

Experimental evidence that density mediates negative frequency-dependent selection on aggression

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Abstract

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.

KEYWORDS

behavioural phenotypes, competition, *Drosophila melanogaster*, Hawk–Dove models, resource defence theory, resource limitation

1 | 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, Boon, & Drent, 1996; Wilson, Grimmer, & Rosenthal, 2013). 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 nonlinear way. Resource defence 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 out-competing 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 (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, Bell, Bolnick, & Ratnieks, 2012; Farine, Montiglio, & Spiegel, 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 to non-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 (Betini, Griswold, & Norris, 2013a, 2013b). 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 c. 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 *Drosophila recens*, *Drosophila subquinaria* and *Drosophila 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.

2 | MATERIALS AND METHODS

2.1 | Experimental system

Drosophila melanogaster was obtained from the Bloomington *Drosophila* Stock Center (<https://bdsc.indiana.edu/>). 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 five pairs of adults ($n = 9$; $M \pm SE$; 380: 76.44 ± 11.41 ; 712: 71.14 ± 7.92 ; t test, $t = 0.39$, $df = 13.67$, $p = .69$), indicating strains are comparable in reproductive output, and adult survival in our experiments (see below) was similar to the survival of outbred flies in similar previous experiments (Betini, McAdam, Griswold, & Norris, 2017).

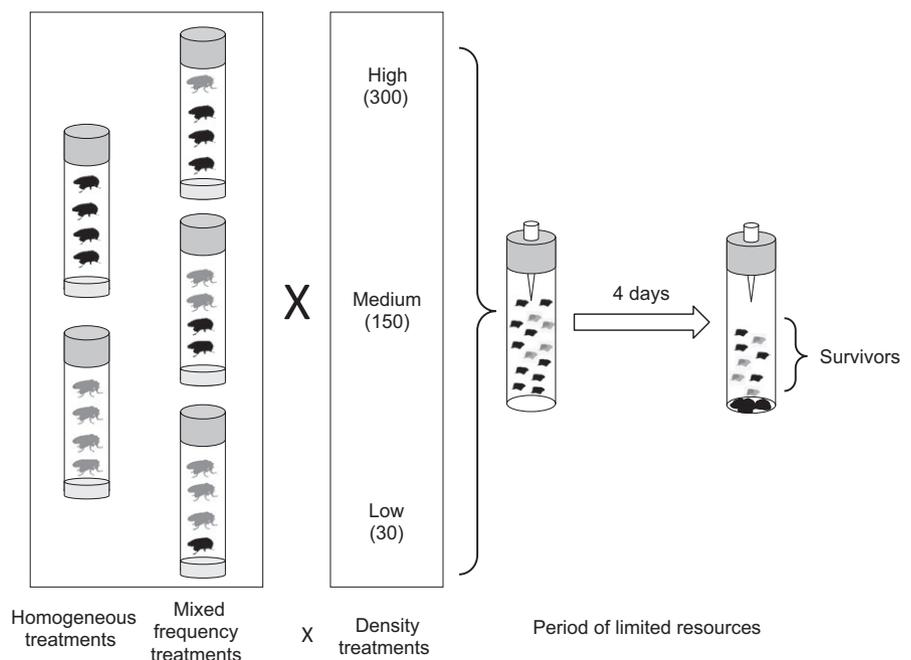
Outside of experimental trials, all flies were kept in 28×95 mm holding vials containing 10 ml of dead yeast-agar-sugar food medium (see Betini et al., 2013a, 2013b for details). Flies were allowed to breed for 3 days and mature for 11 days. Adult flies were removed for breeding within 1 day of emergence.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

2.2 | Experiment 1: Survival assays

To understand how density and frequency affect the survival of aggressive and non-aggressive individuals, we used a fully factorial design of three density treatments and five frequency treatments and 11 replicate populations per treatment (Figure 1). Our three density treatments were low (30 individuals), medium (150 individuals) and high (300 individuals). At each density, we created five 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, USA) 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 hr, 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

FIGURE 1 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



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 4 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 4-day period as our fitness component. We also sampled flies of both strains and sexes, before and after the 4-day period, to assess any differences in body mass.

2.3 | Experiment 2: Aggression assays

To confirm differences in aggression between strains, we video recorded and analysed 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 0.020 ml 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 hr prior to the experiment, as in the survival experiment. We recorded interactions first within 2 hr after being placed in the experimental period and again 15 hr later. Both feeding periods were recorded for 20 min 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, headbutts 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.

2.4 | Statistical analysis

In Experiment 1, we ran three 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 the 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 χ^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 \times 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 \times 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 modelled 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

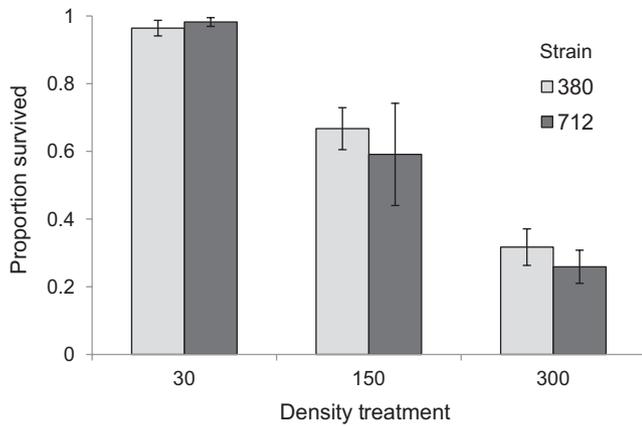


FIGURE 2 The survival of two isogenic, naturally inbred lines of *Drosophila melanogaster* was assessed at three densities (30, 150 or 300 flies per vial). Bar heights represent means and error bars represent standard error (SE)

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 statistically significant at $\alpha = 0.05$. Model fit was assessed using diagnostic plots and scatter plots of residuals and predicted values.

3 | RESULTS

3.1 | 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 ($\beta = -0.01 \pm 0.001$; $\chi^2 = 181.16$, $df = 1$, $p < .01$; Figure 2), there was no evidence of differences in survival between strains ($\chi^2 = 0.87$, $df = 1$, $p = .34$). There was also no evidence of a density \times strain interaction ($\chi^2 = 1.18$, $df = 1$,

$p = .28$; Figure 2), indicating that the two strains were equally food limited during our experimental trials. While females were significantly larger than males ($M \pm SE$: females: $1.24 \text{ mg} \pm 0.008$; males: $0.76 \text{ mg} \pm 0.005$; $\beta = -0.49$, $df = 1211$; $p < .01$), there were no other significant effects (Table S1), 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 ($\chi^2 = 5.18$, $df = 1$, $p = .02$, Table 1), providing evidence for density- and 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 3). As with homogeneous treatments, there was a strong, negative effect of density on survival (Table 1). Similar NFDS was found in the scaled-down trials (Experiment 2), with a significant interaction effect of strain and frequency on survival ($\chi^2 = 4.82$, $df = 1$, $p = .02$), 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. As 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 ($F_{1,90} = 0.76$, $p = .39$) or medium ($F_{1,82} = 2.03$, $p = .15$) population density. Although not significant ($F_{1,264} = 3.19$, $p = .07$), survival increased with homogeneity at medium density relative to high-density treatments (medium density: $\beta = -0.12 \pm 0.08$; high density: $\beta = 0.05 \pm 0.05$).

TABLE 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 ($n = 300$) density 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. Text in bold indicates significance at $\alpha = 0.05$

Variable	$\beta \pm SE$	Wald statistic (χ^2)	p
Density	-0.011 ± 0.002	34.536	<.001
Frequency	-0.003 ± 0.804	0.004	.996
Strain	-0.247 ± 0.394	0.428	.539
Density \times frequency	-0.005 ± 0.003	1.684	.118
Density \times strain	-0.004 ± 0.001	8.422	.003
Frequency \times strain	1.054 ± 0.746	2.071	.158
Density \times frequency \times strain	0.007 ± 0.003	5.181	.022

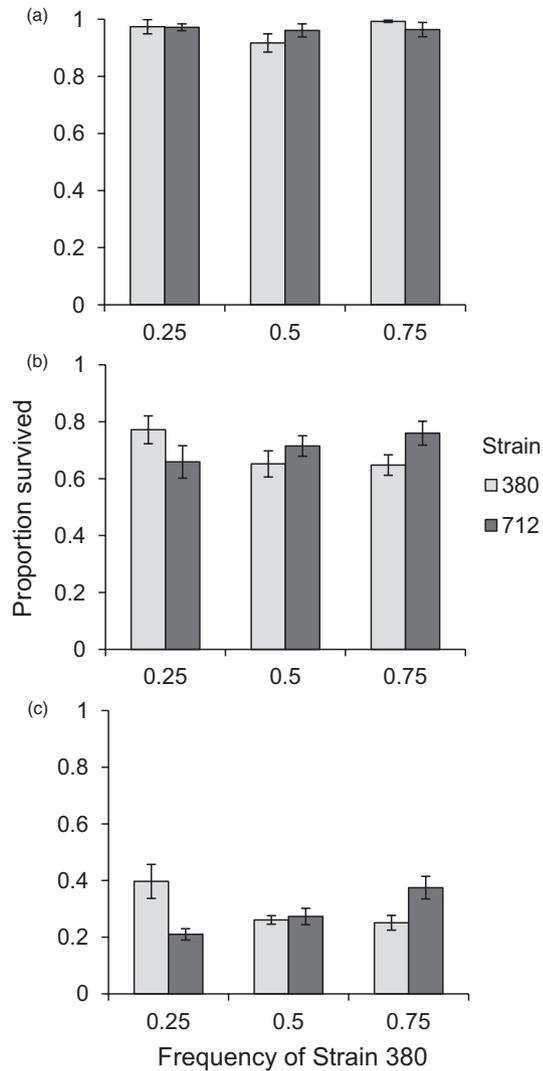


FIGURE 3 The effect of frequency and strain on the proportion of flies survived following a period of limited resources at three density treatments: (a) low (30 individuals), (b) medium (150 individuals), (c) high (300 individuals). Frequency treatments showed each strain and low and high frequency (75%, 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 (SE)

3.2 | Experiment 2: Aggression assays

Aggressive behaviour differed between strains and sexes. Strain 380 was two times more aggressive than strain 712 ($M \pm SE$, 380: 1.92 ± 0.13 , 712: 0.81 ± 0.08 ; $\chi^2 = 33.47$, $df = 1$, $p < .01$, Figure 4) and female aggression was twice as high as males, regardless of strain ($M \pm SE$ females: 1.73 ± 0.14 , males: 1.00 ± 0.08 ; $\chi^2 = 12.69$, $df = 1$, $p < .01$, Figure 4), 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 ($\chi^2 = 10.17$, $df = 1$, $p < .01$), as well as a significant effect of the interaction between strain and frequency ($\chi^2 = 6.91$, $df = 1$, $p < .01$), 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.

4 | 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

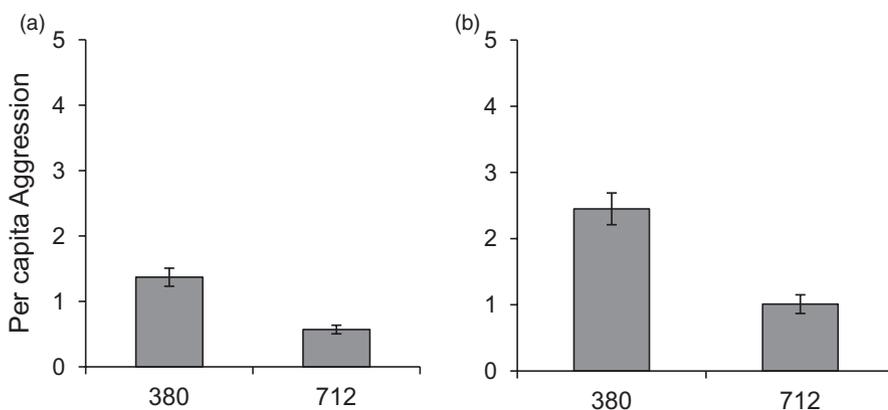


FIGURE 4 Differences in aggression between strains in (a) male and (b) female in the experimental vials. Lunges, shoves and headbutts were recorded for both males and females. Over two feeding periods, aggressive behaviours were recorded for 20 min. In both sexes, flies from strain 380 exhibited more aggression than flies from strain 712. Bar heights represent means and error bars represent standard error (SE) ($n = 150$ per sex and strain)

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 defence 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, Reader, Sol, McDougall, & Dingemanse, 2007; Sih, Bell, Johnson, & Ziemba, 2004).

While the between-strain differences that we observed were consistent with previous studies (Edwards 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, Macias-Ordonez, 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 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), *Drosophila 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 defence 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 defence 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, Sneddon, & Wilson, 2015) and the social environment (Camerlink et al., 2016; Farine et al., 2015), the hypotheses derived from resource defence 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 group 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 depend 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 is 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.

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AUTHORS' CONTRIBUTIONS

R.J.K., A.G.M. and D.R.N. conceived of the study, designed the experiment and interpreted the findings. R.J.K. conducted experimental procedure, collected data and performed the statistical analyses.

G.S.B. provided assistance in experimental logistics and conceptual design. All authors contributed to the writing of the manuscript.

DATA ACCESSIBILITY

Data available from Dryad Digital Repository: <https://doi.org/10.5061/dryad.mv352kc> (Kilgour, McAdam, Betini, & Norris, 2018).

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SUPPORTING INFORMATION

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