females in the population in which males were rarer had shorter mating latencies than those from the population in which males were more abundant (Gotthard *el at*. 1999). Similarly, in sticklebacks, *Gasterosteus aculeatus*, experimental manipulation of the environmental spatial complexity (and thus female search costs), resulted in changes in the amount of time that females spent searching for and evaluating males, and the frequency of mate choice opportunities (Heuschele *et al.* 2012). Such changes in sampling costs can contribute to individual variation in female mate choice, resulting in changes in the strength and/or direction of sexual selection. Such heterogeneity in sexual selection can influence the amount of standing genetic variation present in a population, compared to what would be observed if directional selection was allowed to proceed in an unimpeded fashion (Falconer and Mackay 1996; Jennions and Petrie 1997; Widemo and Sæther 1999). Together, these studies highlight the potentially important role of environmental spatial complexity in mediating the shape of mating systems, and the evolutionary trajectory that a species follows.

The expression of inter-sexual conflict in a population may also be shaped by its environment. As a result of different (and often incompatible) fitness-maximizing strategies over mating rates, males in many species have evolved numerous behavioural and morphological traits that benefit their own fitness by manipulating females (Arnqvist and Rowe 2005; Clutton-Brock and Parker 1995). However, through their selfish actions, these males cause females direct harm or cause them to behave sub-optimally, ultimately reducing their lifetime reproductive success. For instance, in water-striders, *Gerris odontogaster*, males and females often engage in an aggressive pre-copulatory struggle, with females attempting to dislodge males who are attempting to copulate with them (Arnqvist 1989). This resistance is costly to females, and, as the local density of males increases, females adjust their behaviours to exhibit less reluctance to mate to avoid increased harassment-associated costs (Arnqvist 1992). In *D. melanogaster*, males harm females directly through harassment during courtship (Partridge and Fowler 1990), physical damage during copulation (Kamimura 2007), and/or the activity of products transferred in the male's seminal fluid (Fowler and Partridge 1989; Wolfner 2009). This effect is exacerbated when the males that are the most successful at courting and mating also induce the greatest harm to their mates (Pitnick and García-González 2002; Friberg and Arnqvist 2003). Furthermore, when harmful male attention is directed towards those females within a population with the greatest potential fecundity, this can interfere with the process of adaptive evolution (Long *et al.* 2009). The consequences of mating with coercive males may also be reflected in offspring fitness when males pass on alleles with sexually-antagonistic effects, benefitting sons while reducing daughter fitness (Chippindale *et al.* 2001). It is hypothesized that increased environmental spatial complexity may help females avoid these costs by forcing males to spend more time locating a mate, providing potential refuges for females from persistent male courtship and harassment (Byrne *et al.* 2008; Yun *et al.* 2017), and allowing females to invest more energy into feeding and being choosier during mate selection (Heubel and Plath 2008; Köhler *et al.*).

The studies described above strongly suggest that environmental complexity can mediate mating behaviour and sexual selection, and at the same time infers two different (but not mutually exclusive) pathways that contribute to adaptive evolution. In the first scenario, increased environmental complexity may result in stronger inter- and/or intra-sexual selection against males. Those carrying deleterious mutations have an increased selective disadvantage in more complex environment, thereby enhancing the efficiency with which they are purged from the population's gene pool (Whitlock and Agrawal 2009). In the second scenario, increased spatial complexity restricts the ability of males to aggressively court and injure females in the population. Free from (some) of this cost of attractiveness, these females are able to make a larger contribution of offspring to the next generation, potentially resulting in an increased rate that beneficial alleles spread through the population (Long *et al.* 2009). Both these mechanisms result in increased population fitness, either through higher quality offspring or by increased female fecundity. To better understand how environmental complexity mediates sexual selection, it is necessary to conduct an experiment in which both possible pathways are examined. Here, we set out to do so by exploring male-female interactions in *D. melanogaster* with specific attention paid to their role in mediating male-induced harm and remating rates, and how these both potentially contribute to offspring fitness variation in the next generation.

## Materials & Methods

### *Population History and Maintenance*

The source of focal *D. melanogaster* flies used in our assays is the *Ives* (hereafter 'IV'), population, a large, outbred wild-type stock that originated from a sample of 200 mated females caught in Amherst MA, USA in 1975 (Rose 1984). Our assay also used competitor flies from both the IV-*bwD* and IV-*bw* populations, which were created by introgressing (via repeated backcrossing) the dominant brown-eyed allele, *bwD* and the recessive brown-eyed allele, $bw^1$ (respectively) into the IV genetic background.

These populations are maintained at large size (~3500 adults/generation) on a 14-day discrete non-overlapping culture cycle, where flies are cultured *en masse* (under light $CO_2$ anaesthesia) in vials containing 10mL of a banana killed-yeast agar media (Rose 1984). All populations are kept at a density of ~100 eggs/vial and are housed at 25ºC, 60% RH and exposed to a 12hr-L:12hr-D diurnal light cycle (Martin and Long 2015).

### *Measuring female remating rates and offspring production in environments of different complexity*

In this assay, we set out to determine if housing females in environments differing in their spatial complexity influenced remating rates and/or female fecundity. All flies used in this assay were collected as virgins within 8 hours of eclosion and housed in same-sex groups of 10 for 3-4 days prior to the experiment. The experiment began by combining 240 sets of 10 female IV flies (without anesthesia) with an equal number of IV-*bwD* males for a period of 3 hours, which allows sufficient time for all females to mate once (TAFL, pers. obs). Next, using light anesthesia, the IV-*bwD* males were removed and replaced with sets of 10 IV males. Into half of these vials (experimental treatment) we also added a clear strip of acetate (~1.5x13cm) folded accordion-style in order to increase the surface area within the vial (see Figure S1). In the remaining 120 vials (control treatment) no acetate strip was added. On each of the subsequent 4 days, we haphazardly removed females from 30 of the vials in each treatment and transferred them (under light anesthesia) into individual test tubes containing ~3ml of fresh media, the surface of which has been cut to promote oviposition (*see* Rice *et al.* 2005). Females were left to oviposit for 23 hours before being discarded. The test-tubes were incubated under standard conditions for 14 days, at which time the number, and eye-colour, of all offspring in each tube was tallied. Test-tubes that contained wild-type offspring indicate that the female had remated.

### *Assay to examine potential impact of environmental complexity on offspring fitness*

In these assays, we set out to determine if the increased environmental spatial complexity altered the outcome of mate choice, resulting in higher offspring fitness, compared to those females in the control vials. For this experiment we established, using the same protocol described above, a new set of 240 vials each consisting of 10 IV females (that had just been mated to IV-*bwD* males) and 10 IV males. Half of the vials contained a folded strip of acetate, while the other half did not. On each of the next four days we haphazardly selected 30 vials from each treatment and transferred (anesthetized) females into a small egg-laying chamber overnight. The following morning, up to 50 eggs were collected from each of these chambers and transferred to vials containing fresh media. We added a sufficient number of similarly-aged IV-*bw* eggs so that each vial contained 100 eggs (thereby matching typical culture conditions). This experimental set-up was repeated twice, to separately measure both daughter and son fitness using the protocols described below.

*i) Fitness assay of daughters*

For each of the four temporally-offset sets of vials, we collected, 14 days after their creation, five wild-type females from each vial and transferred them to individual test tubes for ~18h to oviposit before being discarded. Test tubes were returned to the incubator for an additional 14 days at which point all eclosed adult flies were removed and counted. This count represents the fitness of daughters produced as a result of their mothers mating with a IV male.

*ii) Fitness assay of sons*

Starting ~9 days after their set-up, we haphazardly collected a virgin wild-type IV male (within 8h of their eclosion) from each vial in our four temporally-offset sets of vials. This male was individually placed into a new vial containing 9 similarly aged IV-*bw* males and 10 IV-*bw* virgin females. Flies were left in these vials for a period of 48h at which time the IV-*bw* females were anesthetized and transferred into individual test tubes for an additional 24 hours before being discarded. Test tubes were incubated for 14 days and the number and phenotype of eclosed adults were tallied in order to determine both the total number of offspring sired by the wild-type male and the total the number of females that the target male had mated with; these represent meaningful indices of the fitness of sons produced as a result of their mothers mating with an IV male.

*Statistical Analysis*

All statistical analyses were performed using R.3.3.2 (R Core Team). In all analyses vial represents the unit of replication and in all models, (unless specified otherwise) spatial environment type, assay day, and their interaction were included as independent variables. The magnitude of differences between groups from different spatial environments on specific days was quantified using Cohen's d or Cliff's delta effect size statistics using functions in the *effsize* package (Torchiano 2017). To analyse the effects of environmental complexity on adult female remating rates over the 4 days of the experiment we constructed generalized linear models (GLMs), with a quasibinomial error distribution, where our response variable was the number of females in a vial that produced offspring whose eye phenotype was *wild-type*. To analyze whether variation in offspring production was associated with different spatial environment and/or day we calculated the mean number of offspring produced by females in each vial and used that as the response variable in a GLM with gaussian error distributions. The significance of independent variables in our GLMs was determined using the *Anova* function from the *car* package (Fox and Weisberg 2011). The location of specific differences between groups was assessed, where necessary using a Tukey HSD post hoc test using the *glht* function in the *multcomp* package (Hothorn *et al.* 2008). We also examined whether there were any differences in sperm displacement/use across treatments/days by computing the mean P2 values (the fraction of offspring sired by the wild-type male(s)) for the females in each vial, both overall, as well as for only those females that were deemed to have remated. As these data had non-normal distributions, we analysed them using the non-parametric Scheirer-Ray-Hare method (Sokal and Rohlf 1995). Effect size for each day was determined using Cliff's delta method.

In our analysis of daughter fitness data, we constructed a GLM with gaussian error distributions where the response variable was the average offspring produced by the five females per vial that were sampled. For sons we quantified fitness in two different ways: first as their success at mating with females (measured as the fraction of females in a vial that produced wild type offspring); and secondly as the total number of grandchildren sired (measured as the sum of all wild type offspring produced by females from the same vial). For both these metrics we constructed GLMs (with quasibinomial, and quasipoisson error distributions, respectively) with spatial environment type, assay day and their interaction as independent variables, with their statistical significance determined using the methods described above.

**Results**

*Remating rates and female offspring production*

In our first assay we measured over the course of 4 days, the potential impact of differences in environmental spatial complexity by evaluating remating rates of females and their fecundity. In our first analysis we found a significant interaction between remating rates of females and the day of the assay (Table 1a), with females in the simpler environment more likely to exhibit evidence of remating in the 1st 24h of the assay (Table 2, Figure 1). This difference between treatments was not detected for females measured 48, 72 or 96h after the start of the assay. When we analyzed the number of offspring produced by females housed in either a simple or complex environment, we saw a significant effect of treatment, of day and their interaction (Table 1b). In both environments females produced, on average more offspring on later days, but, starting on Day 2 of the assay, female offpring production was higher in the complex environment offspring than in the simpler environment (Figure 2, Table 3). The proportion of offspring sired by the initial (*bwD*) male decreased over time under both conditions although a significantly higher proportion persisted in the complex environment (Figure 2S). When examining all offspring produced by females there was a significant effect of both enviroment and day on the fraction of wildtype offspring, but when the analysis was restricted to only remated females, those effects were no longer statistically significant (Table S1b).

### *Offspring Fitness*

The daughters sired by wild-type males in simple and complex spatial chambers produced different numbers of offpsring across the four days of the assay (Table S2), revealed as a significant environment-by-day interaction (Table S3a). Overall, daughters sired on later days were less fecund, especially those sired on the 4th day in the simple environment (Figure S3). We found no significant effect of spatial environment or day on variation on fitness of the sons sired by wild-type males either in terms of how many females they successfully mated with, or the total offspring sired (Table S3b, S3c, S4; Figure S4).

## Discussion

Environmental spatial complexity has the potential to shape a population's evolutionary trajectory by changing the opportunity for sexual selection and/or conflict to be manifested. Here, we show that in *D. melanogaster* – an important model species for the study of sexual conflict and sexual selection – an increase in environmental spatial complexity is associated with increased offspring production in females but has no significant indirect genetic benefits. These patterns are consistent with the prediction that increased spatial complexity frees females from some of male-induced harm that they experience. Our results help provide a better understanding of the factors that shape sexual selection's role as a potential agent of adaptive evolution and provide context for recent studies in this field.

In our first assay, we compared female performance in complex and simple environments by measuring cumulative remating rates and offspring production. Remating rates differed between the treatments, with the largest differences observed on the first day of the assay and decreasing thereafter. Fecundity was higher for females in the simple environment on the first day of the assay, however on every subsequent day of the trial, female fecundity was significantly higher in females in complex environments. In *D. melanogaster*, female fecundity is strongly influenced by the number and timing of matings. Young females may exhibit a short-term boost to their fecundity following remating as a result of the extra dosage of accessory gland proteins (Long *et al.* 2010), so in the simple environment (where there was more remating) females would have had extra short-term stimulation to produce offspring. However, the subsequent, higher fecundity of females in the complex environment is likely the direct and/or indirect result of decreased male-induced harm experienced. If it is harder in the complex environments for males to pursue and harass females, females will incur less damage from unwanted courtship/copulations (Partridge and Fowler 1990), avoid wasting energy evading these males (Long *et al.* 2009), and have more time to feed (where fecundity in *D. melanogaster* females often being limited by food availability (Chapman and Partridge 1996; Linder and Rice 2005; Stewart *et al.* 2005)). In typical laboratory environments (*i.e* the simple environment) male-induced harm is often strongly biased toward females of high potential fitness (Long *et al.* 2009), so if increased spatial complexity interferes with harassment, then these females will be more likely to produce more offspring. The lower average remating rates in experimental females also implies these females may experience less harm through copulation (Kamimura 2007; Chapman *et al.* 1995; Grieshop and Polak 2014), and/or decreased exposure to the physiologically modifying (and toxic) ACPs, than those females in the simple environment (Pischedda *et al.* 2010; Mueller *et al.* 2007; Ram *et al.* 2005; Wigby and Chapman 2005; Wolfner 2002). Although an initial consideration of sperm competition outcomes (fraction of offspring in a vial that were wild-type) suggested a significant impact of day and environment, when analysis was restricted to only those females that were deemed remated, we saw no significant effect of these factors. If changing spatial complexity altered female access to high quality males, then one might reasonably expect differences in the number of offspring sired by such males in her progeny (Snook 2005). The absence of such a pattern (once differences in remated rates are taken into account) is circumstantial evidence against the male-driven model of selection.

When examining the fitness of the offspring produced via remating in both environments, we looked for evidence whether increased spatial complexity resulted in offspring who were of better reproductive competitive ability. In the case of daughters, we saw a significant difference in fecundity between treatments for those produced on day 2 and 3 (Table S2), but not in any consistent manner (Figure S3). Overall, we observed lower fecundity of daughters produced by mothers from the later days of the trial. By the end of the assay, females in both treatments are likely in worse physiological condition than when they started, due to senescence and/or male-induced harm. This might be manifested in the next generation’s phenotype through a maternal effect of decreased resources allocation to offspring (Azevedo *et al.* 1997), by manipulation by their mates to change reproductive investment patterns (Pischedda *et al.* 2010) or some other type of indirect genetic effect (Garcia-Gonzalez and Dowling 2015). Among males, we saw no significant effect of treatment, trial day, or their interaction on individual reproductive success. If increased complexity facilitated females mating with higher quality males we would have predicted some difference in the offspring fitness (the sons in particular). Thus, overall, we saw little evidence that increased spatial complexity enhanced adaptive evolution via indirect effects.

Our results dovetail with recent studies that have examined how spatial ecology can influence sexual selection/conflict dynamics and its consequence for adaptive evolutionary change, (also using *D. melanogaster*). In the first study, Byrne *et al.*(2008) set out to measure whether the presence of a male-free spatial refuge affected female-remating rates and fecundity. Females (which had been raised on limited media and were consequently smaller than usual) were able to access the refuge area, while males (raised at low larval densities and were much larger than usual) could not. The presence of the refuge was associated with a ~25% decrease in remating rate, but no difference in female lifetime fecundity between treatments was detected. However, it is unclear to what extent these observations were influenced by the novel developmental environments used to obtain males and females. Furthermore, the relatively short duration of the assay (48h) may have obscured any differences that would have become apparent with more prolonged exposure, which is often the case with *D. melanogaster* (e.g. Kuijper *et al.* 2006). Next, MacLellan *et al.*(2009) tested the relative competitive performance of a single wild-type male against single males from 10 different populations of *D. melanogaster* that each expressed a visible phenotypic marker (of presumably deleterious effect) in both a small and a large arena (that differed 600x in volume). The reproductive success of the mutant males was greater in the smaller chamber than in the larger chamber in 9 of the 10 assays (3 significantly so). This was interpreted as reflecting the greater challenges posed to mutant males of searching for mates in the more spacious environment, and suggest stronger sexual selection acting on males associated with increased environmental complexity. However, in addition to the issue discussed above related to the short duration of this assay, it was not reported whether there were any differences in the fecundity of females in these two treatments, which potentially differed in their ease of escaping persistently-harassing males. A more recent study by Yun *et al.*(2017) set out to quantify the extent of male behaviour, male-female interactions and female fecundity in groups of flies that housed in two vastly different environments (a simple standard *Drosophila* culture vial and a complex 1650mL arena outfitted with pipe cleaner structures and 5 dishes of food). Females –especially larger females– were courted more frequently by males in the simple environments than in the complex and had greater fecundities. However, there were other notable differences between these two environments that were independent of spatial complexity (*i.e.* humidity, and food availability), so it is possible that differences in fecundity/behaviour could be the byproduct of one of these other factors. These same two types of environmental chambers were also used in an experimental evolution study by Singh *et al.*(2017) to see if deleterious mutations could be more efficiently purged in more spatially complex environments. In this assay, the alleles in question were a group of 22 different gene disruption mutations, whose frequencies were tracked over 8-10 generations of culture. In 18 of the 22 mutation lines surveyed, that allele frequencies dropped more rapidly in the complex chambers, which is suggestive of stronger selection against those carrying the deleterious mutations (possibly though more effective mate choice by females). However, as with Yun *et al.*(2017), it is not possible to rule out the possibility that the changes associated with selection in the complex environment are not the result of the differences in the chamber microhabitats. This potential confound was avoided in the experimental evolution assay by Colpitts *et al.*(2017), who used populations housed in two comparable arenas (a simple 1650ml container and a single petri dish with 10mL of media, or a complex environment of the same volume but containing 5 petri dishes of media and pipe cleaners to add physical complexity) to track the frequency of recessive deleterious alleles (with visible phenotypic effects) over time, Over the course of the assay (8-14 generations), the deleterious allele frequencies decreased more slowly in the simple environment for 2 of the 4 mutant lines tested, which is (partially) consistent with the prediction that selection against deleterious effects is more efficient in the complex environment.

The results of our research are useful for understanding the mechanisms that led to the changes in the frequencies of deleterious mutations that were seen in Singh *et al* (2017) & Colpitts *et al.*'s (2017) studies (and perhaps explain why in some circumstances frequencies did not change). Our assay strongly suggests that (at least in *D. melanogaster*) sexual selection acts more strongly in step with natural selection in more spatially complex environments through a reduction in the gender load (Rice and Chippindale 2002; Long *et al.* 2005) resulting from reduced male-induced harm, rather than via enhanced sexual selection acting directly on males. This is born out of our observation that in more complex environments females remated less and had greater offspring production (consistent with the results of Yun *et al.*(2017)), and there was no increase in the reproductive success of either sons or daughters produced. Thus, the more efficient purging of deleterious mutations in the spatially complex treatments of Singh *et al.*(2017) & Colpitts *et al.*'s(2017) studies is likely to be due to females of greater reproductive potential being able to realize greater fecundities under reduced male harassment (Long *et al.* 2009; Yun *et al.*2017). However, if that is the case, what might explain the *lack* of differences in allele frequences in the complex and simple environment for some of the mutations they assayed? We hypothesize that this may be(in part) due to the specific fitness-associated effects of the mutations used, and the design of their experimental evolution protocol, in which flies were not moved into the experimental chambers until the 11th or 12th day of each generation. Since females begin to eclose as adults starting ~9 days after being laid (and males ~the 10th day), adult flies in their studies spent between 1-3 days interacting (and mating) in their initial culture chamber (a standard, simple vial environment), which our study showed can have significant negative effects on female fitness. However, the intensity of interactions during that phase might be lessened if the deletrious mutation present resulted in slower male developmental rates and/or adults with inferior courting abilities, resulting in fewer and/or less persistant males during those early days the adult phase of each generation. In these cases, females may have experienced realtively less cumulative harm before entering either the simple or complex environments, before the relief of more spatial complexity can have its effect. However, if the mutations have no meaningful impact on male development or courtship, then females may enter the experimental phase of the selection protocol already too physiologically damaged by their early adult experiences for any benefits associated with increased spatial complexity to have a meaningful effect on their fitness. It is worth considering that in Colpitts *et al.*'s (2017) study the two mutations that were more effectively purged in the complex environment were *white* (which results in severely impaired vision) and *plexus* (which changes wing morphology) while those trials that showed no difference were *brown* and *sepia* (where the effect of the eye-phenotype produced may not be as deleterious). Future studies should focus on examining how the relationship between specific mutations, and their phenotypic effects on male development and courtship rates and how this may affect their interactions with females of different potential fecundities. Such studies would help in understanding the situations under which sexual selection acts synergitically with natural selection to purge deleterious alleles from the gene pool.

Our current study has its own set of limitations which are worth considering. First, fruit flies (especially lab-reared populations) are well- known for their expression of inter-sexual conflict (Byrne *et al.* 2008) so the differences we observed, may not nescessarily be similar in other species, and/or other populations that are raised under diffferent conditions. Secondly, the exact nature and magnitude of the change(s) in selective pressues associated with differences in spatial complexity will likely depend on the spcecific characteristics of the structural elements in the environment. Our method of introducing spatial complexity by adding a transparent folded acetate insert produced an effect, but is not clear how much/little environmental modification is needed to have an effect on mating dynamics. Thirdly, in our assay we did not measure egg-to-adult survivorship of the offspring, and thus we could be missing a potentially important sources of indiect benefits. However, flies in our lab population, reared at standard densities, typically have a high survivorship (Long *et al.* 2005), so it is unlikey that this is a major factor. While our four-day assay length was designed to replicate the timeframe of adult male and female encounters that is typical in our laboratory setting, a longer experimental duration might have provided more insight to both direct and indirect effects of spatial complexity on increasingly older flies. Furthermore, while our estimates of offspring reproductive competitive success were designed to replicate (as feasibly as possible) the competitive conditions that present in the normal culture environment IV population, it is possible that a different assay design might have yielded different results.

Environmental variation is ubiquitous in natual systems, so most species have presumably evolved under heterogenous selective pressures. Here, we show how increasing the spatial complexity of the environment in which selection takes place results in decreased remating rates and a corresponding increase in the average female reproductive output. This relationship is likely mediated by changes in male and female encounter rates and the avaliability of refuge sites for females experiencing costly harassment from presistent males. If environmental complexity was correlated with the strength of sexual selection, then successful males in the experimental treatment should have sired better quality offspring than those with less selection pressure; however parental evironmental complexity was not associated with differences in the fitness of their offspring. This research helps us better understand how sexual selection operates and highlights the importance of considering environmental context both in laboratory-reared and in wild populations when measuring the effects of sexual selection.

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## TABLES

Table 1. Results of GLM examining the effects of spatial environment, day and their interaction on female remating rates and mean offspring production in *Drosophila melanogaster*.

| | a) Remating | | | b) Mean offspring | | |
|---|---|---|---|---|---|---|
| Variable | LR $\chi^2$ | df | p | LR $\chi^2$ | df | p |
| Environment | 12.83 | 1 | $3.41 \times 10^{-4}$ | 107.38 | 1 | $<1 \times 10^{-10}$ |
| Day | 95.7 | 3 | $2.2 \times 10^{-16}$ | 1217.96 | 3 | $<1 \times 10^{-10}$ |
| Day x Environment | 17.12 | 3 | $6.67 \times 10^{-4}$ | 88.97 | 3 | $<1 \times 10^{-10}$ |

Table 2. Results of Mann-Whitney and Cliff's delta (*d*) effect size statistics comparing the difference in median remating rates in female *Drosophila melanogaster* housed in simple or in complex spatial environments for each of the 4 days of the assay.

| Assay duration | Mann-Whitney | | Cliff's *d* | 95% CI |
|---|---|---|---|---|
| | W | p | | |
| 24 h | 736.5 | $1.85 \times 10^{-05}$ | 0.64 | 0.36 to 0.81 |
| 48 h | 358 | 0.16 | -0.2 | -0.46 to 0.08 |
| 72 h | 540 | 0.13 | 0.2 | -0.07 to 0.44 |
| 96 h | 494 | 0.45 | 0.10 | -0.16 to 0.34 |

Table 3. Results of Mann-Whitney and Cliff's delta (*d*) effect size statistics comparing the difference in median offspring production in female *Drosophila melanogaster* housed in simple or in complex spatial environments for each of the 4 days of the assay.

| Assay duration | Mann-Whitney | | Cliff's *d* | 95% CI |
|---|---|---|---|---|
| | W | p | | |
| 24 h | 627.5 | $8.84 \times 10^{-3}$ | 0.39 | 0.09 to 0.63 |
| 48 h | 189.5 | $1.23 \times 10^{-4}$ | -0.58 | -0.78 to -0.58 |
| 72 h | 28.5 | $4.82 \times 10^{-10}$ | -0.93 | -0.99 to -0.26 |
| 96 h | 101.5 | $2.67 \times 10^{-7}$ | -0.77 | -0.92 to -0.46 |

**FIGURES**

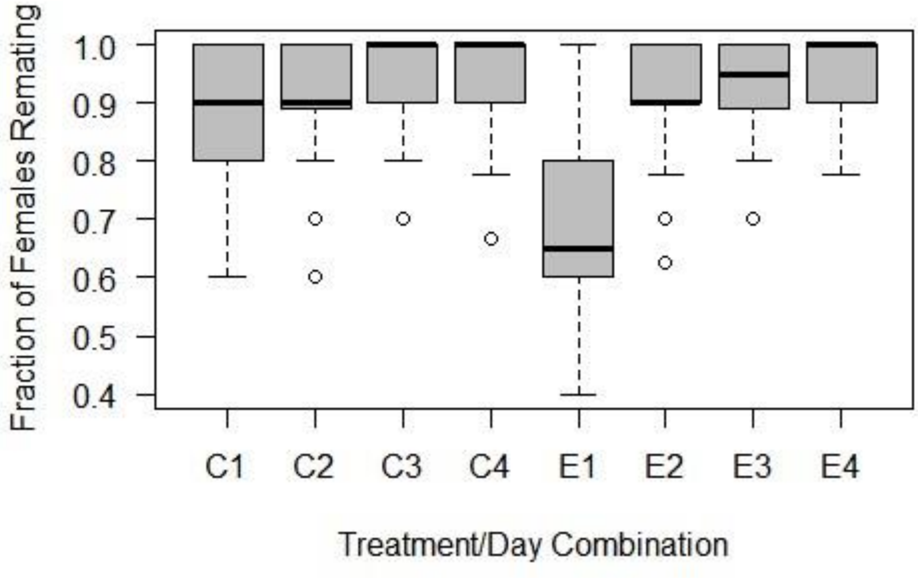

Figure 1. Boxplots illustrating the fraction of female *Drosophila melanogaster* in a vial that remated in simple (C) or environmentally complex (E) chambers measured on each of the 4 days of the assay. The box encloses the middle 50% of data (Inter-Quartile Range, IQR), with the location of the median represented by a horizontal line. Values >±1.5 x the IQR outside the box are considered outliers and depicted as open circles. Whiskers extend to the largest and smallest values that are not outliers.

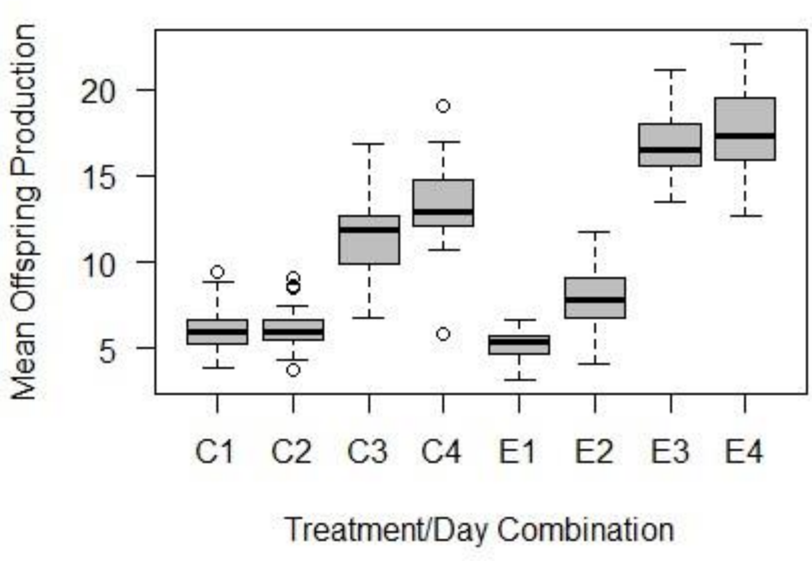

Figure 2. Boxplots illustrating the mean offspring production of female *Drosophila melanogaster* in simple (C) or environmentally complex (E) chambers on each measured on each of the 4 days of the assay. Boxplot components as in Figure 1.

## SUPPLEMENTAL TABLES

Table S1. ANOVA table for Scheirer–Ray–Hare analysis of fraction of wild-type offspring in progeny of a) all female or b) only remated female *Drosophila melanogaster* housed in simple or in complex spatial environments for each of the 4 days of the assay

| | | | a) All Females | | | b) Remated Females | | |
|---|---|---|---|---|---|---|---|---|
| Factor | SS | df | H | p | SS | df | H | P |
| Environment | 44967 | 1 | 9.33 | 0.0022 | 4421 | 1 | 0.9174 | 0.3382 |
| Day | 213585 | 3 | 44.31 | $1.293\text{x}10^{-9}$ | 40253 | 3 | 2.7843 | 0.4261 |
| Day x Environment | 34865 | 3 | 7.231 | 0.0648 | 11308 | 3 | 2.3464 | 0.5037 |
| Residual | 858498 | 232 | | | 1095812 | 232 | | |

Table S2. Results of Mann-Whitney and Cliff's delta (*d*) effect size statistics comparing the difference in median offspring production in daughter *Drosophila melanogaster* from a parent genertion housed in simple or in complex spatial environments for each of the 4 days of the assay.

| Assay duration | Mann-Whitney | | Cliff's *d* | 95% CI |
|---|---|---|---|---|
| | W | p | | |
| 24 h | 491 | 0.55 | 0.09 | -0.20 to 0.37 |
| 48 h | 319 | 0.05 | -0.29 | -0.53 to -0.01 |
| 72 h | 613 | 0.02 | 0.36 | 0.08 to 0.59 |
| 96 h | 334.5 | 0.09 | -0.26 | -0.52 to 0.05 |

Table S3. Results of GLMs examining the effects of spatial environment, day and their interaction on fitness of daughters (number of offpsring) and two metric of fitness in sons (fraction of females mated to male, and total offspring sired) for *Drosophila melanogaster* offpsring whose parents were housed in simple or in complex spatial environments for each of the 4 days of the assay.

**Daughter fitness**

| Variable | LR $\chi^2$ | df | p |
|---|---|---|---|
| Environment | 0.03 | 1 | 0.86 |
| Day | 17.43 | 3 | $5.77\text{x}10^{-4}$ |
| Day x Environment | 11.78 | 3 | $8.17\text{x}10^{-3}$ |

**Fraction of females mated to sons**

| Variable | LR $\chi^2$ | df | p |
|---|---|---|---|
| Environment | 0.95 | 1 | 0.33 |
| Day | 2.38 | 3 | 0.50 |
| Day x Environment | 5.60 | 3 | 0.13 |

**Total offspring sired by sons**

| Variable | LR $\chi^2$ | df | p |
|---|---|---|---|
| Environment | 0.15 | 1 | 0.70 |
| Day | 2.17 | 3 | 0.54 |
| Day x Environment | 4.12 | 3 | 0.25 |

Table S4. Results of Mann-Whitney and Cliff's delta (*d*) effect size statistics comparing the difference in *Drosophila melanogaster* son fitness (measured as the fraction of females mated and in total number offspring sired) whose parents were housed in simple or in complex spatial environments for each of the 4 days of the assay.

**Fraction of females mated to son**

| Assay duration | Mann-Whitney | | Cliff's *d* | 95% CI |
|---|---|---|---|---|
| | W | p | | |
| 24 h | 336.5 | 0.09 | -0.25 | -0.51 to 0.04 |
| 48 h | 404.5 | 0.50 | -0.10 | -0.38 to 0.20 |
| 72 h | 544 | 0.16 | 0.21 | -0.09 to 0.47 |
| 96 h | 407.5 | 0.53 | -0.09 | -0.34 to 0.17 |

**Average offspring sired**

| Assay duration | Mann-Whitney | | Cliff's *d* | 95% CI |
|---|---|---|---|---|
| | W | p | | |
| 24 h | 328.5 | 0.07 | -0.27 | -0.52 to 0.03 |
| 48 h | 448.5 | 0.84 | $-3.3 \times 10^{-3}$ | -0.28 to 0.28 |
| 72 h | 534 | 0.22 | 0.19 | -0.11 to 0.46 |
| 96 h | 494.5 | 0.52 | 0.10 | -0.19 to 0.37 |

## SUPPLEMENTAL FIGURES

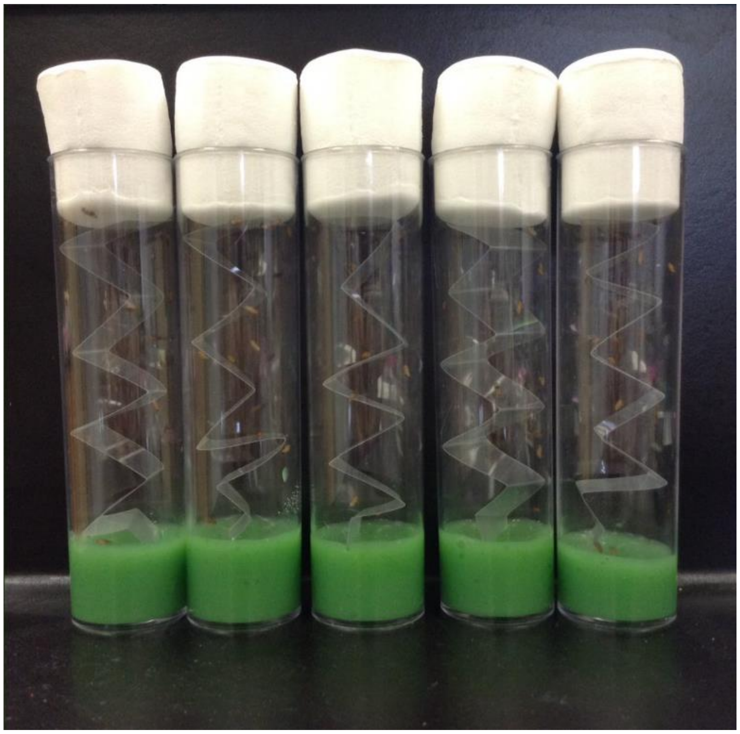

Figure S1. Standard *Drosophila* culture vials used in this assay each containing 10 male and female 10 flies, agar/banana/killed-yeast media and the accordion-style acetate inserts used in our experimental treatments to increase environmental spatial complexity.

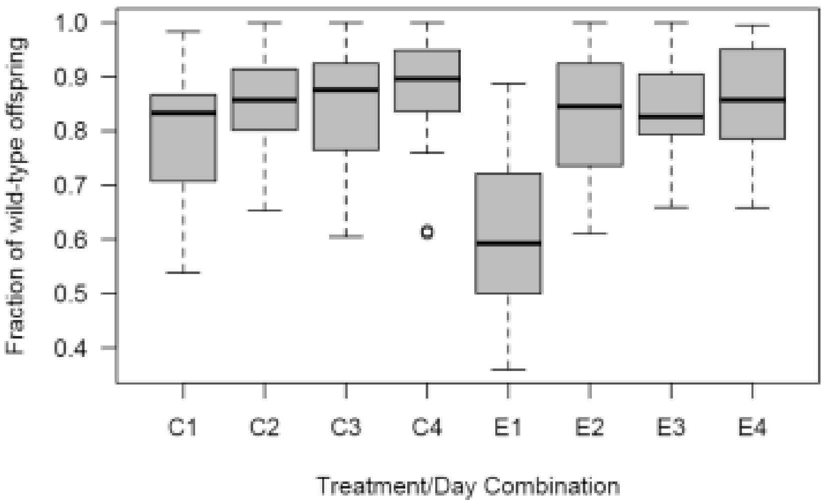

Figure S2. Boxplots illustrating the fraction of wild-type to brown-eyed offspring of *Drosophila melanogaster* in simple (C) or environmentally complex (E) chambers on each measured on each of the 4 days of the assay. The box encloses the middle 50% of data (Inter-Quartile Range, IQR), with the location of the median represented by a horizontal line. Values >±1.5 x the IQR outside the box are considered outliers and depicted as open circles. Whiskers extend to the largest and smallest values that are not outliers.

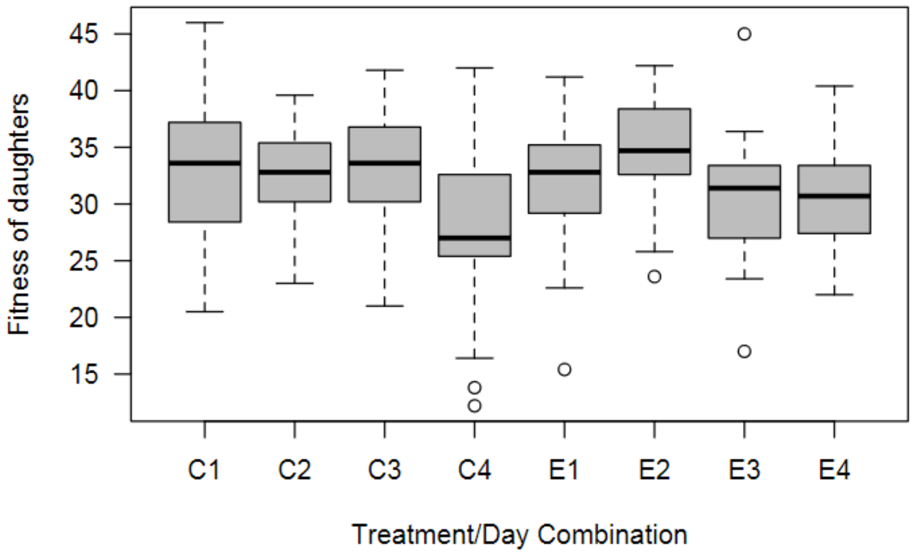

Figure S3. Boxplots illustrating the offspring production of *Drosophila melanogaster* daughters from a parent generation housed for 1 to 4 days in female in simple (C) or environmentally complex (E) chambers measured on each of the 4 days of the assay. Boxplot components are as described in Figure S2.

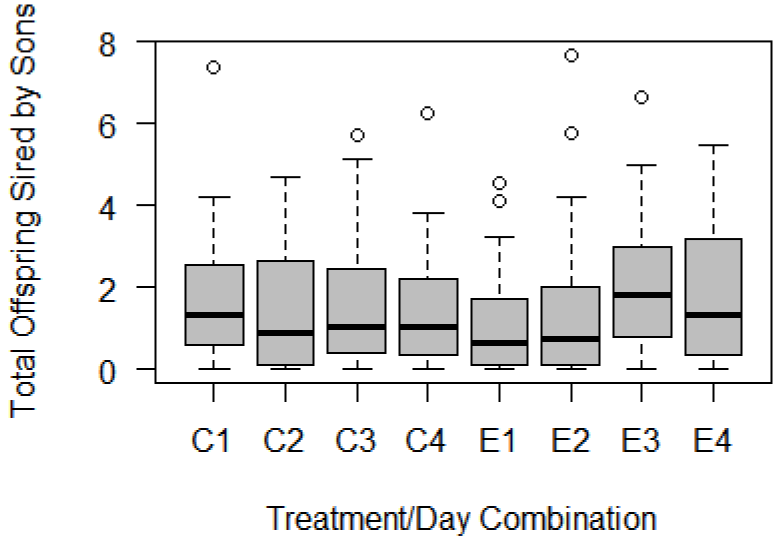

Figure S4. Boxplots illustrating the offspring production of sons from a parent generation housed for 1 to 4 days in simple (C) or environmentally complex (E) chambers measured on each of the 4 days of the assay. Boxplot components are as described in Figure S2.

# Chapter 3

## On the use of social information in oviposition site choice decisions *Drosophila melanogaster.*

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Competing interests: The authors declare no competing interests.

Ethics: This study did not require approval from an ethics committee.

## Abstract

Individuals are faced with decisions during their lifetimes and the choices they make often have important consequences to their fitness. Being able to discern the best available option often incurs sampling costs, which may be largely avoided by copying the behaviour and decisions of others. Although social learning and copying behaviours are widespread, much remains unknown about how effective and adaptive copying behaviour is, and the factors that underlie its expression. Recently, it has been suggested that female fruit flies (*Drosophila melanogaster*) rely heavily on public information when selecting oviposition sites, and may be a promising model system for researching patch-choice copying, and more generally the mechanisms that control decision making. Here, we set out to determine how well *D. melanogaster* females distinguish between a variety of social cues, and if females are using relevant signals when choosing an oviposition site. We found females showed a strong preference for food patches that had been previously used by ovipositing females of the same species and diet over other female outgroups. However, in a separate assay we observed that females favoured media that housed virgin males over media with conspecific signals. Our results confirm that females use the signals left behind by others when choosing between potential oviposition sites, though their prioritization of these signals raises serious questions as to whether this is fruit flies employing copying behaviour, or instead simply responding to attractive signals regardless of their relevance to oviposition site suitability.

*Keywords: Drosophila melanogaster,* behaviour, public information, copying, oviposition,

## Introduction

An individual's fitness is often impacted by the choices it makes throughout its lifetime, be it regarding habitat use, dietary decisions, the nature of its social interactions (including mate choice) and/or where offspring are to be reared. An important part of these decision-making processes is to obtain relevant information through sampling of their environments (Yang *et al.* 2008). To achieve this, individuals must be able to perceive and interpret a variety of different signals, weigh their options and possibly commit them to memory (Drugowitsch *et al*. 2012). This is a potentially expensive endeavour, and species may attempt to avoid these costs by copying the behaviours and decisions expressed by other conspecifics, using public information in lieu of one's own private cost-benefit assessment (Battesti *et al.* 2014, Valone, 1989).

Copying behaviour is employed in a wide range of species in a number of decision-making contexts, when using socially obtained information may be useful in determining suitable habitat or foraging sites; when choosing a mate; and/or deciding where to oviposit eggs and rear offspring. Laland and Williams (1997) found that guppies (*Poecilia reticulata*) used information from previous social interactions when deciding which (of two equivalent) routes to follow to get to a foraging site. In Norway rats (*Rattus norvegicus*) food preference can be altered by experimentally exposing individuals to a conspecific who has been fed a different, novel (but equally palatable) food and letting them smell the scent on their breath. This change in preference could persist for weeks after the exposure and was not seen when rats were exposed to the novel food alone (Galef, 1989). Female sailfin mollies (*Poecilia latipinna*) use copying behaviour during mate choice, where females' assessment of male suitability is influenced not only by observing his successful courtship of a conspecific female, but also from the public information associated with the quality of the female being courted (Hill and Ryan, 2006). Female guppies (*P. reticulata*) reverse their initial mate choice preference if they observe a model female mating with a male of different phenotype (Godin *et al.* 2005). Social egg-laying has been seen across many species, including the collared flycatcher (*Ficedula albicollis*) where both males and females tend to seek new nesting sites the year following a nest failure, unless that site is within a patch with high reproductive success; in this case breeders value the success of their neighbours over their knowledge of past failures (Valone, 2007). While copying the behaviour of another individual can save time and energy associated with sampling and decision-making (Laland, 2004), the potential downside of valuing public over private information comes from trusting that conspecifics have made a suitable decision (Giraldeau *et al*. 2002). In Norway rats (*R. norvegicus)*, copying behaviour can promote preferences for new food sources, but as it can also overwrite prior food aversions developed though personal experiences (Galef, 1989), it might prove costly if the prior aversions benefitted the individual. Thus, when considering social information usage, it may be important to consider both the motivation and the consequences of copying to the individual.

Recently, it has been reported that in the fruit fly, *Drosophila melanogaster*, individuals use social signals to inform their oviposition decisions (Sarin & Dukas 2009, Battesi et al, 2012, Golden & Dukas 2014). In fruit flies, laying one's eggs in proximity to those of conspecifics might be advantageous because of enhanced microbial activity (Wertheim *et al.* 2002) and/or communal foraging behaviour (Dombrovski *et al.* 2017), thus there may be strong advantages to copying the oviposition site choice behaviour of others (Durisko *et al.* 2014). Both Battesti *et al.* (2012) and Sarin and Dukas (2009) observed that a female fruit flies' likelihood of ovipositing on a specific media type was increased if they had previously encountered a demonstrator female ovipositing on a media of the same type. *D. melanogaster*'s disposition to oviposit socially and its reliance on public information was also reported by Golden and Dukas (2014) who measured oviposition site choice preferences by comparing how flies used a reference food (which had not been exposed to larvae and was of high nutritional quality), compared to an experimental food patch of varying nutritional quality (of 100%, 33%, or 11% the nutrition relative to the reference food) but endowed with public information in the form of larval exposure. Females were left to oviposit for 14 hours and the number of eggs laid on each food patch were counted, and larval survivorship was assessed. They observed that females exhibited a strong preference for oviposition on social sites that had contained larvae, even if the food only contained a third of the nutrition of the reference food (which lacked sufficient resources to ensure any offspring survival). A subsequent assay in which larvae were physically removed from the social plates (to avoid potential confounds associated with visual cues from larval presence and the effect of competition against the focal offspring), returned a similar response, indicating that ovipositing females used scent cues (presumably biochemical cues left behind by conspecifics females) when copying. This hypothesis was supported by a parallel study that found that both larvae and adult flies were less likely to visit axenic food than either food that been exposed to larvae with intact microbiomes or from axenic food supplemented with bacterial odors (Venu *et al.* 2014). The implication of these studies is that *D. melanogaster* strongly prioritizes public information over private information (obtained by conducting their own individual cost-benefit analysis of sites) when deciding where to oviposit, so much so that they will ignore (or overlook) large and meaningful differences in food quality. As such, Golden and Dukas (2014) suggested that *D. melanogaster* may serve as a useful model for understanding the factors that shape patch-choice copying, as well as more generally, the mechanisms that control social behavior as well as behavioral decisions.

Our research was inspired by Golden and Dukas' (2014) research, as their results implied that female *D. melanoagaster* are be able to detect and interpret the microbiotic cues left behinds by other flies. However, these signals may show a lot of variation due to differences in species, age, sex, and/or diet (Bing *et al.* 2018; Chandler *et al.* 2011; Han *et al.* 2017). Identifying relevant signals can be a challenge to potential observers and their inability to do so may result in maladaptive behaviours (Depetris-Chauvin *et al.* 2015). As such, we set out to get a better understanding of the specificity and/or limitations of this copying behaviour. We did this in two ways: i) by examining how individual females responded to scents from a range of both conspecific and other species of *Drosophila*; ii) by focusing on how female behaviour differed in response to the scents left behind by *D. melanogaster* differing in their mating status and/or sex and whether these responses are consistent between virgin and mated females. Our overall goal was to better characterize copying behaviour and gain better insight into the factors that influence oviposition site decisions.

## Materials and Methods

### *Population Protocols and Fly Maintenance*

The source of focal individuals for these assays were *D. melanogaster* from the "Ives" (hereafter 'IV'), population, a large, outbred wild-type population that originated from a wild sample caught in Amherst MA, USA in 1975. In addition to the flies from the IV population, we also used flies from i) the IV-*bwD* population, which is genetically similar to the IV population, with the exception that the dominant brown-eyed allele, *bwD* has been introgressed (via repeated backcrossing) into the IV genetic background ii) a population of *D. suzukii* (aka Spotted Wing Fruit Fly, SWD) which was established from individuals eclosing from raspberries that were collected in Cambridge ON, Canada; iii) *D. simulans* individuals from a population that was originally obtained from the University of California San Diego Drosophila Stock Centre (Stock-ID: 14021-0251.064) and were homozygous for the recessive brown-eyed allele, *bw*. These populations were all reared in vials containing ~10ml of a banana/agar/killed-yeast food media (Rose 1984). Flies develop in an incubator at 25˚C and 60% humidity with a 12 L:12 D diurnal light cycle. All stocks (with the exception of *D. suzukii*) develop at an initial density of approximately 100 eggs per vial and are cultured on a non-overlapping 14-day cycle (whereas the *D. suzukii* population is maintained on a 21-day culture cycle, and vials initially contain ~40 eggs each).

### *Can females distinguish between intra- and inter-specific cues for suitable oviposition sites?*

In our first major assay, we set out to determine how well females could discriminate between (presumably) different microbiotic cues left behind by a range of different drosophilids. Target IV females were reared in vials containing media with a 24:1 ratio of protein to carbohydrates (see Lihoreau *et al.* 2016; Young *et al.* 2018), and collected as virgins within 8h of eclosion from their pupae, ~3-4 days before the start of the assay (hereafter "IV-24" flies). These females were housed in vials of 10 then combined with IV males (that were also raised on the same protein-rich media) 24h before the start of the assay. Males were removed (under light CO2 anesthesia) 2 hours prior to the start of the assay, and mated females were returned to the vials.

To measure fly oviposition behaviour, we placed these mated females into "cafeteria-style" choice arenas (described in Young *et al.* 2018) consisting of transparent plastic boxes (KIS Omni Box, 20.3 x 15.9 x 9.6 cm) to which mesh-covered vent holes were added along the upper edges. At the bottom of each chamber we arranged 5 small petri dishes (lids from Kartell 733/4 polyethylene 20 ml sample vials) that each contained 1.5 ml of a standard media. Four of the five dishes presented scents associated with different drosophilids while the fifth had not been exposed to flies and served as a control. Fly-scent dishes had been exposed to mated females for ~18 hours the previous evening in an inverted Kartell 20 ml sample vial with a drilled and foam covered hole (opposite to the lid/media) for air flow. The four fly-scent treatments were as follows: IV-24 females mated to IV-*bwD* males (these were assumed to present the most similar scent to the target IV-24 flies, and would thus provide the most meaningful cues); IV females (raised on standard media) mated to IV-*bwD* males; mated *D. simulans* (*bw*/*bw*) females; and mated *D. suzukii* females. We chose these scents to determine if different food sources could promote a unique microbiome (Bing *et al.* 2018; Blum *et al.* 2013), if different species have different microbial communities (Chandler *et al.* 2011), and whether these factors influenced egg-laying decisions (Schneider *et al.* 2012).

We placed the dishes in the arenas ~2 hours before the introduction of the flies. The arenas, 50 in total, were housed in a well-lit and quiet room. The location of the 10 females were noted every half hour for a 5-hour period and tallied. Petri dishes were then collected, and the media inside was transferred to standard culture vials containing 10ml of media and were placed in the incubator for 14 days. At that time the number and phenotype of all adult flies were counted. The offspring of target IV-24 females were clearly distinguishable from others (as they either possessed brown eyes, or SWD's distinctive phenotype).

To determine whether observed egg-laying biases were due to females associating and/or ovipositing with cues from similar scent cues and not exhibiting a specific preference for the cues associated with flies developing on the high-protein diet we repeated the assay following the same protocols described above with the exception that the target females were raised on a standard media. In this follow-up assay females were observed over 7.5-hour period and left in the chambers overnight to oviposit; the media in each patch was collected (and the offspring counted) ~14 days later.

*Can females distinguish relevant oviposition-site cues?*

In our second set of assays, we set out to measure habitat association (and oviposition behaviour) in two groups (mated and virgin IV females), that were presented with microbiotic scent cues originating from groups of conspecific *D. melanogaster* males or females that were either mated or unmated (virgin). Target females were raised on standard media and collected as virgins within 8h of eclosion from their pupae, ~3-4 days before the start of the assay. Mated females were housed in sets of 10 and combined with males 22 hours prior to the start of the assay. These males were removed (under light CO2 anesthesia) 2 hours prior to the start of the assay, and mated females were returned to the vials. Virgin females were kept in sets of 10 until immediately before the start of the assay and lightly gassed 2 hours prior to the start of the assay to ensure that anaesthesia exposure did not confound behavioural observations). Sets of 10 females were transferred into the cafeteria-style chambers (described above) without anaesthesia. As above, each chamber contained 5 petri dishes that presented different scent cues (four fly-scents and one control dish that had not been exposed to flies). The four fly-scent treatments were as follows: virgin females, previously-mated females, virgin males, and mated males.

Virgin males and females were collected within 8h of their eclosion from pupae, while mated males were collected from mixed-sex vials 24 hours prior to the start of the assay (thus had ~3 days to mate). To distinguish between the offspring oviposited by the target mated females, and the offspring originating from the mated female petri dish (and to remove the potential confounds associated with the presence of live larvae on the media), we mated virgin females to spermless males (described in Kuijper *et al.* 2006, Long *et al.* 2010). These males lack a Y-chromosome, and, while incapable of transferring sperm, do court and transfer normal amounts of seminal proteins (Ingman-Baker & Candido, 1980; Chapman, 1992). Females mated to these spermless males do not produce viable offspring. As above the position of flies on the different media dishes was tallied every 30 minutes for 6 hours. Flies were left in the chambers overnight, and the media from all five dishes in mated female chambers were collected and then transferred to vials of fresh media. The number of flies eclosing from these vials was counted 14 days later. In total, each of the two treatments (mated females and virgin females) was replicated 40 times.

### *Statistical Analyses*

All statistical analyses were performed using R.3.3.2 (R Development Core Team 2017). To analyze the effect of treatment on total patch visits we constructed generalized linear models (GLMs), with a quasibinomial error distribution, where our response variable was the grand number of females observed in each patch in a chamber tallied across each 30-minute interval of the observation period. We used the *Anova* function in the *car* package (Fox & Weisberg 2011) to test whether the treatments means were different from each other using a likelihood-ratio Chisquare test, followed by a Tukey HSD test implemented by the *glht* function in the *multcomp* package (Hothorn *et al.* 2008) to determine the specific location of the differences between treatment. The number of offspring from each patch was also analyzed using the same the procedure, except that the response variable was the total number of wild-type IV individuals that eclosed after 14 days from each patch. We compared the total number of associations with media patches for mated and virgin females using a Mann-Whitney test. As there were significant differences in the overall median number of associations between these two groups, we proceeded by comparing the specific patch usage between these groups by comparing the proportions of visits to each patch type (standardized by individual arena). These comparisons were also made using the Mann-Whitey test and were complimented with the Cliff's delta statistic to determine effect size. To analyse differences in the observation of mated and virgin females we used Mann-Whitney-Wilcoxon test where our variable was the proportion of females on each patch, followed by the Cliff's delta method to determine effect size of female mate status.

## Results

*Can females distinguish between intra- and inter-specific cues for suitable oviposition sites?*

In our first assay we measured the distribution of IV-24 females across treatment patches tallied over 5 hours and the number of offspring eclosing as adults from each media patch. Our analysis found females spent more time associating with scented media plates over control, though a significant difference was only seen in patches previously exposed to *D. suzukii* (Figure 1i). While offspring analysis showed IV-24 females laid more eggs on the media previously used by other IV-24 females (Figure 1ii). In our second assay, we used target IV females raised on standard media measuring their association with treatment patches tallied over 7.5 hours and the number of offspring eclosing as adults from each patch treatment. In our analysis we found females spent more time associating with *D.suzukii* and *D.simulans* treated patches followed by the IV-*bwD* standard media patch, time spent on IV-24 treatments did not differ significantly from control (Figure 1iii). Offspring analysis showed significantly more oviposition activity on

IV-*bwD* plates than any other plate, followed by *D. simulans* as the only other treatment that differed from our control (Figure 1iv).

*Can females distinguish relevant oviposition-site cues?*

In our final assay we measured the distribution of mated and virgin target females across treatment patches tallied over 6 hours and counted the offspring of mated females eclosing as adults from each media patch. We found both mated and virgin females spent significantly more time visiting the patches previously exposed to virgin males followed by mated males, visits to virgin female and mated female patches showed no significant difference from control (Figure 2i). Offspring data showed a similar trend where both virgin and mated male media patches had significantly more oviposition activity than control and both female treatments (Figure 2ii). We found virgin females spent more time on media patches than mated females ($W=426$, $p=8.5\times10^{-4}$). When comparing how virgin and mated females used scented patches we found no significant differences in how virgin and mated females associated with the different media patches (Table 1).

## Discussion

Copying is a strategy used in many species faced with making choices about habitat, food and/or mates (Galef *et al.* 1989; Godin *et al.* 2005; Parejo *et al.* 2006). In doing so, individuals are attempting to reduce the costs incurred with sampling by relying on the observed outcomes of decisions made by others to inform their decisions (Valone, 2007). For copying to be an adaptive strategy, it is essential that those who copy the behaviour of others are able to recognize relevant signals in their environment (Depetris-Chauvin *et al.* 2015). In our study, we set out to examine the nature of oviposition site-copying in *Drosophila melanogaster* (Golden and Dukas,

2014) by examining its specificity and its accuracy. This was done through a series of experiments designed to determine whether ovipositing females would copy the behaviour of individuals whose public information cues provided varying degrees of relevant information about potential egg-site suitability. Our assays revealed that *D. melanogaster* egg-laying behaviour is strongly influenced by the presence and type of signals left behind by others, but that this phenomenon may not be a straight-forward case of copying behaviour. While in our first set of assays females showed affinity for conspecifics who had been raised in a similar environment, in our second assay, where females encountered cues from males and females of different mating statuses, they did not behave in a manner that was consistent with behavioural copying. Here, we discuss how our results fit within the scope of social and public information and what they tell us about the potential for copying behaviour in *D. melanogaster*.

In our first set of assays, we set out to quantify the precision of the oviposition-site copying behaviour of *D. melanogaster* described by Golden and Dukas (2014), by observing how females responded to a variety of social cues originating from other species of *Drosophila* as well from conspecifics that had been raised in different environments. We predicted that if oviposition-site copying behaviour was indeed adaptive (Galef and Giraldeau, 2001), then female flies would utilize socially acquired information produced by those individuals who were the most similar, as this public information would be the most relevant and beneficial to the focal individuals. Our analyses revealed that female choice of oviposition sites in *D. melanogaster* was biased towards using those sites that presented the (presumably) most similar cues to themselves. Although target females showed roughly similar levels of interest in all scented patches, (Figures 1i & 1iii), females oviposited significantly more eggs on those patches that had been exposed to conspecific females that had developed on the same nutritional environment as the targets

(Figures 1ii & 1iv). This pattern was observed in both our first assay where target females had been raised on the high protein as well as in the follow up assay where the females had developed on our standard lab media. While this biased pattern was more pronounced in our first assay compared to our second, this is probably the result of a more distinctive set of cues produced by flies developing on the unusual (for our lab) high-protein media, compared to that produced by the other flies that all developed on media of the same type. These results suggest that *D. melanogaster* are discerning in their use of public information, and are plastic in which cues are used. *Drosophila* display a great sensitivity to the scent cues presented by others, using individual cues associated with diet when making mate choice decisions (Najarro *et al.* 2015), and distinguishing between kin and non-kin, presumably also using scent cues (*e.g.* Lizé *et al.* 2014; Martin and Long 2015). As *D. melanogaster* can develop under a wide-range of conditions (Cavicchi *et al.* 1995; Chippindale *et al.* 1996), which may favour different traits (McCabe and Partridge, 1997), copying the behavioural decisions of individuals whose public information is most similar to oneself may provide the best means of making a decision that will have the greatest fitness benefits (Wagner and Danchin, 2003).

In our second assay, we focused our attention on how females responded to patches that had been exposed to either males or females of differing mating status - the surprising result of which led us to question the fundamental concept of copying behaviours in *D. melanogaster*. As with our previous experiment, we predicted that if oviposition-site copying behaviour was adaptive (Galef and Giraldeau, 2001), then female flies would utilize socially acquired information produced by those individuals who were the most similar, as this public information would be the most relevant. In this case, the most relevant source of information on suitable oviposition sites would presumably have originated from other mated females and consist of a mixture of male pheromonal and female microbial cues that are ejected during oviposition (Duménil *et al.* 2016). Instead, we observed that our mated target females primarily associated on media that had previously kept virgin males (Figure 2i), and laid more eggs on vials that had been exposed to males than they did on other media patches (Figure 2ii). The patches that had been exposed to mated females were amongst the *least* associated and oviposited, and were not significantly different from our unscented control patches. This result would seemingly challenge a key assumption of copying oviposition site behaviour in *D. melanogaster*. A further challenge to this assumption came from our comparison of how virgin and mated females behaved in our assays, in which we observed no significant differences in the frequencies of patch use between these groups. If mated females were engaging in copying behaviour to locate suitable oviposition sites, then this behaviour would presumably differ from virgin females who were not actively seeking oviposition sites. Taken together, this set of assays suggest that if females don't behave differently while looking for oviposition sites or seek out signals originating from other ovipositing females, it may not be appropriate to characterize this as copying behaviour.

The apparent benefit of evolved copying behaviour (in our case, regarding where to oviposit) is that individuals can avoid search costs by using information left by conspecifics who were faced with the same decision, and make advantageous choices (Wagner and Danchin, 2003). If our *D. melanogaster* females did not use relevant signals from other ovipositing females as we saw in our second experiment, what did influence their egg-laying behaviour? There are many chemical signals that could be present to attract females to potential oviposition sites; we are particularly interested in the roles of the pheromone *cis*-vaccenyl acetate (cVA), microbiotic cues, and cuticular hydrocarbons (CHs). The male-specific pheromone cVA is observed across many insect species each able to communicate to females from longer range than most scent-based signals. Although cVA has been shown to encourage aggregation and egg-laying when combined with a food stimulus (Bartelt *et al.*1985; Landolt, 1997; Lin *et al.* 2015), this pheromone is only detectable in sexually active males, and to a lesser extent on mated females via transfer from their mates (Everaerts *et al.* 2010). Since we saw the greatest attraction to virgin males, there must be another male cue involved. Furthermore, it has been shown exposure to aggregation pheromone alone is not enough to encourage a preference for oviposition site scented by cVA (Sarin and Dukas, 2009). When using demonstrator flies that were conditioned to prefer a media type and placed in choice chambers with naïve observer flies Battesti *et al.* (2012) found that the presence of eggs or cVA alone were not enough to influence the behaviour of naïve observer flies who were more inclined to copy the oviposition site of the demonstrator. Based on these cVA results, the male influence on oviposition site decisions was discounted. However, this conclusion may have been premature as it is possible that females may (also) be responding to microbiotic signals and/or CHs, which are influenced by the composition of gut microbiota, can have distinctive phenotypes for males and females, and can vary by both age and individual diet (Fedina *et al.* 2012; Han *et al.* 2017). Furthermore, individual CH phenotypes have been shown to change with mating status (Everaerts *et al.* 2010). Since the females in our experiment did respond strongly to both virgin and mated male associated cues, it is possible that one or both of these phenotypes might be involved in the decision-making process.

Regardless of the specific signals being used, our results are generally consistent with previous studies that female fruit flies use social information when choosing an oviposition site (Battesti *et al.* 2012; Sarin and Dukas, 2009; Golden and Dukas 2014), and further that they exhibit specificity in their perception of cues. However, contrary to our *a priori* prediction that females would use cues from other females when choosing oviposition sites, they instead appeared to value cues from males, and did not change their behaviour depending on their own mating status. If females *are* using social information when making decisions, is that enough to classify this is as copying behaviour? In Valone and Templeton's review of social learning (2002) they stress the differences between public and social information. Social information is obtained *before* an individual visits and evaluates a resource; this is often used to share the location of resources but does little to describe the quality of the resource. In contrast, public information is collected *at a site* by evaluating the attempts and successes of others to better assess the quality of resource. In our experiments the cues present on the patches (eggs, male-derived cVA and/or the CH and microbial cues left behind by males and female conspecifics) may be social, and not public information. By contrast, females may gain public information through direct observation of other females ovipositing (as in Sarin and Dukas, 2009 and Battesti *et al.* 2012), or the presence of larvae (as in our first assay and in Golden and Dukas, 2014) that would alter their own behavioral decisions. Thus, we propose that true copying behaviour may depend on the use of *public* rather than *social* information.

In the wild, there may be considerable heterogeneity in the availability and quality of oviposition sites available to female *D. melanogaster* (Markow 2015; Reaume and Sokolowki 2006), and in the types of signals presented on the surfaces they encounter. The ability to recognize habitat usage by others of the same species, especially ones that developed in a similar nutritional environment, may be of adaptive value to ovipositing females, as this would decrease the probability that their offspring will compete with larvae from another species (Atkinson 1979, Barker 1971). What value might a female gain by choosing to oviposit at a site that had previously been used by males instead of females? On one hand, she may be missing out on suitable developmental environments for her offspring. However, in the context of mate choice copying Nordell and Valone (1998) have argued that for individuals who are unable to make their own assessments, copying others yields the same chance of success as choosing randomly. If males are capable of locating suitable habitats, then females would end up choosing better sites for oviposition, and as a result improve their reproductive success above chance levels. It is possible that in our assay females did not use cues left behind by past females, but, rather, based their decisions on the behaviours of the other target females present in the observation chamber, as has been suggested in previous studies (Sarin and Dukas, 2009; Battesti *et al.* 2012). While interactions between individuals might influence some of the observed patterns of biased patch usage, the consistency of patch usage patterns between individual chambers indicates that cues present on the media surface were being used. If patch use biases arose via positive feedback loops (sensu Wagner & Danchin 2003), then the identity of the preferred patch would be arbitrary and differ from chamber to chamber. Future studies could test the potential effect of social interactions of other ovipositing females by separating females into smaller individual test chambers with the same media options. It may also be worth revisiting earlier experiments by Battesti *et al.* (2012) and Sarin and Dukas (2009) that used isolated aggregate pheromone as male signals and replace it with a more complete male signal as this may have a stronger effect than they found from cVAs alone. These future studies may deepen our understanding of the causes and consequences of why female behaviours differ in response to social cues and through interactions between conspecifics.

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## TABLES

Table 1. Comparisons of frequencies of patch association between virgin and mated *D. melanogaster* females. Medians of proportions were compared using the non-parametric Mann-Whitney test, as well as the Cliff's delta effect size statistic.

| | | | Mann-Whitney | | | |
|---|---|---|---|---|---|---|
| Patch Treatment | Mean proportion of virgin females associating on patch | Mean proportion of mated females associating on patch | W | p | Cliff's Delta | 95% CI |
| Control | 0.05 | 0.07 | 959 | 0.05 | 0.26 | (0.008, 0.484) |
| Mated males | 0.12 | 0.13 | 863 | 0.31 | 0.14 | (-0.122,0.376) |
| Virgin males | 0.72 | 0.65 | 605 | 0.12 | -0.20 | (-0.438,0.055) |
| Mated females | 0.05 | 0.07 | 907 | 0.14 | 0.19 | (-0.069,0.430) |
| Virgin females | 0.06 | 0.07 | 900 | 0.16 | -0.18 | (-0.079,0.423) |

## FIGURES

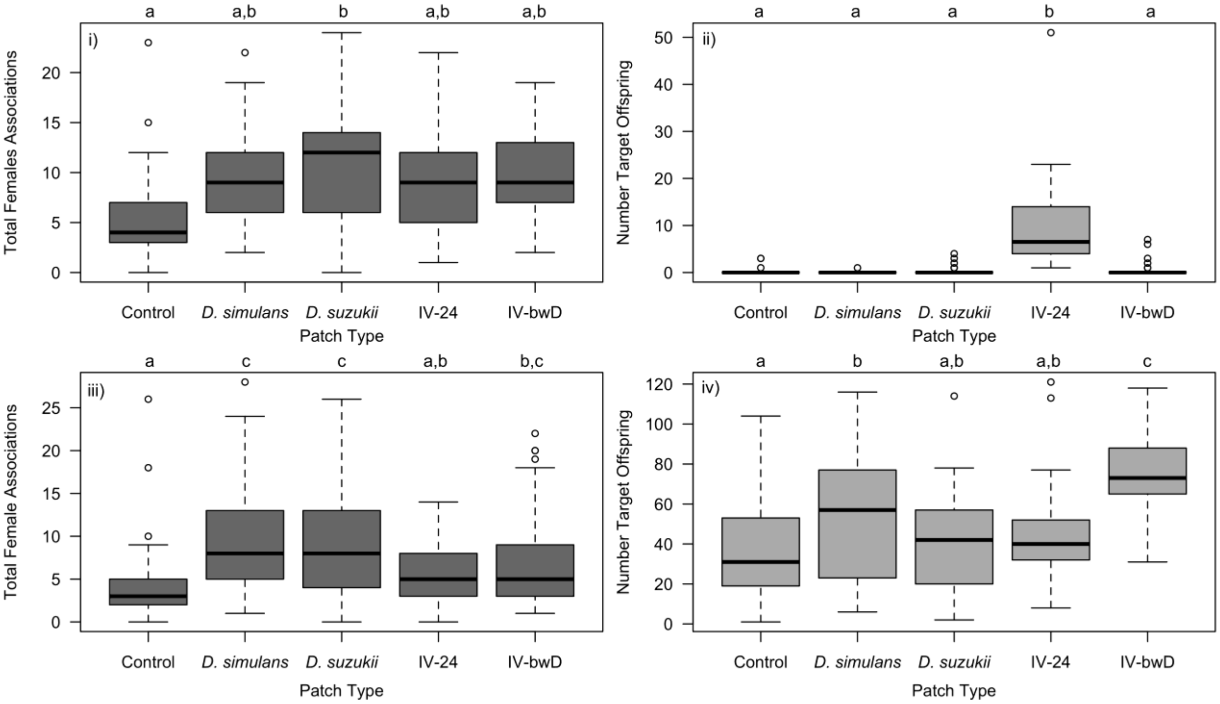

Figure 1. Boxplots illustrating the total number of IV-24 *D. melanogaster* females (reared on a high-protein media) visiting to treatment patches in a chamber, recorded every half hour across a 5-hour period (i), and the total number of IV wild-type offspring that eclosed as adults from media dishes (ii). Media dishes had been previously exposed to either *Drosophila simulans, D. suzukii, D. melanogaster* from the IV population (raised on a high protein media "IV-24"), *D. melanogaster* from the IV-*bwD* population or had not been exposed to any flies (control). The bottom row (iii and iv) is as above except the target female s were IV raised on standard media. The boxes enclose the middle 50% of data (Inter-Quartile Range, IQR), with the location of the median represented by a horizontal line. Values >±1.5 x the IQR outside the box are considered outliers and depicted as open circles. Whiskers extend to the largest and smallest values that are not outliers. Means sharing the same small letters in rows are non-significant at $p<0.05$ according to Tukey's HSD test.

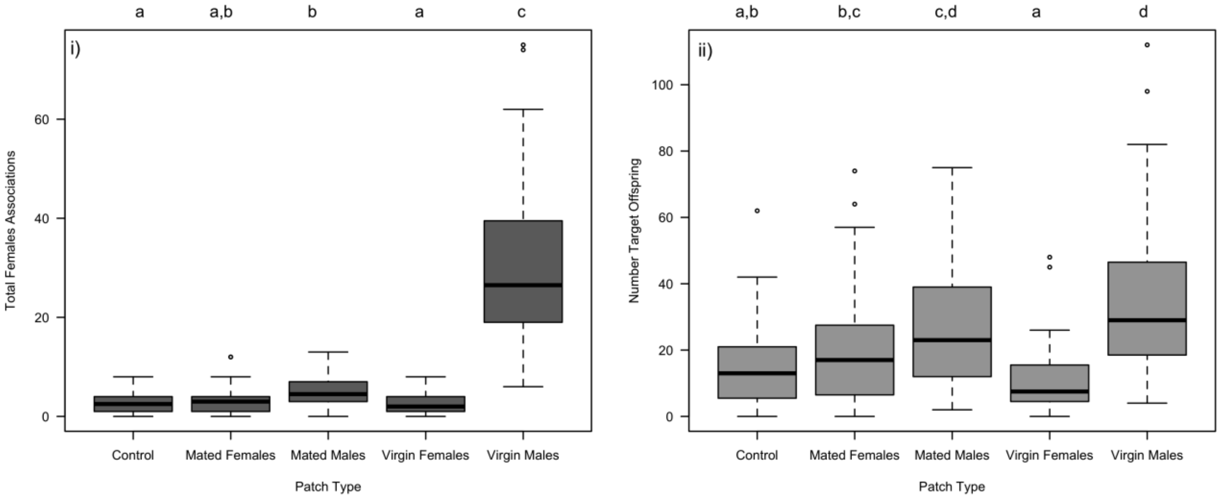

Figure 2. Boxplots illustrating the total number of female *D. melanogaster* visits to each treatment patch recorded every half hour over a 6-hour period (i) and the total number of IV offspring that eclosed as adults from media treatments (ii). Media dishes had been previously exposed to female or male *D. melanogaster* of different mating statuses (mated or virgin) or had not been exposed to any flies (control). Boxplot components as in Figure 1. Means sharing the same small letters in rows are non-significant at $p<0.05$ according to Tukey's HSD.

# Chapter 4

## Environmental context and its role in decision making

Individuals vary considerably in how they behave; this variation can be attributed to benefits and costs associated with different behavioural phenotypes or strategies. In Chapter 1 I set out to explore the context of behavioural strategies and selective pressures, how they can vary in response to environmental heterogeneity and individual variation within species. Behaviours like other phenotypes can be acted upon by selective pressure, where the best behavioural strategy depends on the physical and social environment. Different strategies impose different costs, we can think of an evolutionary stable strategy (ESS) to determine the ideal strategy for individuals to follow. However, changes in environment such as, food availability/quality the social environment, and/or the presence of predators can change the costs and benefits of a behavioural strategy. Furthermore, there is variation between individual condition, which can be influenced by a myriad of factors including genotype, the developmental, social, and physical environment an individual is exposed to. These factors that promote individual variation also contribute to an individual's experience which can shape their future decisions regarding foraging, intrasexual competition and reproduction. These are important areas of research as behavioural isolation is an effective agent of speciation (Boake *et al.* 2002), and although environmental stressors and individual variation also encourage speciation, they also do so in part by influencing the behaviours and decisions of individuals.

The purpose of this thesis was to address how changes in the physical and social environment can influence individual behaviour. In chapter 1 I identified potential reasons for behavioural variation within species and populations. Specifically, I discussed how there can be variation at a population level due to sources of environmental heterogeneity such as food availability, spatial ecology, physical distribution of conspecifics and/or predators), as well as variation between individuals within populations depending on individual condition and experience. For my experimental designs I wanted to look at how environmentally heterogeneity can influence decision making in *Drosophila melanogaster*, specifically mate choice and oviposition behaviour as they are both consequential for individual fitness.

In chapter 2 I was curious to see how environmental complexity would affect social interactions and the consequence this would have to individual fitness. I investigated how spatial complexity influenced remating rates, female fecundity, and offspring fitness as proxy indicators of sexual selection. The results showed there was direct benefit of increasing spatial complexity via decreased remating and increased fecundity, however, no indirect benefits through offspring fitness. These results suggested that females incurred less harm in complex environments when encounter rates would presumably be lower. As discussed in Chapter 1, social interactions can influence behaviour both at the individual and population level depending on the number and nature of those interactions. When considering the number of interactions one can see the similarities between the effect of spatial complexity and density, where both increasing the former and decreasing the latter would decrease the number of encounters individuals experience. For instance, more aggressive behaviour is observed at higher densities in brown trout (*Salmo trutta*) and fire salamanders *Salamandra salamandra* ((Kaspersson *et al.* 2010, Manenti *et al*. 2015). The nature of these interactions can shape future decisions, 'losing' or backing down from previous aggressive encounters influence future behaviours in subsequent contests and even courtships (Otronen, 1990; Tesco *et al.* 2016). In *D. melanogaster*, males that have previously interacted and mated with females were less aggressive to females in future copulation than virgin males (Baxter & Dukas, 2017). Females of *D. melanogaster* who previously mated with aggressive males spend less time associating with males post-copulation in future matings (Filice & Long, 2017). Social interactions can shape selection, although, the direction of this selection relies on the changing costs and benefits of the environment which can vary depending on the individual condition and other factors within the environment.

In chapter 3 I was interested in observing the limitations of copying and social learning in oviposition behaviour. Specifically, how well females were able to discriminate between signals left by individuals of different diets, species, sex, and mate condition, to determine if females behave in a similar way depending on their condition. Copying behaviour, is used by individuals in an attempt to reduce the costs associated with a given behaviour, and as discussed in Chapter 1 and more recently, many factors can influence the perceived costs and benefits of behaviours. How individuals respond to signals within their environment can have significant effects on their fitness, especially when it concerns offspring investment and in the case of *D. melanogaster*, oviposition site. There is a clear evolutionary advantage to being able to interpret signals from predators and appropriately avoid those signals (Grostal & Dicke, 1999); although it is not as imperative to be able to recognize the signals left by competing species or conspecifics. Additionally, the individuals' condition was also of particular interest as we could look at how both diet and mate status affected oviposition choice. In Chapter 1 I discussed how behaviours are subject to change depending one either of these conditions, where diet can influence one's willingness to mate or with whom (Clark *et al* 1997; Fox & Moya-Laraño, 2009; Najarro et al. 2015), while mate status also affects choosiness (Moore and Moore, 2001). However much less work has been done on how these conditions influence oviposition behaviour.

The results for this experiment showed that females were indeed using social signals left behind by others, and that they were able to discriminate between different species and diet treatments. However, when given the option of male-only scents females preferred to oviposit on these sites over those containing cues from other ovipositing females which we would not expect if females were employing copying behaviour. These results were particularly unexpected because males are known to harm females (Partridge and Fowler 1990), and we know recently mated females are less receptive to male courtship, yet mated and virgin females preferred these scents over the smell of females. In this chapter I discuss the implications of these studies and this work going further and highlight the importance of an integrative approach in the study of animal behaviour (Wagner and Danchin, 2003).

## Future directions

There are many potential avenues of research that branch out from these studies, there are numerous sources of behavioural differences, based on the environment or individual variation, which can lead to a range of fitness consequences. In Chapter 2 we used successful remating as a measure of fitness, with the idea that some selection was occurring where either better quality males were locating females or females were able to be more choosy. Although there was no apparent fitness advantage to either the initial mates or wild-type mates, additionally once the first remating took place we lost track of how often matings occurred. More information could be gathered if we ran behavioural assays in our spatial complexity conditions, where we could measure latency to courtship and copulation, the number of courtship attempts and female rejections as well as instances of male induced harm. One potential study that I was curious to see was an integration of some ideas from both experiments in this study. As discussed in chapter 3 there is a strong preference to mate assortatively based on diet (Najarro *et al*. 2015), if environmental complexity is able to influence selection pressure then we may see the strength of assortative mating change under increased spatial complexity. Another interesting study might be to examine these tests again using wild-caught flies, to test i) if fitness is worse in simple environments, especially compared to the lab-reared population which has been kept in that environment for hundreds of generations, ii) if they react any differently to signals from other species as they are presumably more experienced with a variety of cues than lab cultures. Predation is a factor that has shown to influence many decisions by individuals, as discussed in Chapter 1, it may be interesting to see how flies adjust their behaviour in these systems with the introduction of a predator. Would flies fair better in spatially complex environments or would this give predators more ambush opportunities? Would the addition of a predator effect oviposition behaviour? It is likely females would spend less time ovipositing as they may be at an increased risk of predation, whether they still take the time to observe social cues or if actively choose to disperse their offspring in response to predation risk would be interesting to investigate.

## An integrative perspective

This thesis is by its very nature an integrative study, with its purpose to examine decision making within the context of the environment, how individuals' behaviours are interconnected to changes in their surroundings. Animal behaviour is difficult to study out of context, although we may be able to experimentally limit the variables present, this isn't comparable to natural settings where animals interact with a multitude of stimuli. By manipulating both the physical and the biochemical attributes of the environment we were able to see how both these factors may influence behaviour. Since there are so many variables that can result in behavioural variation it is important to consider an integrative approach in future studies of animal behaviour. This work is also integrative in that it can be applied at multiple levels within a population, we can look at these factors and how they influence individual behaviours and fitness, but this can also be examined at a group level, to see the effects on interactions and social learning. Furthermore, we can consider not only how these factors may have consequences to the individual throughout their lifetime but how they might influence the evolutionary trajectory of a population over multiple generations. It is important to think about how behavioural changes may be arising through environmental homogeneity in lab populations, where individuals are very limited in the signals they are exposed to. It may become unnecessarily costly to be able to identify a variety of signals one rarely encounters, thus, we may see individuals be less proficient in discriminating between cues in lab populations. This may bring into question the reliability of using lab populations to measure natural behaviour, and the use of *D. melanogaster* as a model species, though I think it's important to note that the environment at large is becoming less natural and it will always be important to be able to measure the consequence of individual variables. We as humans have removed ourselves from a natural system, typically we are unaffected by day to day changes in the environment, we are not limited by the resources of our own environment and for the most part have little concern for predation risks. If lab populations can be considered unreliable as a natural model, perhaps we can look to them as a model species for innate human behaviours since we have artificial environments in common.

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