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Original Article

Carry-over effects of resource competition and social environment on aggression

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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 (two treatments: high and low per capita resources) and group composition by varying strain frequency (five treatments: homogeneous strains, or 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 competition on aggression. Instead, 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.

Key words: **carry-over effects, density, developmental plasticity, *Drosophila melanogaster*, indirect genetic effects, negative frequency-dependent selection.**

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 et al. 2001), mates (Smith and Blumstein 2008; Baxter et al. 2015), 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 and Elwood 2008; Mohamad et al. 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 behavior, 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 favor 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 et al. 2014), plasticity can also be a costly trait (Nandy et al. 2016). Many different sensory systems are required for individuals to appropriately detect and/or learn environmental cues associated with a specific environmental change (Mery and Burns

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2009; Snell-Rood 2013). Furthermore, extensive study on behavioral syndromes (or animal personalities) and inter-individual variation indicate behavioral 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 et al. 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 behavioral plasticity may, thus, differ between individuals with different personalities (Dingemanse et al. 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 (Hsu et al. 2008; Arnott and Elwood 2009), or the presence of conspecifics, as seen in audience effects (Doutrelant et al. 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 and Travis 2011; Ricci et al. 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 (McGhee and Travis 2011; Herczeg et al. 2016), or during periods of increased resource competition, although little is known on this subject.

Additionally, experiences in one environment can persist to impact behavior in future and different environments. That is, social and environmental effects on behavior can be additionally complex as they can carry-over across time and contexts (Stamps and Groothuis 2010). 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 and Sih 2007), whereas contextual plasticity describes an increase in courtship behavior when an animal is exposed to a high-preference mate (Wagner et al. 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 behavioral 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 (Edwards et al. 2009b; Zwarts et al. 2011; Anholt and Mackay 2012). Previous research has demonstrated that aggression in male *D. melanogaster* is sensitive to their current social group (Saltz and Foley 2011; Carazo et al. 2014; Saltz 2016), but the persistence of social effects on aggression has never been explored.

In this study, 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 behavior 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 behavioral plasticity following the period of fixed resources. We compared 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 NFDS. 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 NFDS is driving the plasticity in aggression. The fitness consequences of behavioral strategies according to frequency is described in hawk-dove theoretical models, wherein the advantages of being aggressive decline with as frequency increases (Maynard Smith and Price 1973). Under hawk-dove hypotheses, therefore, we would expect aggressive individuals to switch to a non-aggressive strategy as their frequency increases, and non-aggressive individuals to increase their aggressiveness when their frequency increases. However, aggressive behavioral 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 et al. 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 *D. 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 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, NC (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 et al. 2009b; 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 et al. 2009a), 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 10 mL of sugar–yeast–agar food (see Betini et al. 2013a for recipe details) in 28- × 95-mm 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 (R.J.K., personal observation), indicating that larval density, and thus larval competition, remained roughly stable for all adults prior to our experiment. Fly populations followed a 14-day life cycle, where adults were allowed to breed for 3 days and larvae allowed to develop for 11 days. 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 h 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 h old) were dusted with fluorescent powder (DayGlo Ltd, Cleveland, OH), and randomly assigned one of three colors 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 CO_2, counted, and placed

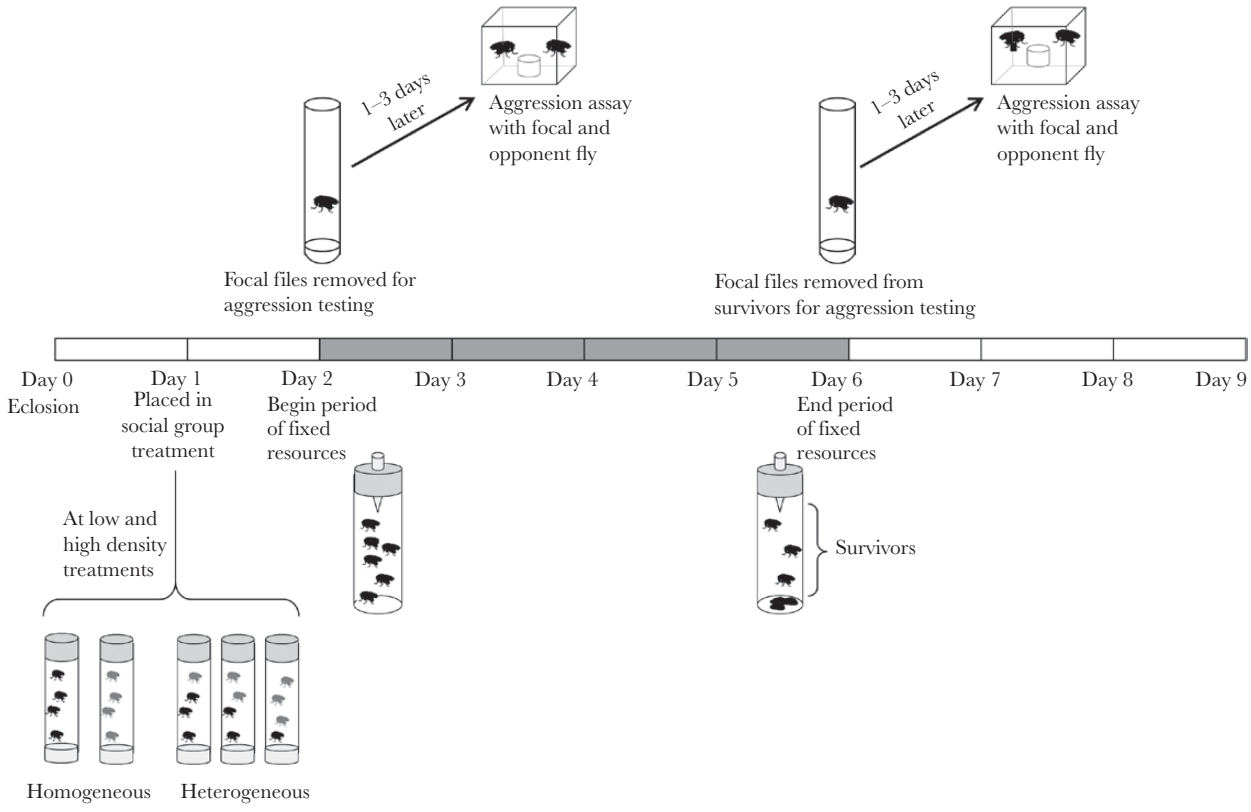

Figure 1
A chronological summary of the experiment. Each bar represents a day, where the bars in gray indicate the treatment social groups were placed in the period of fixed resources. We applied 10 different social treatments across two densities (high and low) and five frequencies (two homogeneous and three 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.

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 was either 30 or 300 individuals when placed in the period of fixed resources. Flies were given 24 h 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 100 µL of 5% sugar water twice per day (between 8:30 and 9:30, and 15:00 and 17:00 h) for 4 days (Betini et al. 2013a, 2013b, Figure 1). Food was dispensed from a single location at the top of the vial using 2.0 mL microcentrifuge tube (Fisherbrand, Waltham, MA) 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 (R.J.K., personal 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 and Blair 1986; Terashima et al. 2005). Following the 4-day period of fixed resources, flies were lightly anesthetized using CO_2 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 (R.J.K., personal observation). We did not record behavior 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 five low-density and five 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 five 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 × 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 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 h following initial creation of social groups, four to six males and four to six females from each strain were selected from each replicate group. Each individual was placed in a 12- × 75-mm glass culture tube (VWR) 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 days before the aggression assay, meaning they were between 3 and 6 days 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 8 days old, we found no effect age on aggressive behavior in aggression assays for either males (generalized linear model [GLM], Aggression ~ Age + Strain; Age: $\beta \pm SE$, -0.06 ± -1.29, $P = 0.20$, $n = 48$) or females (GLM, Age: $\beta \pm SE$, -0.01 ± -0.19, $P = 0.84$, $n = 48$). We repeated this sampling process after the period of fixed resources, wherein four to five 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 h with ad libitum food resources, we could ensure that any observed changes in aggressive behaviors 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 1.5–2 mL 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 days of opponent fly eclosion. Aggression assays followed those described by Mundiyanapurath et al. (2007), where a focal fly and a socially naive opponent fly were placed in a square arena (2.5 × 2.5 cm) with a patch of dead yeast–agar–sugar located in a microcentrifuge tube screwcap (Fisherbrand) 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 and Kidokoro 2002), and Dahomey flies exhibited an intermediate level of aggression between strains 712 and 380 (mean number of aggressive behaviors per trial ± SE; males: 7.47 ± 0.36; females: 7.21 ± 0.43). The aggressive behavior of Dahomey flies was consistent between opponent strains and time of trial (Supplementary Table 1). This type of aggression assays has been repeatedly used in studies of fruit fly aggression of both sexes (Ueda and Kidokoro 2002; Fernández et al. 2010). Each fly was painted with either a blue or yellow dot of acrylic paint on the thorax to allow for individual identification. Paint color was randomly assigned and had no effect on aggression (*t*-test, $t = 0.07$, df = 1,410.2, $P = 0.94$). 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 behaviors 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 behaviors, 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 behaviors were within 90% score accuracy over a minimum of five trial videos. As in other studies (Chen et al. 2002; Yurkovic et al. 2006; Saltz 2013), 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:

$$s_B = \left(\frac{n_{Surv380} \times \overline{Agg}_{380} + n_{Surv712} \times \overline{Agg}_{712}}{n_{Surv}} \right) - \left(\frac{n_{Start380} \times \overline{Agg}_{380} + n_{Start12} \times \overline{Agg}_{712}}{n_{Start}} \right)$$

Where $n_{Surv380}$ and $n_{Surv712}$ refer to the number of surviving individual from strains 380 and 712, respectively; $\overline{Agg}_{380}$ and $\overline{Agg}_{712}$ 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 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

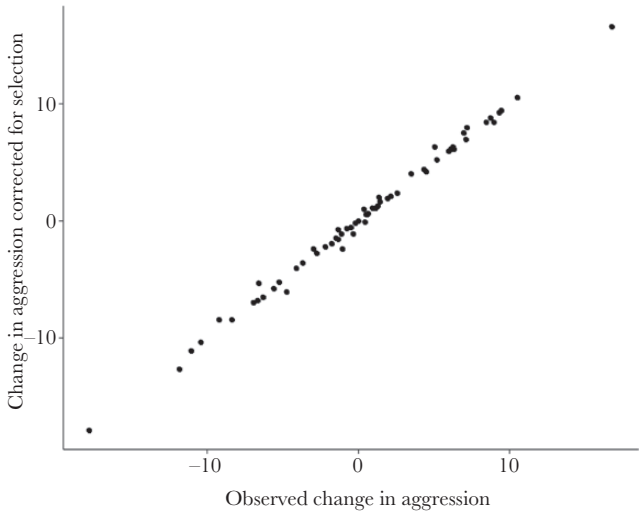

Figure 2
The magnitude of natural selection was much weaker than the magnitude of plasticity so there was close correspondence between the raw observed change in aggression and the change in aggression after correcting for natural selection. Plasticity was the observed change in aggression and was measured 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).

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 four-way interaction. The significance of fixed effects was assessed using Wald's statistic, which is based on maximum likelihood and follows a χ^2 distribution. To assist in interpreting a significant four-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 2019) and GLMMs 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 = 1,211, $P < 0.01$), where females (mean = 1.24 mg, SD = 0.22) were larger than males (mean = 0.76 mg, SD = 0.15; Table 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 h. Therefore, a difference in body mass would not be expected between "before" and "after" aggression assays.

Table 1
The effect of strain, sex, and time on body mass

Fixed effect	β ± SE	*P*
Sex (female)	**−0.49 ± 0.02**	**<0.01**
Time (before)	0.01 ± 0.02	0.75
Strain (380)	0.02 ± 0.02	0.19
Sex*time	−0.01 ± 0.03	0.91
Strain*time	0.01 ± 0.03	0.84
Sex*strain	0.01 ± 0.03	0.79
Sex*time*strain	−0.004 ± 0.04	0.95

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.

Next, we compared changes in the number of aggressive behaviors exhibited between strains in homogeneous groups. We had predicted there would be no change in behavior 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, model results in Supplementary Material). However, we did find significant differences in aggression in males, both before and after the period of fixed resources (Table 2, Figure 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 nonsignificant effect of fly density (Table 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, Table 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).

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 Material). However, in males, we found a significant four-way interaction between strain*density*frequency*time ($\chi^2 = 4.18$, df = 1, $P = 0.04$, complete model results in Supplementary Material). 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 Material). In contrast, at high density, there were significant effects of strain, frequency, and all interactions (Table 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 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.

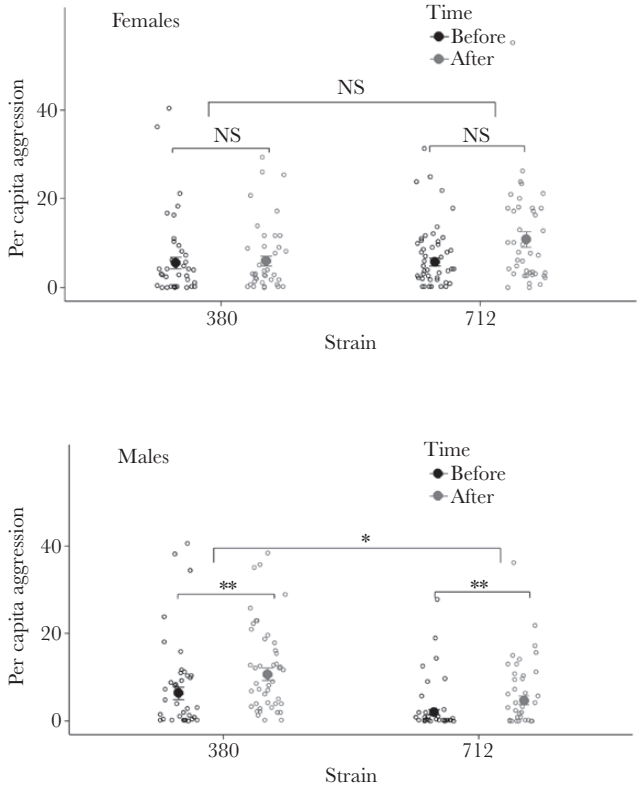

Figure 3

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 SE 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).

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

Table 2

Mixed model results of fly density and strain on aggression

Response variable	Fixed effect	β ± SE	Wald statistic (χ^2)	*P*
Female aggression	Strain (380)	0.70 ± 0.47	2.22	0.14
	Time (before)	−0.05 ± 0.48	0.01	0.90
	Density (low)	0.91 ± 0.49	3.45	0.06
	Strain*time	0.95 ± 0.61	2.45	0.11
	Strain*density	−0.61 ± 0.64	0.90	0.34
	Density*time	0.47 ± 0.63	0.55	0.45
	Strain*time*density	−0.99 ± 0.82	1.43	0.23
Male aggression	**Strain (380)**	**−2.11 ± 0.87**	**5.87**	**0.02**
	Time (before)	**2.09 ± 0.57**	**13.52**	**<0.01**
	Density (low)	0.71 ± 0.81	0.78	0.37
	Strain*time	−0.43 ± 0.89	0.24	0.62
	Strain*density	0.13 ± 1.18	0.01	0.91
	Density*time	−1.49 ± 0.82	3.28	0.07
	Strain*time*density	1.69 ± 1.23	1.86	0.17

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

Mixed model results of social effects on male flies at high density

Fixed effect	β ± SE	Wald statistic (χ^2)	*P*
Strain (712)	**1.32 ± 0.64**	**4.21**	**0.04**
Time (before)	1.25 ± 0.89	2.02	0.15
Frequency	**3.12 ± 0.92**	**11.39**	**<0.01**
Strain*time	**−2.76 ± 1.24**	**4.89**	**0.02**
Strain*frequency	**−5.47 ± 1.41**	**14.96**	**<0.01**
Frequency*time	**−2.99 ± 1.54**	**3.76**	**0.05**
Strain*time*frequency	**6.86 ± 2.31**	**8.80**	**<0.01**

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.

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 low frequency, decreased 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 and Kravitz 2017) and winner effects do not last longer than a few hours (Trannoy et al. 2016; Trannoy and Kravitz 2017). 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 et al. 2004), and the carry-over of environmental effects into different contexts results in fitness costs (Ferrari et al. 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 behavior 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 and 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 behavioral plasticity, there may have been other cues or stimuli during the period of fixed resources that lead to the observed changes in behavior. For example, there may have been aromatic stimuli which varied across the social environments, leading to changes in behavior. In our study, there was a time lag of 1–3 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 et al. 1995). Short-term changes in aggressive behavior in *D. melanogaster* is affected by changes in protein synthesis (Trannoy et al. 2016). In contrast, iterative competitive interactions can result in longer

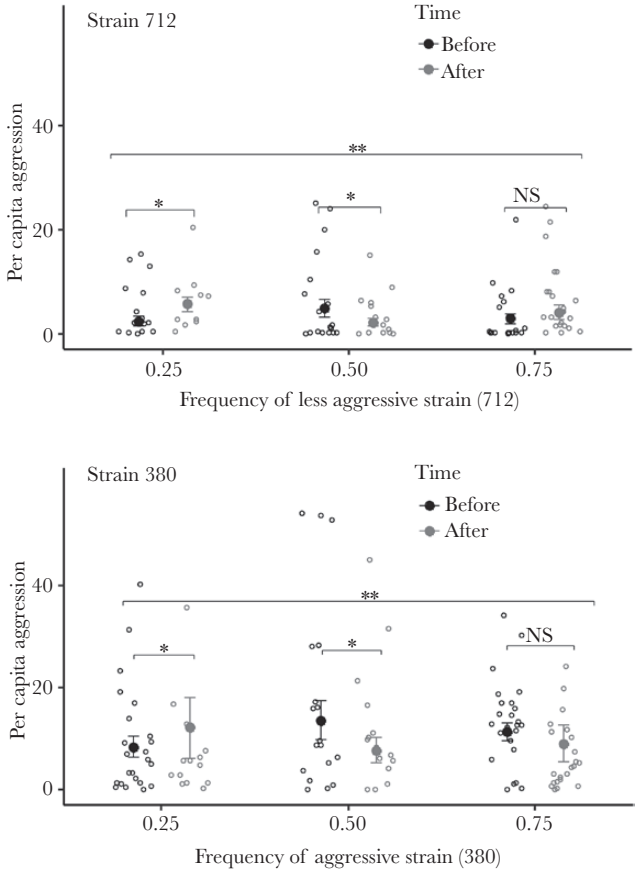

Figure 4
Males of both strains showed an increase in aggression when at low frequency and an increase in aggression at equal frequency. Mean aggression (filled circles) exhibited by males from strain 380 (top) and strain 712 (bottom) at high density, before (black) and after (gray) the period of fixed resources. Vertical bars represent SE.

term (days) changes in aggressive behavior (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 (NiemelÃ and 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 non-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 behavior. 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 behavior. 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; Yurkovic et al. 2006; Saltz 2013) 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' (Ueda and Kidokoro 2002; Nilsen et al. 2004). 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 (Tibbetts 2008; Cain and Langmore 2016). 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 and 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 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.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Kilgour et al. (2019).

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