SPATIAL HETEROGENEITY AND THE EVOLUTION OF ANIMAL WEAPONS

THE ROLE OF SPATIAL HETEROGENEITY IN SEXUAL SELECTION AND THE EVOLUTION OF EXAGGERATED ANIMAL WEAPONS

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LAY ABSTRACT

Sexual selection drives the evolution of extreme animal weapons that are used in competition between individuals for access to mates, however while competition is common, these traits are rare. Theory predicts that animal weapons will evolve when (i) there is intense competition between males for mates, (ii) there is high variance in male reproductive success, and (iii) competitions between males mostly occur as duels. For my research, I created three environments that emulated these conditions to differing extents and used the fruitfly as a model to see how these conditions influence the initial stages of animal weapon evolution. I found that these environments are variable in their effects on sexual selection and create morphological differences that could indicate early weapon evolution. With this research, I provide the initial framework to test whether these conditions can create the circumstances for the initial evolution of exaggerated animal weaponry to occur.

ABSTRACT

Sexually selected male weaponry are among the most diverse and elaborate traits seen in the animal kingdom and often aid males in securing resources and mates, providing a fitness advantage to those that bear them. Yet, while intra-sexual competition between males to secure mates is common, weaponry is a rare trait that is observed in few taxa. Building upon previous theory, Emlen predicted that exaggerated animal weaponry evolves when, (i) there is intense competition between males for access to females, (ii) the biology or behaviour of a species generates high variance in male reproductive success, and (iii) competitions between males promote extreme weapon size evolution. While there are many species bearing weaponry that appear to fit these contexts, it has yet to be tested if these conditions are sufficient to initiate the evolution of exaggerated weaponry. For my research, I created three environmental treatments that emulated the three conditions proposed by Emlen. Using these treatments, I conducted experiments using *Drosophila melanogaster* to examine the effects of spatial structure on natural and sexual selection with a focus on the initial stages of the evolution of exaggerated trait weaponry that arise from this process. From these experiments, I found that the strength of sexual and other components of natural selection vary with environmental complexity and mutational effect. I also found that these environmental treatments generate weak differences in condition dependence and allometry of fly morphological traits that could be indicative of early weapon evolution. With these experiments, I provide the initial framework to test whether the conditions proposed by Emlen are both necessary and sufficient to lead to the initial evolution of exaggerated animal weaponry and demonstrate that these conditions may indeed create the circumstances that allows this evolution to occur.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
FVW	Fenn Valley Winery
NT	Non-territory
OSR	Operational sex ratio
RHP	Resource holding power/potential
SCT	Spatially constrained territory
UCT	Unconstrained territory
VC	Vial, choice
VNC	Vial, no choice

PREFACE

The following manuscript is the basis of chapter 2 of this thesis:

Spatial Heterogeneity in Resources Alters Selective Dynamics in *Drosophila melanogaster*

Authors: Audrey E Wilson, Ali Siddiqui, Dr. Ian Dworkin

Submitted for publication in: Evolution

I am the first author of this manuscript which has been submitted to Evolution and is currently under revision to be resubmitted to this journal. I was involved in the design, acquisition of data, analysis, interpretation, and drafting of this manuscript. I received assistance from Ali Siddiqui, who made a substantial contribution to the acquisition of data. Dr. Ian Dworkin also made contributions to the design, analysis, interpretation, and the drafting of this manuscript.

This work is included in my thesis as the experiments I conducted were a substantiative proportion of work contributing to my research. The objective of this manuscript corroborates the overall theme of this thesis and the conclusions derived from this work provide insight on the role spatial heterogeneity plays in influencing sexual selection and other elements of natural selection.

Chapter 1: Introduction

The environmental context in which an individual or a population evolves in can have drastic effects on phenotype and may ultimately shift selective dynamics. For example, there are several instances of different populations of the same species displaying changes in coat colour to suit their environmental terrain as a means of camouflage (Gibbons and Lillywhite 1981; Karpestam et al. 2016; Mallarino et al. 2016). This example is a clear indication of the link between environment and phenotype as a product of natural selection. Sexual selection has also been shown to have an interaction with environment. When testing how alterations in water turbidity influences three-spined stickleback, Heushele et al. (2009) found that the extent that females relied on visual and olfactory cues to choose mates varied with changing environmental conditions. Additionally, visual and olfactory cues did not result in the same choice of male mates, which could result in females altering their choice in mates depending on the cues available to them within their environment. The dynamics between environment and mate choice was also seen within a population of collared flycatchers in which females paired with highly ornamented males experienced different fitness consequences depending on the level of rainfall during the breeding season (Robinson et al. 2012). Furthermore, genotype-by-environment interactions can also influence the reliability of male sexual signals, which may ultimately lead to changes in mate choice or the indirect benefits gained from mate choice (as reviewed by (Ingleby et al. 2010)). When examining these connections, it is perhaps unsurprising that a theory for how some of the most elaborate traits created by sexual selection came to be, relies heavily on environmental context.

Animal weaponry and ornaments are some of the most recognizable traits seen in nature due to their elaborate sizes or unique colourations that are seemingly detrimental under pure natural selection circumstances. Since their explanation as products of sexual selection by Darwin (1871), our understanding of how these traits evolve and their benefit in natural populations has come a long way. In general, ornaments are used to signal or attract the opposite sex and are products of intersexual selection, while weapons are structures used in intra-sexual competitions (Darwin 1871; Andersson 1994). Both result from selection to maximize reproductive opportunities although may be difficult to distinguish as the functions of these structures sometimes overlap (Berglund et al. 1996; Mccullough et al. 2016). While stemming from similar intentions, ornaments have been studied more broadly as their link to intersexual selection and female preference has historically been a more elusive topic (Andersson 1994; Mccullough et al. 2016) and weaponry and intrasexual selection has not been studied as heavily.

In species that bear weaponry, expressing larger trait sizes often poses a fitness advantage through increasing reproductive opportunities either from securing mates directly or indirectly by securing desirable resources (Sneddon et al. 1997; Fricke et al. 2015; Fea and Holwell 2018). Considering these trends in the context of game theory, weaponry may increase an individuals' fighting ability, also known as resource holding power (RHP, also termed resource holding potential) which in turn may give the bearers of larger weapons a greater ability to defeat oncoming challengers (Parker 1974). When considering the logic of animal contests and strategies used in aggressive encounters, many game theory models include the ability of individuals to assess their rivals' RHP, comparing the fighting ability of rivals to their own, and postulate that the level of asymmetry in RHP (often inferred by differences in overall body size) can predict the outcome of aggressive interactions (Maynard Smith 1974; Parker 1974; Arnott and Elwood 2009). In these cases, individuals can be spared from dangerous contests against unbeatable competitors with noticeably greater RHPs by retreating, resulting in short interactions. However, the ability of individuals to predict the outcome of a contest against a rival decreases with increasing similarity in RHP, resulting in competitions that do occur to be between relatively equally matched rivals and to be longer in duration. While there are several ways individuals assess RHP (Arnott and Elwood 2009), the asymmetry between rivals can predict contest resolution, with traits related to persistence and strength (including weaponry) being of equal importance (Vieira and Peixoto 2013).

With the notions that weaponry can be important for determining contest outcome and reliable indicators of RHP, these traits are also considered to be good indicators of an individuals' overall quality. There are two explanations for why weaponry may indicate an individuals' quality that are often presented together in the literature: the handicap hypothesis and condition dependence (Kotiaho 2000; Cotton et al. 2004; Penn and Számadó 2020). With the handicap hypothesis (sometimes known as the handicap principle), large, sexually selected traits evolve due to their costs because they signal to mates that the individual has passed some kind of test on their survival and hence are honest signals of high quality (Zahavi 1975). Condition dependence proposes that the level of expression of the sexually selected trait changes in accordance with the resources available (either in quality or quantity) to the individual or with their efficiency at using resources, hence the size of the trait correlates with the overall quality of the individual and is difficult to fake (Zahavi 1977; Nur and Hasson 1984; Price et al. 1993; Rowe and Houle 1996). While initially a relatively undefined term, Hill (2011) defined condition as "the capacity to withstand environmental changes" as a means to encompass both phenotypic and genetic elements. It is important to distinguish that although these concepts are often presented together, condition dependent signalling does not inherently invoke any handicap and the correlation of these hypotheses often seen within the literature may be due to confusion of the initial interpretation of the handicap hypothesis (see Penn and Számadó (2020) for a review).

The evidence of the condition dependence of exaggerated weaponry has been explored in several systems often using environmental or developmental manipulation to show individuals raised in poor conditions display smaller versions of these traits compared to their male counterparts raised in better conditions. A feature often tied to these experiments is determining if weaponry displays hyper-allometries, with larger individuals expressing proportionally larger traits. Hyper-allometries (or positive allometries) are expected to evolve when the fitness gains from a relative increase in trait size is greater for larger individuals compared to smaller (Bonduriansky and Day 2003). It is expected that all secondary selected traits can evolve to be hyper-allometric as not only are larger individuals able to allocate more energy to the production of these traits (condition dependence) but also where body size influences mating success, smaller males may already be at a disadvantage for obtaining mates and thus would not gain benefits from allocating resources to the production of these costly traits compared to larger males that would more often employ these traits in competition (Green 1992). Indeed, condition dependence and hyper-allometries have been shown to be a common feature of sexually-selected traits, including weaponry (Kodric-Brown et al. 2006; Voje 2016), although it has been argued that some of these studies are insufficient and biased in terms of traits chosen for study (see Bonduriansky (2007)). Nevertheless, within the rhinoceros beetle, the horns of males display greater changes with nutrition provided during development and also show greater allometric slopes compared to other non-sexually selected traits (Johns et al. 2014). Also in roe deer, antler size scales positively with body size and is additionally dependent on age, with older heavier males displaying the largest antler sizes (Vanpé et al. 2007). It has been further recognized that condition dependence and allometry of weaponry may differ between the sexes as males with larger traits are more likely to obtain mates whereas female mating success is less variable and as such these traits will not influence their mating success as strongly (Bateman 1948). Sex-specific condition dependence has been demonstrated in the neriid fly *Telostylinus angusticollis* (Bonduriansky 2007b), the Asian rhinoceros beetle (Zinna et al. 2018), the insect *Narnia femorata* (Miller et al. 2016), and sepsid flies (Rohner and Blanckenhorn 2018) with individuals raised in lower quality conditions approaching monomorphism, and males displaying increased condition dependence in traits used as weapons.

Sexual selection and male-male competition is prevalent in many species, yet weaponry is seen within a modest set of taxa (Rico-Guevara and Hurme 2019) relative to the ubiquity of such competition. Rico-Guevara and Hurme (2019) highlight in their review of intra-sexually selected weapons the example of primates, in which within this clade high levels of intrasexual selection are observed in strepsirrhines and gorillas, but only the latter display weaponry. Perhaps a reason for this is the metabolic cost of maintaining these traits as has been shown in the insect *Leptoscelis tricolor* in which weaponry in males can account for a large proportion of resting metabolic rate (Somjee et al. 2018a). Additionally, potential trade-offs have been observed in individuals with exaggerated weapons with males showing higher investment in the development in these traits as opposed to other primary sexual traits such as testes (Somjee et al. 2018b). It is also this potential trade-off that is suggested to be the reason why female weaponry, when present, is often much smaller than that of the males as there are greater fitness consequences when females compromise their fecundity (Berglund 2013). However even with these apparent costs, the presence of these structures seen across diverse sets of taxa raises the question; what conditions are these species evolving in that allows the benefits of weapons to outweigh their costs?

Emlen and Oring (1977) provided a vital framework in determining under which conditions benefits of such trait expression outweigh their costs, subsequently leading to the evolution of trait exaggeration. Although not investigating weaponry specifically, Emlen and Oring (1977) provided theory on the evolution of mating systems, with particular focus on what conditions are needed for polygamy to evolve. These key conditions relied heavily on ecological and phylogenetic factors with the two conditions being that the environment provides individuals a way to monopolize multiple mates or their resources and that individuals can afford to allocate energy to their defense. The interpretation of these conditions being that when resources or mates are physically grouped within an ecosystem and males are not hindered by alternate activities that would diminish their time spent to defend these groupings, males are more likely to monopolize multiple females and the probability for polygamy increases. These conditions were later expanded on by Emlen (2014) to apply to the circumstances under which we may see the evolution of exaggerated weaponry, *sensu strictu*. Emlen (2014) hypothesized that three conditions are necessary: (i) there is intense competition for access to mates between males, (ii) the biology or behaviour of the species generates high variance in male reproductive success, and (iii) the intrasexual competitions themselves promote the evolution of exaggerated weapons. As discussed by Emlen (2014), considering these conditions in terms of economics demonstrates how they can influence the evolution of costly weapons. This first condition can be met through male-biased skews in operational sex ratios (OSR), which is the ratio of sexually-receptive females to males (Emlen and Oring 1977), and the spatial or temporal aggregation of females. When there is generally a greater proportion of receptive males than females in the population, males are under greater pressure to secure mates. That is, access to mates is limiting for reproductive capacity. The spatial clumping of females can not only lead to greater competition for access to females, but also influences the second condition, the generation of increased variance in male reproductive success. When females are physically clustered in space, this provides males a greater opportunity to monopolize multiple mates, increasing the potential for polygyny to form. This can occur as resource defence polygyny, in which males guard resources essential to females or female defense polygyny, in which female groups are defended directly (Emlen and Oring 1977). In either form, this may lead to males that are better able to defend females or territories greater reproductive opportunities compared to those that are unable to monopolize potential mates, leading to increased variance in male reproductive success. In these instances, males that bear weaponry may be at an advantage and allow those with larger weaponry to defend multiple resources or mates more easily than males lacking or with smaller traits. Alternatively, if there was no opportunity to monopolize mates, and variance in reproductive success between males was low, there would be little advantage to the males that bear weaponry over other males that do not, leading to wasted energy in producing these exaggerated features (Emlen 2014). Finally, the last condition pertains to the competitions between males themselves, when they do occur. Competition for mates occurs in many forms such as scrambles, endurance rivalries, contests, mate choice, and sperm competition (Andersson 1994), all of which may favour different attributes. For example, weaponry may be disadvantageous in scramble competitions where multiple males fight simultaneously for a resource or mate, as they could be bulky and reduce maneuverability. However, in duels males interact one-on-one and brute strength may be more favourable especially if these duels are occurring in confined spaces that allows weapon size to be the main predicting factor of contest outcome, hence having a larger weapon could give the bearer a distinct advantage over their rival (Emlen 2014). It is proposed that with all three of these conditions, the benefits of bearing weapons are increased to outweigh potential costs, and exaggerated features will evolve.

Species that do display exaggerated weaponry seem to conform to these three conditions, especially within arthropods. For example, dung beetles typically display one of two behavioural conflict strategies; "rollers" encounter scramble competitions when rolling on a ball of dung, typically in open areas whereas "tunnellers" face duels typically in the entranceway of their confined burrows. Emlen and Philips (2006) utilized phylogenetic comparative methods with 46 beetle species and found that beetles that display rolling behaviour generally lacked weaponry, whereas species expressing tunneling behaviour also displayed horn morphologies. Harlequin beetles also have exaggerated forelegs that are used to monopolize oviposition sites as well as guard females (Zeh et al. 1992), and tusked wasps use their weapons to guard female nests whereas untusked males do not (Longair 2004). In bovids, males able to monopolize groups of females display larger horn morphologies (Bro-Jørgensen 2007). When analyzing three sister species of thorny devil stick insects, Boisseau et al. (2020) found that the armoured species fought over resources and mates that were clustered within host trees and the species that lacked weaponry displayed scramble competition as females were more widely distributed. These conditions also appear to apply when examining the magnitude of weaponry as when examining five species of recently diverged Japanese rhinoceros beetle populations, it was found that the populations with longer horn lengths also had rare and concentrated feeding territories whereas food, although still concentrated to specific areas, was more abundant in populations with smaller horns (del Sol et al. 2020). While the apparent trend of animals with exaggerated weaponry fitting the conditions proposed by Emlen (2014) is certainly contributive to the theory (see Emlen (2008a) for a more extensive review), this is not evidence for causality, and can not be ruled out as coincidental without further research. Furthermore, the initial evolution of exaggerated weaponry may be influenced by different mechanisms than those that maintain these traits. The above examples cannot provide conclusive evidence that differentiates whether the weaponry seen in these species originally evolved due to the observed ecological conditions or if they evolved under other circumstances but are maintained under these conditions. These correlations need to be further examined experimentally to determine if these conditions are truly sufficient and necessary to evolve weaponry.

In this thesis, I examine the effects of spatial structure on sexual selection with a focus on the initial stages of the evolution of exaggerated, condition dependent traits that arise from this process. The overall goal of this research was to emulate the three different conditions outlined by Emlen (2014) and lay the initial ground work for determining whether these conditions are both necessary and sufficient for the evolution of weapons. To do this, I used *Drosophila melanogaster* as it has been widely used in many genetic and evolutionary studies (Harshman and Hoffmann 2000; Edwards et al. 2006) and also has a vast literature of inter- and intra-sexual behaviour within laboratory settings (Chen et al. 2002; Baxter et al. 2015). Along with being easy to handle, making experimental evolution with large population sizes and environmental manipulation relatively simple, other species of *Drosophila* exhibit exaggerated structures. *D. prolongata* males have proportionally large forelegs and *D. heteroneura* males have disproportionately large head widths, both traits believed to be the used in intra- or intersexual processes (Spieth 1981; Grimaldi and Fenster 1989; Setoguchi et al. 2014). Other species displaying traits of such fantastic proportions is suggestive that there may be little developmental or genetic restriction that would prevent *D. melanogaster* from evolving weaponry. Most importantly, *D. melanogaster* has also been shown to vary in their antagonistic and mating behaviour depending on resources available to them. In laboratory environments, *D. melanogaster* typically display scramble competition (Spieth 1974) however, territorial behaviour has been observed in this species when environmental and social contexts are altered. Specifically, when given a small, desirable resource, Hoffmann (1987) found that *D. melanogaster* males displayed resource defense and encounters between males were often won by the male that established residence first which often coincided with larger body size. Investigating this further, Hoffmann and Cacoyianni (1990) showed that this resource defensive behaviour occurred more often when, 1) females were present, 2) there was a low density of males, 3) the resources themselves were readily used by females for oviposition, and 4) the resource patch size was around 20mm in diameter. Patches smaller or larger than this size showed a reduction in territorial behaviour possibly due to being less attractive to females when smaller and being too energetically taxing to defend when larger (Hoffmann and Cacoyianni 1990). Further research shows that aggressive interactions between *Drosophila* follow fairly structured patterns that differ between the sexes. In brief, interactions can range from a visual display of the wings as a threat, low level escalation in which individuals tap each other with their legs or one individual chases the other, to high level escalation where holding, lunging, boxing, tussling (more common in males), and head butting (more common in females) can occur between rivals if neither retreats after instigation of the aggressive interaction (Chen et al. 2002; Nilsen et al. 2004).

For my experiments, I created three environments that vary in their degree of accessibility and defensibility of desirable resources based on the work conducted by Hoffmann and Cacoyianni (1990), to emulate the three conditions proposed by Emlen (2014) to different extents. The ultimate purpose of these environments was to create sufficient alterations in resource defensibility and accessibility such that the optimal mode of competition for mates was consistent with the three proposed conditions. Each environment contains two types of resources, a “high quality” and a “low quality” resource. For the “high quality” resources, the food has been optimized for female fecundity based on nutritional geometry studies that examined how dietary components influence female egg production (Lee et al. 2008; Maklakov et al. 2008; Tatar 2011; Reddiex et al. 2013; Jensen et al. 2015), and thus are highly desirable resources for female oviposition. The “low quality” resources are nutritional dilutions of this “high quality” resource and are always provided as large patches. The purpose of the “high quality” resources is to provide a desirable resource for females to use while maximizing their fecundity, potentially leading to males to defend these resources for access to these females. The "low quality" resources provide males unsuccessful in obtaining or defending a "high quality" resource a source of nutrition and relaxes natural selection such that males are not competing for resources to survive. The three environments used are deemed "non-territory" (NT), "unconstrained territory" (UCT), and "spatially constrained territory" (SCT) (Figure 1.1). The NT treatment contains desirable resources that are large (30.25cm^2 surface area) and potentially equally accessible to all individuals, fulfilling only the first condition of competition for mates. In the NT treatment we expected to have males competing for resources in a scramble, as the resource patches are too large defend and monopolize. The UCT treatment contains smaller desirable resources (25mm in diameter, 4.9cm^2 surface area), potentially allowing the opportunity for males to defend, however not easily as these resources are still relatively easy to access for rivals. This environmental treatment was designed to fulfill the first and second conditions of competition for mates and variance in male reproductive success. Finally, the SCT treatment was designed to satisfy all three conditions. The environmental design for this treatment is identical to UCT, except for a modification to the entrance of the desirable resources that only allow for a few individuals at a time. With this modification, it could be potentially used as a point to guard the entrance to the resource and subsequently any females using it, and it may also reduce scramble competition, leading to an increase in one-on-one duels. Using these territorial designs, I conducted two experiments.

In the first experiment, I examined the efficacy of sexual and natural selection and how this varied across environmental treatments. This experiment consisted of two allele purging experiments using a variety of deleterious mutations affecting different *D. melanogaster* traits. While these types of studies have been implemented to determine the efficiency of sexual selection under various environmental and social alterations, the importance of spatial complexity in these experiments has only been a relatively recent focus (Yun et al. 2017a; MacPherson et al. 2018). Even with the recognition of the importance of environmental complexity, the environments used in previous studies are still relatively simplistic compared to mine. In addition to its novelty in the allele purging literature, this experiment served to give insight into the underpinning forces of sexual selection induced by the three novel environments. If the environmental treatments create differences in opportunities for territoriality and access to territories as intended (ie. males in the SCT environment can monopolize territories and mates with the most ease compared to the other environments), this may in turn create differences in the variance in male reproductive success between environments. An increase in variance would be captured by an increase in the purging rate of deleterious alleles as mutant individuals are presumably less likely to hold these territories and contribute to the next generation (assuming that sexual selection and other components of natural selection are aligned) (see chapter 2 for a literature review and more details).

For my second experiment, I experimentally evolved *D. melanogaster* in these different environments and examined these populations for traits that could be indicative of the early evolution of exaggerated weaponry. As previously stated, a common trend seen within animal exaggerated weaponry is they display sexual dimorphism, heightened condition dependence and hyper-allometric relationships with body size, hence these were investigated as they seemed the most likely changes to be detected in the earliest stages of exaggerated weapon evolution. Specifically, we examined changes in condition dependence and allometries for foreleg length, wing length, and head widths. It was expected, that if the conditions proposed by Emlen (2014) result in the evolution of weaponry, the greatest changes in condition dependence and allometries would occur in the SCT treatment (in which all conditions are met) as well as some changes in the UCT treatment. Changes would be very subtle as the populations would have only evolved for a short period of time (~35 generations) when examining differences between treatments. The forelegs, wings, and heads were examined because these traits are often used in intra- and inter-sexual interactions amongst flies. Males often use forelegs in aggressive interactions (boxing and tussling mentioned above) while females are more prone to headbutting their rivals (Chen et al. 2002; Nilsen et al. 2004). As a result of their increased use in intrasexual competitions compared to other traits, it was predicted that male forelegs or female head widths would evolve to exaggerated sizes (see chapter 3 for a literature review and more details).

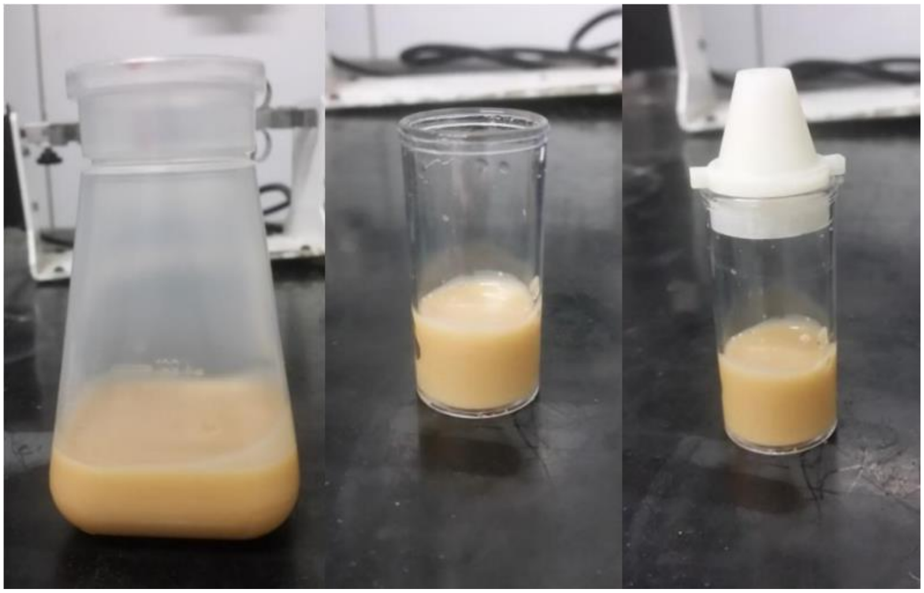

Figure 1.1: Left to right: NT bottle, UCT vial, and SCT vial used in environmental treatments. Note: vials were modified in the second experiment to be 35mm in height, see Chapter 3

Chapter 2: Spatial heterogeneity in resources alters selective dynamics in *Drosophila melanogaster*

Abstract

Environmental features can alter the behaviours and phenotypes of organisms and populations evolving within them including the dynamics between natural and sexual selection. Experimental environmental manipulation, particularly when conducted in experiments where the dynamics of the purging of deleterious alleles are compared, has demonstrated both direct and indirect effects on the strength and direction of selection. However, many of these experiments are conducted with fairly simplistic environments when it is not always clear how or why particular forms of spatial heterogeneity may influence behaviour or selection. Using *Drosophila melanogaster*, we tested three different spatial environments designed to determine if spatial constraint of critical resources influences the efficiency of natural and sexual selection. We conducted two allele purging experiments to 1) assess the effects of these spatial treatments on the selective dynamics of six recessive mutations, and 2) determine how the selective dynamics changed when sexual selection was relaxed. We found that allele purging dynamics depended on spatial environment, however the patterns of purging rates between the environments differed across distinct deleterious mutations. We also found that for two of the mutations, the addition of sexual selection increased the purging rate.

Introduction

Understanding mating systems and the dynamics between the sexes can illuminate how sexual selection acts within populations, driving many organisms' behaviours and phenotypes. Key work in the theory of mating systems conducted by Bateman (1948), Trivers (1972), and Emlen and Oring (1977) has led many studies being dedicated to examining male and female interactions across different species and populations. The mating systems of numerous species have been shown to vary due to local adaption or ecological constraints due to environmental factors (Miller and Svensson 2014). For example, ungulate species that inhabit open environments tend towards group mating systems while those within closed or forested environments tend to adopt small group or pair mating systems as gregariousness of these organisms are influenced by predation and resource distribution in their environment (Carranza 2000; Bowyer et al. 2020). This variation in behaviour can occur within species as well, as seen in the mating system of *Prunella modularis,* which has been shown to shift between polygyny, polygynandry, and polyandry depending on food distribution (Davies and Lundberg 1984). Environmental features such as overall size of the environment, spatial structure, resource abundance, and climate can alter the strength of sexual selection and conflict (both intra- and inter-) on an individual, in turn leading to fitness payoffs for certain phenotypes. In *Sancassania berlesei*, increasing environmental complexity changes the fitness differences between the fighter and scramble male morphs which was believed to be a result of reduced encounters between fighter males (Lukasik et al. 2006). Another example can be found in certain populations of katydid, where sex role reversal occurs under conditions of low resource abundance, placing a greater influence of inter- and intra-sexual selection on females (Gwynne and Simmons 1990). Since environmental variation can impact fitness, it is important to keep environmental context in mind when studying the strength of natural selection, sexual selection and sexual conflict.

Along with the environment, understanding the interaction between sexual selection and other components of natural selection (fecundity and viability) is important for determining an organisms' or a populations' phenotypic and behavioural origins. Since the term was introduced by Darwin (1871), studies have focused on how traits under strong sexual selection (weaponry, ornaments, and mating behaviours) arise and persist within populations. When sexual conflict is present, mutations may be beneficial in one sex but deleterious in the other (antagonistic pleiotropy), allowing for the maintenance of conditionally deleterious alleles. In many species, an extreme case of this is males harming females during copulation, either through mating itself or ejaculates, in order to prevent re-mating, further securing the males' paternity (Johnstone and Keller 2000). However, while natural and sexual selection are often portrayed as being at odds with one another, individuals of higher overall condition will on average receive more mates, resulting in sexual selection working in tandem with other components of natural selection. For instance in ungulates, males of overall higher condition tend to have the largest weaponry and are better able to obtain fertilizations along with access to females themselves (Preston et al. 2003; Hoem et al. 2007; Vanpé et al. 2007; Emlen 2008b).

A common way of determining how various factors influence natural and sexual selection is to conduct allele purging experiments. Within these experiments, deleterious mutations are introduced into populations at a known frequency (or via induced mutations) and the rate they are removed from the populations over time is recorded or populations undergo various fitness assays. Experimental conditions are manipulated (thermal stress, dietary stress, population density, environmental complexity, and mate choice (Sharp and Agrawal 2008; Wang et al. 2009; Young et al. 2009; Hollis et al. 2009; MacLellan et al. 2009; Laffafian et al. 2010; Hollis and Houle 2011; McGuigan et al. 2011; Arbuthnott and Rundle 2012; Clark et al. 2012; Maclellan et al. 2012; Singh et al. 2017; Colpitts et al. 2017) and purging rates (or fitness) are compared to obtain estimates of the effects these conditions have on selective dynamics. While several kinds of these studies have been conducted, many show contrasting results in reference to whether sexual selection aids natural selection in the removal of deleterious alleles. One potential reason for such inconsistencies is that most experiments are performed in small, simple environments (i.e. small vials) at relatively high densities, and it is not clear the degree to which this may influence the strength and orientation of selection. Such simple and high-density environments likely constrain individuals in terms of mating strategies available in more natural conditions. Alternative mating strategies are density-dependent in several species (Greenfield and Shelly 1985; Höglund and Robertson 1988; Kokko and Rankin 2006), and particularly for *Drosophila melanogaster*, territorial defence strategies by males are less likely to occur when the population is at a high density (Hoffmann and Cacoyianni 1990). Simple environments may also influence female strategies in that they may accept more mates due to being unable to seek refuge or escape from constant male harassment (Byrne et al. 2008). Creating a more "complex" environment consisting of a larger space, multiple food cups, and additional spatial structure to alter the interactions between the sexes, Yun et al. (2017) showed that harassment of high quality *D. melanogaster* females was greater in the simple fly vial environments used in many experiments, exaggerating the effects of sexual selection to reduce variance in female fitness.

Since Yun et al.'s (2017) experiment, there have been several studies conducted to determine how natural and sexual selection change within simple (high density in single vials or bottles) versus "complex environments" (lower density cages with multiple resources for interactions to occur). In a later study, Yun et al. (2018) found flies that had mating opportunities within "complex" environments adapted more quickly to novel larval environments as opposed to those mating in simple environments or lacking mate competition. Using a similar environmental design but creating a larger, lower density simple environment, Colpitts et al. (2017) demonstrated that "complex" environments aided the purging of two deleterious mutations that had previously been found to have no difference in purging rate while manipulating opportunity for mate choice (Arbuthnott and Rundle 2012). Singh et al. (2017) showed increased purging rate of deleterious alleles from populations evolving within these "complex" environments, while MacPherson et al. (2018) revealed that low quality females experienced a greater reduction in fitness due to male harm compared to high quality females but only in "complex" relative to simple environments. These studies exemplify that with even modest changes in spatial environment (increasing space and lowering density of individuals), the dynamics of natural and sexual selection can vary vastly. Complexity without the manipulation of overall environment size by inserting dividers into resource vials to increase surface area has been shown to influence female fitness in terms of increased offspring production (Malek and Long 2019), but this has not been used to test overall population fitness. While these studies potentially show how these forces interact in a way that may be more representative of what is seen in nature, the types of environments employed are still simple and largely reflect changes in density. However, it is important for such experiments to explicitly consider factors that are known to influence mating strategy as well, such as territory availability and spatial heterogeneity of resources.

Increasing the environmental complexity in which populations evolve may reveal new patterns of how sexual selection acts, particularly for *D. melanogaster*, which as a species shows considerable variability in mating strategy in different spatial contexts. Typically displaying scramble competition in the lab, territorial behaviours and resource defense polygyny have been observed when *D. melanogaster* males are given a desirable resource (Hoffmann 1987). Males also appear to display resource defense polygyny more often when females are present, when there is a low density of males, and the resource is readily used by females for oviposition and resource patches are about 20mm in diameter (Hoffmann and Cacoyianni 1990). Within laboratory experiments where aggressive interactions amongst *D. melanogaster* males are observed, it is typical that larger males or males that hold residence of a territory first, have greater reproductive success (Hoffmann 1987). Considering this, if populations are within an environment that allows males to benefit from territorial behaviour, these populations may show an increase in overall fitness, and more variation in mating strategies. Yet to date, most experimental evolution and purging experiments have not considered the explicit environmental factors that influence male resource defence polygyny and mating strategies in their design.

While the previous work outlined above has made considerable contributions to our understanding of the interplay between environmental complexity and selective forces, the environments used are relatively simplistic when considering the plasticity of animal mating behaviour. We conducted a series of short-term allele purging experimental evolution assays where environmental complexity and the accessibility of *D. melanogaster* to critical resources were manipulated with these factors in mind. In the first part of this experiment we looked at how differences in resource patch size and accessibility influenced the purging of six recessive deleterious mutations from populations being held within a series of complex environments. Specifically, we provided multiple resource patches of high (to maximize female fecundity) and low quality. In each treatment high quality patch size and accessibility varied according to how they should potentially influence aspects of territoriality. In the second experiment, we examined how the rate of removal for two of these mutations differed between the complex environments and two simple environments in which we additionally manipulated opportunity for mate choice (via forced monogamy). We expected that if natural and sexual selection were aligned, we would see an increase in purging rate as accessibility to resources decreased and that the purging rate overall would be greater when sexual selection in the form of mate choice was present.

Methods

Environmental Manipulations

Images of the environmental treatments and an illustration of general set up are provided in Figure 2.1. Three environments were created to test the effects of desirable resource availability on the removal of deleterious mutations from populations. Within each environment there were both "high quality" resources of a yeast-rich food (see Table S1) and a 15% dilution (in water/carrageenan) of this food as a "low quality" resource. High quality food was determined based on previously published nutritional geometry studies that examined how dietary components influenced various fitness aspects (Lee et al. 2008; Jensen et al. 2015), and that maximized female fecundity (in terms of female egg production). Based on previous studies, the intent of these high quality food resources was to entice females to use these patches for oviposition and potentially lead males to defend these resources to maximize their own mating success. The diluted medium provided resource patches, such that individuals are not competing for survival *per se*, but for the desirable resources that females may prefer to maximize their fecundity. For each replicate environment described below, mesh BugDorm4M1515 cage ($15cm^3$) were used. The "non-territory" treatment environment (NT) consisted of a single *Drosophila* culture bottle (177ml), with a surface area of $30.25cm^2$ (55mm x 55mm base) containing ~50ml of high quality food with the addition of four drops of a yeast-paste and orange juice mixture on top (to attract females (Dweck et al. 2013)), as well as a bottle only with 50ml low quality food. These represent "typical" *Drosophila* lab environments where apparent scramble competition is commonly observed (Spieth 1974), although subtle interference competition may be occurring as well (Baxter et al. 2018). The "unconstrained territory" spatial treatment (UCT) consisted of eight open vials (height of 50mm, 25mm diameter, 4.9 cm^2 surface area) each filled with ~5 ml of high quality food with a single drop of yeast-paste/orange juice mixture on top and a single bottle with the low quality food. Finally, the "spatially constrained territory" treatment (SCT) had the same set-up as the UCT treatment except each vial had a 3D printed funnel cap (22mm bottom diameter, 9.5mm top diameter, 25mm height, 4mm opening, see Supplemental Fig 2.1) to further restrict ease of access to high quality food patches. These 3D printed funnel caps were designed and tested with several specific features in mind. First, that it was relatively difficult to gain access, but would be relatively easy (given positive photo-taxis and negative geo-taxis in *Drosophila* (Markow and Merriam 1977)) for an interloper to be chased out. Second, that the aperture was of sufficient size that two large *D. melanogaster* individuals could pass one another, but one individual could still harass or chase the other in this space. Finally, the funnel cap was designed so that if an individual did display territorial behaviours, it had multiple places to survey or defend (food surface, inner aperture, and outside top of aperture). Pipe cleaners were wrapped around the tops of bottles and vials to provide additional perching substrate for individuals.

Populations

To examine deleterious allele purging rates, six mutations with known morphological defects were used across each of the three spatial treatments. Each allele was picked because of previous work examining the effects of selection on them in the context of either spatial manipulations or varying degrees of sexual selection (Arbuthnott and Rundle 2012; Colpitts et al. 2017). Three of these mutations are autosomal (*brown*^{1}, *vestigial*^{1}, and *plexus*^{1}) and three are X-linked (*white*^{1}, *yellow*^{1}, and *forked*^{1}). The mutations *plexus*^{1}, *white*^{1}, *yellow*^{1}, and *forked*^{1} were obtained from Bloomington stock center while *brown*^{1} and *vestigial*^{1} were obtained from stocks kept in the lab. These alleles were chosen for their wide array of phenotypic effects with two influencing eye colour (*white*^{1} and *brown*^{1}), two influencing wing morphology (*plexus*^{1} and *vestigial*^{1}), one affecting body colour and behaviour (*yellow*^{1}) and one affecting bristle morphology (*forked*^{1}).

To create experimental populations, individuals were backcrossed into a large outbred domesticated lab population (census size of 1200-1600 individuals) originally collected from Fenn Valley Winery (FVW), Michigan (GPS co-ordinates: 42.578919, -86.144936) in 2010. This population was chosen to potentially minimize confounding effects of lab adaptation during this experiment (Harshman and Hoffmann 2000), i.e. it is expected that this population has already had considerable opportunity to adapt to our lab environment (~180 generations prior to initiation of this experiment). To generate experimental populations, the following procedure was used. For autosomal mutations, mutant female virgins were crossed with FVW males. F1 was then crossed to each other and mutant homozygote females were collected. For X-linked mutations, mutant males were crossed with wildtype females. The heterozygous females from this cross were then crossed back to wildtype males, the mutant offspring from this cross were then collected and the process was repeated. For each mutation, backcrossing was conducted for five generations and on the final generation, offspring from the final cross were mated together to create mutant males and females. Fifty pairs were used to generate each cross.

Purging Rates Across Environments

For each mutation, nine replicate populations were created and three of each randomly assigned to one of the three environmental treatments. Initial populations consisted of 100 males and 100 females with starting allele frequencies of 0.7 for their respective mutation. Populations were maintained at 12L:12D cycles at 21°C with 60% relative humidity in a Conviron walk in chamber (CMP6050). Each generation, adults were placed into their respective treatments and allowed to mate and lay eggs for three days. After the three day period, adults were removed from the environments and discarded. Eggs were allowed to develop for 11 days, after which the next generation of adults was collected by bringing the adults to the cold room kept at 4°C and gently knocking them into vials. From these vials, 100 random males and 100 random females from each replicate were phenotyped under light CO_2 and placed into their respective environments with fresh food. This cycle was repeated for 10 generations.

Due to a laboratory bacterial infection in one replicate of the *brown¹* population for the NT treatment, this replicate was discarded after generation 5. A fourth replicate was created with the same starting allele frequencies (0.7) to account for the missing data. This replicate was therefore five generations behind the rest of the experiment and was continued for 10 generations.

In order to get an estimate of allele frequencies for autosomal mutations during this experiment, monogamous pairings of phenotypically wildtype females and mutant males were conducted at generations 3 and 6, for *brown¹* and *plexus¹* populations and at generations 3 and 8 for *vestigial¹* populations. After the collection of adults for the next generation, for each population 50 virgin females were phenotyped over light CO_2. Of the 50 females, those that lacked the mutation (i.e. could be homozygous or heterozygous for the wild type alleles) were placed singularly into vials with a mutant male. Offspring were analyzed from these vials over 3 days after emergence. If a vial contained only wildtype offspring, the female parent was scored as homozygous for lacking the mutation, if the vial contained a mixture of wildtype and mutant offspring, the female parent was scored as heterozygous for the mutation. For the X-linked mutations, allele frequencies were estimated from the frequency of the mutation in males.

Purging Rates with Effects of Mate Choice

To determine the effects of sexual selection on purging rates, we re-ran the experiment using *white¹* and *vestigial¹* with the addition of two new treatments. The first treatment, deemed "vial no choice" (VNC), consisted of randomly assigning 100 individual pairs into vials to mate (i.e forced monogamy). The second treatment, "vial choice" (VC), consisted of randomly assigning 100 male and female adults into vials of five mixed sex pairs. After three days of mating for each treatment, males were removed and females were placed into environments similar to the NT treatment. After three days the females were removed and eggs were allowed to develop for 9-10 days. Emerging female virgins and adult males were collected similar to above and the process was repeated. NT, UCT, and SCT treatments were conducted the same as above except females were collected as virgins and males and females were held separately for three days after collection in order to align with the experimental schedule of the VNC and VC treatments. This experiment was conducted for only four generations as it was disrupted by a lab shutdown brought about by the covid-19 pandemic. One replicate of the SCT *vestigial*^{1} treatment did not have any surviving adults at generation four.

Statistical Analysis

The rate of mutant allele loss in each population over multiple generations for each component of the experiment was analyzed by fitting generalized linear mixed effect models with binomial distribution (i.e. a logistic mixed model). Since each allele was started at a known frequency, and the intercept was known, models were fit without estimating a global intercept (but included offsets). Main effect for allele or treatment were also not included (as all treatments started with the same frequency for a given allele). Fixed effects included in the model were thus generation X mutation type, generation X treatment, and generation X mutant type X treatment. Random slopes for generation was included across replicate lineages, and the intercept was offset to 0.7 for allele frequency (or 0.49 for autosomal and 0.595 for sex-linked mutations when modelling mutant genotypic frequencies). Fixed effects were further examined for significance with a two way ANOVA (type II Wald χ^2 test) and treatment contrasts averaged over mutant type were examined by comparing estimated marginal means within each model. Contrasts were done usings emmeans using Tukey's HSD to adjust for number of comparisons.

For analyzing purging rates across environmental treatments, models were generated with and without the third SCT replicate for the *forked*^{1} mutation due to this replicate having mutant allele frequencies approaching fixation consistently throughout the experiment (Figure S2, Table S2 and Table S3). Results presented exclude this replicate unless otherwise indicated.

Selection coefficients for each mutation were estimated using the allele frequency data. Selection coefficient per generation was calculated as $s = 1 - (q'/q)$, with q representing the previous allele frequency and q' representing the allele frequency of the subsequent generation, and these estimates were then averaged across generation and replicate for each mutant type.

All statistical analyses were performed in R v.3.5.2 (R Core Team 2018) using glmer() (lme4 package v1.1-21 (Bates et al. 2015)), Anova() (car package v3.0-2 (Fox and Weisberg 2011)), and emtrends() (emmeans package v.3.1.1 (Lenth 2019)). All plots were generated with ggplot2 v.3.1.1 (Wickham 2016).

Results

As expected, average allele frequency declined over generations for all six mutations types, indicating these alleles to be deleterious (Figure 2.2). We observed substantial differences in rates of purging (as assessed by genotypic frequencies) based on the identity of the mutation. ANOVA shows significant effects for all interactions of generation with mutant type and treatment, however significant effects may be restricted to certain mutation types as contrasts between treatments among all mutant types are non-significant indicating that there is no substantial difference in purging rates between spatial environments when all mutations are considered collectively (Table 2.1 and Table 2.2). Across the six mutation types, there was no consistent overall pattern in purging rate between the NT, UCT, and SCT environmental treatments. Similar results are shown when analyzing males and females separately. When examining estimated allele frequencies, only the interactions between generation and mutant type, and generation and treatment are significant (Figure 2.3, Table 2.1). However, treatment contrasts are still not significantly different from one another (Table 2.2). Overall trends of significance from ANOVA and treatment contrasts are the same when including the third SCT replicate for the *forked*^{1} mutation. Estimated selection coefficients are of differing strengths for each mutant type suggesting each mutation is affected by selective dynamics to different extents. We observed no consistent pattern in strength of selection of treatment types across mutations with no treatment consistently displaying higher or lower selection coefficients across mutant types (Figure 2.4). Overall, the results suggest that while there are effects of the three spatial treatments on rates of purging (Figure S2.3), they are relatively modest in comparison to the effects of individual mutants and their interactions with the spatial treatment.

In the second experiment, we replicated the above experiment with two of the six alleles and added additional treatments with explicit manipulations of sexual selection. The addition of sexual selection for both *white*^{1} and *vestigial*^{1} mutant populations increased purging rates (Figure 2.5, Table 2.3). The forced monogamy treatment (VNC) that relaxes sexual selection, showed the slowest purging rate for both mutations. However, between the treatments that include sexual selection there is no consistent pattern in purging rate by treatment across the two mutant types. The ANOVA shows significant effects of the interaction between generation and mutant type, and generation and treatment but not for the interaction between all three fixed effects. The addition of sexual selection but not environmental complexity increased purging rates as treatment contrasts show that the VNC (vial no choice) treatment (i.e. forced monogamy) is significantly different from the other treatments but VC, NT, UCT, and SCT are not significantly different from each other. When analyzing the sexes separately, only the interactions between generation and treatment, and generation and mutant type were significant for males whereas the interactions between generation and treatment, and generation, treatment and mutant type were significant for females. Treatment contrasts were similar between male and female models with only the VNC treatment showing a significant difference from other treatment types when looking across all mutation types (Table 2.4).

Discussion

Spatial heterogeneity in the environment can alter many aspects of an organisms' phenotype including mating strategy which in turn influences how selection acts on a population including the degree to which allelic effects may be concordant or antagonistic across fitness components. Analyzing the directions and magnitudes of the components of natural selection has been investigated in many contexts, however many empirical studies teasing apart these elements in varying environments fail to recognise how these environmental changes can influence mating strategies. We created populations with known mutation frequencies and allowed them to evolve in environments differing in spatial constrains for resource accessibility to determine how environmental complexity influences the removal of deleterious mutations. We found environmental complexity did influence purging rates, but these rates depended greatly on mutation type. We reanalyzed the purging rates of two of these mutations in the same environments but also including treatments allowing different opportunities for mate choice within a more "simple" environment. Again, we found that purging rates between treatments varied with mutation type, but for both mutations a lack of mate choice (forced monogamy) decreased purging rates.

For each of the six mutations, we expected that with increased variance in resource accessibility there would be an increase in purging rate and therefore the highest purging rate would be seen in the SCT treatment, with the lowest being in the NT treatment. This prediction rested on several assumptions including that natural and sexual selection are aligned, high quality food patches in the SCT treatment would initiate territorial behaviour within males, and males of the highest quality would be able to hold and defend these food patches with the most success, leading to the most mates. While the SCT treatment showed the highest purging rate among treatment types for *plexus*^{1} populations, this pattern does not hold for other mutant types. This discrepancy between our predictions and the data could be due to inaccurate assumptions or other unknown factors. Despite evidence that *Drosophila melanogaster* among other *Drosophila* species can show context dependent territoriality (Hoffmann 1987; Hoffmann and Cacoyianni 1990), considerable uncertainty exists in the extent of what factors influence it and how it ultimately influences the fitness of an individual. It should also be noted that evolutionary stable strategy theories predict that a behavioural strategy will only be adopted by an individual or population if it is advantageous (Maynard Smith 1974). While our environments were designed based on theory that would suggest our assumptions provide the most advantageous strategy (Emlen and Oring 1977; Emlen 2014), this cannot be known without further empirical testing and observation and other strategies may have been implemented that cause the discrepancy between our expectations and results.

The lack of consistency between mutant alleles and the difference between treatments could be due to populations not using the environments as predicted. The NT environment was designed to resemble environments that promote scramble competition in *Drosophila*, with UCT having characteristics that promote territorial behaviours. The SCT environment was designed to provide greater opportunity for one-on-one contests to occur between individuals due to limited entry to the desirable resource. Individuals in the UCT and SCT environments were provided 25mm diameter high quality food patches with potential densities of 12 males per high quality food patch (if the 100 males within each environment were equally distributed across patches). While these conditions have been shown to increase the rate of territorial behaviour and the success of those males that defend territories (Hoffmann and Cacoyianni 1990), these results were found over short-term experiments (up to 6 hours) and these behaviours may not persist in *D. melanogaster* populations over longer time periods like the three days the adults could mate and lay eggs in our experiment. Although not observed, other unexpected uses of the environments such as the possibility of many copulations occurring outside of food patches, and skewed patch use could have caused the disparity between our predictions and results. Also, the addition of the funnel cap in the SCT treatment was expected to aid males in further defending their resource patches. However due to the novelty of these environments, the behaviours these environments were meant to encourage may not have been used or had the opportunity to evolve. If the behaviours did evolve but at a point in the experiment where the allele frequencies for the mutations were low, genetic drift could have masked their effects.

Although our results do not show any consistent pattern of purging rate across treatment types between mutant types, inconsistent results are common to many purging experiments. Many studies that analyze multiple mutations find that each mutation acts differently to experimental treatments not only in magnitude but also direction and thus mainly focus on the overall patterns among mutation types (Sharp and Agrawal 2008; MacLellan et al. 2009; Arbuthnott and Rundle 2012; Clark et al. 2012; Maclellan et al. 2012; Colpitts et al. 2017; Singh et al. 2017). These differences are also reflected in our calculated selection coefficients, where higher selection coefficients lead to faster purging rates but the environmental treatment that has the highest selection coefficient changes depending on mutation type. Differences between how these mutant individuals interact within their environment can likely explain these variances. For example, the mutant *vestigial* has a wing phenotype that influences both its movement and courtship signalling (Pezzoli et al. 1986) putting it at a greater disadvantage compared to wild-type individuals in the same population, which is likely why it has the most drastic purging rate across environmental treatments among all the mutations analyzed in this study. Furthermore, this mobility disadvantage may have been amplified in the SCT treatment because it is expected to be relatively more difficult to access these resources, although this distinction may have been masked by the strong selection against *vestigial* mutants. Further investigation into the behaviours of these mutant types may give an indication as to why these results differ between mutant types.

While we wanted to explore how resource accessibility and environmental complexity influence populations through purging rates, we also wanted to evaluate how these compared to the purging rates of populations that lacked sexual selection and populations that had simple mating environments. As expected, the addition of sexual selection increased the purging rate for both mutations tested. However, there was no difference between the simple and relatively complex environments in purging rate for either mutation. This contradicts previous work of Colpitts et al. (2017) where polygamous populations of mutant *white*^{1} *D. melanogaster* showed increased purging rates in complex environments. While the overall ideas between our experiments are similar, key differences in experimental design could explain these differences. Firstly, due to the alignment of the experimental schedule, virgins from the VNC and VC treatments were able to mate more quickly than the virgins in the NT, UCT, and SCT treatments that were initially held separately before mating. This difference in waiting times to mate could have caused virgins from the NT, UCT, and SCT treatments to be more receptive to potential mates (Pavković-Lučić and Kekić 2009). This could also explain why we see differences in the overall trends between the NT, UCT, and SCT environments compared to our initial experiment. Secondly, our experiment had a much shorter mating period compared to the work conducted by Colpitts et al. (2017) (3 days versus 6) and all eggs laid during this time period were kept to potentially contribute to the next generation for the NT, UCT, and SCT treatments, but not for the VNC and VC treatments. This could potentially lead to lower quality offspring from early matings with lower quality males being kept within the experiment, decreasing the purging rates within the complex mating treatments.

Overall our study adds to the recently growing body of literature considering "environmental complexity" while breaking down "complexity" further to accommodate for changes in mating strategy by environment.

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Figures and Tables

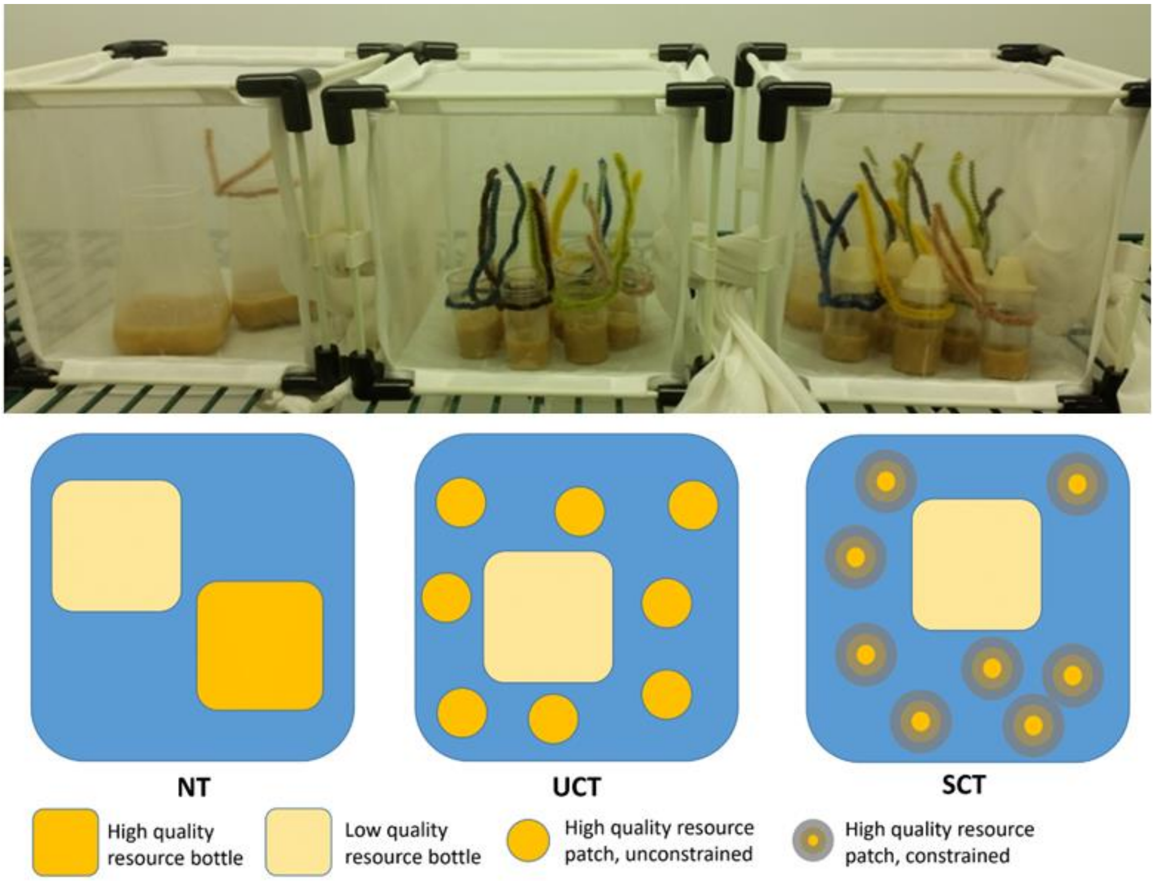

Figure 2.1: Top: Environmental treatment set up for non-territory (NT - left), unconstrained territory (UCT - middle), and spatially constrained territories (SCT - right). Bottom: Overhead schematic of environmental treatment set up

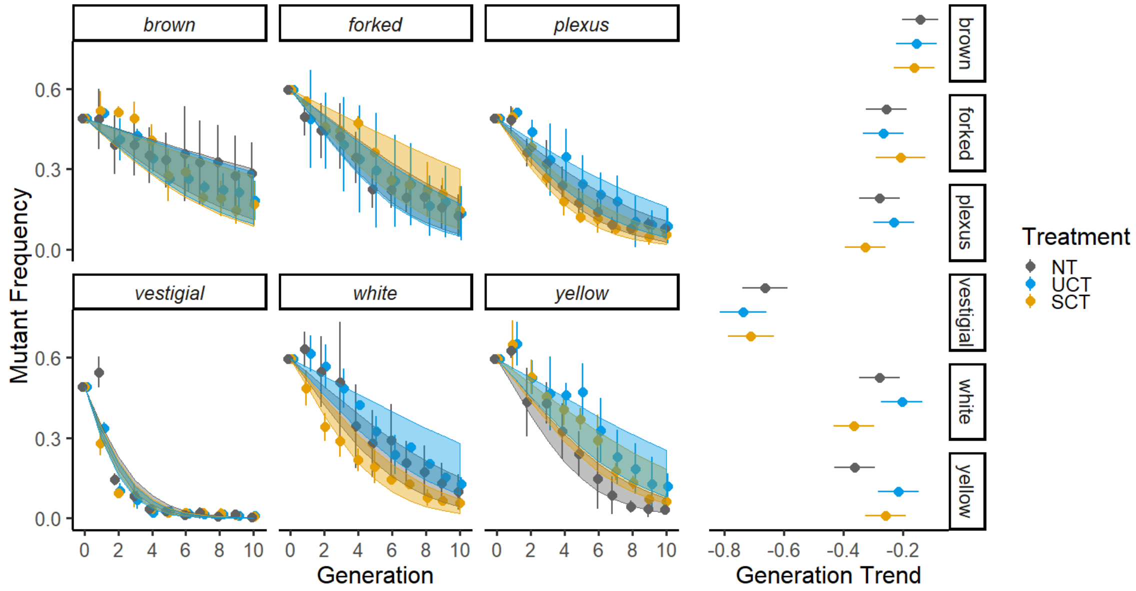

Figure 2.2: Left: Purging rates across the three environmental treatments for each mutant. Data points and error bars represent mean mutant frequency and standard deviation across the three replicates. Confidence bands represent 95% confidence intervals for our generalized linear mixed model. Right: Treatment contrasts for each mutant type based on model estimates. Error bars represent 95% confidence intervals

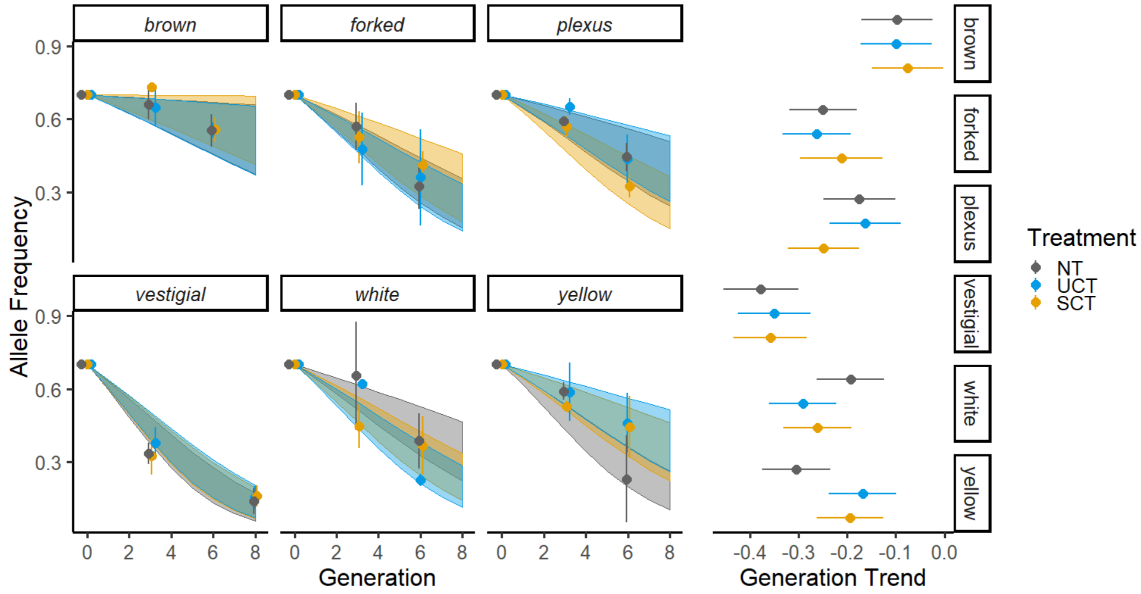

Figure 2.3: Left: Purging rates across the three environmental treatments for each mutant. Data points and error bars represent mean allele frequency and standard deviation across the three replicates. Confidence bands represent 95% confidence intervals for our generalized linear mixed model. Right: Treatment contrasts for each mutant type based on model estimates. Error bars represent 95% confidence intervals

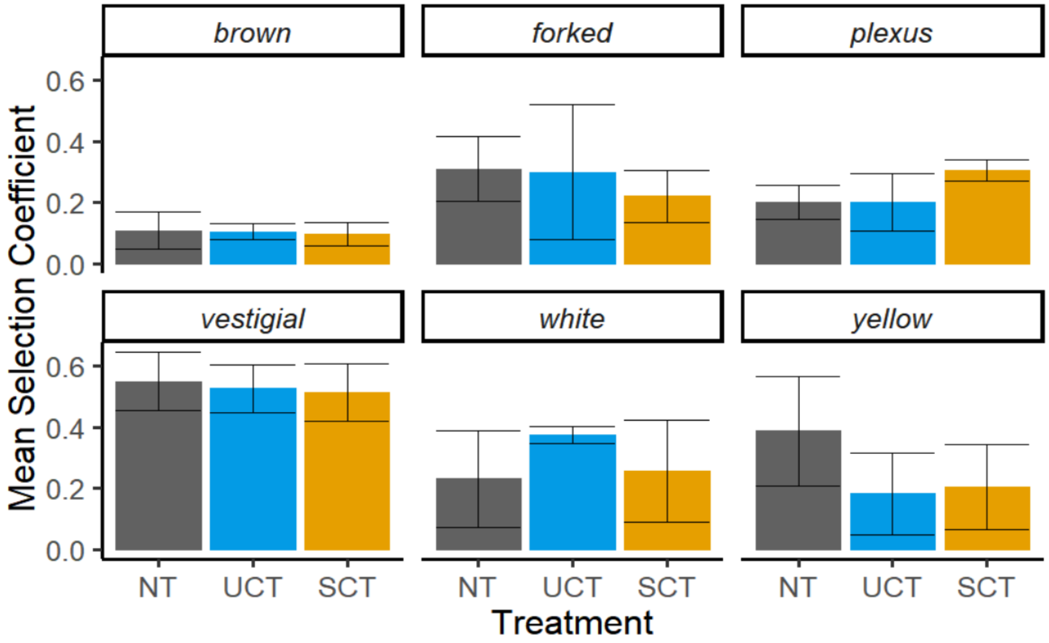

Figure 2.4: Mean selection coefficients for each mutant type across the three environmental treatments. Estimates were created from allele frequency data, error bars represent 95% confidence intervals

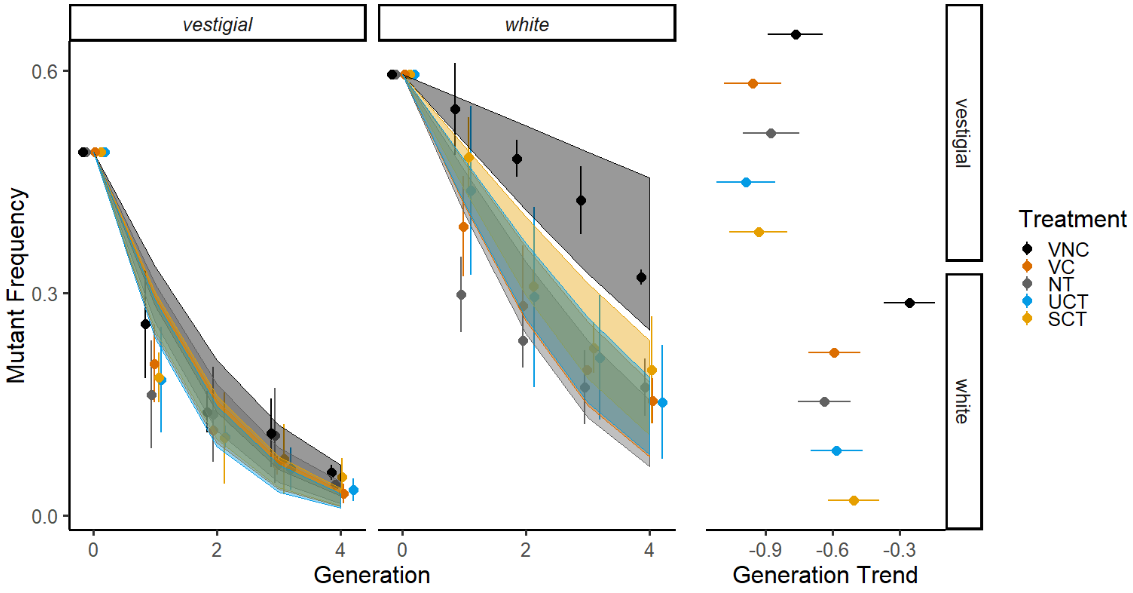

Figure 2.5: Left: Purging rates across the five environmental treatments for each mutant. Data points and error bars represent mean mutant frequency and standard deviation across the three replicates. Confidence bands represent 95% confidence intervals for our generalized linear mixed models. Right: Treatment contrasts for each mutant type based on model estimates. Error bars represent 95% confidence intervals

Table 2.1: ANOVA outputs for fixed effects of four general linear mixed models produced from the six mutant types across the three treatment types

		χ^2	df	P
Both sexes				
	Generation x treatment	66.13	3	**2.88e^{-14}**
	Generation x mutant type	380.81	5	**2.2 e^{-16}**
	Generation x treatment x mutant type	22.05	10	**0.015**
Males only				
	Generation x treatment	79.57	3	**2.2e^{-16}**
	Generation x mutant type	476.51	5	**2.2e^{-16}**
	Generation x treatment x mutant type	24.69	10	**0.0059**
Females only				
	Generation x treatment	36.42	3	**6.09e^{-8}**
	Generation x mutant type	194.25	5	**2.2e^{-16}**
	Generation x treatment x mutant type	24.69	10	**0.0059**
Allele Frequency				
	Generation x treatment	17.98	3	**0.0004**
	Generation x mutant type	80.53	5	**6.49e^{-16}**
	Generation x treatment x mutant type	16.59	10	0.084

Table 2.2: Estimates and significance of treatment contrasts among the six mutation types for four general linear mixed models

	Contrast	Estimate	P
Both Sexes			
	NT – SCT	0.0079	0.9244
	NT – UCT	-0.0292	0.3250
	SCT – UCT	-0.0371	0.1820
Males only			
	NT – SCT	0.0139	0.7444
	NT – UCT	-0.0240	0.4041
	SCT – UCT	-0.0379	0.1209
Females only			
	NT – SCT	-0.0043	0.9882
	NT – UCT	-0.0495	0.1975
	SCT – UCT	-0.0452	0.2772
Allele Frequency			
	NT – SCT	-0.0077	0.9316
	NT – UCT	-0.0104	0.8753
	SCT – UCT	-0.0027	0.9915

Table 2.3: ANOVA outputs for fixed effects of three general linear mixed models produced from the two mutant types across the five treatment types

		χ^2	df	P
Both sexes				
	Generation x treatment	973.56	5	**$2e^{-16}$**
	Generation x mutant type	97.78	1	**$2e^{-16}$**
	Generation x treatment x mutant type	5.21	4	0.27
Males only				
	Generation x treatment	1272.95	5	**$2e^{-16}$**
	Generation x mutant type	460.20	1	**$2e^{-16}$**
	Generation x treatment x mutant type	8.85	4	0.065
Females only				
	Generation x treatment	431.31	5	**$2e^{-16}$**
	Generation x mutant type	1.03	1	0.311
	Generation x treatment x mutant type	12.39	4	**0.015**

Table 2.4: Estimates and significance of treatment contrasts among white1 and vestigial1 mutations for three general linear mixed models

	Contrast	Estimate	P
Both Sexes			
	VNC – NT	0.2451	**0.0007**
	VNC – VC	0.2633	**0.0002**
	VNC – SCT	0.2074	**0.0072**
	VNC – UCT	0.2733	**0.0001**
	NT – VC	0.0183	0.9984
	NT – SCT	-0.0377	0.9750
	NT – UCT	0.0282	0.9917
	VC – SCT	-0.0560	0.9014
	VC – UCT	0.0099	0.9999
	SCT – UCT	0.0659	0.8355
Males Only			
	VNC – NT	0.2142	**0.0010**
	VNC – VC	0.3389	**<0.0001**
	VNC – SCT	0.1712	**0.0197**
	VNC – UCT	0.2844	**<0.0001**
	NT – VC	0.1246	0.2709
	NT – SCT	-0.0430	0.9475
	NT – UCT	0.0701	0.7747
	VC – SCT	-0.1676	0.0639
	VC – UCT	-0.0545	0.9214
	SCT – UCT	0.1131	0.3507
Females Only			
	VNC – NT	0.3555	**0.0001**

	VNC – VC	0.3392	**0.0003**
	VNC – SCT	0.3007	**0.0021**
	VNC – UCT	0.3501	**0.0002**
	NT – VC	-0.0163	0.9997
	NT – SCT	-0.0548	0.9664
	NT – UCT	-0.0055	1.0000
	VC – SCT	-0.0385	0.9908
	VC – UCT	0.0108	0.9999
	SCT – UCT	0.0493	0.9769

Supplementary

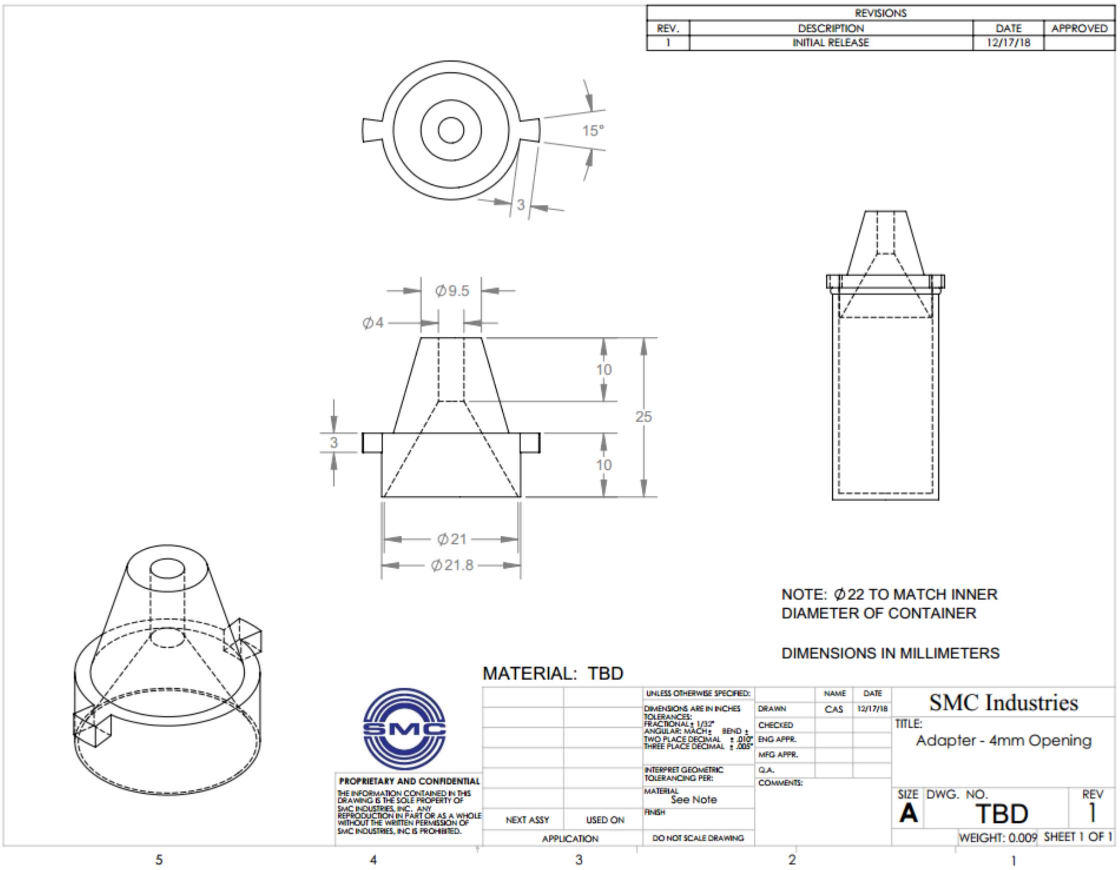

Figure S2.1: Schematic for 3D-printed funnel cap design. Caps were created using filament material

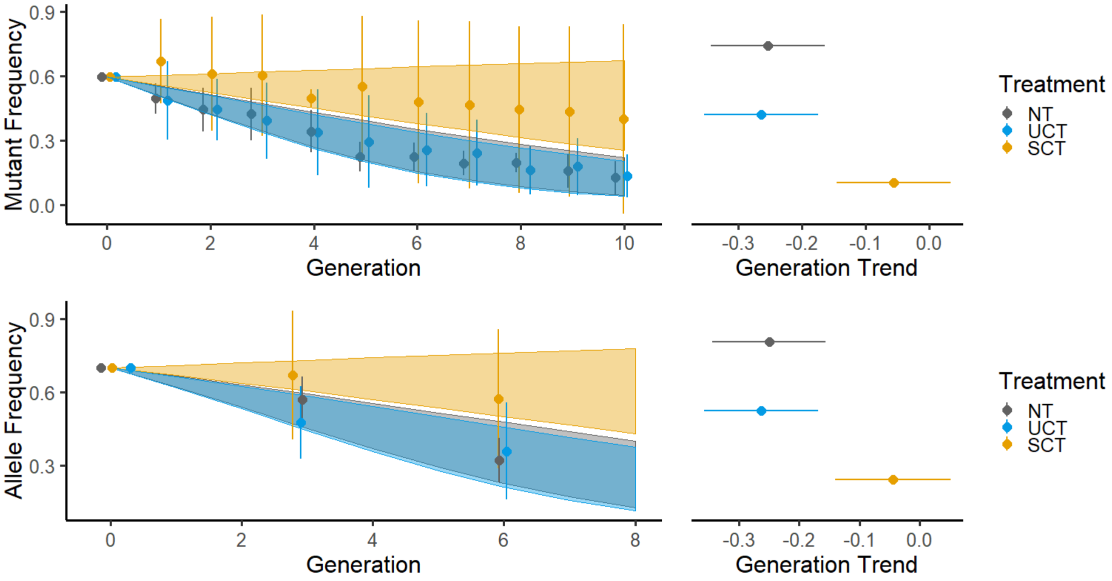

Figure S2.2: Right: Purging rates for forked^1 mutant while including all replicates. Data points and error bars represent average mutant frequency or allele frequency and the standard deviation across all replicates. Confidence bands represent 95% confidence intervals for our generalized linear mixed model. Left: Treatment contrasts for forked^1 mutant while including all replicates based on model estimates. Error bars represent 95% confidence intervals

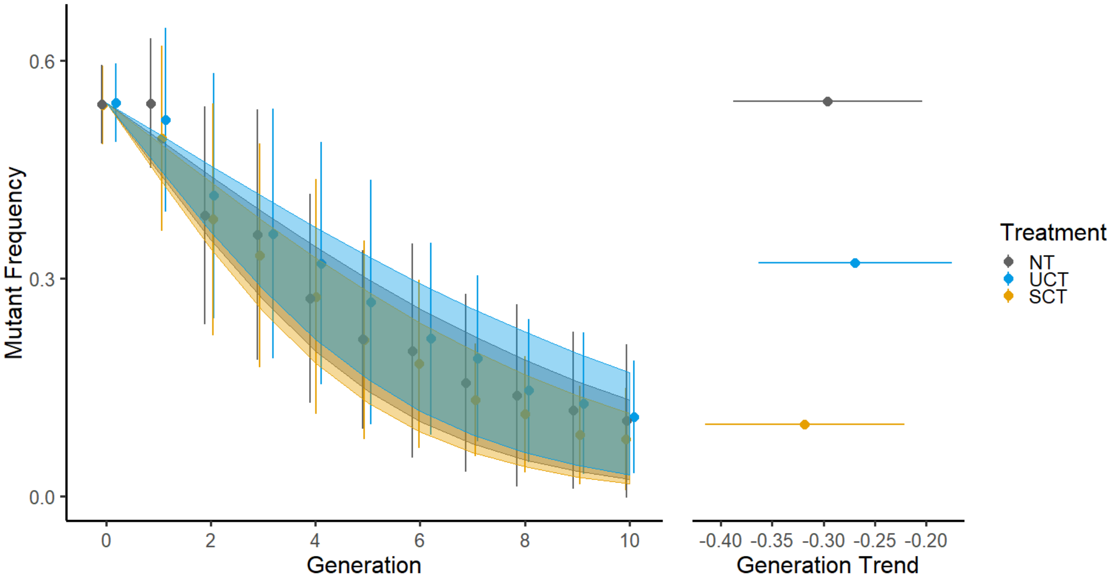

Figure S2.3: Left: Purging rates across the three environmental treatments averaging across mutant types. Data points and error bars represent mean mutant frequency and standard deviation across the three replicates of each mutant type. Confidence bands represent 95% confidence intervals for our generalized linear mixed model treating mutant type as a random effect. Right: Treatment contrasts across mutant types based on model estimates. Error bars represent 95% confidence intervals

Table S2.1: Recipe for high quality food and nutritional contents

Ingredient	Mass (g)	Volume (ml)	Carbohydrates (g)	Protein (g)
Water	4250	4250		
Black strap molasses	40	28	24.40	1.60
Fancy table molasses	40	28	30	0.40
Cornmeal	70	106.06	56.70	4.90
Carrageenan	27	19.71		
Yeast	255	447.37	84.15	127.50
Sucrose	75	46.88	75	0
Propionic acid	11.85	12		
Methylparaben	2.50	1.81		
Ethanol	19.61	25		
TOTAL	4790.97	4964.85	270.25	134.4

Table 2.2: ANOVA outputs for fixed effects of four general linear mixed models produced from the six mutant types across the three treatment types including all replicates

		χ^2	df	P
Both Sexes				
	Generation x treatment	37.99	3	**2.84e^{-8}**
	Generation x mutant type	253.39	5	**2e^{-16}**
	Generation x treatment x mutant type	26.72	10	**0.003**
Males Only				
	Generation x treatment	36.21	3	**6.77e^{-8}**
	Generation x mutant type	295.41	5	**2e^{-16}**
	Generation x treatment x mutant type	26.24	10	**0.003**
Females Only				
	Generation x treatment	25.29	3	**1.34e^{-5}**
	Generation x mutant type	142.61	5	**2e^{-16}**
	Generation x treatment x mutant type	30.93	10	**0.0006**
Allele Frequency				
	Generation x treatment	11.81	3	**0.008**
	Generation x mutant type	47.52	5	**4.46e^{-9}**
	Generation x treatment x mutant type	19.84	10	**0.032**

Table S2.3: Estimates and significance of treatment contrasts among the six mutation types for four general linear mixed models including all replicates

	Contrast	Estimate	P
Both Sexes			
	NT – SCT	-0.0181	0.7741
	NT – UCT	-0.0299	0.4997
	SCT – UCT	-0.0118	0.8998
Males Only			
	NT – SCT	-0.0150	0.8422
	NT – UCT	-0.0258	0.6020
	SCT – UCT	-0.0108	0.9162
Females Only			
	NT – SCT	-0.0328	0.6024
	NT – UCT	-0.0504	0.3034
	SCT – UCT	-0.0176	0.8664
Allele Frequency			
	NT – SCT	-0.0358	0.4162
	NT – UCT	-0.0104	0.9278
	SCT – UCT	0.0254	0.6431

Chapter 3: Initial conditions to evolve exaggerated weaponry: Testing current theories of spatial structure and intrasexual contests

Introduction

Animal ornamentation and weaponry are some of the most conspicuous and elaborate trait expressions seen in the animal kingdom. While much work has been done to understand and uncover the evolutionary forces that generate these unique traits, the mechanisms involved in the initiation of their evolution in populations and different species are yet to be fully accounted for. These mechanisms are likely to vary between ornaments and weaponry due to the different (although sometimes overlapping (Berglund et al. 1996; Mccullough et al. 2016)) functions of these traits. Specifically, ornaments are often used as signals to potential mates, while weaponry is used for intra-sexual competitions between rivals. Focusing on the evolution of exaggerated animal weapons, theory proposed by Emlen (2014) suggests that exaggerated weaponry evolves when the benefits gained by individuals with weaponry (obtaining desirable mates and resources) are greater than the costs of bearing these traits (refer to chapter 1 for more details). Emlen (2014) further indicates that this imbalance between costs and benefits is most likely to be achieved when three conditions are met. The first condition is that there is competition for access to reproductive opportunities, which is the baseline condition for all sexually selected traits to evolve (Emlen 2014). The second is that key resources involved in reproduction are limited and localized such that few individuals can monopolize these resources, creating a source of variation in reproductive success. The final condition is that competition for reproductive resources tends to occur as one-on-one duels, as these types of competitions often result with the individual with the larger weapon succeeding (Sneddon et al. 1997; Fricke et al. 2015; Fea and Holwell 2018) (refer to chapter 1 for how these contexts influence weapon evolution). While "exaggerated" is never clearly defined, here it is assumed Emlen (2014) is referring to structures used in intrasexual competitions that are proportionally larger than other morphological features.

There are many examples in which species that have exaggerated weapons also fit within these ecological conditions (Zeh et al. 1992; Longair 2004; Bro-Jørgensen 2007; Emlen 2008a; Boisseau et al. 2020; del Sol et al. 2020). Perhaps the most striking example is within and among dung beetle species. Emlen and Philips (2006) examined 46 dung beetle species comparing their behaviour and the presence of male horn morphologies. Most of these species studied displayed one of two behaviours, either tunneling or rolling. Species that demonstrated tunneling behaviour had the majority of their reproductive contests occurring in spatially confined burrows, whereas species that demonstrate rolling behaviour had the majority of their reproductive contests occurring on balls of dung in large, open spaces. Using phylogenetic comparative methods (to correct for the effects of phylogeny), Emlen and Philips (2006) found that for the majority of species, those that lacked horns displayed rolling behaviour and beetles that displayed tunneling behaviour tended to have horns. This evolutionary correlation implies that competition occurring in small, confined spaces where duels are likely to occur more often, is significant in the evolution of horns in beetles (Emlen 2000). While this association corroborates the overall theory, it is not empirical evidence that these conditions can lead to the evolution of exaggerated traits. That is, are these conditions sufficient to enable the nascent evolution of exaggerated traits? To our knowledge there has been no studies which implement these conditions in an experimental evolutionary framework, which is a crucial piece in determining the necessity and the sufficiency of these conditions in their contribution to the evolution of exaggerated weaponry.

Two phenotypes stand out as potential candidates to use to detect the initial stages of exaggerated weaponry evolution: heightened condition dependence and hyper-allometry. While all traits show some level of condition dependence, it has been demonstrated that sexually selected traits often display heightened condition dependence (Cotton et al. 2004; Bonduriansky and Rowe 2005). This stems from theory that sexually selected traits (including weaponry) are under directional selection as larger or more exaggerated features often benefit the bearer in obtaining increased mating opportunities. Additionally, this exaggeration is bound by condition as individuals have a limited amount of resources to allocate to their overall development and therefore, only individuals of higher biological quality would be able to bear the costs of such extravagant and resource-intensive traits, reinforcing the honesty of the trait (Zahavi 1977; Nur and Hasson 1984; Price et al. 1993; Rowe and Houle 1996). Ultimately, this is a key element in the evolution of sexual dimorphism in species as these underlying processes may lead to different phenotypic optimums between the sexes (Andersson 1994). Indeed, some exaggerated male traits have been examined and show increased condition dependence, even when compared to nonsexual traits and the corresponding trait in females (Miller et al. 2016; Zinna et al. 2018). When examining condition dependence and sexual dimorphism in the neriid fly, Bonduriansky (2007) found male but not female traits to be highly condition dependent, and secondary sexual traits show condition-dependent sexual dimorphism. Similar findings were seen by Rohner and Blanckenhorn (2018) in several species of the family *Sepsidae* where species with higher degrees of sexual dimorphism also displayed greater sex differences in condition dependence for sexual but not non-sexual traits.

Along with heightened condition dependence, sexually selected traits are also expected to show greater scaling relationships relative to overall size. This relationship is typically represented with the allometric equation, $y = bx^a$, where x is typically body size, y is the size of the trait being analyzed, and b and a are constants (Huxley and Teissier 1936). This equation is usually log transformed to produce a linear relationship in which a becomes the slope of the line and represents the allometric relationship of that trait. Where $a = 1$, the trait scales 1:1 with body size and is deemed isometric, where $a < 1$, the trait is hypo-allometric and scales negatively with body size, and where $a > 1$, the trait positively scales with body size and is hyper-allometric. It is predicted that species will exhibit hyper-allometries when increasing trait size provides a relatively greater advantage to larger individuals (Green 1992). Most traits appear to scale negatively with body size, however many (but not all) traits that show positive allometries are sexually selected traits (Miller et al. 2016; Voje 2016; Rohner and Blanckenhorn 2018; Zinna et al. 2018). Specifically, Kodric-Brown et al. (2006) examined data for 284 species and found that ornaments and weaponry displayed extremely high positive allometries, although it has been argued that these results may be due to sampling bias for traits that are obviously extravagant and ignoring those that are more subtle (see Bonduriansky (2007a)). Nevertheless, Rodríguez and Eberhard (2019) suggest and find evidence for positive allometries of sexually selected traits relying heavily on the function of the trait, with traits that are used to signal body size in intrasexual competitions being most likely to display hyper-allometries, and aggressive traits displaying greater allometries than traits related to courtship. Due to their consistency across sexually selected morphologies, traits displaying heightened condition dependence and hyper-allometries may be indications of these traits functioning as weapons in intrasexual competitions.

In this study, I used *Drosophila melanogaster* as a model organism to determine the effects of the conditions outlined by Emlen (2014) on phenotype and determine if they are sufficient and necessary in the early stages of exaggerated weaponry evolution. To do this, I experimentally evolved populations of *D. melanogaster* in three different environments that were designed to encapsulate the three proposed conditions to different extents. The goal of these environmental designs was to sufficiently alter the accessibility and defensibility of desirable resources, such that the optimal mode of competition for mates fit the conditions proposed by Emlen (2014). The overall changes in accessibility and defensibility of resources in these environments was based on previous research conducted by Hoffmann and Cacoyianni (1990) who demonstrated that *D. melanogaster* can adopt resource defensive polygyny as opposed to their typical scramble competition with subtle interference behaviour (Spieth 1974; Baxter et al. 2018) under certain environmental conditions (see Chapter 1 and 2 for more details).

After the populations evolved within these environments for 35 generations, I then conducted an experiment to see if these environments produced differences in the condition dependence and allometric slopes of traits of these populations. With this, I also examined which traits showed greater allometries and how these trends differed between the sexes. Specifically, I examined thorax size, foreleg segments lengths, head width, and wing length and width. Thorax length was chosen to be used as a proxy for overall body size (see Shingleton et al. (2009)), while the other traits were chosen as likely candidates to demonstrate condition-dependence and heightened allometric slopes. Both males and females of *D. melanogaster* display fairly structured patterns of intrasexual aggression ranging from visual display to highly escalated fights (Chen et al. 2002; Nilsen et al. 2004). Males use their wings as a visual signal of aggression to potentially deter competitors from attempting to compete with them, and also during courtship to produce songs that attract mates. When escalation does occur between individuals, males tend to use their forelegs to push or knock over rivals while females tend to butt heads. As such, all of these traits are expected to be under some level of sexual selection which may be shifted under different environments that facilitate alternate competitive strategies.

Within the time limit of this experiment, it is unreasonable to expect the evolution of large, exaggerated weaponry to evolve via natural selection alone (i.e. without artificial selection on specific traits). Thus, I tested for changes in relative size of traits used in intrasexual interactions of the sexes as an indication of the early stages of weaponry evolution. With increasing levels in which the conditions proposed by Emlen (2014) are provided in the environmental treatments, I predicted that the optimal strategy for maximizing mates would shift from scramble competition to duels as this would correlate with decreased access to desirable resources and increased defensibility. While behavioural changes were not explicitly tested, under these conditions, the initial stages of exaggerated weaponry would be most likely to evolve according to Emlen (2014). This initial evolution could be detected through the subtle changes of increased condition dependence and heightened allometric slope of traits used in intrasexual competitions. The trait most likely to show these features would be male foreleg as males more often interact in intrasexual competition compared to females and this trait is often used in escalated competitions. However, females do sometimes show exaggerated weaponry with heightened allometries (although these traits are not tied to increasing mating opportunities but to obtaining desirable resources (Dalosto et al. 2019)), as such, I predicted that if the initial stages of weaponry evolution occurred in females, this would manifest in female head morphology (ie. exaggerated head widths) as when competition does occur between females for access to resources, this trait is commonly used. While I predicted that male forelegs and female heads would be the most likely traits to evolve to exaggerated sizes based on their usage in intrasexual competitions, I only expected trait exaggeration to occur in one of the sexes as weaponry is often sexually dimorphic in its' presence (although not always) (Emlen 2008b; Rico-Guevara and Hurme 2019).

Methods

Environmental Treatments

Three environmental treatments were created in order to encapsulate the ideas of the three conditions for weapon evolution proposed by Emlen (2014). The first environment, the "non-territory" (NT) treatment, was designed to have large, open, easily accessible food patches that are potentially difficult to defend as territories. The second environment, the "unconstrained territory" (UCT) treatment, was designed to have smaller, but still relatively accessible patches. These patches would ideally be easier for individuals to defend compared to the NT treatment, but difficult to hold due to the ease of access. The third environment, the "spatially-constrained territory" (SCT) treatment, would have the same small patches as the UCT treatment, making them easier to defend compared to the NT treatment, but would have a modified funnel cap that would restrict the opening (accessibility) to the resource. The addition of this funnel cap would allow for the opportunity of defending and holding the resource to occur more easily as well as to provide additional physical spaces that could encourage antagonistic encounters for the resource to occur as one-on-one battles (see Chapter 1 and 2 more descriptions and figures of environmental designs).

Each environmental treatment was set up in mesh BugDorm-4F3030 cages with the specific set up as follows; the NT treatment had four *Drosophila* culture bottles (177ml, surface area of 30.25cm^2) containing ~50ml of the high quality food resource with four drops of yeast-paste and orange juice mixture placed on top (to attract females (Dweck et al. 2013)). Four 177ml *Drosophila* culture bottles filled with 50ml of a 25% dilution of the high quality food resource were also included. The purpose of these diluted food resources was to allow individuals that could not access the high quality resources refuge. In this way, individuals were not necessarily competing for resources for basic survival but were competing for the most desirable resources culture bottles. This feature was included in each environmental treatment. The UCT treatment had 25 open vials (height of 32mm, 25mm diameter, 4.9 cm^2 surface area) that are close to the optimal 20mm size to promote resource defence polygyny determined by Hoffmann and Cacoyianni (1990) each with a single drop of the yeast-paste and orange juice mixture placed on the food surface. The SCT treatment had the same set-up as the UCT treatment except each vial had a 3D-printed funnel cap (22mm diameter, 25mm height, 4mm opening) to restrict the opening of the vial to a smaller entrance (Note: the vials for the UCT and SCT treatments were implemented at generation 10 of experimental evolution to reduce the amount of space between the top of the funnel caps and the surface of the resource in the SCT treatment. This was done to increase defensibility of the resources as initial monitoring of these vials showed high adult densities. Original vials used in this experiment differ in height and are identical to those used in chapter 2, see supplementary Figure 3.1 for comparison). Pipe cleaners were also wrapped around the tops of the bottles and vials containing the high quality food resource in order to add an additional layer of complexity and provide a perching area (as similar to set up used by Yun et al. (2017), see chapter 2 for details).

Experimental Evolution Population Maintenance

Populations were created by collecting virgin females and males from a large outbred domesticated lab population originally collected from Fenn Valley Winery (FVW), Michigan in 2010 (GPS co-ordinates: 42.578919, -86.144936). It is assumed that this population has already adapted to the lab environment (~160 generations in the lab prior to the initiation to this experiment), thus any confounding effects of concurrent selection for lab adaptation would be minimized (Harshman and Hoffmann 2000). From this population, 300 males and 300 females were placed into a cage set up with one of the three environmental treatments. This was done with 4 replicate cages for each environment, resulting in 12 populations total. These populations were maintained at 12L:12D cycles at 21°C with 60% relative humidity in a Conviron walk-in chamber (CMP6050).

The populations were kept on a 13-15 day schedule depending on emergence times, such that each population had about an equal amount of adults contributing to the next generation (census size was not measured directly). After the initial populations were placed into their respective treatments, adults were allowed to mate and lay eggs for three days. After this period, the media with eggs and larvae was removed from these cages and placed into new cages to allow for development and eclosion. The adults were kept in their original cages for another 3 days to lay eggs on a molasses-rich food medium to serve as a back-up generation before being discarded. Development and eclosion occurred over a 10-12 day period. Once the new generation of adults emerged, new food was placed in these cages with the set-up described above, and the cycle was repeated. For each generation, the new food was placed into the cages in a random distribution and the cages were placed onto racks in a random order such that each population was not kept in the same position every generation in the walk-in chamber.

Initially for each treatment, replicates 1 and 2 were 10 days ahead of replicates 3 and 4 on this maintenance schedule. Due to a lab shut-down brought about by the COVID-19 pandemic, the schedules for the replicates were shifted such that replicates 3 and 4 were exactly one generation behind replicates 1 and 2 so the feeding schedule could be completed on a minimal amount of days.

Condition Manipulation

At generation 35, two 177ml *Drosophila* food culture bottles containing high quality food were placed in each environmental treatment after the initial 3 day egg laying period for population maintenance. These bottles were removed after 7 hours in order to keep egg density low, and were kept at 21°C. Upon emergence of adults, 20-25 pairs were placed in containers with a 2% apple-juice agar plate with a drop of yeast paste on the surface for egg laying. Eggs were collected and placed into vials containing the high-quality food resource at a density of 50 eggs per vial. For each of the 12 populations, 16 vials of eggs were collected and were split into three condition cohorts to undergo a starvation protocol (Stillwell et al. 2011). The purpose of this starvation protocol was to generate size differences (and by extension, differences in condition) by limiting the nutritional content available to the larvae during growth phases of development. Specifically, the first condition cohort has normal food availability through-out larval development, while condition cohorts 2 and 3 each have successively reduced food availability for larger proportions of larval development. Condition cohorts 1, 2, and 3 consisted of 4, 5, and 7 replicate vials and the eggs collected in these vials developed at 21°C for 6, 5, and 4 days, respectively. After these time periods the larvae were removed by adding 5ml of a 40% sucrose solution to each vial and shaking for 20 minutes. Once the larvae were loose from the food, they were collected using a fine paintbrush and placed into a new vial containing a moist cotton ball. The larvae continued development at 21°C and upon eclosion and sclerotization, 50 individuals of each sex amongst all vials from each condition cohort and population, were collected and stored in 70% ethanol for morphometric measurements.

Of the flies collected, 20 individuals of each sex of each cohort and treatment combination were dissected for imaging and subsequent measurement. Flies were dissected and images of the head, thorax, wing, and foreleg were taken with a Leica M125 stereoscope with a Leica DFC400 digital camera at magnifications of 50X or 63X, depending on the trait. Measurements of head width, thorax, wing length, wing width, femur, and tibia were conducted using ImageJ (1.53e) software (Rasband 1997).

Statistical Analyses

The change in size of each trait between the different treatments and cohorts was analyzed by fitting a series of linear mixed models. Before creating the model, thorax size was centered about the mean to allow intercept estimates to be easily interpreted. Models were generated for each trait with fixed effect predictors including treatment and sex;

continuous predictors of condition cohort and centered thorax size and allowing for two-way interactions amongst all predictors. Random effects for replicate lineage nested within treatments including random slopes for cohort and sex were included.

To analyze the allometries of each trait, similar linear mixed effect models were created. These models predicted the logarithmic size of each trait with fixed effect predictors of treatment and sex and the continuous predictor of logarithmic thorax size and three-way interaction term amongst all predictors. These models included random effects for replicate lineage nested within treatments with random slopes for sex. To examine if overall thorax size was influencing the evolution of the allometric slopes of traits, another model was created to estimate log thorax size with the varying predictors. This model included treatment and sex as fixed effect predictors with starvation cohort as a continuous predictor and allowing for three-way interaction amongst all predictors. The random effects for this model were the same as the models created for examining the allometries of each trait, but also included random slopes for starvation cohort.

Fixed effects for all models were further examined for significance using a two-way ANOVA (type II Wald χ^2 test). To account for multiple comparisons with each model, p-values were adjusted using the Bonferroni correction. All statistical analyses were performed in R v.3.5.2 (R Core Team 2018) using lmer() (lme4 package v1.1-21 (Bates et al. 2015)), Anova() (car package v3.0-2 (Fox and Weisberg 2011)), predictorEffects() (effects package v4.1-0 (Fox and Weisberg 2019). All plots were generated with ggplot2 v3.1.1 (Wickham 2016).

During the condition manipulation procedure, two of the populations were one day behind in the protocol at the point of dividing eggs into condition cohorts due to the lack of eggs produced (starvation period for each condition cohort was the same, just conducted one day later). Upon emergence of the condition cohorts for these populations, it was noted that condition cohort 3 for both populations had a greater reduction in survival compared to the populations one day ahead. As such, the data was analyzed with and without these two populations for all models. The results presented below are in accordance with the full dataset, however differences in the significance of model effects were noted when analyzing the change in size of each trait between the different treatments and cohorts. These differences in effects between the datasets are presented in Table S3.1.

Results

Initial analyses of trait sizes did not indicate any differences in overall trait size between the environmental treatments demonstrating that there was no consistently detectable selection for changes in trait size between treatment types after 35 generations of experimental evolution (Figure 3.1). Analysis of the raw trait values also demonstrates that female traits are typically larger than male traits (although this dimorphism is substantially reduced in leg measurements), as expected since *D. melanogaster* displays overall female-biased sexual size dimorphism. As expected, the manipulation of condition through starvation created a reduction in size for each trait across treatments and sexes as the amount of time with insufficient nutrients during development increased.

When analyzing the models for overall trait size, all five demonstrate significant effects for cohort, further signifying the success of condition manipulation (Table 1, Figure 3.2). All models also showed significant effects of sex and thorax size as expected since these effects correlate with female-biased sexual size dimorphism and overall size, respectively. Thus, the models conform to expectations that females are larger and overall trait size scales with body size. The interaction between cohort and thorax size was also significant for all trait models, indicating that each trait is condition dependent. Furthermore, all trait models except the tibia showed significant effects of the interaction between sex and cohort indicating that this condition dependence is sexually dimorphic, with females having a greater reduction in trait size compared to males (Figure 3.2).

When examining the effects of evolutionary treatment, none of the models show significant effects for treatment on its own nor with its interaction with cohort indicating condition manipulation produced relatively the same size reduction of the specified trait between environment types. However, the models for tibia, head, and wing width show significance for the interaction between treatment and thorax size demonstrating that the reduction in size for these traits is dependent on the overall size of the individual and which treatment they are placed in. For the head model, this trend is shown to be further influenced by the interaction of treatment and sex, demonstrating sexually dimorphic reductions in head size according to treatment (Table 3.1, Figure 3.2).

When examining the models used to estimate allometric relationships, all models showed significant effects of thorax size (i.e. size allometry) and sex as expected due to these representing the relationship to overall size and female-biased sexual size dimorphism as stated previously (Table 3.2). If the environmental treatments generate differences in allometric slopes between traits, with evolution towards more positive-allometric slopes for traits that are potentially used in one-on-one combat, this would be shown through significant effects of treatment and its interaction with overall size, and the interaction between treatment and sex if these traits are only or preferentially used by one sex. This would also appear as significant for the interaction between all three of these predictors demonstrating that all else being equal, the allometric slope for a given trait of individuals of the same size will differ with both treatment and sex. However, all models showed no significant effects for treatment, the interactions between treatment and sex, nor the three-way interaction between treatment, sex, and thorax size. Only the model for tibia showed significant effects of the interaction between treatment and thorax size. Additionally, all traits continue to demonstrate hypo-allometric relationships (although some approaching isometry) and none of the traits showed significant differences in allometric slopes between treatments nor the sexes (Table 3.3, Figure 3.3). However, these relationships could be influenced by a treatment effect on the evolution of overall size. This is evident when examining the model created to estimate proportional thorax size as there is a significant effect of the interaction between treatment, sex, and cohort (Table 3.4, Figure 3.4).

Discussion

Emlen (2014) proposed three conditions that lead to the evolution of exaggerated animal weaponry. Many species that exhibit these impressive features have also been linked to evolving within ecological niches that conform to the three environmental conditions. Additionally, exaggerated animal weaponry tend to show increased condition dependence compared to non-sexually selected traits and also show hyper-allometries. I created three different environments that encapsulated the three different conditions proposed by Emlen (2014) to differing extents and tested for differences in condition dependence and allometries of traits as possible indications for evidence of the early evolution of animal weaponry. Based on previous studies of *D. melanogaster* mating behaviour and theory of trait evolution, I predicted that I would see subtle changes in condition dependence and allometric slope between the sexes for traits used in intrasexual competition with these changes increasing as more of the conditions were met within the given environmental treatment.

Analysis of overall trait size after 35 generations of experimental evolution across condition, sex, and condition cohort exhibited the expected biological outcomes seen within *D. melanogaster*. All traits were female-biased in size and reduced in size as time without nutritional resources during development increased (i.e. reduced condition). At this point, not seeing any differences amongst trait sizes within treatments was not unexpected. Within the time constraints of this experiment (35 generations), noticeable evolution in trait size may have not yet occurred, hence the further examination of condition-dependence and changes to allometries as these could be precursors leading to the eventual change in overall trait size.

Two of the traits examined in this study demonstrated significant effects for multiple interactions of treatment with other predictors; the tibia and head width. Head width only showed significant effects when analyzing overall trait size models for condition dependence but did show effects for both the interaction between treatment and thorax size and treatment and sex. This suggests that individuals of the same size will display different head widths depending on treatment and that this difference is sexually dimorphic. These trends appear to stem from the NT treatment creating smaller head widths between treatments and females experiencing greater reductions in head size. This follows with my prediction and may be indicative that females are using their heads in intra-sexual competitions less frequently in the least-defensible environment and thus head size in this environment is not being selected upon as strongly as in the other two environments. Although, not one of the most likely predictions, it is not surprising that head size is one of the traits showing increased condition dependence as several *Drosophilidae* species display exaggerated head widths (although more commonly in males (Spieth 1981; Grimaldi and Fenster 1989)). It is important to note that the model for head width in terms of allometry showed none of these predictors as significant, however these trait allometries may be masked by the differences in overall thorax size between the treatments as shown by the thorax model (Table 3.4).

Models for the tibia in terms of both condition dependence and allometry demonstrated significant effects of treatment and size, suggesting that for both phenotypes individuals of the same size would differ in tibia length between treatments. Intriguingly, tibia was also the only trait that did not show significance in sexually dimorphic condition dependence. The femur, although anatomically connected to the tibia, and thus expected to demonstrate some correlation, does not show any significance for these effects. While the tibia shows difference in trait size across treatments, this is in the opposite trend expected as the allometry of the NT treatment appears to be greatest with SCT and UCT at shallower trajectories (although all still hypo-allometric).

Evolving changes in allometric slope has shown to be notoriously difficult. Although some have demonstrated changes in allometry within a relatively few number of generations via artificial selection (Frankino et al. 2005; Houle et al. 2019), this may be due to the relative ease of evolving the relative trait size (the intercept) and not the slope of the allometric relationship (Egset et al. 2012; Voje et al. 2013; Stillwell et al. 2016) Several theories have been postulated to determine why this trend occurs. When examining allometric constraints in butterfly wings, Frankino et al. (2005) suggest that there are no internal developmental constraints preventing allometry evolution, but rather strong stabilizing selection. This has been argued and suggested that the stasis of allometric relationships within species is also due to pleotropic genetic constraints (Houle et al. 2019). Alternatively, it has been stressed that static allometry must be examined as two components, genetic and environmental (Stillwell et al. 2016) and these allometries may show different slopes for the same trait. In addition, in terms of environmental static allometry, the evolution of the slope may be extremely difficult without also influencing the intercept as these could be closed related to each other if trait and size variation are influenced by environment. Finally, the evolution of exaggerated traits may be hindered by intralocus sexual conflict. Traits such as weaponry that display extreme sexual dimorphism also have lower intersexual genetic correlations (Stewart and Rice 2018), however intralocus sexual conflict can be difficult to overcome even when the environment prefers different phenotypic optimums between the sexes. While Bird and Schaffer (1972) demonstrated that changes in sexual dimorphism can occur within a relatively short amount of time, this focused on within family differences and is likely to not occur as easily in natural populations resulting in prevented or slowed evolution (Stewart and Rice 2018).

While most traits showed no evidence of differences in condition dependence of allometric slope between treatments, there are several possible elements that may have to be overcome before these changes can arise which likely could not have been achieved within the relatively short time span (35 generations) of this experiment. In terms of allometry, species are expected to show positive allometries when an increase in trait size creates a greater relative advantage for larger individuals and these traits are under sexual selection with no associated viability costs (Bonduriansky and Day 2003). The environmental designs were created to influence *D. melanogaster* behaviour in such a way that optimal mating strategies would differ and drive the benefits between individuals to differ such that low condition males experienced less benefits compared to high condition males with the increasing degree in which the 3 conditions proposed are met (ie: in the NT treatment males of low and high condition about equally likely to obtain mates and resources with or without exaggerated traits while in the SCT treatment high quality males are much more likely to hold and obtain resources and the associated mates especially with the addition of an exaggerated trait). However, this relies on the environmental designs not only providing the correct "substrate" for the behaviours to evolve, but the evolution of the actual behavioral differences. If the environments are not creating these behavioural differences or are not creating the intended optimal mating strategies, phenotypic differences between treatments will not manifest through condition dependence nor allometry. I believe this to be unlikely as the results do show some treatment differences in trait models and the overall environmental design was based on previous work that demonstrate the environmental designs change the mating strategy adopted by male *D. melanogaster* (although these experiments were for much shorter time periods and not as large environments). It is more likely that the time period for this experiment was too short to see the expected behavioural differences to occur, even when only expecting very subtle change or there are unrecognized viability costs preventing trait exaggeration.

Nevertheless, even if the traits analyzed demonstrated strong changes in the trends of condition dependence and allometric slopes across the different environmental treatments, further experimentation in terms of behaviour needs to be conducted. As stated previously, all sexually selected traits are predicted to have heightened condition dependence and are more likely to display hyper-allometries, this includes weaponry, ornaments, and dual-utility traits. While the conditions these environmental treatments emulated were specific to the evolution of exaggerated weaponry, it cannot be ruled out that other sexually selected traits may have evolved within these environments without further testing. Behavioural experimentation is needed to determine how these traits are being used, both between the different environments and between the sexes to not only determine what type of trait may be evolving but also what behavioural strategies are being employed between the different treatments to give rise to these features. One key experiment that may illuminate what strategies are being used between treatments would be to directly observe mating and aggressive behaviour within smaller versions of these environments. I suggest introducing a few virgin males and females from each population into smaller versions of their respective treatments and directly observing intrasexual competitions, keeping track of how many interactions occur and where. This would include recording what traits are used within aggressive encounters for both male-male and female-female interactions. Differences in strategies between treatments could be determined through changes in where aggressive interactions are occurring within the environment (ie. directly on the resource or at the entrance to the resource) and changes in escalation of these encounters. Additionally, whether the tibia or the head is being used as a weapon may also be inferred through the increase of its use in intrasexual competitions observed.

Overall, this experiment demonstrates that these specific conditions altering resource accessibility and defensibility may lead to the evolution of sexually selected exaggerated traits. Although trends at this stage are subtle, this experiment provides evidence that with time, we may be able to see greater effects.

Figures and Tables

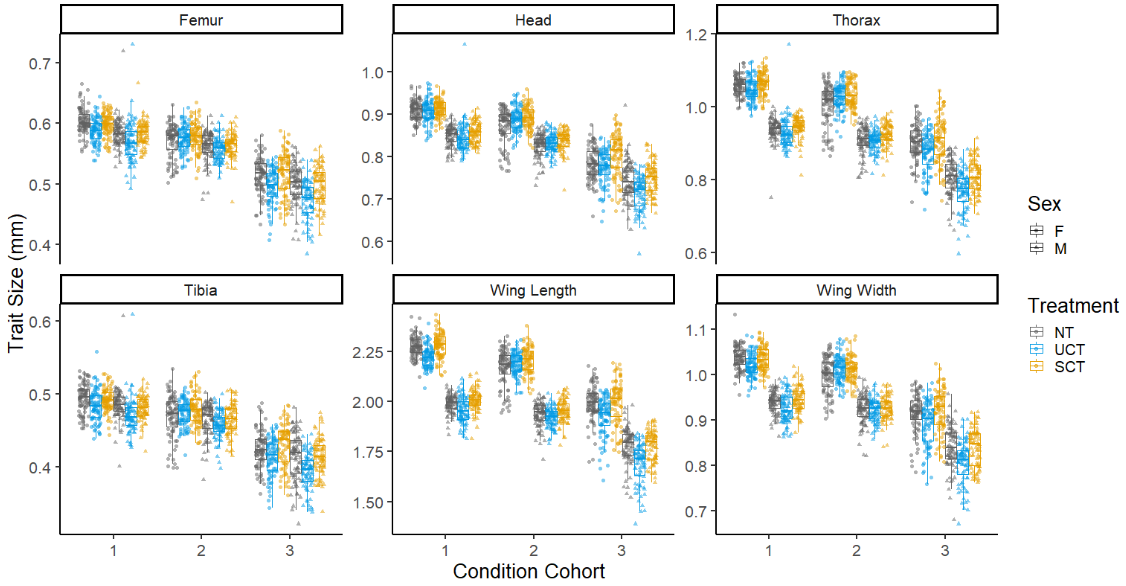

Figure 3.1: Trait size across condition cohorts for each treatment and sex. Note the difference in size for each trait between the sexes due to female biased sexual size dimorphism of Drosophila melanogaster

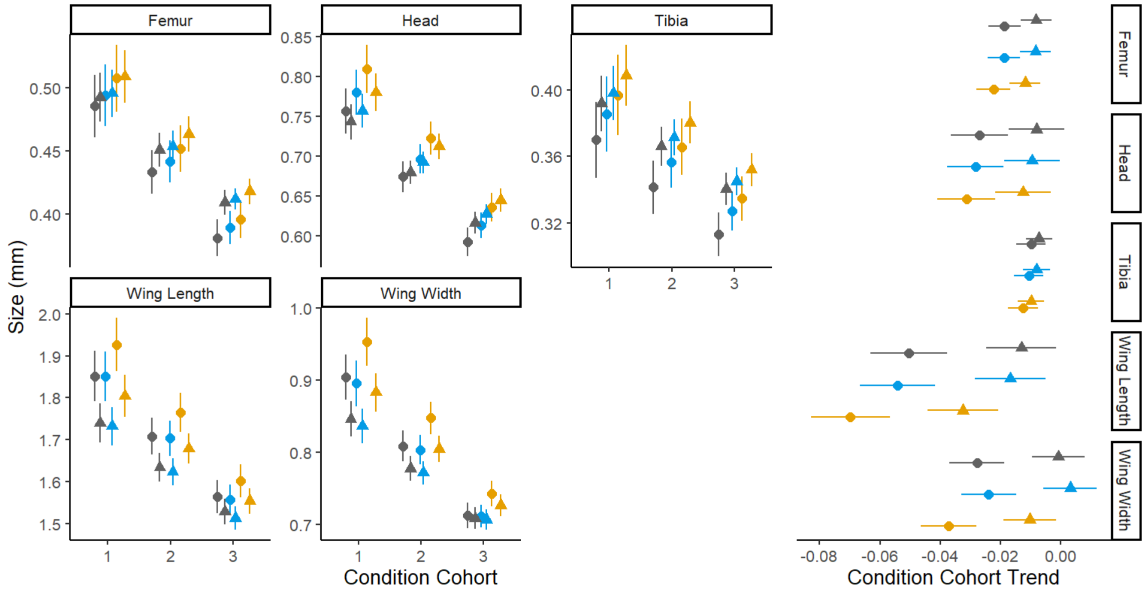

Figure 3.2: Left: Condition dependence of flies between each treatment based on model estimates (adjusted for overall size (thorax)). Data points and error bars represent model estimates and 95% confidence intervals, respectively. Right: Treatment contrasts for each trait based on model estimates. In both figures, circles represent estimates for females while triangles represent estimates for males. The colours grey, blue, and yellow represent treatments NT, UCT, and SCT, respectively. Errors bars are 95% CI's

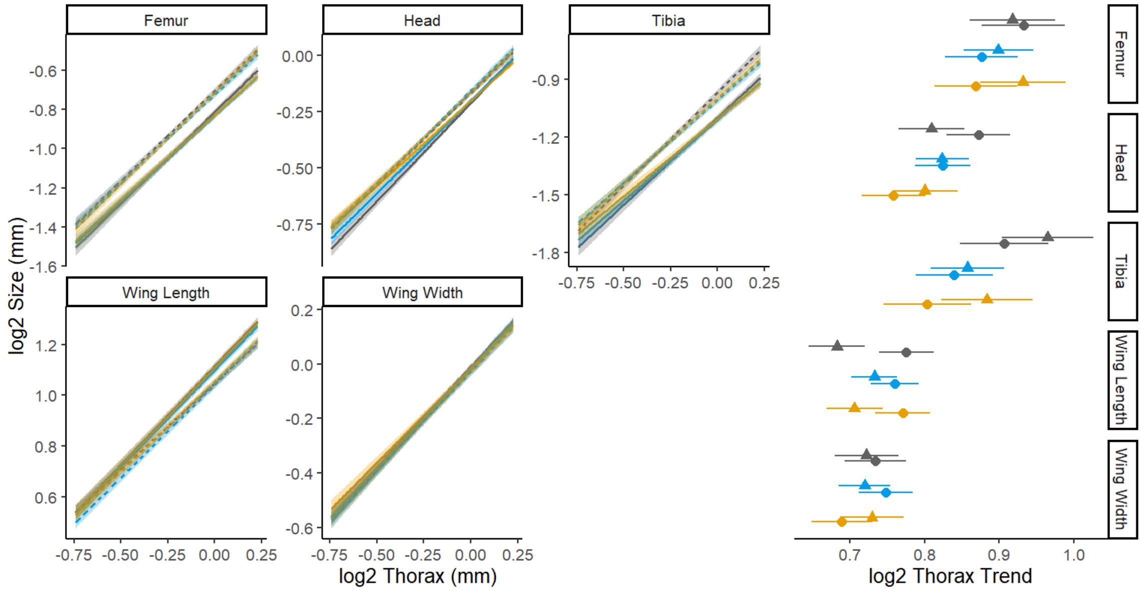

Figure 3.3: Left: Allometric relationships of each trait based on model estimates. Dotted lines represent males while solid lines represent females. Right: Treatment contrasts for each trait based on model estimates. In both figures, circles represent estimates for females while triangles represent estimates for males. In both figures, colour indicates treatment with NT, UCT, and SCT represented by grey, blue, and yellow, respectively. Confidence bands represent 95% confidence intervals for model estimates

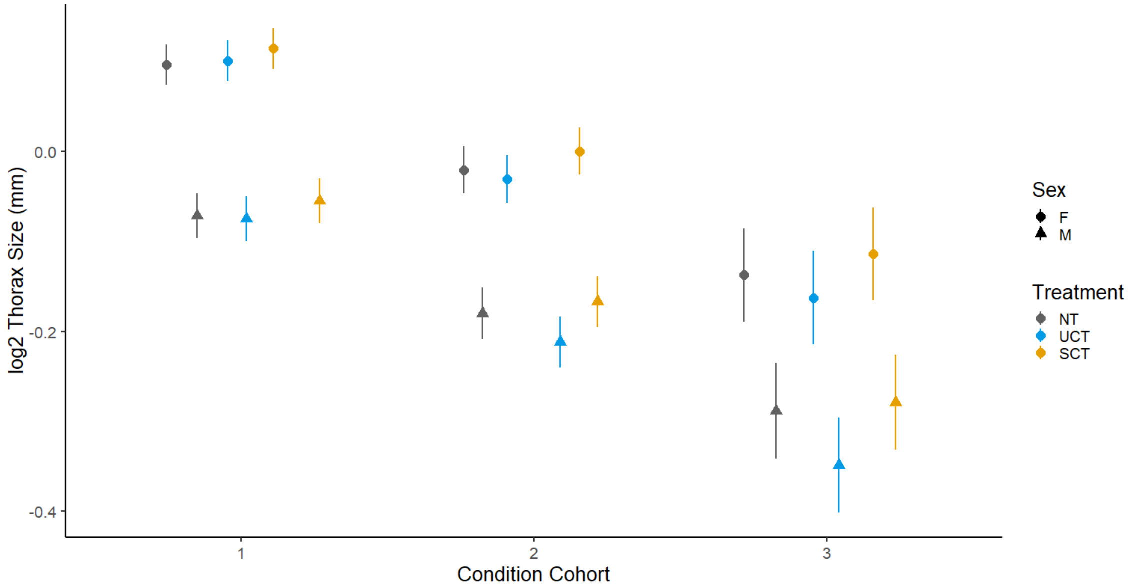

Figure 3.4: Proportional change in thorax size with condition cohort between the sexes and treatments. Error bars indicate 95% confidence intervals

Table 3.1: Chi-square for each predictor for each model based on trait including cohort with p-values in parentheses. Significant p-values are shown in bold

	Femur	Tibia	Head	Wing Length	Wing Width
Treatment	3.42 (0.18)	4.86 (0.09)	1.97 (0.37)	5.63 (0.06)	0.49 (0.78)
Sex	142.97 (**<2.2e^{-16}**)	188.38 (**<2.2e^{-16}**)	7.63 **(0.005)**	404.80 (**<2.2e^{-16}**)	92.63 (**<2.2e^{-16}**)
Cohort	74.63 (**<2.2e^{-16}**)	46.76 **(8.01e^{-12})**	32.57 **(1.15e^{-8})**	98.61 (**<2.2e^{-16}**)	15.41 (**8.62e^{-5}**)
Thorax	1566.02 (**<2.2e^{-16}**)	1282.16 (**<2.2e^{-16}**)	2394.98 (**<2.2e^{-16}**)	2683.73 (**<2.2e^{-16}**)	2291.10 (**<2.2e^{-16}**)
Treatment:Sex	1.66 (0.44)	8.28 (0.02)	11.83 **(0.003)**	0.73 (0.69)	6.04 (0.049)
Treatment:Cohort	1.69 (0.43)	0.99 (0.61)	0.58 (0.75)	7.84 (0.02)	6.08 (0.048)
Treatment:Thorax	5.30 (0.07)	8.44 **(0.01)**	11.71 **(0.003)**	5.89 (0.053)	14.49 (**0.0007)**
Sex:Cohort	18.04 (**2.17e^{-16}**)	1.38 (0.25)	47.94 **(4.39e^{-12})**	41.24 (**1.34e^{-10}**)	80.16 (**<2.2e^{-16}**)
Sex:Thorax	1.75 (0.19)	0.52 (0.47)	0 (0.99)	9.82 (**0.002)**	0.10 (0.75)
Cohort:Thorax	82.98 (**<2.2e^{-16}**)	31.18 **(2.36e^{-8})**	182.08 (**<2.2e^{-16}**)	115.56 (**<2.2e^{-16}**)	229.23 (**<2.2e^{-16}**)

Table 3.2: Chi-square for each predictor for each model based on trait to predict allometries with p-values in parentheses. Significant p-values are shown in bold

	Femur	Tibia	Head	Wing Length	Wing Width
Treatment	5.34 (0.07)	3.21 (0.20)	3.30 (0.19)	2.95 (0.23)	0.90 (0.64)
Sex	576.03 **(<2.2e^{-16})**	761.81 **(<2.2e^{-16})**	243.01 **(<2.2e^{-16})**	268.24 **(<2.2e^{-16})**	11.53 **(0.0007)**
log$_2$(Thorax)	6742.05 **(<2.2e^{-16})**	5546.86 **(<2.2e^{-16})**	9403.68 **(<2.2e^{-16})**	10367.58 **(<2.2e^{-16})**	7929.99 **(<2.2e^{-16})**
Treatment:Sex	0.15 (0.93)	7.29 (0.03)	5.67 (0.06)	0.73 (0.70)	0.30 (0.86)
Treatment:log$_2$(Thorax)	2.12 (0.35)	12.20 **(0.002)**	8.96 (0.011)	0.99 (0.61)	1.69 (0.43)
Sex:log$_2$(Thorax)	1.18 (0.28)	4.31 (0.04)	0.14 (0.71)	15.71 **(7.4e^{-5})**	0.04 (0.85)
Treatment: Sex:log$_2$(Thorax)	1.87 (0.39)	1.33 (0.52)	5.87 (0.05)	3.65 (0.16)	3.19 (0.20)

Table 3.3: Allometric slopes based on model estimates, 95% CI's are in parentheses

Sex	Treatment	Femur	Tibia	Head	Wing Length	Wing Width
Male	NT	0.918 (0.784-1.053)	0.965 (0.823-1.108)	0.809 (0.708-0.912)	0.682 (0.594-0.771)	0.722 (0.623-0.822)
	UCT	0.899 (0.589-1.213)	0.857 (0.528-1.188)	0.824 (0.587-1.060)	0.733 (0.528-0.939)	0.720 (0.489-0.952)
	SCT	0.932 (0.605-1.258)	0.884 (0.540-1.227)	0.800 (0.553-1.048)	0.706 (0.493-0.920)	0.730 (0.489-0.970)
Female	NT	0.933 (0.876-0.987)	0.906 (0.847-0.965)	0.873 (0.829-0.914)	0.775 (0.738-0.812)	0.734 (0.691-0.773)
	UCT	0.876 (0.746-1.005)	0.840 (0.702-0.976)	0.824 (0.726-0.923)	0.760 (0.674-0.845)	0.748 (0.652-0.844)
	SCT	0.868 (0.736-1.005)	0.803 (0.663-0.947)	0.759 (0.659-0.862)	0.771 (0.683-0.859)	0.689 (0.652-0.789)

*Table 3.4: Chi-square for each predictor based on model estimates of log thorax size. P-values are in parentheses with significant values in bold. *Note, the Bonferroni correction was not applied when examining the effects from this model as multiple comparisons were not made*

Treatment	3.44 (0.17)
Sex	989.01 **(<2.2e^{-16})**
Cohort	186.28 **(<2.2e^{-16})**
Treatment:Sex	2.67 (0.26)
Treatment:Cohort	1.33 (0.51)
Sex:Cohort	0.78 (0.38)
Treatment:Sex:Cohort	7.47 **(0.02)**

Supplementary

Figure S3.1: Comparison of vials used in chapter 3 (left) versus chapter 2 (right) with modified funnel cap. Vials used in chapter 3 experiment measured 32mm in height while vials in chapter 2 were 50mm. All other dimensions of the vials were the same

Table S3.1: Differences in model outputs after removal of data due to reduced survival of two populations. Table correlates with model output in Table 1, with red text indicating significant effects

	Femur	Tibia	Head	Wing Length	Wing Width
Treatment	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Sex	Unchanged	Unchanged	Lost significance	Unchanged	Unchanged
Cohort	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Thorax	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Treatment:Sex	Unchanged	Became significant	Unchanged	Unchanged	Unchanged
Treatment:Cohort	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Treatment:Thorax	Became significant	Unchanged	Unchanged	Became significant	Unchanged
Sex:Cohort	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Sex:Thorax	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Cohort:Thorax	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged

Chapter 4: Conclusion

The processes of natural and sexual selection on an organism can change with environmental context, generating plastic responses within a species, and a plethora of defining features among taxa. Animal exaggerated weaponry while shocking in their grandeur, are no exception and are shaped by selection, genetics, and environment although the influential degree of these factors on the extent of weaponry evolution is argued. Emlen (2014) proposed three environmental conditions that must be met for these highly exaggerated traits to evolve, however no studies have attempted to employ these conditions in an experimental evolutionary framework. The goal of this thesis was to examine the effects of spatial structure on sexual selection, with a particular focus on the initial stages of the evolution of exaggerated traits used in intrasexual competition. To meet this goal, I emulated the conditions outlined by Emlen (2014) to differing extents in three different environmental designs and conducted two experiments using *D. melanogaster* as a model.

Within the first experiment, I examined the efficacy of sexual selection and its' interaction with natural selection to determine if these change between environmental treatments. Additionally, I compared my results to similar studies previously conducted, highlighting the importance of environmental "complexity" in the analysis of the directions and magnitudes of components of natural selection. From this experiment, I determined that the interaction between natural and sexual selection did differ between environmental treatments, however this relied heavily on genetic background as there was no consistent relationship in purging rate amongst treatments between mutant types. I also demonstrated that the addition of sexual selection increases the rate at which mutations are removed from populations, however, the addition of environmental "complexity" in terms of increased space did not alter these trajectories relative to simpler environments, an unexpected result that contradicts previous findings.

In the second experiment, I experimentally evolved *D. melanogaster* in the three environmental treatments and after 35 generations of experimental evolution, tested for changes in condition dependence and allometric slopes of traits to identify potential indications of the early stages of weaponry evolution. While the effects were subtle, tibia length and head width both showed differences in size between treatments. For head width, this varied with treatment interactions with sex, and thorax size, with the non-territory treatment showing the greatest overall reduction in head size compared to the unconstrained territory and spatially constrained territory treatments. The tibia also demonstrated effects of a treatment and thorax size interaction, demonstrating that individuals of the same size would display different tibia lengths between treatments. However, even with these effects, none of the traits tested showed overall differences in condition dependence according to treatment. When examining allometry, only the tibia displayed different trajectories between environmental treatments, however all traits remained hypo-allometric. While no traits showed differences in condition dependence with treatment and all displayed hypo-allometries, the length of the experiment was relatively short and these subtle differences in tibia and head width sizes may be early indications of some form of evolution of these traits.

With these two experiments, I demonstrate that spatial structure can influence sexual selection, which can ultimately change the phenotypes of species at the population level. In terms of the conditions proposed by Emlen (2014) and their influence on exaggerated weaponry evolution, the extent of the necessity and sufficiency of these conditions are still uncertain. While these conditions appear to change the phenotypes of populations, how these differences arise and whether they conform to the theory in question, is unclear. Further experimentation is needed to truly determine; (i) the environments create different optimal behaviours associated with the spatial environment, (ii) the traits that appear to be influenced by these conditions (tibia and head width) are being used as weapons and not as other sexually selected traits (ie. ornaments or pure signals), and (iii) all conditions are necessary in the evolution of exaggerated trait weaponry.

These experiments relied heavily on the assumption that the environments created produce different optimal strategies between individuals which would manifest into specific phenotypic characteristics across treatments. However, this assumption was not directly studied with behavioural experimentation. Determining whether these environments provide the substrate for the evolution of different optimal mating strategies could be done through behavioural experiments examining how territories are used (if at all) and the benefits of territorial behaviour employed (number of mates/copulations gained compared to others). Within this type of experiment, determining how traits are used within these mating strategies could also give insight into whether weaponry may be evolving, especially if there is a correlation between increased use of the tibia and head between treatments, conforming to traits I have already shown to be possible candidates for evidence of weapon evolution. Behavioural experiments could also uncover if all of these conditions are needed for weaponry evolution to occur. Throughout both of my experiments, both vial environments (UCT and SCT) showed similarities while it was often the larger bottle environment (NT) that differed. This could be an indication that only the conditions of strong intrasexual competition and high variance in reproductive success caused by the behaviour or biology that is needed to evolve exaggerated weaponry. Although it is important to note that this study can only comment on the initial evolution of these traits and whether or not the third condition, increased intrasexual competition promoted exaggerated trait evolution, is needed to maintain these traits once evolved cannot be tested at this point.

At the end of Chapter 3, I suggested an observational experiment to determine how individuals are interacting within their evolutionary environment and what traits are often being used in intrasexual encounters. However, two other experiments could also be important to unravelling the underlying selective forces occurring between the three environmental treatments. The first experiment I conducted and completed, but the results have yet to be examined and are not provided within this thesis. For this experiment, I examined how variance in male reproductive success differed between the three environments with the expectation that the SCT environment would have the greatest variance as this environment had the highest potential for larger males to dominate a resource compared to smaller males. After manipulating males nutritionally to create high quality (large) and low quality (small) individuals, I paired these males against a genetically marked common competitor for access to a female within environments that had a single resource of one of the environmental treatment types. After three days of mating, I discarded the adults and upon eclosion of offspring from the matings that occurred, I counted and scored progeny. Reproductive success for a high or low quality male was measured as the percentage of wildtype progeny and these values were compared between male types and across treatments. While the results of this study still need to be analyzed, this experiment will show whether the environments created produce the variance in reproductive success (in a population similar to the ancestral population used for experimental evolution) that is required by the conditions proposed by Emlen (2014). If the environments are in accordance with the conditions, both the UCT and SCT environments should display higher variance in male reproductive success compared to NT as these were both designed to have increased defensibility of the resource and a decrease of the occurrence of scramble competition. The SCT environment was expected to have the greatest variance in male reproductive success between the high and low quality males because this environment had the addition of decreased accessibility, potentially allowing larger individuals a greater opportunity to hold territories over smaller individuals.

A second experiment that can be conducted to determine if males from the different environmental treatments vary in their ability to hold desirable resources. In this experiment, males from the evolving populations would be removed and put into an environment with a single resource representing either a UCT or SCT environment and allowed to settle. A female and then competitor male that has been nutritionally manipulated to be high or low quality (estimated by size, large or small respectively) will then be introduced. Once all individuals are within the environments, scans can be conducted to determine which individuals are on the desirable resource. With this experiment, the aggression and ability of individuals to hold resources between the different environments they evolved in can be estimated by their ability to evict competitor males from the resource.

Overall further experimentation is needed to solidify these results to determine whether the three conditions proposed can lead to the evolution of exaggerated trait weaponry. However, this work met the goal of providing the initial framework to answer this question and shows that indeed these conditions may create the circumstances needed for these impressive traits to evolve and provides additional avenues to be explored experimentally.

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