The Role of Group Composition and Resource Availability on Selection for Aggression

by

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ABSTRACT

THE ROLE OF GROUP COMPOSITION AND RESOURCE AVAILABILITY ON SELECTION FOR AGGRESSION

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Traditionally, aggression has been considered an indicator of competitive ability, such that selection in competitive environments should favour aggressive individuals. However, variation in aggression persists in natural populations. An obvious question then becomes: what maintains variation in aggression? Aggression is considered both a plastic trait and a “static” one, as we observe both intra- and inter-individual variation. Plasticity in aggression occurs when individuals modify their behaviour according to the environment, and static differences occur when individuals differ consistently in their aggressive phenotypes across environments. In this thesis, I examined three components – variation in resource availability, variation in the social environment, and differences in aggressive phenotype – and their interactions to understand how variation in aggression persists across generations. The general experimental approach employed aggressive and less-aggressive strains of fruit flies (Drosophila melanogaster) mixed at different ratios and placed in environments of varying resource availability to understand how resource competition and the social environment influence the evolution of aggression. Interestingly, I found that, in a low resource environment, individual survival was greatest for the low-frequency strain, which is expected to lead to the maintenance of aggressive and less-aggressive phenotypes.
by negative frequency-dependent selection (NFDS), in Chapter 1. I then uncovered a novel
behavioural mechanism which can drive NFDS through a combination of disruptive selection
and social plasticity (Chapter 2). Lastly, I demonstrate how the social environment experienced
during periods of food limitation carries over to future environments (Chapter 3). My thesis
demonstrates that selection can act on a population of aggressive and less-aggressive phenotypes,
such that aggressiveness is not always the most fit phenotype. Further, I show how social
plasticity plays a critical role in determining the fitness of individuals, as well as how individuals
will behave in the future. Taken together, I provide novel insight into why there is variation in
aggression.
DEDICATION

This thesis is dedicated to all the womxn scientists who have come before me, whose contributions were often unrecognized and their names rarely mentioned. Their strength and bravery have paved the way for womxn, like me, who they never met. I have no doubt that this thesis would not exist without them. The passion fueled by a love of science is not bound by personal identity and nor should access to it be.
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First, I must acknowledge the traditional territories upon which my research took place. The work presented in this thesis was conducted on the unceded ancestral lands of the Attawandaron people and the treaty lands and territory of the Mississauga of the Credit. I recognize the significance of the Dish with One Spoon Covenant to this land, and I offer further respect to our Anishnaabe, Haudenosaunee and Metis neighbours. Although the scientific method is rooted in a colonial history, the research we conduct today could not occur without the knowledge provided by indigenous peoples, past and present, and their vast understanding of our natural world.

I am thankful to my supervisors, Ryan Norris and Andrew McAdam, for their guidance, mentorship, and, especially, their continuous support. During the times when I was struggling, they were always able to provide the help I needed. I’m grateful to have such extraordinary advisors, who sat with me while I strove to normalize “crying in science” and stood by me while I pushed my feminist agenda.

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I am thankful to my mentors and collaborators, including my committee members, Elena Choleris and Cort Griswald. Gustavo Betini, in particular, spent many hours teaching me the ins and outs of Drosophila research, and provided enormous support during challenging research times. These individuals helped with project design, data analysis and interpreting results.

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Prologue

Aggression is widespread across the animal kingdom and the behavioural components that entail aggressive acts vary substantially across taxa (Lorenz, 1963; Tinbergen, 1953). The disparate forms of aggression preclude the creation of a broad definition, but instead require consideration of both the species-specific form and its function, given the context wherein those behaviours are applied (Archer, 1988). Aggression has been defined as, “any combative behaviour involving a struggle or contest among individuals of the same species” (King 1973), specifically involving both the initiation and/or the attack phases of agonistic encounters (Hinde, 1970). Additionally, the contexts wherein an individual might display aggression are highly varied, giving range to broad types: territorial aggression, dominance aggression, sexual aggression, antipredator aggression, and others (Wilson, 1975). Competitive aggression is a functional form of intraspecific aggression that historically was considered to be the most “important” (Archer, 1988; Wilson, 1975). A key component of any aggressive interaction is that it necessarily involves more than one individual, thus emphasizing the importance of the social environment. An animal’s social environment refers to the phenotypic makeup of its social group and is crucial when even basic questions on the nature of aggression are addressed.

Most animals experience social interactions at some point in their lives, whether in reproduction, development, or during competitive interactions. The outcomes of competitive aggression are often cited as relating to access to limiting resources, particularly with respect to dominance hierarches (Clutton-Brock & Harvey, 1976). Indeed, classically, an individual’s predisposed level of aggression was considered to be synonymous with its competitive ability (Rudin & Briffa, 2012; Syme, 1974; Wilson, Grimmer, & Rosenthal, 2013). A critical aspect of aggression is the variety of forms it can take: most animals are equipped with the ability to express different forms of aggression that vary in intensity and risk. Low intensity behaviours, such as displays or calls, do not require any contact between the players involved, whereas high intensity behaviours are typically based on physical contact, potentially resulting in damage or death (Archer, 1988). Individuals can choose which behaviours to exhibit, depending on the context, the resource, and the competitors (Clutton-Brock, Albon, Gibson, & Guinness, 1979; Maynard Smith & Price, 1973; Wilson, 1975). Therefore, while aggression can, and often will,
result in the acquisition and monopolization of limited resources, the costs associated with such high intensity behaviours leads to question why variation in aggression occurs in nature. However, despite the potential costs, many animals will nonetheless exhibit aggression in contexts wherein it may not be optimal or advantageous.

Animals often exhibit a predisposition to a specific intensity of aggression, where some individuals may be less aggressive and others more so when placed in the same context (Harvey & Freeberg, 2007; Kralj-Fišer & Schneider, 2012; Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Verbeek, Boon, & Drent, 1996). Such tendencies have been reported for decades (Huntingford, 1976) and this cross-contextual behavioural consistency forms the basis of animal personality (Sih, Bell, Johnson, & Ziemb E, Bell, Johnson, & Ziemb, 2004; Wilson, Clark, Coleman, & Dearstyne, 1994). The genetic basis of aggression demonstrates some proximate mechanisms to individual variation (Anholt & Mackay, 2012; Edwards, Rollmann, Morgan, & Mackay, 2006; Edwards, Zwarts, Yamamoto, Callaerts, & Mackay, 2009) and the heritability of aggression has been observed for decades (McClearn, 1970; Wilson, 1975). This genetic basis indicates that selection can act on different aggressive phenotypes. However, aggression is not a perfectly “static” trait, as individuals are further able to learn from experiences and respond to environments, thus modifying their expression of aggression.

In addition to the abundance of research documenting individual consistency in aggression, within-individual variation exists, indicating that the expression of aggression can also be a plastic trait (Wagner, 1989). Intra-individual variation in behaviour, not limited to aggressiveness, is often triggered by changes in the social environment, as different individuals can have different effects on others (Been, Gibbons, & Meisel, 2019). For example, male Pacific tree frogs (Hyla regilla) modify the intensity of their aggressive calls depending on the calls received by their neighbours, demonstrating social plasticity (Brenowitz & Rose, 1994). The influence of social context is also demonstrated in audience effects, wherein individuals modify their behaviour when a conspecific is watching, and depend on the phenotype of the audience member(s) (Desjardins, Hofmann, & Fernald, 2012). A pivotal paper by Evans and Marler (1994) found that food calling rate by male chickens (Gallus gallus) increased in the presence of hens compared to no audience. Similarly, the intrasexual displays by male Siamese fighting fish
(Betta splendens) included more aggressive biting in the presence of a male than a female audience (Matos & McGregor, 2002). Plasticity in behavioural phenotypes, including aggression, is often perceived as the ability for individuals to behave optimally in different situations.

When aggression is exhibited in an adaptive manner, an individual can balance the risks and rewards. The benefits of aggression with respect to competitive ability are clear, when aggression is linked to the ability to acquire and maintain access to limited resources (Clutton-Brock et al., 1979; Rudin & Briffa, 2012), but in the same environment, the possible benefits associated with reduced aggression are more challenging to understand. With competitive behaviour, individuals may reap more benefits from aggression when doing so increases the likelihood of gaining or maintaining access to a limited resource. In contrast, balancing the costs of competition by reducing aggressiveness when the likelihood of accessing resources is low is also an adaptive strategy (Maynard Smith & Price, 1973). Furthermore, adaptive differences in aggressive behaviour may be state-dependent (Wolf & Weissing, 2010). Individuals may modify the intensity of their behaviour given the value of the resource and of their current state: a starving animal may fight more fiercely over low quality food than if she were well nourished. Optimal use of aggression may further vary depending on the social context, as contests against stronger or more dominant opponents could encourage typically aggressive individuals to be less aggressive. Examples of individuals modifying aggressiveness based on characteristics of the social environment demonstrate its importance on fitness (Desjardins et al., 2012; Humfeld, Marshall, & Bee, 2009; Saltz, 2013). Therefore, attributes of the social environment can have profound effects on individual fitness.

Individuals can simultaneously exhibit a predisposition to a certain degree of aggression as well as cross-contextual social plasticity, particularly across competitive environments (Briffa, Rundle, & Fryer, 2008; Briffa, Sneddon, & Wilson, 2015). For example, an animal may exhibit more intense aggression in the presence of a subordinate than in the presence of a dominant conspecific, while that individual may still display greater than average aggressiveness (Francis, 1988). Inter-individual behavioural variability may also be a result of the social context, where the relative difference in personality predicts the level of social plasticity exhibited by an individual. For example, less exploratory zebrafish (Durio rerio) exhibit greater social plasticity.
when placed with individuals who are more exploratory (Guayasamin, Couzin, & Miller, 2017). The combined effects of an individual’s predisposition to aggression as well as social plasticity can persist over time. Such variability and their persistence over time are well demonstrated in the animal contest literature in winner/loser effects, wherein the consequence of one contest (winning or losing) can carry-over into future contests, resulting in the subsequent modification of aggression or competitive behaviour, such that losers keep losing and winners keep winning (Hsu, Earley, & Wolf, 2006). As such, the social environment both past and present must be considered in understanding individual aggression. For example, the social environment experienced during ontogeny, such as the presence of territorial adults (Liebgold, 2014), or the mere presence of a conspecific (Herczeg, Ghani, & Merilä, 2016), can also influence an animal’s aggressiveness as an adult. Often, social effects are exacerbated by features of the non-social environment, particularly resource availability. For example, antipredator behaviour in rainbow trout (Onchorhynchus mykiss) is influenced by the relative rank (more or less dominant than the focal individual) as well as the availability of food resources (Brown, Harvey, Leduc, Ferrari, & Chivers, 2009). The persistence of contest outcomes on behaviour demonstrates how social effects can carry-over across time, and potentially contexts, although few studies have explored this phenomenon across time and space.

As an inherently social behaviour, aggression and aggressive interactions have the potential to act as a selective force, particularly in competitive interactions where resource acquisition is limited. Some hypotheses, such as proposed in Resource Defense Theory (Grant 1993), theorize that aggression is often adaptive in competitive environments. However, the persistence of phenotypic variation across generations indicates that selection on aggression may be more complex. The phenotypic makeup of a social group (i.e. the social environment) can influence the fitness consequences, including the direction of selection, of its group members (Farine, Montiglio, & Spiegel, 2015; Webster & Ward, 2011). Social interactions between individuals can have fitness consequences for all individuals involved. For example, in water striders (Aquarius remiges), all individuals experienced reduced mating activity when in groups which included hyperaggressive males (Sih and Watters 2005). Classic game theoretic Hawk-Dove models predict how alternative aggressive phenotypes can coexist based on the costs and
benefits of aggressive encounters (Maynard Smith, 1974; Maynard Smith & Price, 1973). Quantitative genetic approaches describe how heritable phenotypes, including aggressive phenotypes, influence the phenotype of interacting individuals, ultimately affecting the direction of selection through processes of social selection (West-Eberhard, 1979) or indirect genetic effects (IGEs; Moore et al. 1997; Wolf et al. 1998). For example, the combination of aggressive genetically-based phenotypes in triads of male fruit flies (Drosophila melanogaster) results in changes in the aggressive behaviour of the flies as well as their reproductive success (Saltz, 2013). These mechanisms help to explain how variation in aggressive phenotypes might persist across generations.

Given the complexity of heritable and environmental influences shaping aggression in animals, it is not surprising that both phenotypic and environmental factors are typically assessed in isolation. There has been research on how personality and plasticity intersect (Adriaenssens & Johnsson, 2013; Frost, Winrow-Giffen, Ashley, & Sneddon, 2007; Grace & Anderson, 2014), on the effect of social composition on individual behaviour and fitness (Cameron & Du Toit, 2005; Farine et al., 2015; Niemela & Santostefano, 2015), as well as on how different aggressive phenotypes interact with competitive environments (Briffa et al., 2015; David, Auclair, & Cézilly, 2011; N. J. Dingemanse & de Goede, 2004; McGhee & Travis, 2010), but the combination of factors remains unexplored. Furthermore, aggressive predispositions combined with social plasticity add complication to understanding how selection acts on aggressive phenotypes. For instance, when aggressive individuals are interacting, the positive feedback of aggression eliciting increased aggression in partners can further exacerbate individual differences in aggression (Wilson, Gelin, Perron, & Réale, 2009). As such, the question remains: how and to what extent does social composition interact with competition to influence selection on aggression?

In this thesis, I explore how the social environment influences individuals during and subsequent to a period of limited food resources, both in terms of their expression of aggression and their ultimate survival. For each experiment, I used strains of aggressive and less-aggressive fruit flies, Drosophila melanogaster, and created experimental groups of different densities and strain frequencies. Each experiment involved the same general methodology, wherein groups of
flies were exposed to a period of fixed resources. By varying density, I was able to test the effects of competition (i.e. per capita resource availability) on both survival and behaviour. In addition to limiting resource abundance, I also restricted resource availability by dispensing food from a single location thereby creating a localized area of competition. *D. melanogaster* offers an excellent opportunity to examine the social effects of competition at both the population and the individual level. Using this system, I was able to incorporate naturally inbred genotypes which were artificially selected to exhibit differences in aggressiveness (Anholt & Mackay, 2015) and place groups in experimental treatments where only 40% of individuals survived. In addition, I could uniquely mark individuals and record aggressive interactions. In each chapter, I apply one or both of these techniques to address the fundamental evolutionary question: under what circumstances is aggression beneficial or costly and how is it influenced by the social environment? In identifying the balancing social forces or context-dependent costs and benefits of aggression, we can better understand the persistence of variation in this fitness-related trait.

In Chapter 1, I explore how density and the frequency of aggressive and less-aggressive phenotypes interact to affect survival. In this study, I made predictions rooted in two prominent hypotheses to address how group composition and competitive environments might favour only aggressive individuals or the coexistence of both phenotypes in a population. Aggression may be favoured if it yields a competitive advantage, a fundamental component of Resource Defense Theory. Alternatively, the selective advantage of both aggressive and less-aggressive phenotypes may depend more on the relative frequencies of each type in the population and costs and benefits of within- and between-morph interactions, as described in Hawk-Dove models. In examining the survival of aggressive and less-aggressive flies, I was able to assess how the social and environmental contexts influence individual fitness.

In my second chapter, I seek to understand the behavioural mechanisms behind negative frequency-dependent selection (NFDS) on aggression. In this study, I tested two leading hypotheses: NFDS resulting from non-random interactions, and NFDS resulting from differential between and within morph interaction costs. Using individually-marked flies placed in mixed-strain groups where each strain was common or rare, I examined aggressive interactions in a
food-limited environment. Additionally, by tracking individuals, I could assess if and how survival was predicted by aggressiveness.

Chapter 3 further examines how the social group influences plastic changes in aggressive behaviour. Unlike in the previous experiments, I was interested in carry-over effects of the social environment on individual aggression and if the effects differed between aggressive and less-aggressive phenotypes. Specifically, this chapter examines the impact of resource competition and group composition on aggressive behaviour in future environments. While winner/loser effects describe carry-over of competitive outcomes over time, there is little understanding of how the effects may persist over time and across contexts. In this “before and after” experiment, I examined how aggression changes following a fixed resource period, given an individual’s social experience. I varied the density and frequency of aggressive and less-aggressive strains to assess the effects of competition and group composition on individual aggressive behaviour in a future, and different, environment. Additionally, I compared the carry-over effects of the social environment in both males and females. Determining how social effects persist across time and across contexts is critical in our understanding of how aggressive behaviour develops.

Each chapter addresses a fundamental component in understanding the variation in aggression that we consistently observe in natural populations. First, social composition interacts with competitive periods resulting in the selection of both aggressive and less-aggressive phenotypes, as described in NFDS. Secondly, social interactions and plastic adjustments of aggression to the social environment, together with differential costs of aggression, may be the causal factors ultimately resulting in NFDS. Lastly, the aggression of individuals is impacted by competitive periods and the specific social environment experienced during competition, resulting in modifications of aggressiveness that do not always enhance survival. Each of these three conclusions provides critical insight into our understanding of why we observe variation in aggression within populations.
Chapter publications and author contributions

Chapter 1 is published in the Journal of Animal Ecology (Kilgour et al. 2018) and was coauthored by Andrew G. McAdam, Gustavo S. Betini and D. Ryan Norris. All authors contributed to study design. RJK conducted the experiment, statistical analyses and wrote the manuscript with AGM and DRN. All authors provided feedback and comments on the manuscript.

Chapter 2 is not yet out for review but will be coauthored by Andrew G. McAdam and D. Ryan Norris. All authors contributed to study design. RJK conducted the experiment, statistical analyses. RJK wrote the manuscript with support from AGM and DRN.

Chapter 3 is published in Behavioral Ecology (Kilgour et al. 2019) and was coauthored by D. Ryan Norris and Andrew G. McAdam. All authors contributed to study design. RJK conducted the experiment, statistical analyses and wrote the manuscript with AGM and DRN. All authors provided feedback and comments on the manuscript.
Chapter 1: experimental evidence that density mediates negative frequency-dependent selection on aggression

Abstract

Aggression can be beneficial in competitive environments if aggressive individuals are more likely to access resources than non-aggressive individuals. However, variation in aggressive behaviour persists within populations, suggesting that high levels of aggression might not always be favoured. The goal of this study was to experimentally assess the effects of population density and phenotypic frequency on selection on aggression in a competitive environment. We compared survival of two strains of *Drosophila melanogaster* that differ in aggression across three density treatments and five frequency treatments (single strain groups, equal numbers of each strain, and strains mixed at 3:1 and 1:3 ratios) during a period of limited resources. While there was no difference in survival across single-strain treatments, survival was strongly density-dependent, with declining survival as density increased. Furthermore, at medium and high densities, there was evidence of negative frequency-dependent selection, where rare strains experienced greater survival than common strains. However, there was no evidence of negative frequency-dependent selection at low density. Our results indicate that the benefits of aggression during periods of limited resources can depend on the interaction between the phenotypic composition of populations and population density, both of which are mechanisms that could maintain variation in aggressive behaviours within natural populations.

Introduction

Individual aggression level may reflect the likelihood that an animal will engage in competition (Brown, 1964; Camerlink, Turner, Farish, & Arnott, 2015), and thus has the potential to yield fitness benefits because aggressive behaviours can allow individuals to acquire, or maintain preferential access to, limited resources (Eccles & Shackleton, 1986; Syme, 1974; Verbeek et al.,
However, in spite of the competitive advantages gained through aggression, there is often a diversity of aggressive phenotypes observed within populations. Indeed, consistent individual differences in aggression have been observed across a wide range of taxa including insects (Kortet & Hedrick, 2007; Lichtenstein & Pruitt, 2015), fish (Huntingford, 1976; McGhee & Travis, 2010), birds (Both, Dingemanse, Drent, & Tinbergen, 2005; Kralj-Fiser, Weiss, & Kotrschal, 2010), and mammals (Bergvall, Schapers, Kjellander, & Weiss, 2011; Boon, Réale, & Boutin, 2007; Gosling, 1998). Therefore, two major questions remain unanswered: how is variation in aggression maintained and what are the consequences of behavioural diversity for populations?

The competitive advantages and fitness benefits that are gained through aggressive behaviours may depend on population density in a non-linear way. Resource defense theory predicts that at low density, aggression is often unnecessary because resources are not limited, whereas at high densities, competitive interactions can become so numerous that aggression becomes uneconomical (Grant, 1993) due to the costs of competition being elevated beyond the value of the disputed resource. Therefore, it has been proposed that aggression is most beneficial at moderate densities (Grant, 1993). Experiments exploring the density-dependent advantages of aggression have demonstrated an increase in the frequency and intensity of aggressive interactions or displays as the foraging patch size decreases (Johnson, Grant, & Giraldeau, 2004) or density increases (Yoon, Sillett, Morrison, & Ghalambor, 2012).

In addition to density, frequency-dependent selection may also be a mechanism that allows the maintenance of variation in aggression within a population (Dall, Houston, & McNamara, 2004; Wolf & McNamara, 2012). Negative frequency-dependent selection (NFDS) occurs when the fitness advantages of a phenotype increases as it becomes less common in a population and has been shown to be a mechanism by which alternative morphological (Bots et al., 2015; Le Rouzic, Hansen, Gosden, & Svensson, 2015; Svanbäck & Bolnick, 2007) and behavioural phenotypes (Sinervo & Lively, 1996) can be maintained across generations. The competitive advantage of aggressive individuals when rare is believed to occur as a result of aggressive individuals outcompeting non-aggressive individuals, whereas when aggressive individuals are common, the advantage to non-aggressive individuals results from costs saved by
not engaging in aggressive interactions (Maynard Smith & Parker, 1976). That is, consistent with the elevated costs of competition at high densities, the advantage experienced by non-aggressive individuals when rare is due to the lower cost of employing their resource acquisition strategy relative to aggressive individuals. Game theoretic models have demonstrated that NFDS can maintain variation in consistent individual differences in behaviour, such as aggression, over generations (Dall et al., 2004; Wolf & McNamara, 2012), but empirical evidence that aggressive phenotypes can be maintained through NFDS is rare (but see Lichtenstein & Pruitt 2015).

The maintenance of phenotypic variation through NFDS can also have evolutionary and ecological consequences (Bolnick et al., 2003; Dall, S, R, Bell, Bolnick, & Ratnieks, 2012; Farine et al., 2015). For example, groups containing both social and asocial morphs of the temperate social spider (Anelosimus studiosus) showed increased foraging efficiency and overall greater mean fitness than either phenotype in homogeneous groups (Pruitt & Riechert, 2011). It has also been proposed that behaviourally heterogeneous populations can have greater longevity than homogeneous populations (Wolf & Weissing, 2012). Environmental heterogeneity, through nutrient availability, or, potentially, social heterogeneity, can impact how selection acts on a population. Experimental populations of D. melanogaster adapted to heterogeneous environments have greater genetic variation in fitness traits than populations originating from homogeneous environments (Huang, Stinchcombe, & Agrawal, 2015). Over longer time scales, environmental heterogeneity, might lead to variability in natural selection which could maintain the evolutionary adaptability of populations (Huang, Tran, & Agrawal, 2016).

The goal of this study was to empirically test the roles of density and frequency on the survival of alternative behavioural phenotypes. We used naturally inbred strains of Drosophila melanogaster that exhibit consistent differences in aggression (Shorter et al., 2015) to understand how density and frequency affect the survival of individuals from aggressive and less-aggressive strains during a period of limited resources. In the wild, many animal populations experience periods of limited resources wherein both sexes are present but reproduction does not occur (i.e. a non-breeding season). In this context, fitness can be estimated through individual survival, not reproductive metrics. Thus, we replicated this period by creating mixed-sex groups in enclosed environments with limited food resources and assessed the relative survival of each strain
We hypothesized that density would impact the survival benefits of aggression because aggression is commonly used to gain access to limited resources. Previous studies using *D. melanogaster* during an identical non-breeding period found a mean carrying capacity of approximately 200 individuals (Betini et al., 2013a). From resource defence theory, territorial aggression at food patches is expected to be most advantageous at intermediate densities (Grant, 1993). Therefore, we predicted the greatest survival of aggressive individuals at a medium density treatment (150 individuals), relative to lower (30 individuals) and higher (300 individuals) density treatments.

*Drosophila* species are often used as a model system to explore social dynamics in general (Schneider, Atallah, & Levine, 2012), and aggression specifically (Penn, Zito, & Kravitz, 2010; Saltz, 2013; Zwarts, Magwire, Anna, Versteven, & Herteleer, 2011). Fruit flies live socially, aggregating at discrete patches on rotting fruit (Wertheim, Allemand, Vet, & Dicke, 2006), where males engage in competitive interactions (Hoffmann, 1987a, 1988). Density-dependent competition also occurs in *D. recens*, *D. subquinaria*, *D neotestacea*, which breed in patchy environments, such as on mushrooms (Heard, 1998). In *D. melanogaster*, wild-caught males will actively, and aggressively, defend food patches in the laboratory, mimicking behaviour observed in the wild (Hoffmann, 1987b). In natural populations, aggression is heritable in fruit flies (Hoffmann, 1988) and considerable variation in this trait exists within populations (Hoffmann, 1987a). To examine the effect of frequency, we established experimental populations with different ratios of two strains of *Drosophila* that differed in their aggression. Given that aggression was consistent within strains (Shorter et al., 2015), each strain represented an alternative behavioural phenotype. We hypothesized that selection experienced by each behavioural type would depend on its relative frequency in the population, specifically that each type would have the highest survival when rare, as in NFDS (Wolf & Weissing, 2010). Thus, we predicted that, in competitive environments, less-aggressive individuals would show greater survival when highly aggressive individuals were common but lower survival when less-aggressive individuals were common. Additionally, we were interested in how group composition impacted population-level dynamics. In this part of our experiment, we tested whether per-capita survival was affected by group composition. We predicted that heterogeneous
groups composed of both highly aggressive and less-aggressive strains of *D. melanogaster* would exhibit greater per-capita survival than behaviourally homogeneous groups.

**Methods**

**Experimental System**

*Drosophila melanogaster* were obtained from the Bloomington Drosophila Stock Center ([http://flystocks.bio.indiana.edu/bloomhome.htm](http://flystocks.bio.indiana.edu/bloomhome.htm)). The two strains of homozygous, isogenic, naturally inbred lines that were used in this study were originally bred as part of the Drosophila Genetic Reference Panel (DGRP; Mackay *et al.* 2012). These strains (DGRP 380 and DGRP 712, hereafter “380” and “712”, respectively) were selected for previous use in aggression studies (genetics of aggression; Shorter *et al.* 2015); indirect genetic effects of aggression (Saltz, 2013), and their adaptability to current laboratory conditions. Although strains were inbred, preliminary analysis found no significant difference between strains in number of pupae following 3 days of breeding per 5 pairs of adults (*n* = 9; mean ± SE; 380: 76.44 ± 11.41; 712: 71.14 ± 7.92; t-test, *t* = 0.39, *df* = 13.67, *p* = 0.69), indicating strains are comparable in reproductive output, and adult survival in our experiments (see below) was similar to survival of outbred flies in similar previous experiments (Betini, McAdam, Griswold, & Norris, 2017).

Outside of experimental trials, all flies were kept in 28 x 95mm holding vials containing 10mL of dead yeast-agar-sugar food medium (see Betini *et al.* 2013a; b for details). Flies were allowed to breed for 3 days and mature for 11 days. Adult flies were removed for breeding within one day of emergence. All flies, including experimental treatments, were held at a 12L:12D light cycle, 25°C and humidity held between 30-50%.
**Experiment 1: Survival Assays**

To understand how density and frequency affect the survival of aggressive and less-aggressive individuals, we used a fully factorial design of three density treatments and five frequency treatments, and 11 replicate populations per treatment (Figure 1.1). Our three density treatments were low (30 individuals), medium (150 individuals) and high (300 individuals). At each density, we created 5 frequency treatments using the two isogenic strains described above: two homogeneous treatments (all 380 or all 712) and three mixed-strain treatments (75% 380 and 25% 712; 50% 380 and 50% 712; 25% 380 and 75% 712). Therefore, these five treatments represented frequencies of each strain that ranged from 0 to 100%. Prior to placement in treatment groups, day-old flies were dusted with fluorescent pigment (DayGlo Ltd, Cleveland OH) where strains were randomly assigned one of three colours. Treatment groups were established and placed in holding vials, where all individuals could interact and familiarize to social partners. Given that groups were made up of both sexes, we assume mating occurred during this period.

After 24 hours, treatment groups were placed in the ‘non-breeding season’, wherein flies were placed in an empty vial and 0.200 mL of 5% sugar water was dispensed each day from a pipette tip fixed at the top of the vial (Betini et al., 2013a, 2013b). This experimental scenario is ideal for assessing competitive dynamics as food is limited and dispensed from a single location, preventing all flies from feeding simultaneously. In this context, while males and females are able to interact, females do not produce eggs due to the lack of nutritional protein (Bownes & Blair, 1986; Terashima, Takaki, Sakurai, & Bownes, 2005). After four days, those flies that survived were separated by strain based on their fluorescent pigment colour and counted. The observer did not know which colour corresponded to which strain when sorting and counting. When sorting, we did not observe any flies without pigment, nor any flies with multiple colours of fluorescent pigment, indicating that pigment application was effective and not transferred among individuals during the experimental period. We considered individual survival through this four-day period as our fitness component. We also sampled flies of both strains and sexes, before and after the four day period, to assess any differences in body mass.
Experiment 2: Aggression Assays

To confirm differences in aggression between strains, we video recorded and analyzed aggressive behaviours during feeding in a similar period of limited resources, but which involved a smaller number of flies that could be individually marked and tracked. Groups of 30 flies were provided with 0.020mL of 5% sugar water per day, thus mimicking the amount of food resources per fly as in the high density treatment. In other words, this assay represented a scaled-down version of the most competitive treatment applied in our main experiment in which the number of flies per vial was much lower, but where we maintained the same per capita food availability (i.e. functional density) as our high density treatment. We ran 10 replicates each of two alternative mixed-strain treatments: one composed of 25% 712 and 75% 380 and the second with 75% 712 and 25% 380. That is, we applied two frequency treatments, where each strain was either common or rare, allowing us to observe NFDS. Although not the goal of this experiment, we also measured survival of the two strains and found that the patterns of survival in this experiment were the same as in the larger survival experiment described above. Groups were evenly composed of males and females and all individuals were uniquely marked with acrylic paint, enabling us to identify aggressive behaviours exhibited by sex and strain. Groups were established and placed in a holding vial for 24 hours prior to the experiment, as in the survival experiment. We recorded interactions first within 2 hours after being placed in the experimental period and again 15 hours later. Both feeding periods were recorded for 20 minutes and videos were subsequently analysed for aggressive behaviours occurring within one body length of the food tip. In this experiment, we recorded the number of shoves, head-butts and lunges exhibited by both males and females. During video analysis, the observer (RJK) had no knowledge of the strain identity of each individual. Populations were kept in the period of limited resources for 4 days (as in main experiment) and survival was assessed by individual identification to test for NFDS as above.

Statistical Analysis

In Experiment 1, we ran 3 sets of models to (1) isolate how mixed-frequency treatments influenced survival of each strain, (2) identify any overall differences in strain survival from
homogeneous treatments and (3) to compare per capita survival based on degree of heterogeneity. We used generalized linear mixed-effects models (GLMMs) to address goals (1) and (2), and a generalized linear model for goal (3). For all GLMMs, we assessed the significance of fixed effects with the Wald statistic, which is calculated using maximum likelihood and is distributed as $\chi^2$ for each term (McGowan, Sharp, Simeoni, & Hatchwell, 2006). We found no evidence of overdispersion in models (dispersion parameter $>1$, Bolker et al. 2009; Harrison 2014) unless otherwise described.

To test for changes in group composition following the non-breeding season in mixed frequency treatments, we examined the proportion of flies that survived for each strain using a GLMM with a binomial error distribution and a logit link function. The density and frequency treatments as well as strain were fitted as fixed effects, as well as their three-way interaction and all component two-way interactions, with vial as a random effect. The three-way interaction term was included to determine whether frequency-dependent survival was affected by density. The frequency*strain interaction tested for NFDS overall. We also used a linear model to assess the effects of sex, strain and time (before and after the period of limited resources) on body mass, as well as all interactions therein.

We ran separate models for homogeneous treatments, which allowed us to test for any differences between strains that might impact survival. To measure differences between the homogeneous social treatments across densities, we ran a similar GLMM survival model, examining only the relationship between density, strain and the density*strain interaction, with vial as a random effect.

We grouped frequency treatments based on the degree of homogeneity of strains to explore differences in per capita survival between homogeneous and heterogeneous groups. Therefore, we used a single three-level factor: entirely homogeneous (100% of either strain), equally mixed groups (50% of each strain) and unequally mixed groups (75% DGRP 380 or 75% DGRP 712). There were no random effects, as we had no within vial replicates, so we used a generalized linear model with a quasibinomial distribution to examine how diversity in group composition (frequencies of 100%, 50:50 and 25:75) and density affected per-capita survival in
each factor. A quasibinomial distribution was used because the dispersion parameter for this model was substantial (dispersion parameter: 9.47). Per-capita survival was measured as the overall proportion of surviving flies per vial, regardless of strain.

For Experiment 2, we confirmed strain-based differences in aggression using a GLMM with Poisson error distribution and a log link function. We modeled aggression (number of aggressive behaviours exhibited per feeding period) with strain, frequency and sex and the interactions between sex and strain, and strain and frequency as fixed effects. We incorporated vial and paint pattern as random effects to account for any within-vial effects or effects due to paint pattern.

All analyses were performed using R version 3.2.2 (R Core Team, 2015), with GLMM conducted using R package lme4 (Bates, Mächler, Bolker, & Walker, 2014). Fixed effects were considered significant at α = 0.05. Model fit was assessed using diagnostic plots and scatterplots of residuals and predicted values.

**Results**

**Experiment 1: Survival Assays**

To assess overall differences in survival between strains in the main experiment, we compared the survival of homogeneous strain treatments across three densities. While there was a significantly negative effect of density on survival ($\beta = -0.01 \pm 0.001; \chi^2 = 181.16, df = 1, p < 0.01$; Figure 1.3), there was no evidence of differences in survival between strains ($\chi^2 = 0.87, df = 1, p = 0.34$). There was also no evidence of a density*strain interaction ($\chi^2 = 1.18, df = 1, p = 0.28$; Figure 1.3), indicating that the two strains were equally food limited during our experimental trials. While females were significantly larger than males (mean ± SE: females: 1.24mg ± 0.008; males: 0.76mg ± 0.005; $\beta = -0.49, df = 1211; p < 0.01$), there were no other significant effects (Supplementary S1.1), suggesting no difference in body size between strains within sex or before and after the period of limited resources.
In comparing the survival of each strain in mixed-strain groups, we found a significant three-way interaction between density, frequency and strain ($\chi^2 = 5.18$, df = 1, $p = 0.02$, Table 1.1), providing evidence for density-dependent frequency-dependent survival. At high density, the survival of strains depended on their relative frequency in the group, where the rare strain experienced higher survival. For example, at high density, the survival of strain 380 was 40% when rare, but only 25% when common. This trend was also observed in the medium density treatment, but there was no frequency-dependent survival at low density, as almost all individuals survived the period of limited resources (Figure 1.4). As with homogeneous treatments, there was a strong, negative effect of density on survival (Table 1.1). Similar NFDS was found in the scaled-down trials (Experiment 2), with a significant interaction effect of strain and frequency on survival ($\chi^2 = 4.82$, df = 1, $p = 0.02$), with a comparable effect strength to the results in Experiment 1 (Experiment 1: $\beta = 1.05 \pm 0.75$; Experiment 2: $\beta = 1.80 \pm 0.82$).

We examined how group composition influenced per-capita survival and if this relationship was affected by density. Because survival was almost 100% in the low-density treatment, we excluded this level from our analysis. We compared survival between homogeneous (100% one strain) with equally mixed heterogeneous groups and unequally mixed heterogeneous groups (strain compositions of 50:50, and 25:75 or 75:25 respectively) and found no difference in per-capita survival among these three levels of population heterogeneity at either high ($F_{1,90} = 0.76$, $p = 0.39$) or medium ($F_{1,82} = 2.03$, $p = 0.15$) population density. Although not significant ($F_{1, 264} = 3.19$, $p = 0.07$), survival increased with homogeneity at medium density relative to high density treatments (medium density: $\beta = -0.12 \pm 0.08$; high density: $\beta = 0.05 \pm 0.05$).

**Experiment 2: Aggression Assays**

Aggressive behaviour differed between strains and sexes. Strain 380 was two times more aggressive than strain 712 (mean ± SE, 380: 1.92 ± 0.13, 712: 0.81 ± 0.08; $\chi^2 =33.47$, df = 1, $p < 0.01$, Figure 1.2) and female aggression was twice as high as males, regardless of strain (mean ± SE, females: 1.73 ± 0.14, males: 1.00 ± 0.08; $\chi^2 = 12.69$, df = 1, $p < 0.01$, Figure 1.2), although the effect between strains was stronger than the effect between sexes (strain: $\beta = -1.78 \pm 0.3$; sex:
\( \beta = -0.51 \pm 0.14; \). There was a significant effect of frequency on aggression, with decreased aggression when common \( (\chi^2 = 10.17, \text{df} = 1, p < 0.01) \), as well as a significant effect of the interaction between strain and frequency \( (\chi^2 = 6.91, \text{df} = 1, p < 0.01) \), where strain 380 showed increased aggression when rare and strain 712 showed a slight decline in aggression when rare. However, even in the presence of this interaction, 380 exhibited greater aggression than 712 at both frequencies.

**Discussion**

Our study provides an explanation for how consistent between-individual differences in aggression, which are commonly found in populations (Kortet & Hedrick, 2007; Rudin & Briffa, 2012; Sih, Chang, & Wey, 2014), could be maintained over time. While NFDS on aggressive and non-aggressive phenotypes has been described theoretically as a potential mechanism of maintaining phenotypic variation (Dingemanse & Wolf, 2010; Wolf & Weissing, 2010), it has never been empirically demonstrated. Our results indicate the occurrence of frequency-dependent survival at high densities. Our results suggest that this frequency-dependent survival resulted from differences in aggression between the two strains, but we cannot rule out the possibility that other strain differences also contributed to the NFDS that we observed. The inclusion of additional replicate strains would have helped to more conclusively identify the importance of aggression in the NFDS but this was not feasible within the context of our experimental design. Notwithstanding these limitations, our results imply that differences in survival of alternative aggressive phenotypes can occur and promote the maintenance of behavioural variation.

Traditionally, aggression has been interpreted as a main component predicting an individual’s resource holding potential, an indicator of dominance and contest outcomes (Parker, 1974), but our results suggest that aggression may not always be beneficial in competitive environments. Our data demonstrate that more aggressive individuals did not experience increased survival in all treatment groups. Aggression may be advantageous within contests, but
costs associated with aggressive interactions may lead to reduced fitness. Within contests, recent studies in juvenile pigs (Sus scrofa) indicate that individual aggressiveness may be a signal of intent to escalate interactions to fighting, but is not always a predictor of who “wins” in contests, and, therefore, not necessarily a component of resource holding potential (Camerlink et al., 2015). Aggression is also not necessary to “win” a competitive interaction, as many other non-contact agonistic behaviours, such as displays, can be involved (Camerlink, Arnott, Farish, & Turner, 2016). Alternatively, different environments may select for different phenotypes depending on resource attributes. For example, resource defense theory hypothesizes that the benefits of aggression in competitive environments are determined based on the spatial and temporal distribution of resources (Grant, 1993; Robb & Grant, 1998), where aggression is less advantageous when resources are extremely spatially clumped (as with one resource patch) and temporally dispersed. In our study, resource quality and distribution remained consistently clumped and temporally predictable throughout the experiment, which may have enabled NFDS on both more and less-aggressive phenotypes.

Our results also demonstrate the importance of incorporating group composition in understanding the adaptive nature of aggression. Although contests involving multiple individuals may be difficult to measure, the composition of the entire social group can alter the outcomes of competitive interactions and ultimately the evolutionary trajectory of aggression (Saltz, 2013). Even if contests themselves are between two, or a few, individuals, other group members can influence outcomes through audience effects (Dzieweczynski, Earley, Green, & Rowland, 2005) or assessment strategies (Yasuda, Takeshita, & Wada, 2012). Our study design allowed for multiple individuals to be present at and around a spatially clumped resource, allowing any individual fly to observe competitive interactions among other flies. Contests allowing iterative interactions between multiple group members can also alter competitive dynamics and ultimately individual resource holding potential compared to dyadic contests (Chase, Tovey, Spangler-Martin, & Manfredonia, 2002). Flies in our study were held in the treatment vials for 4 days, allowing surviving individuals to repeatedly interact with each other. Quantitative genetic models emphasize the importance of incorporating the phenotypes of all group members into predictions of the strength and direction of selection, as different group
composition can dramatically influence how selection acts on different phenotypes (Farine et al., 2015). The results of our study demonstrate the necessity of incorporating group composition in studies on aggression, as selection for more and less aggressive phenotypes may depend on the phenotypic makeup of the social environment (also see Saltz 2013).

Although aggression is often consistent within a context (Grace & Anderson, 2014), individuals can demonstrate considerable variability in aggression between contexts (Hewitt, Macdonald, & Dugdale, 2009). As with many behavioural phenotypes, aggression can be a plastic trait and the expression of aggression in competition can be influenced from a variety of ephemeral and social factors, such as previous competitions (as with winner/loser effects: Chase, Bartolomeo & Dugatkin 1994) or social context (as with audience effects: Dzieweczynski et al. 2005). We observed this plasticity in our experiment: aggression exhibited by individuals of the same strain was influenced by their frequency but direction of the effect was opposite between the two strains. Despite the plasticity exhibited within-strains, the differences in aggression between strains remained consistent. As such, we considered aggression as a fixed trait of each strain, and aggression is commonly found to be a repeatable behaviour (Bell, Hankison, & Laskowski, 2009). While still exhibiting plasticity around their aggressive tendencies, individuals of many species demonstrate overall consistency in aggression (Bell et al., 2009). This behavioural consistency between strains could be considered similar to behavioural phenotypes explored in studies of consistent individual differences and animal personality (or behavioural syndromes) (Réale et al., 2007; Sih, Bell, Johnson, et al., 2004).

While the between-strain differences that we observed were consistent with previous studies (Edwards, Ayroles, et al., 2009), changes in context can have important consequences for the expression of aggressive behaviour. Indeed, another study found the opposite order of aggression in these strains (Shorter et al., 2015). While individuals are often repeatable in their aggression within a context, even small changes in the social and physical environment can alter behavioural expression. For example, alterations of food type and location may result in different expression of aggression, given that D. melanogaster exhibits strong geo- and phototaxis (Strauss & Heisenberg, 1993), and that nutritional content can alter the expression of behaviour (Kaspi, Taylor, & Yuval, 2000), particularly for females (Ueda & Kidokoro, 2002). With respect
to the social environment, individuals may express more or less aggression depending on the identity of the competitor (such as differences in dominance rank, Meese & Ewbank 1973) or the social context (such as differences in group size, Johnson et al. 2004, or group composition, Saltz 2013). In same-sex dyadic assays, few studies on *D. melanogaster* have examined female behaviour, and those that have document lower aggression in females than males (Nilsen, Chan, Huber, & Kravitz, 2004). Our study showed greater aggression in females than males, potentially as a result of the different social and physical contexts created in this experiment. Females often demonstrate different patterns of aggressive behaviours than males (Nilsen et al., 2004), and competitive behaviour in females may be more influenced by the value of the resource than competition among males (Cain & Langmore, 2016; Draud, Macías-Ordóñez, Verga, & Itzkowitz, 2004; Tibbetts, 2008). Furthermore, female aggression increases after mating (Bath et al., 2017), which may have accounted for higher aggression in females compared to males, as it is unlikely any females were virgins at the time of our trials.

Although we simulated a non-breeding period for our experiment, there is evidence that this is an ecologically relevant period of *Drosophila*. Wild flies do experience periods of reduced reproduction, often in spring and fall when photoperiods are short (Zhai et al., 2016). Many species experience variation in resource abundance during the breeding periods (Carson & Stalker, 1951), and reports of reproductive diapause, where females are not reproductively active, have been documented in *D. robusta* (Carson & Stalker, 1948), *D. suzukii* (Zhai et al., 2016), and *D. melanogaster* (Schmidt & Paaby, 2008). These periods of reproductive diapause in late summer have been referred to as non-breeding seasons (Carson & Stalker, 1948). Furthermore, *Drosophila* species do defend food resources in the wild (Hoffmann, 1987a) and many species forage and reproduce on patchy and ephemeral food resources, and competition may be high when those resources become limited (Heard, 1998; Hoffmann, 1987a), particularly outside of the breeding season. Given the variability in aggressiveness observed in wild populations (Hoffmann, 1987a), this period could be when NFDS on aggressive phenotypes occurs in natural populations.

While our work may provide an explanation for how variation in aggressive behaviour could be maintained within a population, we did not find any support for resource defense
theory. This theory predicts that aggression should be most advantageous at intermediate densities, where the costs of aggressive interactions are less than the benefits they provide in accessing limited resources (Grant, 1993). One reason why we did not find support for this prediction is that resource defense theory is rooted in the assumption that aggression is a proxy for competitive ability and that individuals will express aggressive behaviours based on trade-offs, given the benefits of accessing limited resources and the time and energy costs of aggressive interactions. While aggression may reflect competitive ability, the increasing costs associated with increasing density ultimately lead to reduced survival. Additionally, given the other factors that can influence aggression, such as an individual’s personality (Bell et al., 2009; Briffa et al., 2015) and the social environment (Camerlink et al., 2016; Farine et al., 2015), the hypotheses derived from resource defense theory may offer an oversimplified understanding of aggression. Although changes in density and frequency of strains occurred over the duration of the period of limited resources, it is the density and frequency at the beginning that was most important. This is because the benefits of being rare decline as strains become less rare (i.e. as a result of NFDS). Further studies of individually marked flies will be needed to determine how the composition of the population and natural selection change through time as populations approach their carrying capacity.

Interestingly, our work did not provide evidence for the hypothesis that phenotypic diversity of groups leads to higher overall group performance (Wolf & Weissing, 2012). There was no difference in mean survival between treatments made up of mixed-groups (both strains) and homogeneous groups (only strain 380 or strain 712). Other studies exploring the effect of aggressive individuals on group dynamics have shown contrasting results. For example, research on social spiders demonstrated that females had higher egg-case mass when there was a heterogeneous groups of aggressive and docile individuals, compared to homogeneous groups (Pruitt & Riechert, 2011). Additionally, the presence of hyper-aggressive male water striders reduced the mating success of all individuals in the group (Sih et al., 2014), indicating that extreme variation in group aggressive behaviour may be detrimental to group performance. Previous studies exploring the benefits of phenotypic diversity at the group level suggest that heterogeneity among individuals enhances niche exploitation, as individuals with alternative
phenotypes are not competing for resources. In our study, all individuals were competing for the same food resource, thus facilitating direct competition between phenotypes. In this case, character convergence might be predicted, where a single competitive trait is selected (Abrams, 1996; Abrams & Matsuda, 1994). It is also possible that using survival as a fitness metric in this study may not have been sufficiently sensitive to observe any effect of individual phenotypes on group-level performance.

Our study shows negative frequency-dependent selection may be acting on alternative aggressive phenotypes in competitive environments and that the presence and strength of NFDS depends on density. While aggression is believed to be advantageous in competitive environments, our findings illustrate that viability selection may not universally favour aggression, but instead might favour rare behavioural phenotypes when competition for resources is strong. To better understand how variation in aggression in maintained in populations, it will be important to identify the mechanisms by which NFDS acts on aggression, to understand whether NFDS is sufficient to maintain variation in aggressive phenotypes, and to investigate how this might interact with behavioural plasticity.
### Tables

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<td>5.181</td>
<td>0.022</td>
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</table>

**Table 1.1.** The effects of aggression on survival depended on the density and the frequency of aggressive individuals. Survival was compared across low- (n = 30), medium- (n = 150) and high-density (n = 300) treatments using a generalized linear mixed effect model. Frequency treatments involved altering the relative ratio of each strain (75% 380 and 25% 712; 50% 380 and 50% 712; 25% 380 and 75% 712) at each density treatment. The three-way interaction demonstrates density-dependent NFDS.
Figures

Mixed Frequency Treatments

Homogeneous Treatments

Density Treatments

High (300)

Medium (150)

Low (30)

4 days

Survivors

Period of Limited Resources
Figure 1.1 A summary of methods. Black and grey flies represent the two strains used in this study. In addition to homogeneous treatments, we created mixed frequency treatments of strains at 1:3, 1:1 and 3:1 ratios. All homogeneous and mixed frequency treatment groups were tested at three density treatments (high, medium and low). Each of the 15 treatments was placed through a limited resource period for 4 days, where flies were fed 5% sugar water dispensed at the top of the vial. Following the limited resource period, survivors were sorted by strain and counted.
Figure 1.2 Differences in aggression between strains in A) male and B) female in the experimental vials. Lunges, shoves and head-butts were recorded for both males and females. Over two feeding periods, aggressive behaviours were recorded for 20 minutes. In both sexes, flies from strain 380 exhibited more aggression than flies from strain 712. Bar heights represent means and error bars represent standard error ($n = 150$ flies per sex and strain).
Figure 1.3. The survival of two isogenic, naturally inbred lines of *D. melanogaster* was assessed at three densities (30, 150 or 300 flies per vial). Bar heights represent means and error bars represent standard error.
Strain Frequency of Strain 380

Proportion Survived

Frequency of Strain 380
Figure 1.4. The effect of frequency and strain on the proportion of flies survived following a period of limited resources at three density treatments: A: low (30 individuals), B: medium (150 individuals), C: high (300 individuals). Frequency treatments showed each strain and low and high frequency (75% 380 and 25% 712; 25% 380 and 75% 712), and strains at equal frequency (50% 380 and 50% 712). Bar heights represent means and error bars represent standard error.
Chapter 2: social plasticity and differential interactions costs can drive negative frequency-dependent selection

Abstract

Despite the potential competitive advantages to aggressive individuals, variation in aggression persists in populations. Theoretical and empirical evidence demonstrate that both aggressive and non-aggressive strategies can be maintained through negative frequency-dependent selection (NFDS). However, the behavioural mechanism through which this occurs remains unclear. We compared two leading hypotheses addressing the behavioural mechanism of NFDS. The first hypothesis suggests that fitness costs associated with biased, within-morph competition results in decreased fitness as morph frequency increases. Alternatively, random within- and between-morph encounters might impose different costs to each morph resulting in increased fitness when rare, similar to classic Hawk-Dove games. We created groups of 30 individually marked *Drosophila melanogaster* from aggressive and less-aggressive strains mixed at 1:3 and 3:1 ratios, and video-recorded interactions under food limitation, with survival as the fitness metric. We found no support for either hypothesis. Instead, our results showed that individuals interacted at random and that aggressive interactions positively affected the survival of flies from the aggressive strain, while negatively affecting the survival of flies from the less-aggressive strain. We also observed social plasticity in aggression. In particular, when common, less-aggressive flies increased their aggression and aggressive flies decreased their aggression. These maladaptive plastic responses to the composition of the social environment reduced survival when common. Although not hypothesized *a priori*, the combination of social plasticity in aggression and differential costs of aggression that we documented represents a novel behavioural mechanism that can result in NFDS.
Introduction

While it is commonly predicted that natural selection should favour the evolution of one optimal morph, polymorphisms persist in nature. The occurrence of polymorphisms is often thought to be maintained through selective forces, such as negative frequency-dependent selection (NFDS; Ayala and Campbell 1974; Takahashi and Kawata 2013). Through this process, the relative fitness of a morph decreases as its relative abundance (or frequency) increases compared to other morphs in the population. In this manner, morphs experience higher fitness compared to other morphs when at low frequency (or when rare) and lower fitness compared to other morphs when at high frequency (or when common). For example, the mating success of two genotypes of *Drosophila* in a single population was inversely related to their relative abundance in a population (Ayala, 1972). Studies have shown that NFDS can maintain variation in body colouration (Fitzpatrick, Feder, Rowe, & Sokolowski, 2007; Nosil et al., 2018; Takahashi & Kawata, 2013), alternative reproductive strategies (Gross, 1991; Sinervo & Lively, 1996), predator-prey systems (Koskella & Lively, 2009), and can, in some circumstances, ultimately result in speciation (Dijkstra, Seehausen, Pierotti, & Groothuis, 2007; Svanbäck & Bolnick, 2007). NFDS can also explain variation in aggressive behaviour within a social group, such as differences in dominance ranks (Dijkstra et al., 2010) and levels of aggression (Fitzpatrick et al., 2007). While NFDS is considered one of the strongest forces in maintaining diversity at the species and genetic level (Ayala & Campbell, 1974; Fitzpatrick et al., 2007; Takahashi & Kawata, 2013), the behavioural mechanisms driving NFDS can be challenging to identify (Brisson, 2018) and may not be consistent across all polymorphisms or species and thus are often left unexplored.

One mechanism for NFDS is when the number of competitive interactions per unit time per individual is different between a high-frequency versus low-frequency morph. For example, morphs may be biased to initiate a competitive interaction with individuals of the same morph versus the other morph (hereafter, “Like competes with Like”). Under this scenario, a high-frequency morph is more likely to experience an interaction that is competitive than a low-frequency morph. If fitness consequences accumulate across competitive interactions for an
individual, then a high-frequency morph will accumulate more deleterious competitive interactions than a low frequency morph, causing a high-frequency morph to have lower fitness than a low-frequency morph, on average. This process assumes that the costs associated with competitive interactions are the same for both morphs, or at least not too divergent. Therefore, when a morph is at high frequency, the number of competitive interactions experienced per common individual increases at a faster rate than their frequency and imposes competitive costs. As such, per capita fitness declines with increasing frequency. In contrast, when a morph is at low frequency, the probability of negative interactions is lower and thus the rare morph engages in fewer competitive interactions. This rare morph advantage enables them to increase in frequency in the population. The maintenance of alternative reproductive strategies can occur in this manner (Brockmann, 2001; Gross, 1991; Taborsky, 1994). For example, in bluegill sunfish (*Lepomis macrochirus*), males employ one of two reproductive strategies: territory holders or sneaker males are smaller, do not court females and reproduce through sneak copulations. Both males are competing for access to females, and within-morph competition occurs more often than between-morph competition. For example, sneaker males are more successful at achieving sneak copulations when there are fewer sneaker males. Territorial males, in contrast, are able to acquire better nesting habitats and successfully defend nests from other males when there are fewer territorial males in the population (Gross, 1991). Therefore, the most influential competitive interactions are within-morphs and fitness costs to common morphs occur because of the culmination of competitive interactions. Similar effects are found when polymorphic males exhibit within-morph aggression biases, such that each morph is likely to experience greater competitive aggression when common (Dijkstra et al., 2010; Dijkstra & Border, 2018).

A second mechanism by which NFDS can maintain variation in behaviour is where individuals interact at random, but the fitness outcomes of within- and between-morph interactions differ, forming the basis of the classic Hawk-Dove game (Maynard Smith and Price 1973; hereafter "Hawk-Dove"). In its essence, two types can be maintained if the type that experiences greater costs from within-type interactions incurs greater benefits from between-type interactions, and the other morph experiences greater costs from between-type than within-type
interactions. In other words, when the fitness consequences of encounters between two morphs, A and B (where the notation A-B refers to the fitness of A when interacting with B), are ranked such that A-B > B-B and B-A < A-A, then NFDS will maintain the two morphs. In the Hawk-Dove metaphor, when faced with a limited resource, hawks fight and doves display. Hawk-hawk interactions always involve fights, resulting in a shared resource but also fitness costs to both individuals, while dove-dove interactions result in a shared resource and no fitness costs. Between-morph Hawk-Dove interactions result in the resource going to the hawk while the dove gets no resource but also incurs no cost. In this game, hawks act as the A morph in the conceptual game above and doves act as the B morph. It is important to note that independent of the Hawk-Dove characterization, provided that payoffs of interactions follow the relationships of the A/B morph game, NFDS is expected to occur.

NFDS resulting from different interaction costs as predicted by Hawk-Dove models have been observed in real world animal systems. For example, outcomes consistent with Hawk-Dove models were observed with respect to head colour polymorphisms in Gouldian finches (Erythrura gouldiae) (Kokko, Griffith, & Pryke, 2014) and competitive behaviour in house sparrows (Passer domesticus) (Johnson et al. 2004). While some studies suggest that aggressive interactions can be consistent with Hawk-Dove predictions (Kilgour, McAdam, Betini, & Norris, 2018; Lichtenstein & Pruitt, 2015), there is no clear connection between aggressive behaviour and the Hawk-Dove paradigm resulting in NFDS.

In this study, we sought to understand how within- and between-morph aggressive interactions and their associated fitness consequences could lead to NFDS. We placed individually marked fruit flies (Drosophila melanogaster) in groups that differed in composition, such that individuals from a more aggressive strain were common (and paired with a rarer less-aggressive strain) or rare (and paired with a more common less-aggressive strain). We quantified aggressive interactions within each group and subsequent survival of individuals from aggressive and less-aggressive strains during a subsequent period of limited food resources.

This set of experiments follow up previous work that demonstrated NFDS on aggressive and less-aggressive strains in this species during periods of food limitation (Kilgour et al., 2018).
Generally, *D. melanogaster* offers a unique opportunity to empirically test proximate behavioural mechanisms driving NFDS, as the more-aggressive and less-aggressive strains have separate genotypes that exhibit substantial differences in aggressive behaviour (Shorter et al., 2015).

We examined two hypotheses to understand how aggressive interactions among flies results in the previously documented NFDS, using a similar methodology to our previous study (Kilgour et al. 2018). We placed flies from either a more-aggressive or less-aggressive strain in a limited resource environment, wherein only 40% of individuals survived. However, in this experiment we proportionally reduced food availability and the number of individuals in the vial to be able to document individual aggressive events between marked individuals. By observing individual interactions, we were able to measure the behaviour of each individual and how aggressive interactions impacted individual survival. Direct examination of aggressive interactions in this study allowed us to test the two hypotheses (predictions described in Figure 2.1). Following the Like-Competes-with-Like hypothesis, we predicted that within-strain interactions would occur more frequently than expected by chance and that within-strain interactions would be more costly to survival than between-strain interactions (Figure 2.1). For our second hypothesis, Hawk-Dove, we predicted that the aggressive strain would mimic hawks such that within-strain encounters would be more costly than between-strain encounters, while the less-aggressive strain would mimic doves such that between-strain encounters would be more costly than within-strain encounters (Figure 2.1).

**Methods**

To test the underlying behavioural mechanisms of NFDS on aggression, we placed individually marked flies in a 4-day period of limited resources and video recorded behavioural interactions during eight 15-minute-long feeding periods, twice daily throughout the experimental time period. Treatment groups were composed of mixed ratios of two strains of flies that differ in their aggression, each at 1:3 or 3:1 ratios. Previous studies have found that these two strains
experience negative-frequency dependent survival when placed in a period of limited food (Kilgour et al., 2018). In the current study, we were interested in the aggressive behaviour of individuals, how they interacted during periods of limited resources, and the survival consequences of behavioural interactions.

**Study species and strains**

We used the fruit fly *Drosophila melanogaster* as our study species because it has previously been used to study various aspects of social evolution, such as the evolutionary consequences of mate choice (Billeter, Jagadeesh, Stepek, Azanchi, & Levine, 2012), intrasexual competition (Baxter, Barnett, & Dukas, 2015), and social group preferences (Saltz & Foley, 2011). In this study, as in other social behaviour studies (Anderson, Scott, & Dukas, 2016; Saltz, 2013), we used naturally inbred strains selected from the *Drosophila* Genetic Reference Panel (DGRP). DGRP strains were originally derived from a population in Raleigh, N.C. (Mackay et al., 2012) and developed for use in genome-wide association mapping for use in quantitative trait loci analyses of behaviour, such as aggression (Shorter et al., 2015). In our study, we selected two strains (DGRP 380 and DGRP 712, hereafter 380 and 712) that previously had exhibited different levels of aggression (Edwards, Zwarts, et al., 2009; Kilgour et al., 2018; Shorter et al., 2015). In our study, flies from strain 380 exhibited higher levels of aggression than flies from strain 712 (Supplementary S2.2). Flies were obtained from the Bloomington *Drosophila* Stock Center (BDSC, https://bdsc.indiana.edu).

Replicate single-strain populations were maintained in separate 28 x 95mm vials with 10mL of sugar-yeast-agar food at a density of approximately 100 individuals per vial with approximately equal sex ratios prior to the experiment. All vials were maintained in consistent laboratory conditions (12:12 light:dark cycle, at 25°C with 40% humidity) and all flies were adapted to our lab conditions over 40 generations. The general consistency in adult density suggests that larval density was also relatively stable across generations, although we did not measure this. All fly populations followed a 14d life cycle: adults bred for 3d and larvae were allowed to develop for 11d. Adult flies were removed within 24 hours of eclosion and placed in fresh breeding vials.
Social groups

In this study, we ran 10 replicates of two treatment groups: one composed of approximately 23% 712 strain and 77% 380 strain, and another composed of approximately 77% 712 strain and 23% 380 strain. Each social group contained 30 flies: 7 individuals of the strain at low frequency and 23 individuals of the strain at high frequency. Sex ratios were kept even within the strains by alternating odd or even numbers between replicates, such that there were 11 or 12 males or females at high frequency and 3 or 4 males or females at low frequency. Social groups were created from flies no older than 24hrs post-eclosion. We applied 1 of 15 different colour combinations to the thorax of each fly using two colours of acrylic paint to males and the same 15 combinations to females, as males and females are easily distinguished on camera based on body size and different coloured abdomen. Individuals used in our experiments were randomly selected from natal population vials and lightly anesthetized using carbon dioxide and painted with a unique paint pattern with acrylic paint. Afterward, each fly was placed in individual glass vials containing 1.5-2mL of sugar-yeast-agar food medium. Following application of the unique markings, each fly was returned to their glass vial and allowed to recover for 24h before being placed in their social group. Social groups were allowed to acclimatize in a 28 x 95mm vial with 5mL of sugar-year-agar food for another 24h before commencing the period of limited resources.

Period of limited resources

We placed all social groups in a period of limited resources for 4d to create an environment of intense food competition. Our previous study demonstrated that negative-frequency dependent survival occurs between strains when provided with a limited amount of food, dispensed from a single location (Kilgour et al., 2018). Treatment groups of 30 flies were placed in a 28 x 95mm vial wherein 10µL of 5% sugar water was dispensed from a single location at the top of the vial twice daily. A single patch of food was created using a 2.0mL microcentrifuge tube (Fisherbrand, USA) with a hole pierced in the bottom. Pilot studies found that 30 flies fed 10µL of sugar water twice per day experienced approximately 40% survival, indicating that 0.67µL of 5% sugar water per fly per day could create an environment wherein individuals might compete for food resources. Treatment groups were fed twice per day (between 8:30 – 9:30 and 15:00 -
17:00h) for 4d (Betini et al., 2013a, 2013b; Kilgour et al., 2018), for a total of 8 feeding periods. In this environment, females are unable to produce eggs as no protein medium was available (Bownes & Blair, 1986; Terashima et al., 2005). Therefore, although males and females could interact, court and potentially mate, successful reproduction did not occur. Video recordings (described below) indicated that copulation did not occur, although males would occasionally attempt to court females (RJK, pers. obs.). Following the period of limited resources, surviving flies were identified based on individual paint pattern.

**Behavioural analysis**

By examining aggressive interactions while foraging, we were able to gauge how aggressive behaviours, both exhibited and received, impacted survival and resulted in negative-frequency dependent survival, as well as the identity of both interactants. All 8 feeding periods were recorded for 20min using a Sony Handicam (DCR-SR12, San Diego CA, USA).

Data were extracted from videos following completion of the experiment. Behavioural data were scored from videos starting as soon as food became available to the flies and lasted for 15min following presentation of the food because food was consumed within 5-12min, depending on the feeding period. Behavioural observations were recorded when individuals were within one body width of the food tip, indicating that the individual could be feeding. Therefore, the area within one body length of the food patch was designated the ‘observation zone’. The camera was placed directly in front of the vial, focused directly at the feeding tip, allowing us to view one half of the feeding patch. Given that we could only view behaviour from the angle at which the camera was placed, as soon as an individual was not visible to the camera, it was marked as having left the food patch. For logistical reasons, we could not determine exactly if an individual was feeding while it was present in the observation zone. We recorded when an individual arrived at the food patch and when it departed, as well as the frequency of visits to the food patch.

While in the observation zone, we recorded all incidences of head butts and lunges, as well as the identity of the actor and the recipient. Lunges and headbutts are often used as
behavioural expressions of aggression in *Drosophila* (Chen, Lee, Bowens, Huber, & Kravitz, 2002; Nilsen et al., 2004; Penn et al., 2010; Ueda & Kidokoro, 2002). We tracked use of both behaviours in both sexes, as our experimental environment was different from most aggression assays (typically via dyadic assays; Fernández et al. 2010a; Saltz 2013; Kilgour et al. 2019) and flies may behave somewhat differently in this different context. Videos were analysed by a single observer (RJK) and observations were conducted with no knowledge of the identity or strain of individual flies. Data were collected using BORIS software (http://www.boris.unito.it/).

For each of the 20 replicates, we recorded behavioural data across all feeding periods. A "feeding period" refers to the time period during which foraging occurred, when food was available. Therefore, we recorded 8 feeding periods during this experiment. During feeding period 1, there were very few behavioural interactions and almost no aggression, as flies were likely not food-motivated at this time, having experienced the limited resource period for only 1-2hrs. The greatest mortality was observed during the first 24hrs of the experiment (Supplementary S2.1), indicating that events occurring during feeding period 2 may have been the most influential for surviving individuals, as competition was strongest. Additionally, given that group size decreased following feeding period 2, the relative frequency of each strain also changed. Therefore, to control for density and frequency, we only considered behavioural data from feeding period 2 for all behavioural analyses.

**Statistical Analyses**

Before conducting behavioural analyses, we tested for frequency-dependent mortality following the period of limited resources. We used a binomially distributed Generalized Linear Model (GLM), with strain, frequency and their interaction as predictors of survival (binary response), where a frequency*strain interaction provided evidence for NFDS.

Prior to testing predictions from the two hypotheses, we analyzed the aggressive behaviour of flies from each strain, to address the premise that individuals within each strain behave consistently regardless of their social environment. That is, we expected individuals to exhibit consistent levels of aggressive behaviour as expected by their strain regardless of their
frequency. That is, at every interaction, flies from the aggressive strain and the less-aggressive strain should exhibit similar levels of aggression regardless of whether the strain is common or rare. To investigate this, we examined the aggressive behaviour of flies from the aggressive and less-aggressive strain at both frequencies: when common and when rare. Using a Poisson-distributed GLMM with log link function, we examined the effects of frequency, strain and their interaction on the number of aggressive events given and, in a separate model, aggression received. Our response variable (aggression given) was measured at the level of the individual. In these models, vial and individual identity were incorporated as random effects. This individual-level random effects was included to account for overdispersion in this Poisson model (Harrison, 2014).

Our first hypothesis, Like-Competes-with-Like, predicted that within-strain encounters would occur more often than expected by chance. In our frequency treatments, one strain was approximately three times more common than the rare strain, and thus the expected number of interactions within and between strains depended on their frequency in the vial. To determine the prevalence of within- and between-strain encounters, we extracted data of all dyadic encounters: wherein the identity of the giver of the aggression and the receiver could be identified. It should be noted that in many observations of aggression, we were unable to identify the recipient, as flies typically clustered at the food tip during feeding. Therefore, all analyses involving dyadic encounters represented a subset of the aggressive behaviours observed where the identity of both flies was known. To test encounter rates, we used 2x2 Chi-square tests on all dyadic encounters (df = 1).

In both hypotheses, we were interested in the fitness consequences of within- and between-strain interactions. In Like-Competes-with-Like, we expected within-strain interactions to negatively affect survival for both strains, whereas between-strain interactions would incur lower costs, as they would occur less often than expected by chance. Under the Hawk-Dove hypothesis, the intensity of fitness costs was predicted to differ depending on which flies were interacting. As described in the classic Hawk-Dove payoff matrix, interactions between hawks incur costs, whereas interactions between doves incur shared resources with no costs. Therefore, we first tested how encounters within and between strains impacted overall survival. Similar to
testing for encounter frequency, above, we used the subset of dyadic encounters wherein the identity of both individuals involved in an interaction was known. To test how within- and between-strain encounters ultimately impacted fly survival (a binary response), we used a binomially distributed GLMM with a logit link function. We tested whether the survival of individual flies was predicted based on the frequency of within strain encounters and between-strain encounters. The strains were modelled separately, and var was incorporated as a random effect.

All analyses were conducted in R version 3.5.3 (R Core Team, 2015), using the packages lme4 (Bates et al., 2014) for mixed models and DHARMa (Hartig, 2019) to examine model fit. Fixed effects were considered significant at $\alpha = 0.05$. We have noted situations where p values were greater than 0.5, but less than 0.1.

**Results**

The survival of flies in our experimental social groups was influenced by an interaction between strain and frequency on survival ($\beta = -0.84 \pm 0.41, p = 0.04$), providing evidence that survival was negatively frequency-dependent, where strains experienced greater survival when rare. Unlike our previous study, we observed significantly greater overall survival among individuals from strain 712 ($\beta = 1.09 \pm 0.36, p < 0.01$). Interestingly, we also found that females experienced greater survival than males (mean proportion survived ± SE, females: 0.60 ± 0.03; males: 0.21 ± 0.02), another observation not found in a previous study (Kilgour et al., 2018).

Of the 600 flies included in the experiment, we observed 335 individuals in the second feeding period. Flies spent an average of 283s in the observation zone (range: 1 – 1,456s). We counted a total 826 incidents of aggression given (average 2.5/fly; range 0-24) and 323 incidents of aggression received (average 1.0/fly; range 0-18). There were 80 dyadic encounters wherein both individuals could be identified.

Prior to testing predictions from the two main hypotheses, we assessed whether the aggressive and less-aggressive strains behaved consistently across frequency treatments or if they were influenced by their social environment. Overall, flies from both strains behaved as
expected, with 380 flies exhibiting more aggression than flies from strain 712 (Supplementary S2.2), but the aggression of each strain was also affected by the social environment. Results of GLMM on aggression given by flies demonstrated a significant effect of strain, frequency and their interaction (Table 2.1). The significant strain*frequency interaction indicated that flies from the two strains behaved differently in the two frequency treatments (Figure 2.2; Table 2.1). Individuals from strain 380 (the more aggressive strain) reduced their aggression substantially when at high frequency, whereas the flies from strain 712 (the less-aggressive strain) exhibited a marginal increase in aggressiveness at high frequency (Figure 2.2). In contrast, there was no effect of aggression received by individuals across frequency treatments (Figure 2.2 bottom panel; Table 2.1).

To test our hypotheses, we examined encounter frequencies both within and between strains, as well as how encounters influenced survival. In contrast to the prediction of the Like-Competes-with-Like hypothesis, we found no evidence of non-random interactions in frequency treatments when strain 380 was common ($\chi^2 = 0.09$, df = 1, $p = 0.77$), nor when strain 712 was common ($\chi^2 = 0.21$, df = 1, $p = 0.64$; Figure 2.3). The Like-Competes-with-Like hypothesis predicted that within-strain encounters would negatively impact survival and between-strain encounters would have no effect (Figure 2.1). However, our model results showed no evidence that within-strain encounters were associated with fitness costs (Table 2.2). The Hawk-Dove hypothesis predicted that the fitness consequences of competitive encounters would depend on the strain of both players. For strain 380, the aggressive strain, we expected within-strain encounters to have a negative influence on survival, whereas between-strain encounters would have a positive influence on survival. For strain 712, the less-aggressive strain, we expected within-strain encounters to have a positive influence on survival, whereas between-strain encounters would have a negative effect on survival. However, our results were not consistent with either prediction (Table 2.2). In strain 380 flies, we found that within-strain encounters had marginally statistically-significant positive effects on survival. In contrast, for strain 712, between and within-strain encounters had a negative effect on survival, although not statistically significant (Table 2.2). Together, these results suggest that, overall, a greater number of aggressive encounters increased the survival of flies from the more aggressive strain (380), but
might reduce the survival of flies from the less-aggressive strain (712; Figure 2.4), and that flies altered their aggressive behaviour in response to their social environment.

**Discussion**

We used experimentally manipulated social groups to examine leading hypotheses addressing the proximate behavioural mechanisms behind NFDS on aggression. Previous hypotheses have suggested that NFDS can result from interaction biases within morphs (as described in the Like-Competes-with-Like hypothesis), or different consequences of within- and between-morph interactions, mirroring Hawk-Dove models. However, we found no support for either of our a priori hypotheses. Instead, we propose a post-hoc hypothesis describing an entirely new mechanism based on random interactions, social plasticity and disruptive selection.

When encounters are random, as we found, classic Hawk-Dove models predict that coexistence between hawks and doves can occur through NFDS when the costs of within-strain interactions are greater than between-strain interactions for one morph, and the reverse for the second morph (Maynard Smith & Price, 1973). Contrary to the predictions of Hawk-Dove, we found interactions between flies from the less-aggressive strain (“doves”) decreased mean fitness, whereas interactions with and between flies from the aggressive strain (“hawks”) increased mean fitness, although our results were based on marginal statistical significance. The difference between our expected and observed results may be a result of how encounters are classified. In classic Hawk-Dove models, encounters are defined as “meetings”, where interacting individuals either exhibit aggression or not. In our study, interactions were recorded based on aggression given and received by flies, and therefore, all encounters, including those between flies from the less-aggressive strain, were rooted in aggression. Although less aggressive than the aggressive strain (Figure 2.2; Supplementary S2.2), flies from strain 712 still exhibited some aggression. However, regardless of how encounters were classified, our resulting payoff matrix differed from the expected results of Hawk-Dove game theoretic models.

The negative effects of aggressive interactions on the survival of flies from the less-aggressive strain suggest that aggressive interactions themselves may have been more costly for
flies from this strain. In groups of dominant and subordinate individuals, the use of aggressive behaviours by subordinate individuals can impose costs of fighting without the benefits of gaining access to the resource in question (Maynard Smith, 1974, 1982; Senar, Camerino, & Metcalfe, 1989). If a subordinate individual engages in physical combat with a more dominant individual, the fitness consequences of that interaction are much greater than if it had retreated (Maynard Smith, 1974). In contrast, phenotypic traits of dominant individuals may be more suited for physical combat such that the utility of aggression is beneficial (Clutton-Brock et al., 1979). A similar pattern may be occurring between flies of the two strains in our study. However, if one strain has a competitive advantage in its use of aggression, then NFDS, and ultimately the coexistence of the two strains, would not occur, as aggressive individuals would consistently outcompete less-aggressive individuals. In other words, our data suggest that individuals from the aggressive strain had higher survival when they exhibited greater aggression and that individuals from the less-aggressive strain may have had higher survival when they exhibited less aggression.

We found that flies from the aggressive strain (380) incurred benefits from aggression, whereas flies from the less-aggressive strain (712) may have incurred costs from the use of aggression (Figure 2.4). This association between levels of aggression and the direction of selection on aggression is consistent with disruptive natural selection (Brodie et al. 1995). Disruptive selection occurs when multiple phenotypes are favoured, maintaining polymorphisms as each have equal or comparable fitness (Mather, 1954). Disruptive selection on aggression in male-male competition has been identified as an essential mechanism in speciation (Dijkstra & Border, 2018). Furthermore, experimental study of the social niches of D. melanogaster have revealed that aggressive individuals typically form smaller groups than less-aggressive individuals and that flies with the highest mating success were those whose social niche was best suited to their phenotype (Saltz & Foley, 2011). The observations that individuals exhibiting different levels of aggressiveness create different social groups and that individuals create social groups that best facilitate their mating success, demonstrate how the social environment can act as a selective force, potentially resulting in the coexistence of aggressive and less-aggressive phenotypes by disruptive selection. However, on its own, disruptive selection on the fly strains
included in our study would not necessarily result in the maintenance of both strains in the population with random interactions. For example, if less-aggressive encounters are beneficial for flies from strain 712, then interactions with flies from strain 380 would result in fitness costs. As such, when rare, flies from both strains would experience fitness costs, preventing coexistence. Therefore, there must have been additional factors which led to reduced survival of individuals when their strains occurred at high frequency.

Our empirical results suggest a potentially new mechanism driving NFDS on aggression through a combination of disruptive selection on aggression and social plasticity, although future experiments are necessary to test this hypothesis. Individuals often display plasticity in aggression in response to changes in social group composition (Dyer et al. 2008; Webster and Ward 2011, Kilgour et al. 2019). Although aggressive flies in our study remained more aggressive and less-aggressive flies remained less aggressive overall (Supplementary S2.2; Chapter 3), flies from both strains altered their aggression in response to the composition of the social environment. In other words, both strains induced a social effect, although in different directions. Flies from the more aggressive strain (380) induced a general reduction in aggression in flies from both strains, whereas flies from the less-aggressive strain (712) induced a general increase in aggression in other flies. This suggests a negative association between the direct effect of each strain (the aggressiveness of the strain) and its social effect (a strain’s effect on the aggression of other individuals in the social group). As a result, when fly strains were common, their behaviour was modified to become less extreme through social effects: flies from the aggressive strain became less aggressive and flies from the less-aggressive strain became more aggressive. In contrast, when strains were rare, the negative association between the direct and social effects resulted in the amplification of their inherent aggressiveness. Since these negative social effects occurred in association with disruptive selection where more extreme phenotypes were favoured, this social plasticity enhanced the fitness of the rare strain while reducing the fitness of the common strain.

The strains in this study were distinct genotypes that have been used in combination with other DGRP strains to study the genetic basis of aggression (Shorter et al., 2015). As unique genotypes, we might expect limited within-strain variation in aggression. However, the relative
aggressiveness of the strains used in our study (380 and 712) has differed among previous studies (Edwards, Zwarts, et al., 2009; Shorter et al., 2015), indicating that strain behaviour is not constant across all environments. Furthermore, a previous study comparing the two strains demonstrated plasticity in aggression resulting from variation in the social environment, although the relative aggressiveness of the strains remained consistent (Kilgour et al. 2019; Saltz 2013). Plasticity in aggression is typical across taxa, where expression depends on the value of a resource (Brown, 1964) or differences in hierarchical ranks of individuals (McGhee & Travis, 2010). Given the potential costs of engaging in aggressive interactions, including the potential for injury or death, it is expected that individuals should employ aggressive tactics optimally (Maynard Smith, 1974). The phenotypic composition of a group, based on different aggressive phenotypes, has been shown to result in modifications of individual aggressiveness in water striders, *Aquarius remigis* (Sih & Watters, 2005). Using quantitative genetic analyses, Wilson et al. (2009) found that aggression in mice (*Mus musculus*) is highly influenced by the aggression of the opponent as well as an individual’s own level of aggressiveness, demonstrating the intensity of social influences on aggression. In our study, flies from both strains differed both in their inherent levels of aggression and in their social effect on other flies: flies from the aggressive strain induced a decrease in aggression in both strains and flies from the less-aggressive strain induced an increase in aggression in both strains. Plasticity in response to group phenotypes is common and can influence the direction of selection (Farine et al., 2015). At high frequency, we found that each strain modified their aggressiveness in a manner which increased their fitness costs and ultimately resulted in reduced survival.

We found variation in fitness consequences arising from both within- and between-strain interactions, as well as social plasticity in aggression, and the combination of these two factors may result in NFDS. In both strains, individual aggressive behaviour shifted in a maladaptive direction when common based on the consequences of within-strain encounters. Although not statistically significant, our data suggested that aggressive encounters for less-aggressive flies (strain 712) incurred survival costs. If this were the case, then flies from the less-aggressive strain should further reduce their aggressiveness, and when rare, less-aggressive flies did exhibit a reduction in their aggression, resulting in improved fitness. However, less-aggressive flies
caused an increase in aggression in other flies, including among flies from the less-aggressive strain. This social effect had detrimental consequences when common, as the increase in aggression in flies from strain 712 resulted in their reduced survival. In contrast, individuals from the more-aggressive strain (strain 380) benefited from aggressive encounters, suggesting that the use of aggression increased the survival of flies of this strain. Indeed, flies from strain 380 increased their aggression when rare, but reduced their aggressive behaviour when common. The presence of aggressive flies caused a reduction in aggression among flies, regardless of their strain. Therefore, when common, a reduction in aggression in flies from strain 380 could potentially result in reduced mean strain survival, given that aggressive interactions are beneficial for 380 flies. It is possible, therefore, that NFDS in aggression results from a combination of disruptive selection and a negative association between a strain’s aggression level and its effect on the aggression of other flies. Although no other study has yet documented NFDS on aggression resulting from the same mechanisms as we observed, a combination of individual and social factors resulting in NFDS has been observed in other species. For example, in *Pundamilia* cichlids, red males exhibit interaction biases, wherein they preferentially interact with their own morph (an individual factor), while simultaneously having social dominance over blue morph males when blue males are common (a social factor; Dijkstra et al. 2010). Future research is needed to test this potential new mechanism, wherein a combination of disruptive selection and social plasticity result in NFDS on aggressive phenotypes. In systems where disruptive selection has been observed in alternative aggressive phenotypes, as in the *Pundamilia* cichlids, further exploration into social plasticity and the within and between-morph consequences could provide evidence into when disruptive selection results in speciation, as observed, and when it results in the maintenance of alternative aggressive phenotypes within species. Additionally, mathematical models can provide insight into the extent of the social plasticity required for disruptive selection on behavioural traits to result in NFDS, and not niche specialization (Montiglio, Ferrari, Réale, & B, 2013) or speciation (Dijkstra & Border, 2018).

In our study, we compared two leading hypotheses to understand the behavioural mechanisms explain how aggression could lead to NFDS between two ‘types’ of individuals. While interaction biases and Hawk-Dove dynamics are known drivers of NFDS, we found no
support for these hypotheses. Instead, we found support for a novel and potentially general mechanism, through a combination of social plasticity and disruptive selection. Although neither phenomenon could result in NFDS on its own, the combination allows for aggressive and less-aggressive phenotypes to coexist across generations in competitive environments.
Tables

**Table 2.1.** The aggression exhibited by flies was significantly influenced by the frequency of strain in the group, wherein aggressive flies elicited more aggression when common than rare (Figure 2.2). There was no difference in aggression received based on frequency (Figure 2.2). Model results from Poisson-distributed GLMM with a log link function show significant effects of strain, frequency and their interaction on aggression given. Fixed effects in bold are significant at p < 0.05 and reference categories for fixed effects are in parentheses. Frequency was incorporated as a factorial variable (“common” or “rare”). Vial and Individual ID were included as random effects; Individual ID was included as a random effect to address overdispersion. Analyses were based on counts of aggression given and received (n = 335 flies) from 10 replicate vials per treatment.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Fixed Effect</th>
<th>B ± SE</th>
<th>Wald’s Statistic (χ²)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggression Given</td>
<td>Strain (712)</td>
<td>-1.71 ± 0.36</td>
<td>22.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Frequency (common)</td>
<td>-0.57 ± 0.28</td>
<td>4.25</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Strain (712) * Frequency (common)</td>
<td>1.02 ± 0.51</td>
<td>4.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Aggression Received</td>
<td>Strain (712)</td>
<td>-0.16 ± 0.48</td>
<td>0.11</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Frequency (common)</td>
<td>-0.15 ± 0.41</td>
<td>0.14</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Strain (712) * Frequency (common)</td>
<td>0.03 ± 0.69</td>
<td>&gt;0.01</td>
<td>0.97</td>
</tr>
</tbody>
</table>
Table 2.2. The survival of flies from strain 380 (the aggressive strain) was positively influenced by both within-strain and between-strain encounters, whereas the survival of individuals from strain 712 (the less-aggressive strain) was negatively influenced by within-strain encounters but positively influenced by between-strain encounters. Within-strain effects were marginally significant in predicting survival based on binomially-distributed GLMM with logit link. Survival was measured at the level of the individual, and within and between strain encounters were based on incidences where the giver and receiver of aggression were identified (n=80). Fixed effects underlined indicate statistical significance at $p < 0.1$. Vial was incorporated as a random effect.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Fixed Effect</th>
<th>B ± SE</th>
<th>Wald’s Statistic $\left(\chi^2\right)$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain 380</td>
<td>Within-strain encounters</td>
<td>0.15 ± 0.09</td>
<td>3.03</td>
<td>0.08</td>
</tr>
<tr>
<td>Survival</td>
<td>Between-strain encounters</td>
<td>0.02 ± 0.12</td>
<td>0.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Strain 712</td>
<td>Within-strain encounters</td>
<td>-0.05 ± 0.13</td>
<td>0.17</td>
<td>0.67</td>
</tr>
<tr>
<td>Survival</td>
<td>Between-strain encounters</td>
<td>-0.02 ± 0.15</td>
<td>0.02</td>
<td>0.88</td>
</tr>
</tbody>
</table>
Figures

<table>
<thead>
<tr>
<th>Focal fly</th>
<th>Interacting fly</th>
<th>Like-Competes-with-Like</th>
<th>Hawk-Dove</th>
</tr>
</thead>
<tbody>
<tr>
<td>380</td>
<td>712</td>
<td></td>
<td></td>
</tr>
<tr>
<td>380</td>
<td>Negative</td>
<td>Neutral</td>
<td></td>
</tr>
<tr>
<td>712</td>
<td>Neutral</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.1.** Qualitative predictions of fitness payoffs as outlined in our two hypotheses: Like-Competes-with-Like (left matrix) and Hawk-Dove (right matrix). In Like-Competes-with-Like, interactions are assumed to be non-random, where individuals express within-strain biases. In Hawk-Dove, each strain experiences different fitness outcomes in within- and between-strain interactions, assuming random interactions. Strain 380 is the aggressive strain, strain 712 is the less-aggressive strain. Fitness payoffs are based on the identities of the two interacting flies, the focal and the interacting fly. Grey boxes indicate within-strain interactions; white boxes represent between-strain interactions.
Figure 2.2. Aggression given by flies depended on their frequency (top panel), however there was no difference in aggression received based on frequency. Flies from strain 380 exhibited significantly more aggression when rare than when common and flies from strain 712 showed a
marginal change in their aggressiveness by frequency (model results in Table 2.1). As expected, strain 380 was more aggressive than strain 712, regardless of the frequency treatment. Diamonds represents the mean. Results are from all recorded incidents of aggression per fly.
Figure 2.3. Interaction rates between and within strains occurred as expected based on frequency (top panel = when strain 380 was common; bottom panel = strain 712 was common). Results are
from dyadic encounters wherein both aggressor and receiver were identified: both strain 380 and strain 712 exhibited aggression 3X as often toward individuals from the common strain. Black dashed lines represent expected between-strain encounter frequencies based on within-strain encounter frequency. In the top panel, the observed encounter frequency between strain encounters for flies from strain 712 was equal to the expected encounter frequency.
Figure 2.4. The payoff matrix indicates that within-strain and between-strain encounters incurred costs to survival for strain 712, whereas within- and between-strain encounters positively affected survival in strain 380. Estimates are from GLMM model (Table 2.2) with 95% confidence intervals. Grey boxes indicate within-strain interactions, white boxes represent between-strain interactions. In all cases, the confidence intervals overlap zero.
Chapter 3: carry-over effects of resource competition and social environment on aggression

Abstract

Aggressive behavior is common in many species and is often adaptive because it enables individuals to gain access to limited resources. However, aggression is also highly plastic and the degree of plasticity could be influenced by factors such as resource limitation and the social environment. In this study, we examined how the effects of social experience and resource limitation could persist to affect future aggressive interactions. Using naturally inbred strains of *Drosophila melanogaster* that differ in aggressiveness, we manipulated the level of available resources by varying fly density (2 treatments: high and low per capita resources) and group composition by varying strain frequency (5 treatments: a population composed of a homogeneous strain, or strains mixed at 1:3, 1:1 or 3:1 ratios of the more aggressive to less-aggressive strain). For each treatment group, we measured aggression before and after flies were placed through a 4-day period of fixed resources. There was no consistent effect of resource availability on aggression. Instead, carry-over effects and the resulting changes in aggression depended on resource availability in combination with group composition. In homogeneous groups made up of only one strain, all males became more aggressive following the fixed resource period, regardless of fly density. In mixed strain treatments at high density, we observed plastic shifts in aggression of males from both strains, but the direction of plastic responses depended on social composition. Our results show that aggression may not only be influenced by the intensity of previous competitive experiences caused by resource limitation, but also through social effects caused by the composition of the group.
Introduction

Aggression is a common behavior observed in almost all animal species and is often associated with resource defense or resource competition. Aggressive behaviors can yield fitness benefits by improving access to food (Goldberg, Grant, & Lefebvre, 2001), mates (Baxter et al., 2015; Smith & Blumstein, 2008), or nesting sites (Duckworth, 2006). In competition or defense, animals can use a variety of aggressive behaviors, ranging from low cost signals to high cost contact behaviors. Because some aggressive interactions can be harmful or fatal, animals will often reserve these potentially costly behaviors for situations where the perceived risk, or loss of a resource, is high (Arnott & Elwood, 2008; Mohamad, Monge, & Goubault, 2010). In competitive interactions over novel or “un-owned” resources, individuals who engage in more intense levels of aggression are more likely to successfully gain access to the resource, and thus are often considered to have a greater competitive ability (Brown 1964; Syme 1974; though see Camerlink et al. 2015). Therefore, an individual’s ability to use aggression in competitive environments can provide preferential access to limited resources. However, given the potential costs of aggressive behavior, it may be important for animals to vary their behavior to maximize the difference between benefits and possible costs.

Aggression is often a highly plastic behaviour, and the degree or intensity of aggression expressed by an individual is often determined by the environmental context. Animals typically exhibit more aggressive behaviors in resource-limited environments than in resource-rich environments (Brown, 1964). If a scarce resource is easily defendable, such as an isolated food patch or discrete nesting locations, this may also increase the use of aggression in gaining and maintaining access to that resource (Sol et al., 2005). As resource abundance and distribution can fluctuate over time, selection should favour animals that are successful at gauging situations when aggression will most likely be beneficial and when it is risky. Although individuals who can exhibit plasticity in aggression may incur fitness benefits in unpredictable or fluctuating environments (Herborn, Heidinger, Alexander, & Arnold, 2014), plasticity can also be a costly trait (Nandy, Dasgupta, Halder, & Verma, 2016). Many different sensory systems are required for individuals to appropriately detect and/or learn environmental cues associated with a specific
environmental change (Mery & Burns, 2009; Snell-Rood, 2013). Furthermore, extensive study on behavioural syndromes (or animal personalities) and inter-individual variation indicate behavioural plasticity is constrained (Stamps, 2016). Certain aggressive phenotypes may be more constrained in their ability to exhibit behavioral plasticity, such as described through coping mechanisms (Coppens, de Boer, & Koolhaas, 2010). Along a proactive-reactive axis, proactive individuals tend to be bolder and more aggressive while also less sensitive to their surroundings (Koolhaas et al., 1999). Reactive individuals, in contrast, are highly sensitive to changes in their surrounding environment and may exhibit greater plasticity as a result (Koolhaas et al., 1999). The extent of behavioural plasticity may, thus, differ between individuals with different personalities (N. J. Dingemanse, Kazem, Reale, & Wright, 2010). Therefore, an individual’s behavior likely relates to multiple internal state characteristics as well as features in the environment, such as group composition (Stamps, 2016).

Aggression is an inherently social behavior, and as such, can also be heavily influenced by social context. In dyadic interactions, aggression can be influenced by the attributes of a social partner and this effect is often exacerbated in competitive environments (Briffa et al., 2015). For example, the intensity of aggressive behavior in both the elicitor and the responder can vary with body size or display of a partner (Arnott & Elwood, 2009; Hsu, Lee, Chen, Yang, & Cheng, 2008), or the presence of conspecifics, as seen in audience effects (Doutrelant, McGregor, & Oliviera, 2001; Dzieweczynski et al., 2005). The intensity of aggression an individual will exhibit can also depend on whether it is competing with a more or less dominant individual (McGhee & Travis, 2011; Ricci, Summers, Larson, O’Malley, & Melloni, 2013). In more complex groupings, group phenotype can also influence aggression (Farine et al., 2015). The timing of these effects can also be pivotal, wherein the social composition may influence an individual’s aggression during specific periods of life, such as during ontogeny (Herczeg et al., 2016; McGhee & Travis, 2011), or during periods of increased resource competition, although little is known on this subject.

Additionally, experiences in one environment can persist to impact behaviour in future and different environments. That is, social and environmental effects on behaviour can be additionally complex as they can carry-over across time and contexts (Stamps & Groothuis,
Developmental plasticity describes phenotypic variation as a result of external or environmental conditions experienced in the past. This form of plasticity is distinct from contextual plasticity, wherein within individual phenotypes varies in response to the immediate (or present) context or conditions, and from ontogenetic plasticity, which refers to variation in phenotypes resulting from experiences during specific age or life stage (Stamps, 2016). For example, developmental plasticity describes an increase in boldness following a risky experience with predators (Bell & Sih, 2007), whereas contextual plasticity describes an increase in courtship behaviour when an animal is exposed to a high preference mate (Wagner, Murray, & Cade, 1995). In contrast, an example of ontogenetic plasticity is when group density experienced as a juvenile alters the social tendencies (e.g. shoaling behavior, Chapman et al. 2008) in adulthood. Developmental plasticity thus describes how experiences in the past can carry over into the present, regardless of the similarities of the environments past and present. Variation in behavioural phenotypes may, therefore, be a result of previous experiences which alter the internal state of an individual.

In this study, we sought to understand how resource limitation interacts with group composition to impact aggression in two strains of fruit fly, *Drosophila melanogaster*, that differ in aggression level. Fruit flies are an ideal system for studying aggression. Studies on *D. melanogaster* have provided insight into the neurobiological and genetic processes involved in aggressive behaviors (Anholt & Mackay, 2012; Edwards, Zwarts, et al., 2009; Zwarts et al., 2011). Previous research has demonstrated that aggression in male *D. melanogaster* is sensitive to their current social group (Carazo, Tan, Allen, Wigby, & Pizzari, 2014; Saltz, 2016; Saltz & Foley, 2011), but the persistence of social effects on aggression has never been explored.

We measured aggression before and after groups of flies were exposed to a period of fixed resources across different social treatment groups and resource levels. In a study using the same strains, Kilgour et al. (2018) found that when flies are placed in groups at high fly density, both aggressive and less-aggressive strains follow a pattern of negative frequency-dependent survival (NFDS) following the period of fixed resources, such that rare strains experience greater survival than common strains. That is, both aggressive and non-aggressive strains experience positive fitness benefits when at low frequency in a social group when resources are limited. In
the same study, there was no difference in survival between the strains in homogeneous treatments, indicating neither strain had a survival advantage (Kilgour et al. 2018). From these results, it was clear that relative frequencies impacted survival, but it was unclear how the social experience impacted the aggressive behaviour of the surviving individuals. Here, we report plasticity in aggression in these two strains of flies before and after this period of fixed resources.

Given the effects of resource availability and competition on aggressiveness, we expected flies to exhibit behavioural plasticity following the period of fixed resources. We induced competitive and non-competitive environments by varying fly density and social composition by varying strain frequency to estimate their effects on plasticity in aggression. In doing so, we examined two alternative hypotheses that describe how plasticity in aggression could result in the NFDS that we observed.

First, flies could exhibit adaptive social plasticity, wherein some individuals (the surviving individuals) of the common strain adaptively adjust their aggression to mimic the rare strain, which has higher survival. In this case, the differences in survival between the frequency treatments are driving the plasticity in aggression. The fitness consequences of behavioural strategies according to the interacting phenotypes is described in Hawk-Dove theoretical models, wherein the advantages of being aggressive decline with as frequency increases (Maynard Smith & Price, 1973). If our results were consistent with Hawk-Dove hypotheses and aggressive behaviour is only advantageous when rare, we would expect the surviving aggressive individuals to switch to a less-aggressive strategy at higher frequency, and less-aggressive individuals to increase their aggressiveness when their frequency increases, thus reducing their chance of survival. However, aggressive behavioural strategies may amplify due to increased within-strategy interactions when at high frequency. For example, increasing within strategy (or strain) interactions cause individuals to double-down on their aggressive strategy, such that the behavior of a particular strain is amplified when that strain is more common. Therefore, when aggressive individuals are common, they become increasingly aggressive, and flies from the less-aggressive strain further reduce their aggression when common.
Our second hypotheses reflects reactive aggression, wherein individuals are more likely to exhibit aggression when they experience (or receive) aggression (Branch, Kozlovsky, & Pravosudov, 2015). Social plasticity resulting from aggressive (or non-aggressive) reactions to the aggressive strategy of interacting individuals could increase the strength of NFDS. However, this would represent maladaptive plasticity because rare individuals are shifting their aggression toward the more common phenotype, thereby further reducing their survival. Under both hypotheses, we expect social plasticity in aggression to result from resource limitation, and thus competition. As such, we predict social effects to occur in high-density treatments, but no changes in aggression in low-density treatments.

Methods

In this experiment, we measured the aggressive behavior of two strains of Drosophila melanogaster before and after they were subjected to experimental treatments. Treatment groups varied by fly density and the frequency of strains, allowing us to assess the effects of both group size and group composition on aggressive behavior. All treatment groups were placed in a period of fixed resources for 4 days, and flies were tested for aggression before and after this period. The experimental protocol can be found in Figure 3.1.

Fruit flies were obtained from the Bloomington Drosophila Stock Center (https://bdsc.indiana.edu) and were adapted to our lab conditions for over 20 generations. Strains were selected from the Drosophila Genetic Reference Panel (DGRP), originally derived from a population in Raleigh, N.C. (Mackay et al., 2012). DGRP strains are naturally inbred strains which were developed for use in genome-wide association mapping, and have been applied in studies on the genetic basis of behaviors, including aggression (Shorter et al., 2015). The two strains used in this study, DGRP 380 and DGRP 712 (hereafter, 380 and 712) were selected for this study based on differences in previous aggression assays (Edwards, Zwarts, et al., 2009; Shorter et al., 2015), although we did not necessarily expect that these differences would be the
same under our assay conditions. In our study system, as in others (Edwards, Ayroles, et al., 2009), strain 380 exhibited greater levels of aggression than strain 712.

Prior to the experiment, both strains were maintained in groups of approximately 100 individuals per vial and were fed 10mL of sugar-yeast-agar food (see Betini et al. 2013a for recipe details) in 28 x 95mm polypropylene vials (VWR, Radnor PA). The sugar-yeast-agar medium acts as both food for adults as well as serving as an egg laying medium and a nutrition source for larvae. All groups were held in constant laboratory conditions of 12:12 light:dark cycle, at 25°C with 40% humidity. Population density remained relatively consistent (RJK pers. observation), indicating that larval density, and thus larval competition, remained roughly stable for all adults prior to our experiment. Fly populations followed a 14 d life cycle, where adults were allowed to breed for 3 d and larvae allowed to develop for 11 d. Sex ratios of populations were approximately equal throughout the duration of the experiment, as determined through periodic counting of populations. Breeding flies were removed from their natal vials within 24 hrs of eclosion.

Creation of social groups

A subset of the newly emerged adult flies from stock vials was used to create social groups for the 10 treatment groups. Prior to placement in their social groups, newly emerged flies (no more than 24 hrs old) were dusted with fluorescent powder (DayGlo Ltd, Cleveland OH), and randomly assigned one of three colours for later identification. Social groups were established using flies from multiple vials of stock populations for both strains. After flies were dusted, groups were lightly anesthetized using CO₂, counted and placed in one of the 10 social group treatments. During this time, sexes were counted to ensure equal sex ratios in treatment vials. Because social groups contained males and females, females were most likely mated prior to the start of the experiment.

Given that focal flies were removed from social groups for aggression testing prior to starting the period of fixed resources, groups were created with an extra 10-20 flies (depending on if the social composition was homogenous or heterogeneous). This ensured that group density
when place in the period of fixed resources was either 30 or 300 individuals. Flies were given 24 hrs to acclimatize to their social group treatment prior to beginning the period of fixed resources (see below), during which they were given ad libitum access to sugar-agar-yeast food medium. Social groups of flies were lightly anesthetized for transfer from their acclimatization vial into the vials used in the period of fixed resources. During this time, focal individuals were removed for use in “before” aggression assays.

**Fixed-Resource Period**

Animals tend to show higher levels of competition when resources are limited and only available in clumped patches (Grant, 1993). Therefore, social groups of flies were placed in a vial where a fixed quantity of food was provided in a single location. In this environment, flies were fed 100µL of 5% sugar water twice per day (between 8:30 – 9:30 and 15:00 - 17:00h) for 4 days (Betini et al. 2013a, 2013b, Figure 3.1). Food was dispensed from a single location at the top of the vial using 2.0mL microcentrifuge tube (Fisherbrand, USA) with a hole placed in the bottom, thus allowing only a few flies to feed at a time and creating a more competitive environment, as access to food resources were restricted (Grant, 1993; Johnson et al., 2004). This feeding system only allows approximately 8-10 individuals for forage at the same time (RJK pers. observation). During this period of fixed resources, males and females could interact but successful breeding did not occur in this environment as females were not provided with sufficient protein medium to produce eggs (Bownes & Blair, 1986; Terashima et al., 2005). Following the 4 d period of fixed resources, flies were lightly anesthetized using carbon dioxide during which they were counted and sorted by strain before aggression was assayed. The sex ratio of surviving individuals was assessed and confirmed as consistent based on visual assessment (RJK *pers. observation*). We did not record behaviour of flies during the period of fixed resources.

**Social Density Treatment**

We compared the effects of a competitive and non-competitive environment using high and low density treatments. In varying group size, instead of food quantity, we were able to directly examine the effect of group size and competition. We recently demonstrated negative frequency-
dependent survival at high density when these two strains were placed in a period of fixed food resources (Kilgour et al., 2018). Both high- and low-density treatments received the same amount of food per day, allowing us to create a treatment group where survival was limited by food availability (high fly density, mean survival 29.5%) and a treatment group where survival was not limited by food availability (low fly density, mean survival 96.4%) (Kilgour et al., 2018). Thus, experimental flies were placed in one of two density treatments: low-density groups of 30 individuals and high-density groups of 300 individuals. The low-density treatment can be considered a control treatment when assessing the effect of competition on individual aggressive behavior. We created 5 low-density and 5 high-density groups for each of the frequency treatments, described below.

**Social Composition Treatment**

In addition to estimating the effect of fly density (group size) on individual aggression, we also estimated the effect of group composition, as described in Kilgour et al. (2018). The impact of group composition was assessed by altering the frequency of each strain. We created 5 group composition treatment groups that were homogeneous, composed entirely of strain 380 or entirely strain 712, or heterogeneous. Three heterogeneous treatment levels were established where strains were mixed at an equal ratio (1:1) or unequal ratios of the two strains (3:1 and 1:3), representing scenarios where each strain was common and rare. In combination with the fly density treatments, we used a full factorial design, providing a total of 10 different treatments (2 densities x 5 frequencies) with 5 replicate groups per treatment, providing a total of 50 social groups. All replicates were established with an approximately equal sex ratio. In measuring the effect of group composition, the homogenous treatments were considered controls as they account for behavioral changes as a result of fly density with no strain frequency variation. Any inherent differences in the strains could be observed in homogenous social groups.

**Measuring Changes in Aggression**

We measured aggression in flies prior to, and just following, the period of fixed resources (Figure 3.1) to assess social plasticity in each strain. We define social plasticity as the effects of
social composition, or the density and the composition of a social group, on an individual’s aggressive expression. Prior to the period of fixed resources, and 24 hrs following initial creation of social groups, 4-6 males and 4-6 females from each strain were selected from each replicate group. Each individual was placed in a 12 x 75mm glass culture tube (VWR, Radnor PA) containing 1.5-2 mL of dead yeast-agar-sugar food medium. Due to logistical constraints, flies were held in glass vials for 1-3 d before the aggression assay, meaning they were between 3- to 6-d old during the first round of aggression assays. In a separate experiment using flies of the same strains where we assayed aggression of flies between 3 and 8d old, we found no effect age on aggressive behaviour in aggression assays for either males (Generalized linear model, Aggression ~ Age + Strain; Age: β ± SE, -0.06 ± 1.29, p = 0.20, n=48) or females (GLM. Age: β ± SE, -0.01 ± 0.19, p = 0.84, n=48). We repeated this sampling process after the period of fixed resources, wherein 4-5 individuals from both sexes and strains (in heterogeneous social groups) were placed in individual vials for 1-3 days, after which aggression assays were conducted. Therefore, we used different individuals to measure aggression “before” and “after” the period of fixed resources. In affording individuals a minimum of 24 hrs with ad libitum food resources, we could ensure that any observed changes in aggressive behaviours were not a result of food restriction. At both sampling periods, we tested approximately 10 flies from each homogeneous social group and approximately 20 flies from each heterogeneous group (10 from each strain). We included males and females in our experiment to assess any sex-related differences in plasticity. There was some variation sample size per treatment group due to incidental mortality of focal flies during the experimental protocol.

Aggression Assay

Aggression assays constituted measuring aggressive behavior from a focal fly when paired with an opponent of the same sex. All opponent flies were from an outbred population of D. melanogaster that has been maintained in cage culture at laboratory conditions since 1970, originally collected in Dahomey (now Benin). For opponent flies, pupae were isolated in their own glass vials containing 1.5-2mL of dead yeast-agar-sugar feed medium and eclosed adults remain in isolated vials until aggression assays, and thus female opponent flies were virgin. Trials were conducted within 3 - 6 d of opponent fly eclosion. Aggression assays followed those
described by Mundiyannapurath et al. (2007), where a focal fly and a socially-naïve opponent fly were placed in a square arena (2.5cm x 2.5cm) with a patch of dead yeast-agar-sugar located in a microcentrifuge tube screwcap (Fisherbrand, USA) and placed in the center. In isolating the opponent Dahomey flies as pupae, we were able to control the social experience of adults. Isolation as pupae can promote increased aggression in adult flies (Ueda & Kidokoro, 2002), and Dahomey flies exhibited an intermediate level of aggression between strains 712 and 380 (mean number of aggressive behaviours per trial ± SE; males: 7.47 ± 0.36; females: 7.21 ± 0.43). The aggressive behaviour of Dahomey flies was consistent between opponent strains and time of trial (Supplementary S3.1). This type of aggression assays has been repeatedly used in studies of fruit fly aggression of both sexes (Fernández et al., 2010; Ueda & Kidokoro, 2002). Each fly was painted with either a blue or yellow dot of acrylic paint on the thorax to allow for individual identification. Paint colour was randomly assigned and had no effect on aggression (t-test, t = 0.07, df = 1410.2, p = 0.94). Following 5 min of acclimatization, all behaviors were video-recorded for 30 min. Fresh weights were obtained from focal individuals following aggression assays. When trials were completed, videos were watched and the number of head-butts (females) and lunges (males) exhibited by each individual per 30-min trial were recorded. Therefore, individual aggression was determined based on the number of aggressive behaviours exhibited toward the opponent fly. This aggression assay was not meant to mimic contest interactions between the flies, and therefore we did not record data on defensive or retreat behaviours, nor did we determine any “winner” or “loser” from the aggression assays. Data from video recordings was recorded blindly, as observers were unaware as to the identity of the focal fly or its strain. Inter-observer reliability was assessed by testing observers with trial videos until the scored tallies of aggressive behaviours were within 90% score accuracy over a minimum of 5 trial videos. As in other studies (Chen et al., 2002; Saltz, 2013; Yurkovic, Wang, Basu, & Kravitz, 2006), only aggressive interactions that occurred on the food patch were considered in this analysis. All assays between males occurred between 0800 and 1100 and assays between females all occurred between 1300 and 1600. Following the aggression assays, individuals were returned to their glass vials and body mass was measured using fresh weight.
**Distinguishing Plasticity from Selection**

As stated previously, we did not measure the same individuals before and after the period of fixed resources. Instead, we inferred plasticity based on the difference in aggression between individuals from the same strain sampled before and after the period of fixed resource. However, given that not all individuals survived through the period of fixed resources, particularly in the high-density treatment, changes in mean aggression following the period of fixed resources could also result from natural selection (i.e. non-random survival based on aggression). That is, individuals exhibiting a certain amount of aggression were more likely to survive than others. To account for potential changes in aggression caused by natural selection, we calculated the between-strain selection differential based on between-strain differences in survival and aggression. For each vial in the mixed-strain frequency treatments, we calculated the between-strain selection differential as:

\[
S_B = \left( \frac{n_{Surv380} \times \overline{Agg380} + n_{Surv712} \times \overline{Agg712}}{n_{Surv}} \right) - \left( \frac{n_{Start380} \times \overline{Agg380} + n_{Start12} \times \overline{Agg712}}{n_{Start}} \right)
\]

Where \( n_{Surv380} \) and \( n_{Surv712} \) refer to the number of surviving individual from strains 380 and 712, respectively; \( \overline{Agg380} \) and \( \overline{Agg712} \) are the average aggression of each strain exhibited before the period of fixed resources; \( n_{Surv} \) is the total number of surviving individuals per vial; \( n_{Start} \) is the number of individuals in the vial at the start of the experiment. The second term in the equation, therefore, represents the mean aggression in the vial prior to the period of fixed resources, which is calculated based on the frequency of the two strains in the vial and their mean aggression. The first term in the equation is the expected mean aggression in the vial (i.e. in the absence of plasticity) based on the observed frequencies of the two strains following the period of fixed resources and their mean aggression prior to the period of fixed resources. We
then assumed that the within-strain selection differential was equal to this calculated between-strain selection differential and used the selection differential calculated for each vial to determine whether observed changes in aggression (plasticity and selection) exceeded the change that could be explained by natural selection alone. The observed average magnitude of plasticity per vial (absolute value of the change in aggression per vial; 4.71; regression slope: 0.99) was an order of magnitude larger than the average magnitude of selection (0.19; regression slope: 1.00). As such, observed changes in aggression (plasticity and selection) were very similar to those corrected for selection (slope = 0.99; Figure 3.2) and thus relatively insignificant, so we did not further consider the effect of selection on changes in aggression. Therefore, using the analysis above, we were able to assess the influence of natural selection on any observed changes in aggression and determined that this process was negligible relative to plasticity.

**Statistical Analyses**

Based on previous studies, we expected differences in aggression between the sexes (Nilsen et al., 2004; Penn et al., 2010). Therefore, all analyses were separated by sex. Body mass can also have an influence on aggression and, because we were interested in examining competitive aggressive behaviors, it is possible that changes in body mass might impact the aggression of individuals. To examine this, we fitted a general linear model, regressing strain, sex and time and all interactions therein on body mass. In all models, “time” is a dichotomous variable identifying when the trial occurred, either before or after the period of fixed resources.

Homogeneous treatments allowed us to examine whether there were any differences in aggression between strains based on fly density. A Poisson-distributed generalized linear mixed effect model (GLMM) with a log-link function was applied to homogeneous treatments, where fly density, strain and time and all interactions therein were incorporated as fixed effects. Vial, Observer and Fly ID were included as random effects. Observer refers to the person who extracted the aggression data from the video recording. Although there was only one observation per fly, we included an observation-level random effect (here, the identity of the individual fly, or “Fly ID”) to account for over-dispersion in the model (Harrison, 2014).
To measure the effect of fly density and strain frequency on the changes in aggressive behavior of flies, we used a GLMM with a Poisson error distribution and a log link function on heterogeneous treatments. With the data separated by sex, we estimated the effects of fly density, strain frequency, strain and time (before and after) and all subsequent interactions on aggression exhibited in aggression assays, including the 4-way interaction. The significance of fixed effects was assessed using Wald’s statistic, which is based on maximum likelihood and follows a $\chi^2$ distribution. To assist in interpreting a significant 4-way interaction, we further subsetted the data by fly density and ran a GLMM with frequency, strain and time as fixed effects. As with homogeneous models, we incorporated vial, observer, and observation as random effects.

All analyses were performed using R version 3.2.2 (R Core Team, 2015) and GLMM’s were constructed using R package lme4 (Bates et al., 2014). Fixed effects were considered significant at $\alpha = 0.05$. Model fit was assessed using diagnostic plots and scatterplots of residuals and predicted values and confirmed using R package DHARMa (Hartig, 2019).

Results

We did not find any significant predictors of body mass other than sex ($\beta = -0.49 \pm 0.02$, df = 1211, $p < 0.01$), where females (mean = 1.24 mg, SD = 0.22) were larger than males (mean = 0.76 mg, SD = 0.15; Table 3.1). The lack of a significant sex*time and strain*time interactions indicated there was no change in body mass in different sexes or strain types over the duration of the experiment. Although a reduction in body mass might be expected following the period of fixed resources, body mass was measured after flies had been placed in the glass vials containing dead yeast-sugar-agar food medium for a minimum of 24 hours. Therefore, a difference in body mass would not be expected between “before” and “after” aggression assays.

Next, we compared changes in the number of aggressive behaviours exhibited between strains in homogeneous groups. We had predicted there would be no change in behaviour in low density treatments, given that food was not limited and thus flies were not competing for access
to food resources. In comparing aggression between strains across the sexes, there was no difference in aggression between the strains in females (Figure 3.3, model results in Supplementary S3.2). However, we did find significant differences in aggression in males, both before and after the period of fixed resources (Table 3.2, Figure 3.3). In females, there was no evidence of any changes in aggression, either between strains or differences before and after the period of fixed resources, although there was a marginally non-significant effect of fly density (Table 3.2). While it appears that females from strain 712 increased in aggression over time (i.e. following the period of fixed resources), this effect was not statistically significant (Figure 3.3, Table 3.2). However, in male homogeneous groups, we found a significant effect of time, where males of both strains showed an increase in aggression following the period of fixed resources, but there was no effect of fly density on aggression (Figure 3.3).

In heterogeneous treatments, we were interested in how group composition influenced the change in the number of aggressive behaviors exhibited in both males and females. We found no evidence for an effect of group composition (frequency) or group size (density) on female aggression (Supplementary S3.2). However, in males, we found a significant 4-way interaction between strain*density*frequency*time ($\chi^2 = 4.18$, df = 1, $p = 0.04$, complete model results in Supplementary S3.3). We further subsetted the data by fly density to elucidate how aggression changed over time in each strain based on their frequency. In low-density treatments, there was no evidence that any of the fixed effects (strain, frequency, and time) or their interactions influenced aggression (see Supplementary S3.4). In contrast, at high density, there were significant effects of strain, frequency, and all interactions (Table 3.3). Strain 380 demonstrated consistently higher levels of aggression than strain 712, although strain 380 exhibited greater variance in aggression, both before and after the period of fixed resources (mean, variance; before: 380 = 11.05, 165.04; 712 = 3.36, 37.99; after: 380 = 9.44, 355.13; 712 = 4.01, 32.33). Both strains exhibited a decline in aggression in evenly mixed groups (Figure 3.4). Additionally, both strains exhibited an increase in aggression when rare (mean ± SE: 380 before = 8.36 ± 2.10; 380 after = 12.11 ± 5.89; 712 before = 2.40 ± 0.97; 712 after = 5.61 ± 1.31). There was no change in aggression in either strain when common.
Discussion

Our study demonstrates how the social effects of group size and composition in one environment can carry-over to affect aggressive behavior in different environments. In this study, we sought to examine whether experiencing periods where resources are limited influence aggression and if that effect is further impacted by social composition. We found that males of both strains modified their aggression in the aggression assays based on social experience in a past environment, which were the vials where groups were held with limited quantities of food. In other words, we observed plasticity in males according to their social experience, although we did not observe plasticity in females. This demonstrates how social influences can carry over from one context, where flies were in groups and may be experiencing competition due to resource limitation, into another, the dyadic aggression assays. These results indicate that periods where resources are limited can impact aggression in future contexts, although resource limitation is not always necessary to induce changes in aggression. Further, we found the direction of this behavioral plasticity is impacted by the density and composition of social environment experienced during that period.

Our results were inconsistent with both of our a priori hypotheses. We predicted that the strains would be affected by their social environment differently, but instead the social environment and resource availability affected each strain in a similar way among males. Our hypotheses were based on the premise of differences in survivability between the strains depending on their frequencies. Therefore, we predicted that any resulting changes in aggression would reflect differential survival of strains in mixed frequency treatments, such that shifts in aggression would occur when strains were at high or low frequencies. Although we did observe modification in aggression levels at different density and frequency treatments, our results indicate that the observed shifts are not a reflection of the negative frequency-dependent survival of strains. Instead, we found that specific social experiences result in changes in aggression, but not in ways predicted by NFDS.

We found that, at high density, group composition was a significant predictor of aggressiveness in males. In both strains, aggression increased when at a low frequency, showed a
reduction when strains were at equal frequencies, but showed no change when at high frequency. This differed from both of our hypotheses, in that males of the less-aggressive strain did increase their aggression when at low frequency (i.e. more similar to the common strain), the more aggressive strain also increased their aggression when at low frequency (i.e. more different from the common strain). Considering the more aggressive strain, it is possible that the competitive environment and subsequent greater survival of the rare strain led to some kind of winner effect, wherein winning a contest makes an individual more likely to win subsequent contests (Dugatkin, 1997). Acquiring access to the food resource and thus increasing chances of survival may reflect a winner effect, where results from interactions that led to increased access to the limited food resource are perpetuated in future interactions. Among dyadic contests, winner and loser effects do occur in *D. melanogaster*, although loser effects are often stronger than winner effects (Penn et al., 2010; Trannoy & Kravitz, 2017) and winner effects do not last longer than a few hours (Trannoy & Kravitz, 2017; Trannoy, Penn, Lucey, Popovic, & Kravitz, 2016). Furthermore, a winner effect would not explain why both strains decreased aggression when at equal ratios, where both strains exhibited equal survival. It is also possible that experiences of competition or specific social environments induced a carry-over effect that may not be adaptive, but are a result of changes in internal stimuli based on between-individual differences in aggressiveness (Sih, Bell, & Johnson, 2004), and the carry-over of environmental effects into different contexts results in fitness costs (Ferrari, Warren, McCormick, & Chivers, 2019). It is worth noting that we did not measure the aggression of flies during the period of fixed resources, and therefore cannot assume that aggressiveness related to any competitive advantage or that contests occurred during this period. However, previous research using the same strains under a similar period of fixed resources confirmed that aggression levels between the strains occurred as expected (Kilgour et al., 2018). More research is necessary to understand the mechanisms of social influences on future aggressive behaviour and the carry-over across contexts, as well as the effects of negative frequency-dependent survival, as we observed following the period of fixed resources (Kilgour et al., 2018).

Developmental plasticity describes how, and to what extent, past experiences can influence an individual’s current behavior (Stamps & Groothuis, 2010). We observed
development of aggressive behavior, as we found that experience in a specific social environment (i.e. fly density and frequency of each strain) produced a behavioral shift in a different environment. Interestingly, the observed developmental plasticity was independent of initial aggression levels. The carry-over effects of a social environment in one context to another, as observed in this study, provide insight into developmental plasticity of aggression, separately from ontogenetic processes. Such shifts in behavior resulting from behavioral plasticity have been shown in a variety of species. For example, the experiences of genetically identical mice alter the expression of exploratory behavior between individuals (Freund et al., 2013), demonstrating how unique experiences shape the development of individual behavior. Developmental plasticity resulting from a cross-context carry-over effect demonstrates a key feature in understanding variation in aggression within populations.

Past experiences can alter genetic, neural and hormonal states of an individual at the current time (Hsu et al., 2006; Stamps, 2016) and these physiological alterations may not change at the same rate as attributes of the environment, such as the social context. If the delay in carry-over effects were the case, then the consequential neural or hormonal responses may be expressed, not in the current context, but at a future time. In our study, the stimuli that triggered changes in aggressive behavior are unknown. Since differences in survival did not appear to be the main driver of the behavioural plasticity, there may have been other cues or stimuli during the period of fixed resources that lead to the observed changes in behaviour. For example, there may have been aromatic stimuli which varied across the social environments, leading to changes in behaviour. In our study, there was a time lag of 1 to 3 days between when the flies completed the period of fixed resources and when they underwent the second aggression assay, indicating that the carry-over effect was relatively long lasting. Carry-over effects caused by modifications in hormone levels or neurotransmitters are typically short-lasting (minutes to hours), as observed with octopamine levels in Field crickets, Gryllus bimaculatus (Adamo, Linn, & Hoy, 1995). Short-term changes in aggressive behaviour in D. melanogaster is affected by changes in protein synthesis (Trannoy et al., 2016). In contrast, iterative competitive interactions can result in longer term (days) changes in aggressive behaviour (Wilson et al., 2013). Long-term carry-over effects can result from changes in hormonal profiles, physiological changes such as body mass or
organ size, or even slow-changing neurological shifts (Niemela & Santostefano, 2015). Although our experiments were performed within a single generation and did not examine cross-generational effects, epigenetic modifications may also explain long-lasting changes in aggressive behavior in other scenarios (Crews, 2008). Further, while it is possible for certain phenotypes to exhibit plasticity in aggression while others cannot (see Dingemanse et al. 2010 for review), we found no evidence that aggressive or less-aggressive phenotypes were constrained in their ability to exhibit plasticity in males. Our results demonstrate the sensitivity of aggressive expression can be based on both past and present experiences. This pattern provides some insight into why animals may exhibit aggressive behavior, even in contexts where it is not beneficial.

We found somewhat inconsistent results between socially heterogeneous and homogenous treatments among males. When males experienced a homogenous social group, that is, composed only of their own strain, we observed an increase in their aggression, regardless of the fly density, indicating that food limitation (and thus competition) did not induce the change in behaviour. In contrast, when males were placed in mixed-strain groups, observed shifts in aggression occurred only when food was limited and when they were at low or equal frequency. In other words, when strains were at the high frequency, a social composition most closely resembling the homogenous treatments, we observed no change in aggressive behaviour. These results demonstrate the complexity of social experiences. Aggression in male *D. melanogaster* is affected by the presence of kin, as males exhibit reduced aggression to related males compared to unrelated males when in the presence of females (Carazo et al., 2014). In our experiment, males of the same strain have high genetic similarity, and thus, males of the same strain may be more genetically similar than full-siblings. Our results are inconsistent with those of Carazo et al.(2014), as males increased their aggression following experiences in homogenous groups, that is, groups with the highest degree of genetic similarity. Although we did not measure the social effects occurring during the period of fixed resources, it is clear that social makeup during that time influenced male aggression.

Our results showed considerable effects of the social environment on male aggression, but little to no effect on female aggression. One possible reason is that females lack the ability to
exhibit plasticity in aggression, as plasticity is a trait with costs. Another possibility is that the aggression assay used in this study is typically applied to males (Chen et al., 2002; Saltz, 2013; Yurkovic et al., 2006) and may not be suitable to measure female aggression. Although female aggression has been assessed using this type of assay, aggression levels are typically lower than males’ (Nilsen et al., 2004; Ueda & Kidokoro, 2002). Therefore, despite its wide usage, this assay may be geared toward eliciting male, and not female, aggression. In the context of our dyadic aggression assay design, females would be competing for egg-laying sites. Female aggression may be better elicited where individuals are competing for survival resources and not reproductive resources source (Cain & Langmore, 2016; Tibbetts, 2008). Indeed, when competing directly for access to limited food resources, we have observed higher levels of aggression in female *D. melanogaster* than males (Kilgour et al. 2018). Therefore, if our metric of female aggression was not, in fact, conducive to eliciting variation in female aggression, we could not expect to observe any changes in female aggressive behavior.

Established dominance hierarchies can have lasting effects on aggressive behavior. When aggressive behavior patterns of *D. melanogaster* have been compared between males and females, Nilsen et al. (2004) observed not only different behaviors used between the sexes, but male fights also led to the development of hierarchies, whereas female fights never led to hierarchy formation. The formation of dominance hierarchies requires individuals to have some kind of assessment ability, either of their own competitive quality or a memory of previous competitors (Mesterton-Gibbons & Dugatkin, 1995). While in our study, the opponent flies in the before and after assays were different individuals, there may have been ranks established within social treatment groups during the fixed resource period that altered the subsequent intensity of aggression in our assays. If females do not establish rank dominance, there may be less behavioral carry-over between the treatment environment and the aggression assays.

Our results also suggest that fly density can change social dynamics and alter future social influences on behavior in different contexts. We predicted that changes in behavior would not occur at low-density treatments because flies were not food limited. In contrast, we expected changes in behavior in high-density treatments because of more competitive interactions related to food limitation. At high density, flies experienced an average of 70% mortality indicating a

69
high intensity of competition, whereas flies in low density experienced an average of 4% mortality (Kilgour et al., 2018). In addition to having a greater number of per capita interactions at high density, the competitive nature of encounters at high density would not be experienced by flies in low-density treatments, where flies were not food limited and thus unlikely to experience competition for food resources. Thus, the intensity of competition and repetitive nature of interactions occurring at high fly density may result in physiological changes that persist in subsequent environments (Hsu et al., 2006). Consistent with this, we observed significant changes in aggressive behavior in high-density treatments. In addition to potential competitive effects, groups at high density might have a greater impact on individual behavior due to the increased number of interactions between individuals and that food resources were located at a single dispensing location in the vial. The increased frequency of interactions may have a compounding effect on the social influence, thus resulting in a pronounced carry-over into aggressive behavior in a subsequent, and changing, environment.

Our results have important implications for our understanding of the development of aggression and the role of social composition and resource competition in the expression of aggression. We show how social experiences during a resource-stressed period can alter the expression of aggression in future contexts. Furthermore, our data demonstrate how developmental plasticity stemming from past experiences in one context carry-over into future, and different, contexts, providing a source of variation in aggression observed in natural populations.
Table 3.1. The effect of strain, sex and time on body mass. Body mass varied between the sexes but did not change over time, nor were there any differences between strains. Body mass was compared across time (before and after the period of fixed resources) and strains (more and less aggressive) using a linear model. Statistical significance estimated at $\alpha = 0.05$, as indicated by fixed effects in bold. The reference categories for fixed effects are shown in parentheses.
<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Fixed effect</th>
<th>$\beta \pm \text{s.e.}$</th>
<th>Wald statistic ($\chi^2$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain (380)</td>
<td>$0.70 \pm 0.47$</td>
<td>2.22</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Time (Before)</td>
<td>$-0.05 \pm 0.48$</td>
<td>0.01</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Density (Low)</td>
<td>$0.91 \pm 0.49$</td>
<td>3.45</td>
<td>0.06</td>
</tr>
<tr>
<td>Female aggression</td>
<td>Strain*Time</td>
<td>$0.95 \pm 0.61$</td>
<td>2.45</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Strain*Density</td>
<td>$-0.61 \pm 0.64$</td>
<td>0.90</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Density*Time</td>
<td>$0.47 \pm 0.63$</td>
<td>0.55</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Strain<em>Time</em>Density</td>
<td>$-0.99 \pm 0.82$</td>
<td>1.43</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Strain (380)</td>
<td>$-2.11 \pm 0.87$</td>
<td>5.87</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Time (Before)</td>
<td>$2.09 \pm 0.57$</td>
<td>13.52</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Density (Low)</td>
<td>$0.71 \pm 0.81$</td>
<td>0.78</td>
<td>0.37</td>
</tr>
<tr>
<td>Male aggression</td>
<td>Strain*Time</td>
<td>$-0.43 \pm 0.89$</td>
<td>0.24</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Strain*Density</td>
<td>$0.13 \pm 1.18$</td>
<td>0.01</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Density*Time</td>
<td>$-1.49 \pm 0.82$</td>
<td>3.28</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Strain<em>Time</em>Density</td>
<td>$1.69 \pm 1.23$</td>
<td>1.86</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 3.2. There was no significant change in female aggression of either strain in homogeneous treatments. Aggression in homogenous male treatments significantly depended on time (before and after the period of fixed resources) and strain, regardless of density treatment. Fixed effects in bold are statistically significant at $p < 0.05$. Trial, Vial and Observer were incorporated as random effects. The reference categories for fixed effects are shown in parentheses.
Table 3.3. Effects of strain, and social environment on the change in male *Drosophila melanogaster* aggression in heterogeneous social environments at high density. Time indicates the difference in aggression between before and after the period of fixed resources. Frequency refers to treatments where strains were mixed at 1:3, 1:1, or 3:1 ratios. Fixed effects in bold indicate statistically significance at $p \leq 0.05$. Vial, Trial and Observer were incorporated as random effects. The reference categories for fixed effects are shown in parentheses.

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>$\beta \pm$ s.e.</th>
<th>Wald statistic ($\chi^2$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain (712)</td>
<td>1.32 ± 0.64</td>
<td>4.21</td>
<td>0.04</td>
</tr>
<tr>
<td>Time (Before)</td>
<td>1.25 ± 0.89</td>
<td>2.02</td>
<td>0.15</td>
</tr>
<tr>
<td>Frequency</td>
<td>3.12 ± 0.92</td>
<td>11.39</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Strain*Time</td>
<td>-2.76 ± 1.24</td>
<td>4.89</td>
<td>0.02</td>
</tr>
<tr>
<td>Strain*Frequency</td>
<td>-5.47 ± 1.41</td>
<td>14.96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Frequency*Time</td>
<td>-2.99 ± 1.54</td>
<td>3.76</td>
<td>0.05</td>
</tr>
<tr>
<td>Strain<em>Time</em>Frequency</td>
<td>6.86 ± 2.31</td>
<td>8.80</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
**Figure 3.1.** A chronological summary of the experiment. Each bar represents a day, where the bars in grey indicate the treatment social groups were placed in the period of fixed resources. We applied 10 different social treatments across 2 densities (high and low) and 5 frequencies (2 homogeneous and 3 heterogeneous; see text for details). Aggression assays occurred 1-3 days following the removal of focal individuals on Day 2 and Day 6. The two strains are not depicted in this figure.
Figure 3.2. The magnitude of natural selection was much weaker than the magnitude of plasticity so there was close correspondence between the raw observed change in aggression and the change in aggression after correcting for natural selection. Plasticity was the observed change in aggression and was measured as the change in mean aggression from before to after the period of fixed resources. To account for selection, we subtracted the between-strain selection differential measured separately for each vial, from the observed plasticity value calculated for each vial (see text for details).
**Figure 3.3.** Aggression exhibited by females (top) and males (bottom) in homogenous treatment groups, where social groups were composed entirely of the same strain. Filled circles represent mean number of aggressive events during a 30-min assay with standard error bars, and includes data from both high and low density treatments, as fly density was not found to be a significant effect (see Table 2).
Figure 3.4. Males of both strains showed an increase in aggression when at low frequency and an increase in aggression at equal frequency. Mean aggression (filled circles) exhibited by males from strain 380 (top) and strain 712 (bottom) at high density, before (black) and after (grey) the period of fixed resources. Vertical bars represent standard error.
Epilogue

My thesis explores how natural selection and social plasticity interact to explain why we consistently observe variation in aggression in animal populations. While aggression can provide considerable fitness benefits, such as in reproductive contexts or maintaining access to a shared and limited resource (Archer, 1988; Clutton-Brock et al., 1979; Tinbergen, 1953; Wilson, 1975), variation in aggressive phenotypes persists in populations across generations. The field of animal personality has demonstrated that consistent individual differences in aggression exist in a broad range of taxa, including fish (Bell & Sih, 2007; Huntingford, 1976), birds (Aplin et al., 2013; Carere, Drent, Privitera, Koolhaas, & Groothuis, 2005), mammals (Careau et al., 2015; Mella, Ward, Banks, & McArthur, 2015; Zipser, Kaiser, & Sachser, 2013), and invertebrates (Kortet & Hedrick, 2007; Rudin & Briffa, 2012). However, while individuals may be predisposed to exhibit greater (or lesser) intensity of aggressive behaviours, aggression is also a highly plastic trait and animals may opt to use aggression in specific contexts and not in others (Been et al., 2019; King, 1973; Wagner, 1989). Natural selection can also shape the variation that we observe, and negative frequency-dependent selection (NFDS) is one mechanism through which polymorphisms can be maintained across generations (Ayala & Campbell, 1974). My research has demonstrated how an individual’s expression of aggression is affected not only by its inherited predisposition, but also by current and previous social environments. Additionally, while other studies have identified NFDS on aggression (Lichtenstein & Pruitt, 2015; Sinervo & Lively, 1996), I have demonstrated that NFDS acts on aggressive and non-aggressive phenotypes through a previously undocumented combination of disruptive selection and social effects.

Aggression is a context-dependent social behaviour wherein individuals will often vary their aggressiveness according to the social environment. The social effects can range from the specific behavioural phenotypes of the interacting individuals (Desjardins et al., 2012; Wagner, 1989) to the phenotypic makeup of the social group (Farine et al., 2015). Furthermore, social effects may be more prominent as the fitness consequences of social interactions increase, such as during periods of resource limitation. While many papers have hypothesized that all individuals ought to increase aggressive behaviour during competition (Goldberg et al., 2001;
Grant, 1993), my results indicate that the direction of social effects depends on both the focal and the group phenotypes. In Chapter 2, flies from both the aggressive and less-aggressive strains significantly reduced their expression of aggression when groups were composed of mostly aggressive individuals. In contrast, when groups were composed mostly of less-aggressive individuals, all flies significantly increased their aggression. Given how these social effects related to individual survival, group composition clearly resulted in varied fitness consequences depending on the phenotype of a focal individual. That is, social effects in an individual’s current environment can result in modifications of aggression.

Developmental plasticity of behaviour describes how previous experiences (outside of ontogeny) can influence behavioural expression in the future (Stamps, 2016). The nature of developmental plasticity can alter the direction of evolution, providing novel traits for selection to act upon (Moczek et al., 2011). I found that aggression in male flies was significantly affected by their previous social experience (Chapter 3). My results further demonstrated how some social effects carry-over across environments, where others do not. Flies from strains which were at low frequency in a population were subsequently affected by the group composition, whereas flies from the common strain experienced no future social effects. Additionally, the observed persistence of the social effects occurred over time and context, where the future effects were observed in a very different social environment. The social effect occurred when focal flies were in groups of 300 mixed-sex individuals, whereas the aggression in the subsequent environment occurred in same-sex dyadic pairs. It is important to note that the direction of the in situ social plasticity observed in Chapter 2 was not consistent with the social plasticity observed in Chapter 3. During the period of resource limitation, social effects from flies from the aggressive strain resulted in increased aggression and social effects from flies from the less-aggressive strain resulted in reduced aggression. In contrast, the results described in Chapter 3 indicate that the social effects which persisted into a future and novel environment induced an increase in aggression in flies from both strains when paired with an unfamiliar test fly. Therefore, the changes in aggression observed within a social context did not persist into the new social context for flies from the common strain. In males, socially-induced changes in aggression are mediated through volatile cuticular hydrocarbons (11-cis-vaccenyl acetate, hereafter cVa), altering
aggression toward males (Wang & Anderson, 2010) and females (Fernández et al., 2010), although its influence on males depends on exposure. When males are exposed to acute increasing concentrations of cVa, their aggression increases (Wang & Anderson, 2010). In contrast, exposure to high cVa concentrations for 48h correlates with a decrease in aggression (Liu et al., 2011). However, in Chapter 3, where flies would have experienced a chronic exposure to cVa, neither strain exhibited a subsequent decrease in their aggression.

Negative frequency-dependent selection (NFDS) is cited as a main driver of phenotypic variation (Ayala & Campbell, 1974), although the mechanisms by which this occurs can vary (Dijkstra et al., 2010; Gross, 1991; Sinervo & Lively, 1996). In Chapter 1, I found that negative frequency-dependent survival, following a period of limited resources, can result in greater survival to morphs when rare, or at low frequency. While NFDS describes patterns of varied fitness consequences depending on the relative frequencies of each morph, there are several established behavioural mechanisms by which this can occur. For example, in alternative mating strategies, morphs undergo NFDS resulting from non-random competitive interactions resulting in fitness advantages to morphs when rare (Gross, 1991). In Lake Victoria cichlids (Pundamilia spp.), aggressive competitive interactions resulting in NFDS is based on interaction biases coupled with differences in dominance ranks, allowing for the coexistence of aggressive and less-aggressive morphs (Dijkstra et al., 2010). Alternatively, classic game theoretic Hawk-Dove models (Maynard Smith & Price, 1973) describe NFDS resulting from random interactions and differential between- and within-phenotype interaction costs and benefits. Unlike in any previously described mechanism, in Chapter 2, I found that aggressive and less-aggressive phenotypes undergo disruptive selection and when combined with social plasticity, resulted in NFDS. Although not statistically significant, we found trends indicating that flies from the aggressive strain benefited from aggressive interactions, while flies from the less-aggressive strain incurred costs from aggressive interactions. These results suggested that disruptive selection should favour greater aggression in individuals from the aggressive strain, and less aggression in individuals from the less-aggressive strain. Disruptive selection between aggressive and non-aggressive morphs is hypothesized to drive speciation in cichlids (Dijkstra & Border, 2018). Disruptive selection on its own, however, would not result in NFDS, given
random interactions. Nonetheless, individuals of both phenotypes exhibited significant social plasticity, as flies from both strains induced a social effect resulting in changes in aggressive behaviour for all flies within the social group. The directionality of the behavioural changes depended on the frequencies of both strains, as groups composed mostly of flies from the aggressive strain resulted in individuals from both strains reducing their aggression, and groups composed mostly of flies from the less-aggressive strain resulted in individuals of both strains increasing their aggression. In other words, individuals were influenced by their social composition in a manner that induced an adaptive shift in behaviour when rare, and a maladaptive shift in aggression when common. My results propose a completely novel 2-part behavioural mechanism driving NFDS of disruptive selection and social plasticity, further showing the complexity of how social effects interact with natural selection.

Aggressive behaviour is often linked to an individual’s competitive ability and, subsequently, its dominance status. My thesis adds to a growing body of evidence demonstrating that aggression can be independent from competitive ability, indicating that more dominant individuals may not be the most aggressive in a population while still experiencing greater fitness and thus increased survival. Wild fruit flies are known to exhibit territorial aggression on patches of rotting fruit (Hoffmann, 1987b). In Chapters 1 and 2, I found evidence that aggressiveness is not necessarily an indicator of competitive ability. My results are consistent with findings in many other species, where the most aggressive individuals may be exhibiting this intense behaviour in a maladaptive way (Camerlink et al., 2015; Kilgour & Brigham, 2013). In each experiment in my thesis, I incorporated food competition as a major facet as each experiment included treatments with limited food availability, which resulted in low survival. The results in Chapter 1 demonstrated how the increased survival of rare phenotypes can only occur in the context of food competition, as observed in the high density treatment, a context mirrored in Chapter 2. If aggression were an indicator of competitive ability, then flies from the aggressive strain would consistently out-compete flies from the less-aggressive strain. However, NFDS demonstrates that aggressiveness does not necessarily indicate that an individual is more likely to gain access to a limited resource. Camerlink (2015) found that aggressive in pigs (Sus scrofa) were not the most likely to win in dyadic contests. Instead, consistent with Hawk-Dove
predictions, pigs who opted for low intensity displays and only increased their aggression when necessary where those with the greatest competitive abilities. In social groups, dominant individuals who attempt to acquire or defend access to limited resources may selectively apply aggressive defenses when appropriate, and, as I have demonstrated, the efficacy of such a tactic will depend on the phenotypic composition of the competitors. Therefore, although increased aggression may not be advantageous in gaining access to limited resources, it remains a prevalent component of competitive interactions.

Studies examining aggressive behaviour and its evolutionary consequences are often based solely in males across species, but experiments in my thesis incorporated both males and females. *D. melanogaster* is a model organism in research on aggression, although the vast majority of research focuses only on males. Biasing studies against one sex is a disservice to research, and in doing so, we are ignoring a critical component needed for a complete understanding of aggression. Intra- and inter-sexual female aggression is prevalent in natural populations (Been et al., 2019; Draud et al., 2004; Finkler & Terkel, 2010; Ueda & Kidokoro, 2002) and can have profound fitness consequences. Studies on wild spotted hyenas (*Crocuta crocuta*) examine aggression in both sexes during feeding periods; limited access to prey can have profound fitness consequences. In Chapters 2 and 3, I purposely included females in my experiments, and found different results between the sexes and also between experiments. When aggression was studied in groups during the period of food limitation, females demonstrated considerably more aggression than males, regardless of the strain. In contrast, the social effects experienced during the period of fixed resources did not persist into dyadic contests in females in treatments where the social effects did carry-over in males (Chapter 3). My results demonstrate that female aggression may be affected more during periods of food limitation than in males, consistent with hypotheses on the evolution of female ornamentation (LeBas, 2006; Tobias, Montgomerie, & Lyon, 2012). In spite of this, the vast majority of experimental studies on aggression relating to resource competition were conducted using males competing for egg-laying sites (Kravitz & Fernandez, 2015; Mundiyapanapurath et al., 2007; Wang & Anderson, 2010) or access to mates (Fernández et al., 2010; Fernandez & Kravitz, 2012; Martin & Long,
It is likely that environments which induce increased aggression in females may differ from those which induce aggression in males. For example, females may be more aggressive in competition for food resources, whereas males may be more aggressive in competition for reproductive contexts (Cain & Langmore, 2016; Tibbetts, 2008). Despite the obvious importance of aggression in females, general interest and research of female aggression in resource competition is disappointingly sparse.

While my results provide critical insights into our understanding of the maintenance of aggression in populations, there were several limitations to my work. For example, while using *D. melanogaster* to address these questions has many advantages, applications of this work to other animal systems may be more challenging. The strains of fruit flies in this study were two unique genotypes, indicating genetic dissimilarity between strains and high genetic similarity within strains, such that all flies from one strain could be classified as “aggressive” or “less-aggressive”. Natural populations of animals are made up of interbreeding, heterozygous individuals, and the variation of aggressiveness within the population would fall along a continuous spectrum, such that most individuals could not be classified as “aggressive” or “non-aggressive”. Therefore, repeating this study in populations where aggression is a continuous trait might yield different results. A second limitation relates to the unrealistic environment for competition used in my experiments. *D. melanogaster* interact in social groups in the wild, and will aggregate at food patches, where they will engage in aggressive interactions. In my experiments, flies could only forage from a single food patch, and, in competitive treatments, their survival depended on their ability to access food resources. In contrast, natural populations of flies are unlikely to be limited by food, as rotting fruit and vegetable matter are prevalent in the environment. Further, while flies will defend food patches (Hoffmann, 1987b; Wertheim et al., 2006), their survival is unlikely to depend on their access to a single food patch. Lastly, extrapolation of my results to other animal populations may be limited by the use of inbred lines in my experiments. Other studies have used these strains in studies of aggression (Anderson et al., 2016; Saltz, 2013; Shorter et al., 2015), but strains are either crossed to create heterozygous individuals used in experiments and/or multiple strains are used as replicates (Saltz, 2013). In other words, it is possible that the results found in my experiments are unique to these strains,
suggesting that NFDS may not be a common means of maintaining variation in aggression. However, my results indicate that NFDS is, at least, one possible mechanism through which variation in aggression is maintained.

In this thesis, I have made advances in our understanding of the nature of individual aggression and the maintenance of variation in competitive environments, although many questions remain. Where I demonstrated that an individual’s expression of aggression is influenced by past and present social environments, how long these social effects persist is unknown. I found evidence that social effects influence survival, but it remains unknown if or how the social effects alter reproductive ability. If that variation in aggression is maintained through disruptive selection and social effects, this raises questions regarding the nature of the interaction of these two processes. For example, if interactions were non-random and only within strain interactions occurred, or strains mated assortatively, would we still observe NFDS? Alternatively, are social effects powerful enough that NFDS could result from social effects influencing behaviour of only one strain? My results were based on within-generational effects. Therefore, an essential question remains as to whether NFDS would continue across multiple generations. Research is needed to determine the reproductive consequences of both social effects and NFDS. Additionally, the proximate mechanisms underlying the social effects observed in my experiments remain unclear. Although it is likely that cVa influenced male behaviour (see above), the chemical or pheromonal influences on female aggression remain unknown. Aggressive behaviour in *Drosophila* is correlated with the hormone octopamine, the invertebrate equivalent of noradrenaline (Hoyer et al., 2008). Competitive interactions can result in modification of circulating hormones, although rarely, if ever, are the effects of group composition assessed with respect to individual hormone levels. Exploration of reproductive consequences, multi-generational patterns of selection, and proximate physiological mechanisms is necessary to truly further our understanding of social effects and competition on the evolution of aggression.

In combining evolutionary and ecological principles, I have provided critical insight into answering the question: why do we observe variation in aggression? I have demonstrated that individual expression of aggression is a result of intra-individual variation depending on the
social context, and variation in fitness based on inter-individual differences. That is, variation in aggression is rooted in the combination of individual plasticity, an individual’s predisposition, and its social experiences. Individual plasticity occurs in response to both the past and present social environment, as well as its own predisposed level of aggression. Additionally, genetically based inter-individual differences in aggression incur changing fitness costs and benefits in response to the phenotypic composition of the social group during periods of intense competition. Therefore, the persistent variation that we observe in aggression in wild populations is likely a result of social plasticity, and the phenotypic makeup of past and present social groups.
Table S1.1. The effects of sex, strain and time period on body mass from a linear model. Time period refers to measurements taken before or after the period of limited resources. As expected, females were significantly larger than males. However, there was no significant difference in body size between strain 380 and strain 712 within sexes, nor did we observe any significant change in body size following the period of limited resources. Fixed effects were considered significant at $\alpha = 0.05$. 

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta$</th>
<th>SE</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
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<td>0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>Time (before, after)</td>
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</tr>
<tr>
<td>Strain</td>
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<td>0.02</td>
<td>0.19</td>
</tr>
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</tr>
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</tr>
<tr>
<td>Strain*Time</td>
<td>0.006</td>
<td>0.03</td>
<td>0.84</td>
</tr>
<tr>
<td>Sex<em>Time</em>Strain</td>
<td>-0.004</td>
<td>0.04</td>
<td>0.93</td>
</tr>
</tbody>
</table>
Figure S2.1. Histogram depicting mortality count following each feeding period. Mortality was not evenly distributed across the experiment, and the greatest mortality occurred following feeding period 2. We estimated mortality per individual per vial based on which feeding period during which an individual was last observed and did not survive. Of the 600 flies used in this experiment, only 2 survived with no data recorded throughout the experiment. These data were extracted across 30 individuals in 20 vials (600 individuals total). The majority of flies died before after feeding period 2. Individuals who survived the experiment were not included in this analysis.
Figure S2.2. Strain 380 exhibited significantly more aggression than strain 712, and females were more aggressive than males, although not significantly so. Aggression counts were based on the number of lunges and headbutts counted when an individual was present in the observation zone (see text for details). Circles represent means with standard error bars.

To confirm the strains were behaving differently and as expected, we analysed differences in behaviour by strain using a Poisson-distributed GLMM. We regressed sex and strain on aggression given, with vial and Individual ID as random effects. We incorporated an individual level random effect to account for overdispersion in the data. Vial was included as a random effect to account for any potential differences that could be occurring between vials.

Aggression Given ~ Sex*Strain + (1|Vial) + (1|Ind.ID)
We found that aggression was significantly affected by strain (β ± Std. error: -1.07 ± 0.21, \( p < 0.01 \)), but there were no significant effects of sex (β ± Std. error: 0.25 ± 0.19, \( p = 0.19 \)) or the interaction between sex and strain (β ± Std. error: -0.10 ± 0.31, \( p = 0.74 \)). As we observed in previous studies, we found strain 380 to exhibit more aggression than strain 712 (Kilgour et al., 2018). However, unlike results from dyadic assays (Nilsen et al., 2004; Ueda & Kidokoro, 2002), females were more aggressive than males, although not significantly.
Supplementary Material: Chapter 3

<table>
<thead>
<tr>
<th>Response variable</th>
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<th>Wald statistic $(\chi^2)$</th>
<th>$p$</th>
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<tr>
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<td>Time*Strain</td>
<td>$0.14 \pm 0.38$</td>
<td>0.13</td>
<td>0.72</td>
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</tbody>
</table>

Table S3.1. Dahomey flies acted as opponents in aggression assays and behaved consistently regardless of the strain of the focal fly. We used a GLMM, where Dahomey aggression was the response variable with the strain of the focal fly as well as the time of the trial (before or after) as fixed effects. As with other models, sexes were analyzed separately. Vial and trial were incorporated as random effects. Fixed effects in bold are statistically significant at $p < 0.05$. Trials, Vial and Observer were incorporated as random effects. The reference categories for fixed effects, “before” assays (Time) and strain 380 (Strain).
<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Fixed effect</th>
<th>$\beta \pm \text{s.e.}$</th>
<th>Wald statistic ($\chi^2$)</th>
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<td>Frequency</td>
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<td>0.77</td>
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<tr>
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<td>Density*Frequency</td>
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<td>Strain<em>Time</em>Density*Frequency</td>
<td>-0.34 ± 2.43</td>
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**Table S3.2.** Mixed model results of fly density, frequency, strain and time on female aggression. Aggression in female heterogeneous treatments did not significantly depended on time (before and after the period of fixed resources), fly density, strain frequency, or strain. Frequency refers to treatments where strains were mixed at 1:3, 1:1, or 3:1 ratios. Fixed effects in bold are statistically significant at $p < 0.05$. Trial, Vial and Observer were incorporated as random effects. The reference categories for fixed effects are low density (Density), “before” assays (Time) and strain 380 (Strain).
<table>
<thead>
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<td>Time</td>
<td>0.88 ± 0.91</td>
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<tr>
<td></td>
<td>Density</td>
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<td>Strain*Time</td>
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<td>0.48</td>
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<td>Strain*Density</td>
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<td>0.31</td>
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<tr>
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<td>Strain*Frequency</td>
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<td>Frequency*Time</td>
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<tr>
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<td>Density<em>Time</em>Frequency</td>
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<tr>
<td></td>
<td><strong>Strain<em>Time</em>Density*Frequency</strong></td>
<td><strong>6.94 ± 3.32</strong></td>
<td><strong>4.38</strong></td>
<td><strong>0.03</strong></td>
</tr>
</tbody>
</table>

**Table S3.3.** Mixed model results of fly density, frequency, strain and time on male aggression. Aggression in male heterogeneous treatments was significantly influenced by the interaction between time (before and after the period of fixed resources), fly density, strain frequency, and strain. Frequency refers to treatments where strains were mixed at 1:3, 1:1, or 3:1 ratios. Fixed effects in bold are statistically significant at $p < 0.05$. Trial, Vial and Observer were incorporated as random effects. The reference categories for fixed effects are low density (Density), “before” assays (Time) and strain 380 (Strain).
Table S3.4. Mixed model results of social effects on male flies at low density. There were no significant effects of strain, and social environment on the change in male *Drosophila melanogaster* aggression in heterogeneous social environments at low density. Time indicates the difference in aggression between before and after the period of fixed resources. Frequency refers to treatments where strains were mixed at 1:3, 1:1, or 3:1 ratios. Fixed effects in bold indicate statistically significance at $p \leq 0.05$. Vial, Trial and Observer were incorporated as random effects. The reference categories for fixed effects are “before” assays (Time) and strain 380 (Strain).

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>$\beta \pm$ s.e.</th>
<th>Wald statistic $\left(\chi^2\right)$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.40</td>
</tr>
<tr>
<td>Time</td>
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<td>0.33</td>
</tr>
<tr>
<td>Frequency</td>
<td>1.58 $\pm$ 1.08</td>
<td>2.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Strain*Time</td>
<td>0.94 $\pm$ 1.27</td>
<td>0.54</td>
<td>0.45</td>
</tr>
<tr>
<td>Strain*Frequency</td>
<td>-2.50 $\pm$ 1.60</td>
<td>2.44</td>
<td>0.11</td>
</tr>
<tr>
<td>Frequency*Time</td>
<td>-0.89 $\pm$ 1.58</td>
<td>0.32</td>
<td>0.57</td>
</tr>
<tr>
<td>Strain<em>Time</em>Frequency</td>
<td>-0.75 $\pm$ 2.35</td>
<td>0.10</td>
<td>0.75</td>
</tr>
</tbody>
</table>
References


http://florianhartig.github.io/DHARMa/


Huang, Y., Tran, I., & Agrawal, A. F. (2016). Does genetic variation maintained by environmental heterogeneity facilitate adaptation to novel selection? The American


the American Philosophical Society, 123(4), 222–234.


