

Genetic Variation in Male Aggression Is Influenced by Genotype of Prior Social Partners in *Drosophila melanogaster*

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ABSTRACT: Social behaviors can be influenced by the genotypes of interacting individuals through indirect genetic effects (IGEs) and can also display developmental plasticity. We investigated how developmental IGEs, which describe the effects of a prior social partner's genotype on later behavior, can influence aggression in male *Drosophila melanogaster*. We predicted that developmental IGEs cannot be estimated by simply extending the effects of contextual IGEs over time and instead have their own unique effects on behavior. On day 1 of the experiment, we measured aggressive behavior in 15 genotypic pairings ($n = 600$ males). On day 2, each of the males was paired with a new opponent, and aggressive behavior was again measured. We found contextual IGEs on day 1 of the experiment and developmental IGEs on day 2 of the experiment: the influence of the day 1 partner's genotype on the focal individual's day 2 behavior depended on the genotypic identity of both the day 1 partner and the focal male. Importantly, the developmental IGEs in our system produced fundamentally different dynamics than the contextual IGEs, as the presence of IGEs was altered over time. These findings represent some of the first empirical evidence demonstrating developmental IGEs, a first step toward incorporating developmental IGEs into our understanding of behavioral evolution.

Keywords: indirect genetic effects (IGEs), developmental plasticity, *Drosophila melanogaster*, genotype-by-genotype epistasis, aggression.

Introduction

An individual's phenotype can be influenced by its own genotype (i.e., direct genetic effects [DGEs]) and also by the genotypes of social partners, a phenomenon termed "indirect genetic effects" (IGEs; Griffing 1967; Moore et al. 1997; Wolf et al. 1998). A classic example of IGEs is maternal genetic effects (Wilson and Réale 2006), which describes when an offspring's phenotype is influenced by the maternal genotype beyond the effect of the alleles inherited. How-

ever, IGEs have been found in a variety of contexts and behaviors, such as communication (Kent et al. 2008), dominance (Wilson et al. 2011), antipredator behavior (Bleakley and Brodie 2009), and sexual attractiveness (Danielson-François et al. 2009; Saltz 2013).

The presence, directionality, and magnitude of IGEs can vary depending on the interaction between the genotype of the focal individual and the genotype of its social partner; this phenomenon, termed "genotype-by-genotype ($G \times G$) epistasis" (Culumber et al. 2018; Jaffe et al. 2020; Walsh et al. 2022), is a special case of genotype-by-environment interaction. Here, "genotype" refers to DGEs, and the relevant environment is the genotype (and phenotype) of one or more social partners. For example, in the pharaoh ant *Monomorium pharaonis*, groups of ant workers altered their exploratory behavior based on the genotypic composition of their social group (Walsh et al. 2022). In other words, the effect of specific genotypic combinations in the social group explained more variation in exploratory behavior than the main effect of individual genotypes. IGEs and $G \times G$ epistasis, when present, indicate that social interactions can create an evolving, heritable social environment that could impose new selection pressures on plastic traits (Wolf et al. 1998; Bailey et al. 2018; Jaffe et al. 2020).

IGEs and $G \times G$ epistasis have been well documented; however, most of the current work on IGEs focuses on its role in contextual plasticity (i.e., how an individual responds to immediate or current external stimuli; Stamps 2016). This snapshot picture of IGEs limits our understanding of the impact of IGEs on evolutionary processes. Indeed, at the behavioral level, we often see that how an individual behaves is a function of its past experiences, not only its current context. This type of longer-term plasticity, called "developmental plasticity," is when an individual's current behavioral response varies as a result of past stimuli or experiences. Here, the word "development" refers to the

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effects of prior experiences over any time frame. Behavioral traits that display developmental plasticity are common, and this plasticity creates variation in behavioral phenotypes that may significantly impact the behavior and lifetime fitness of an individual (reviewed in Frost et al. 2006; Snell-Rood 2013; Nettle and Bateson 2015; Stamps 2016). Mechanisms through which prior experiences can affect future behavior often involve changes at the neural or hormonal level (McBride et al. 1999; Oliveira et al. 2001). Despite this wealth of knowledge on developmental plasticity, the effect of the genotype of a previously experienced social partner on a focal individual's later behavior has scarcely been studied or even described. To our knowledge, only two studies have considered how the genotypes of prior social partners may influence later behavior: Filice and Long (2017) studied the persistence of IGEs over time in the context of female *Drosophila melanogaster* mate choice, and Makowicz et al. (2022) investigated how contextual IGEs could extend to influence new social partners in *Poecilia formosa*. We propose to refer to the ability of prior social partner genotype to alter behavior at a later time as “developmental IGEs.”

Studying developmental IGEs is important because they may produce different behavioral and evolutionary dynamics than contextual IGEs. For example, a common modeling approach used to estimate IGEs is to average the trait values of all of an individual's social partners over its lifetime (Moore et al. 1997; McGlothlin et al. 2010; Montiglio et al. 2018). If every individual reacts similarly to interactions with social partners across different time points, then this method would produce accurate predictions of social dynamics. However, developmental plasticity theory suggests that how individuals respond to social interactions is determined in part by their past experiences (Clark et al. 1999). A well-known example of this is seen in winner/loser effects, which explains how prior aggressive contests can influence an individual's fighting behavior, thus impacting the likelihood of winning or losing subsequent contests (Hsu et al. 2006; Trannoy et al. 2016). This example highlights an additional, more subtle consequence of developmental plasticity in social behaviors: individuals can modify their behavior based on their own prior experiences, and their behavioral modification may cause their future social partners to change their own reactions in response (Moretz et al. 2006). By extension, the presence, magnitude, or direction of contextual IGEs at a particular time point is predicted to be dependent on the history of all participants' previous interactions, but this hypothesis has not been tested. Empirical studies on developmental IGEs are needed to determine whether current IGEs models are effective in capturing and predicting how developmental IGEs alter behavioral, social, and evolutionary dynamics (Bijma 2013; Marjanovic et al. 2022).

Aggression is a common behavior displayed across a wide range of organisms and is often associated with the ability of

an individual to secure access to and defend resources, such as mating partners or territory (Huntingford and Turner 1987; Baxter et al. 2015). Aggression, being an inherently social behavior, is heavily influenced by the social environment in which these interactions occur (Fitzsimmons and Bertram 2013). Contextual IGEs have been well documented in aggressive contests, with individuals modifying their aggressive behaviors based on the phenotypes and genotypes of their current partner (Wilson et al. 2009; Camerlink et al. 2013; Saltz 2013). Additionally, the timing and order in which aggressive encounters occur during an individual's life span can be crucial for determining that individual's later behavioral responses (e.g., winner/loser effects; Hsu et al. 2006; Trannoy et al. 2016). However, we are unaware of any research into this intersection of social partner genotype and developmental plasticity on aggressive interactions—that is, developmental IGEs. Using aggressive behavior to analyze how IGEs and developmental plasticity are linked will illuminate the unique role, if any, that developmental IGEs may play in shaping trait variation.

In this study, we conducted an experiment to test how prior social experiences and IGEs can influence an aggressive contest (fig. 1). By manipulating the genotypes of opponents and having individuals participate in a series of dyadic aggressive contexts, we were able to test how prior experience with different genotypes influenced individual behavior in subsequent contests (i.e., developmental IGEs). We were also able to investigate how developmental plasticity influences the dynamics of future social interactions (i.e., differences between contextual and developmental IGEs). Specifically, we tested the following nonexclusive hypotheses:

Hypothesis 1 (H1): contextual IGEs. We predict that the genotype of an individual's current partner will influence the focal individual's aggression during the contest.

Hypothesis 2 (H2): developmental IGEs. We predict that the genotype of the focal individual's prior social partner will influence the focal individual's aggression at a later time.

Hypothesis 3 (H3): fundamental differences between contextual IGEs and developmental IGEs. We predict that developmental IGEs (H2) will not be a simple extension of contextual IGEs (H1) through time. Instead, we predict that developmental IGEs will produce novel behavioral patterns—for example, changes in the presence, magnitude, and/or direction of contextual IGEs—in an aggressive contest at a later time, compared with the previous contest.

Methods

Study System

We used *Drosophila melanogaster*, the fruit fly, to determine whether and how the genotype of previous social partners can affect an individual's later behavior. Fruit

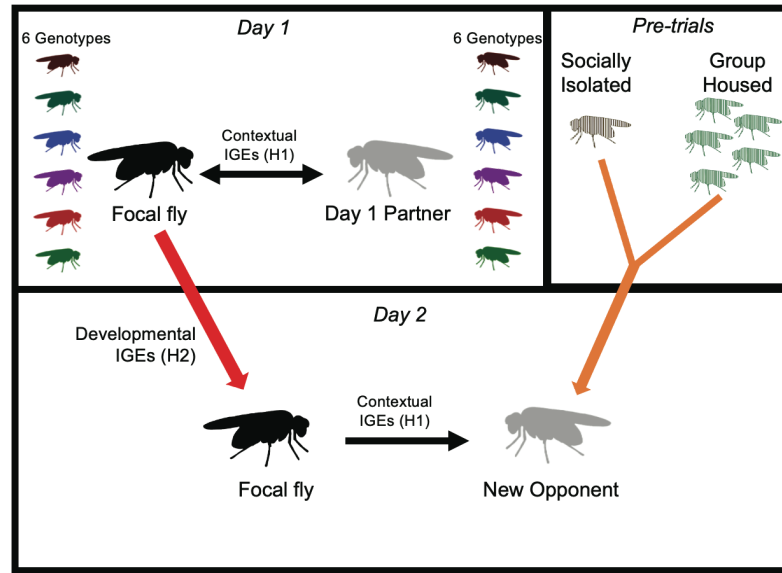


Figure 1: Overview of experimental trials for measuring developmental indirect genetic effects (IGEs) in aggression. A focal male fly ($n = 6$ inbred genotypes) was measured for aggressive behaviors with a partner fly on day 1. The genotype of the partner fly was varied ($n = 6$ inbred genotypes). Every possible genotype combination (except same-genotype combinations) was tested ($n = 15$ genotypic pairings) for a total of 300 trials. The same focal male fly from day 1 was again measured for aggressive behaviors against a new opponent on day 2 of the experiment. The new opponent's genotype was kept constant, but its prior social experience was varied before the experimental trials (pretrials). The black arrows represent contextual IGEs, the orange arrow represents developmental plasticity, and the red arrow represents developmental plasticity that is mediated by IGEs (i.e., developmental IGEs).

flies readily form social groups on their rotting fruit food sources, both in the laboratory and in the field (Powell 1997; Wertheim et al. 2002, 2006; Saltz and Foley 2011; Dukas 2020). A number of social interactions occur within these social groups, including aggressive encounters (reviewed in Chen et al. 2002; Nilsen et al. 2004). IGEs in developmental plasticity would require us to examine not only the past experiences of an individual but also the genotype of prior interactants. Fruit flies are amenable to this complex experimental design, as they are well suited for laboratory use and we can easily obtain distinct genotypes for experimental testing (Stamps et al. 2005b; Saltz and Foley 2011; Stamps 2016). Additionally, fruit flies have been shown before to be an ideal study system for investigating IGEs, as demonstrated by the number of contextual IGE studies that have previously been conducted (Cabral et al. 2008; Saltz 2013; Filice and Long 2017; Kilgour et al. 2020).

Overview

Our study aimed to assess how DGEs, IGEs, and prior social experiences can interact to influence aggressive contest dynamics across time (fig. 1). To accomplish this, on day 1 of the experiment, we paired individuals from six distinct genotypes to engage in an aggressive contest, creating a total

of 15 unique genotypic pairings. During day 1 of the experimental trials, we measured contextual IGEs on aggressive behavior (H1 and H3; i.e., the extent to which the genotype of a focal fly's social partner influenced his aggression). On day 2 of the experiment, both day 1 participants were paired with a new opponent from a standard stimulus genotype. We manipulated the prior experience of the day 2 new opponents: new opponents were either group housed or socially isolated before the day 2 trials. These two treatments were chosen according to previous work that has shown how social experience (or lack thereof) can influence aggressive behaviors (Hoffmann 1990; Yurkovic et al. 2006; Wang et al. 2008). Day 2 experimental trials also allowed us to measure developmental IGEs (H2; i.e., the extent to which the genotype of a focal fly's day 1 partner influenced his later aggression against the new opponent on day 2). Furthermore, measuring social dynamics on day 2 allowed us to identify any novel behavioral patterns that may have arisen because of the developmental IGEs (H3).

Genotypes

Flies used in this study were created by repeated crosses of inbred parental lines. The parental lines were originally wild derived from a population in Raleigh, North Carolina

(MacKay et al. 2012), representing a sample of natural variation, plus Canton-S (CS), which is the standard wild-type genotype that is widely used in studies of fly behavior (Chen et al. 2002; Nilsen et al. 2004; Trannoy et al. 2016). The Raleigh genotypes used were 208, 335, 360, 637, and 707 (the genotype names/numbers are arbitrary and have no meaning other than providing a name for each genotype). Genotype CS was used both as a focal genotype and as a new opponent for the day 2 experiments.

Rearing

Flies were reared on approximately 10 mL of standard fly food. Each vial was founded by 10 males and 10 virgin females to create standard conditions that minimized variability in larval densities. All experimental flies were collected within 8 h of eclosion. To identify individual males during the trials, each fly was anesthetized with CO₂, and a small dot of paint was placed on its back (Stamps et al. 2005a; Saltz 2013). The paint color was chosen randomly both for focal males and for day 2 new opponents. Focal male flies were housed individually in vials and were maintained under a 12L:12D cycle, with lights turning on at 0900 hours. Experimental trials began 5 days after eclosion.

Aggression Arenas

Aggressive contests were conducted in arenas that were assembled by taping two Petri dishes together (3 cm in diameter, 1 cm high) and adding a small blue cap (1.5 cm in diameter, 1 cm high) in the middle of the arena that was filled with standard fly food medium (Chen et al. 2002). The cap was topped with a small dot of yeast paste (made by combining live Fleishman's yeast with a small amount of water).

Trials

Trials took place from October 2016 to November 2017. All trials were conducted in the morning at subjective dawn (0900–1000 hours), reflecting peak activity time for the flies (Partridge et al. 1987).

Day 1. On day 1 of the experimental trials, two focal males were added to the arena without anesthesia and allowed to acclimate to the new environment for 30 min (Chen et al. 2002). After the acclimation period, an observer recorded how many times each of the males performed one of our five measures of aggressive behavior: lunging (one fly rearing on his back legs and pouncing on his opponent), fencing (both flies extending and engaging with each other's legs by tapping and/or pushing), wing threat (one fly lifting and holding his wings at a 45° angle while facing his opponent), chasing (one fly closely following/running behind his opponent), and boxing (a high-level behavior characterized

by both flies rearing on their hind legs and engaging each other with their front legs by pushing and/or hitting; Chen et al. 2002; Nilsen et al. 2004). Trials were conducted for 30-min periods.

Day 2. On day 2 of the experiment, each focal male who participated in a day 1 contest was then paired against a new opponent. All day 2 new opponent males were genotype CS. We used two treatments of opponent males: group housed and socially isolated. Upon eclosion, new opponent males were placed into vials either in groups of five or individually, depending on their treatment, and aged in those vials for 6 days. Each male focal fly from day 1 was placed into an identical arena along with his assigned new opponent to test how prior social experience and IGEs influence developmental plasticity in aggression. Again, after a 30-min acclimation period, an observer recorded five measures of aggressive behavior for each of the two males for 30 min.

Experimental Design and Replication

On day 1, each genotype ($n = 6$) was paired and observed for aggressive behaviors with each other genotype in a round-robin design (focal males of the same genotype did not fight each other). Thus, we tested a total of 15 unique genotypic pairings. We conducted 361 day 1 trials; however, about 20% of individual flies either escaped or died before day 2. In trials where escapes occurred, we retained the remaining male and also conducted make-up trials, resulting in 20 trials for each genotypic pairing. In total, we had observations of 600 individual focal flies for day 1.

Next, males tested on day 1 of experimental trials were assigned to one of two treatments for day 2 of trials, corresponding to different prior experiences of their new opponents: focal male flies faced a CS male who was either socially isolated ($n = 300$) or group housed ($n = 300$). We conducted a total of 600 trials on day 2, using the 600 individual focal flies from day 1 against 600 new opponents.

Analysis

Approach

To quantify aggression, we summed the number of lunges, wing threats, fencing, and chasing behaviors performed by each fly on each day; we refer to this measure as "total aggression." Boxing was excluded from this measure because of its low rate of occurrence (day 1 boxing: $n = 15$, mean = 0.025; day 2 boxing: $n = 12$, mean = 0.02).

All analyses were performed using R (ver. 4.1.0; R Core Team 2021). To investigate how previous social experience, genotype, and current social partners could modify an individual's aggression, we created generalized linear mixed models in a Bayesian framework using the brms package

in R (Bürkner 2018), which is an interface to the MCMC sampler Stan (Carpenter et al. 2017). Generalized linear mixed models were used to model the nonnormal error distribution and account for random effects (Bolker et al. 2009). Our response variables were total aggression of the focal male on day 1 and total aggression of the focal male on day 2 (see table S1 [tables S1–S6 are available online] for a model with a response variable of new opponent aggression on day 2). For all of our models, we specified a negative binomial error distribution with a logit link function, which is appropriate for overdispersed count data (for a histogram of the raw data, see figs. S2–S4; figs. S1–S4 are available online). In all models, for β values of fixed effects, we specified weakly noninformative priors centered on zero.

Model Building and Model Selection

We modeled the genotype of the focal individual and the genotype of his day 1 partner as random effects in all three of our models. By using random effects, we were able to infer variance in male aggressive behavior due to genetic differences. Additional fixed effects and their interactions were determined through hypothesis-led model building. The full results of the models are given in tables 1 and 2.

We used a variance-based approach to model the effects that IGEs had on aggressive behavior (McGlothlin and Brodie 2009). The variance-based modeling approach considers how the genotype of a focal individual's current or prior social partner affects focal behavior. Thus, the variance-based approach captures the indirect effects of all traits that vary among genotypes in this sample, quantifying the overall influence of interacting with individuals of different genotypes on a focal individual's aggressive behavior over time.

The first model, focal day 1 model, was used to test the hypothesis that the genotype of the focal individual's day 1 partner influenced his aggression during the contest (H1). We modeled the focal individual's total aggression

on day 1 of the experiment as our response variable in this model (table 1). A nonzero effect of partner genotype on focal aggression would indicate that we observed contextual IGEs. In addition to the random effects of the genotypes of both focal flies and partner flies, we also included an interaction term between the two, to test for $G \times G$ epistasis. Our full model for the aggressive response of the focal male, i , against a partner, m , was as follows:

$$Y_{im} = \mu_0 + \beta_1 c_i + (d_i + t_i + g_i + g_m + g_{im}) + \varepsilon_{im}.$$

Thus, β_1 describes the fixed effect of focal paint color, d_i is the random effect of date, t_i is the random effect of trial ID, g_i is the random effect of focal genotype, g_m is the random effect of partner genotype, and g_{im} is the interaction between partner and focal genotype.

Our second model, the focal day 2 model, assessed how the focal individuals' aggression on day 2 of the experiment varied as a function of his own and his opponents' past social experiences, thus testing for the effects of developmental IGEs on focal aggression (H2). Additionally, by comparing how IGEs influenced focal aggression in this day 2 model compared with the day 1 model, we could test the hypothesis that developmental IGEs are not simply an extension of contextual IGEs and rather could create new or altered behavioral patterns (H3). The focal individual's total aggression on day 2 of the experiment was the response variable for this model (table 2).

The prior social experience of the new opponent was a fixed effect. We included a random effect of the genotype of the focal male's partner on day 1; a nonzero estimate for this term would provide support for developmental IGEs (H2). In addition, we tested several interactions in our focal day 2 model to further investigate our proposed hypotheses. We tested the interaction between the genotype of the focal male and the genotype of the focal male's partner from day 1 as a random effect to investigate $G \times G$ epistasis and to test whether developmental IGEs were different in their presence, magnitude, and/or direction compared

Table 1: Generalized linear mixed model assessing focal aggression on day 1 of the experiment

Predictor variable	Description	Estimate (95% CI)	Proportion total variance (95% CI)	Permutation <i>P</i> value
Focal genotype	Direct genetic effects	.75 (.39–1.83)	.202 (.04–.1)	<.001
Day 1 partner genotype	Contextual indirect genetic effects (variance based)	.27 (.05–.81)	.03 (0–.14)	.009
Focal genotype $\times$ day 1 partner genotype	$G \times G$ epistasis on day 1 of experimental trials	.12 (.01–.35)	0 (0–.03)	.4
Trial ID	Nonindependence of two interacting males in each arena	.93 (.72–1.12)	.29 (.14–.42)	...
Date	Day the trial was conducted	.31 (.32–.58)	.03 (0–.1)	...

Note: CI = credible interval; $G \times G$ = genotype by genotype.

Table 2: Generalized linear mixed model assessing focal aggression on day 2 of the experiment

Predictor variable	Description	Estimate (95% CI)	Proportion total variance (95% CI)	Permutation <i>P</i> value
Focal genotype	Direct genetic effects	.48 (.19 to 1.28)	.19 (0 to .53)	.001
Day 1 partner genotype	Effect of developmental IGEs on focal individual aggression	.1 (.00 to .45)	.01 (.00 to .09)	.5
Focal genotype × day 1 partner genotype	G × G epistasis in developmental IGEs	.3 (.06 to .53)	.07 (0 to .18)	.006
New opponent treatment	Effect of prior social experience on focal individual	−.16 (−.37 to .05) ^a	...	...
Date	Day the trial was conducted	.34 (.09 to .54)	.08 (0 to .18)	...

Note: CI = credible interval; G × G = genotype by genotype; IGE = indirect genetic effect.

^a Solo.

with the contextual IGEs from day 1 (H3). Our full model for the aggressive response of the focal male, i , against a new opponent, n , and a prior partner, m , was as follows:

$$Y_{imn} = \mu_0 + \beta_1 c_i + \beta_2 s_n + (d_i + g_i + g_m + g_{im}) + \varepsilon_{imn}.$$

Thus, β_1 describes the fixed effect of focal paint color, β_2 describes the fixed effect of new opponent treatment, d_i is the random effect of date, g_i is the random effect of focal genotype, g_m is the random effect of day 1 partner genotype, and g_{im} is the interaction between partner and focal genotype.

In principle, it is possible for DGEs and IGEs to be correlated (Wolf 2003; Fisher et al. 2019). To test whether this might be important in our case, we fitted preliminary models for each day, in which we either estimated the DGE-IGE correlation or set it to zero. We found that including a nonzero DGE-IGE correlation in the model substantially degraded the fit of the model for day 1 ($\Delta\text{WAIC} = 9.4$ in favor of the model without the covariance) and had no major effect on model fit for day 2 ($\Delta\text{WAIC} = 1.9$ in favor of the model with the covariance). Furthermore, all estimated DGE-IGE correlations were small and had credible intervals (CIs) overlapping zero (tables S2, S3). Therefore, our final models did not include a correlation between DGEs and IGEs.

Inference

Our main focus was on the variance components (i.e., random effects) of each model, as these capture DGEs and IGEs and their interaction. Because variance components are bounded at zero (i.e., a variance cannot be negative), inspection of CIs for variance components is not a reliable guide for determining whether a particular variance is meaningfully different from zero. Following recent guidance for using simulated data to improve statistical inference (Pick et al. 2022), we computed additional measures to determine support for nonzero group-level variance.

To calculate P values (i.e., in the frequentist's sense) for the random effects, we used a permutation approach (Araya-Ajoy and Dingemanse 2017; Pick et al. 2022). For each dataset, day 1 or day 2, we randomly shuffled the values of the focal genotype and day 1 partner genotype pairing 1,000 times. Therefore, all of the same genotype pairings in our real data, in the same sample sizes, were present, but they were disassociated from the relevant behavioral data (for another example of using this approach to estimate IGEs and G × G epistasis, see Wice and Saltz 2023).

To preserve the structure of the data and facilitate direct comparison to the real variance estimates, we randomized genotype-pair information within trial dates. Next, each permuted dataset was analyzed using the relevant generalized linear mixed model: either the focal day 1 model for the permuted day 1 datasets or the focal day 2 model for the permuted day 2 datasets. Following each analysis, we retained the median value of the posterior parameter estimate for each random effect of interest (i.e., DGEs, IGEs, and their interactions). This approach allowed us to generate a null distribution of 1,000 medians of the posterior parameter estimate for each effect of interest on each day, describing the expected value of the median for the posterior parameter estimate if a fly's aggressive behavior was randomized with respect to his own genotype and that of his partner. We then computed the proportion of these null estimates that were greater than or equal to the median of the posterior distributions from analysis of our real data to compute a P value (Pick et al. 2022). Variance components from analysis of our real data (assessed as the median of the posterior parameter distribution) were considered to be nonzero if their magnitude was greater than 95% or more of the corresponding estimates in the null distribution.

To assess the magnitude of the variance components, we report the model parameter estimate as well as estimates of the proportion of variance “explained” by each random effect (similar to Bowdring et al. 2021). The model parameter

estimate, standard deviation (SD) parameter estimate, is the square root of the variance of random effect slopes; thus, greater values indicate greater differences among levels of the relevant parameter (e.g., for the random effect of genotype, greater values of the SD parameter estimate mean greater differences among genotypes). To estimate the variance “explained” by each random effect, we calculated the posterior medians and highest-density continuous intervals for each random effect and residual variance (Nakagawa and Schielzeth 2013) by taking draws from the posterior distribution, a method used to calculate a Bayesian version of R^2 (Gelman et al. 2019). We report estimates of variance on the log-odds scale (i.e., the scale of the model rather than the scale on which the data were originally collected). While there is some dispute about which scale is more relevant for evolutionary analysis, our focus here was on directly comparing these magnitudes across days to test H3.

Results

H1: Direct and Indirect Genetic Effects on Aggression on Days 1 and 2

We found support for both DGEs and contextual IGEs. We observed that genotypes differed in their aggression levels both on day 1 (focal day 1 model: SD parameter estimate, 0.75 [0.39–1.83 95% CI]; proportion total variance, 0.20 [0.04–0.52 95% CI]; zero permuted values were greater than or equal to this estimate, $P < .001$) and day 2 (focal day 2 model: SD parameter estimate, 0.48 [0.19–1.28 95% CI]; proportion total variance, 0.19 [0.00–0.53 95% CI]; zero permuted values were greater than or equal to this estimate, $P < .001$), indicating evidence of DGEs. The genotype of the day 1 opponent had an effect on the focal male’s aggression on day 1 (focal day 1 model: SD parameter estimate, 0.27 [0.05–0.81 95% CI]; proportion total variance, 0.03 [0.00–0.14 95% CI]; nine permuted values were greater than or equal to this estimate, $P = .009$), indicating evidence of contextual IGEs.

H2: Genotype of a Previous Social Partner Influenced Later Aggressive Behavior

We found evidence for developmental IGEs that affected focal male behavior on day 2. While we did not see a main effect of day 1 partner genotype (focal day 2 model: SD parameter estimate, 0.1 [0.00–0.45 95% CI]; proportion total variance, 0.01 [0.00–0.09]; 501 permuted values were greater than or equal to this estimate, $P = .5$), the interaction term between focal genotype and day 1 partner genotype predicted focal male aggression on day 2 (focal day 2 model: SD parameter estimate, 0.30 [0.06–0.53 95% CI]; proportion total variance, 0.07 [0.00–0.18 95% CI]; six permuted values

were greater than or equal to this estimate, $P = .006$). This result indicates that how IGEs from day 1 influenced focal behavior on day 2 of the experiment depended on the genotype of both the focal male and his day 1 partner (fig. 2).

H3: Developmental IGEs Differed from Contextual IGEs

Developmental IGEs produced distinct effects on focal male behavior over time compared with contextual IGEs. In the focal day 1 model, we found evidence for a main effect of day 1 partner genotype in predicting the aggression of the focal male (reported above under H1). While we found that the median of the posterior parameter estimate for partner genotype differed from the distribution of medians derived from the permuted datasets on day 1, the CI for the proportion of total variance explained by this grouping factor was very wide. Therefore, while we found support that this variance component is nonzero, we are unable to draw firm conclusions about its exact magnitude.

In contrast, we did not see a main effect of day 1 partner genotype in predicting aggression of the focal male on day 2 (reported above under H2). Additionally, in the focal day 1 model, we did not find evidence that the variance component for $G \times G$ epistasis differed meaningfully from zero (focal day 1 model: SD parameter estimate, 0.12 [0.01–0.35 95% CI]; proportion total variance, 0.00 [0.00–0.03 95% CI]; 375 permuted values were greater than or equal to this estimate, $P = .4$). However, we did see support for $G \times G$ epistasis in predicting focal male aggression on day 2 of the experiment (reported above under H2). Specifically, our permutation tests showed support for differences between our null and real estimates for $G \times G$ epistasis only on day 2. Again, given the wide CIs for the proportion of total variance estimates, we are unable to draw firm conclusions about the exact magnitude of these variance components.

Full results from each model can be found in tables 1 and 2, and the model covariate results as well as the results from the new opponent model are presented in the supplemental PDF. Altogether, these results indicate that developmental IGEs produce novel dynamics in male aggressive behaviors over time compared with contextual IGEs.

Discussion

Despite the wealth of knowledge on both developmental plasticity and IGEs, developmental IGEs, which describe the effect of the genotype of a previously experienced social partner on a focal individual’s later behavior, have only recently been described. Studying IGEs purely within the immediate interaction in which they occur results in empirical studies and theoretical models that are unable to answer how IGEs operate on longer timescales. IGEs could interact with prior experience to produce novel or distinct effects on

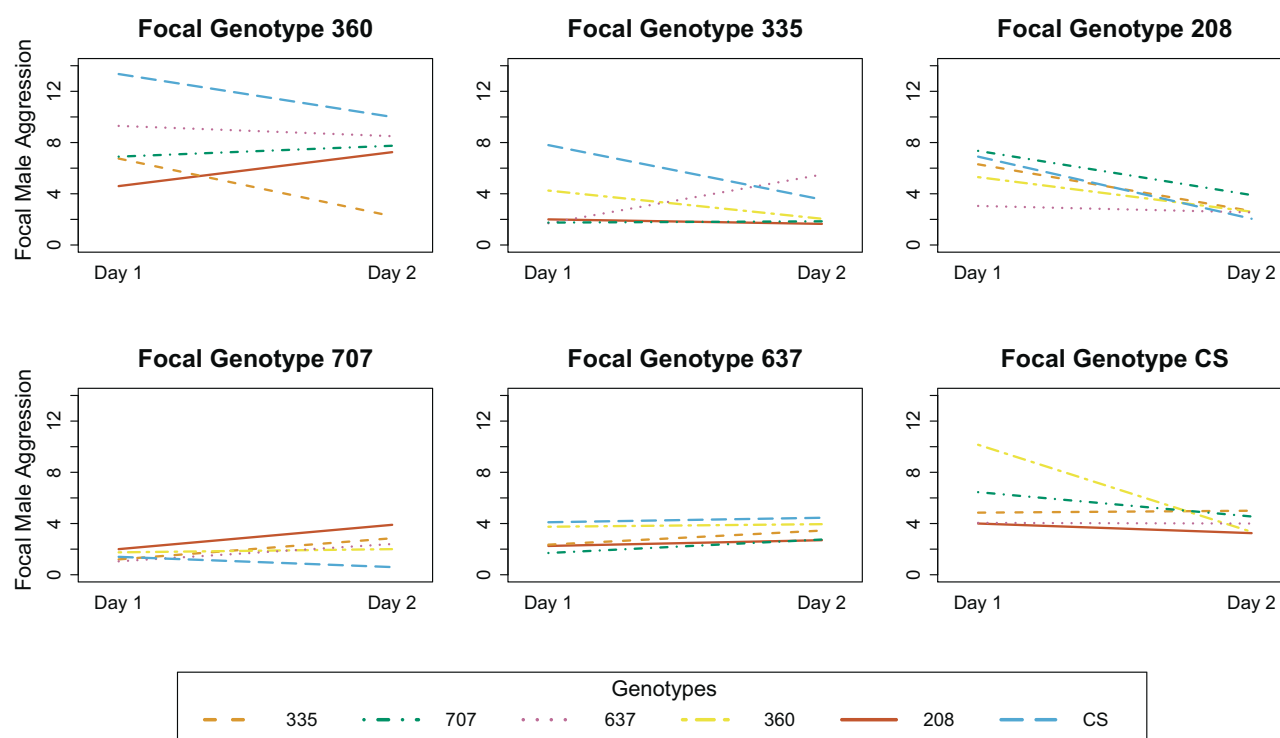


Figure 2: Genotype-by-genotype ($G \times G$) epistasis in developmental indirect genetic effects. Each graph represents the focal male's aggression (y-axis) toward his day 1 and day 2 opponents (x-axis), and each line in the graph represents a day 1 partner genotype. The genotype of the day 2 opponent was always Canton-S (CS). We identified $G \times G$ epistasis even after controlling for the new day 2 opponent's prior social experience (see text). The genotype of the focal male's day 1 partner significantly influenced focal male behavior on both day 1 and day 2 of the experimental trials. On day 2 of the experiment, the effect of the day 1 partner genotype depended on the genotype of both the day 1 partner and the focal male (see text). A color version of this figure is available online.

individuals, but this has yet to be demonstrated. In this study, we used *Drosophila melanogaster* to investigate how prior social experience and IGEs can influence aggressive contests through developmental IGEs. We found support for our first hypothesis, as contextual IGEs did affect focal male aggressive behavior. We also found evidence that IGEs continued to affect focal male aggressive behavior a day after the initial interaction, representing developmental IGEs. Finally, developmental IGEs had distinct effects on behavior compared with contextual IGEs. These results support our second and third hypotheses and represent one of the first experimental demonstrations of developmental IGEs (fig. 2).

We found that developmental IGEs can produce fundamentally distinct dynamics from predictions that are formed from contextual IGEs alone. Most importantly, we identified $G \times G$ epistasis on day 2 of the experiment but not on day 1. In other words, on day 1 the influence of a partner's genotype on the focal individual's aggression (i.e., contextual IGEs) was similar across all focal genotypes. In contrast, the effect that day 1 partner genotypes had on focal individuals' aggression the next day (i.e., developmental IGEs) was

dependent on the genotypic identity of both the focal male and the partner male. Our finding of $G \times G$ epistasis during day 2 of the experiment suggests that the presence of IGEs was altered from day 1 to day 2 in a genotypic-specific way. Additionally, our finding of $G \times G$ epistasis for developmental, but not contextual, IGEs indicates that developmental IGEs are not simply an extension of contextual IGEs but instead produce distinct effects on behavior. Social behaviors are flexible and depend heavily on the environment, and researchers have long suggested that the most relevant way to study these behaviors is through the lens of interacting phenotypes and genotypes (Moore et al. 1997). Our results affirm this view and suggest that IGE models should incorporate $G \times G$ epistasis and other forms of non-additive variance when studying any individual phenotype, but especially behavior, to make accurate estimations of IGEs in social groups across time (Meffert et al. 2015; Culumber et al. 2018; Jaffe et al. 2020).

There are several mechanisms that may be driving the observed genetic variation in developmental IGEs (i.e., the $G \times G$ epistasis we observed on day 2). Individuals engaged in fights can utilize learning and memory to adjust

their later behavior based on this prior social experience (including in flies; Yurkovic et al. 2006). Thus, the presence of $G \times G$ epistasis during the second set of interactions in this experiment could arise because some genotypes did not remember the social interactions that happened to them the day before. As a result, these genotypes would have adjusted their behavior in response to their social partner on day 1 but then returned to their genotype-specific “baseline” on day 2, regardless of what had happened to them the day before. Other neurobiological mechanisms, such as peptide and amine systems, have also been heavily implicated in influencing aggression levels through several complex pathways and could also contribute to genetic differences in aggressive response (reviewed in Kravitz and Fernandez 2015). Factors that contribute to developmental IGEs and $G \times G$ epistasis, especially those concerning aggression, are likely to be complicated and multifaceted, incorporating various molecular, neuronal, and sensory mechanisms. Further studies into the mechanisms through which developmental IGEs can influence future contest dynamics could help us better understand the long-lasting effects of social encounters.

There are a few caveats to acknowledge within this study. The first is that we used inbred lines of *D. melanogaster* in our experiments. While using inbred lines allowed us to answer our questions about how genetic variance and its effects can influence developmental plasticity, inbred lines do not always reflect the genetic diversity that we may see in the wild. In addition, we unexpectedly saw a nonzero effect of paint color on aggression on days 1 and 2 (tables S4–S6). Since paint colors were assigned randomly, and we adjusted for the unexpected effect of paint color in the model, this effect did not interfere with our ability to test our hypotheses about IGEs over development. However, it reinforces the fact that individual aggressive behavior can be shaped by all prior experiences, such as handling stress, not just social experiences. Experimentalists continue to seek the best methods for marking individuals in species such as fruit flies, where individuals look mostly indistinguishable to the human eye.

Additionally, we experimentally manipulated the social experiences of focal individuals to address our research questions. However, in nature, individuals can choose who they interact with, a process that may further influence the dynamics and fitness outcomes of social interactions (Partridge 1980; Wolf et al. 1999; Weidt et al. 2008; Saltz and Nuzhdin 2014; Saltz 2016). Subsequent studies into developmental IGEs would benefit from allowing individuals to select their own social partners, which could uncover how assortative interactions can alter the effects and evolutionary outcomes of developmental IGEs (Agrawal et al. 2001).

Our results confirm that prior social experience and IGEs can interact together to influence developmental plasticity

in an aggressive context. To the best of our knowledge, we are among the first to demonstrate an empirical example of developmental IGEs. Continuing to examine developmental IGEs in a variety of traits and social contexts will further illuminate the many ways in which IGEs can influence behavior and evolution.

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Statement of Authorship

T.D. and J.B.S. were responsible for conceptualization and experimental design. T.D. collected data. M.H., L.P., and J.B.S. analyzed data. M.H., L.P., and J.B.S. wrote the manuscript.

Data and Code Availability

Data and code have been uploaded to figshare (https://figshare.com/projects/Genetic_variation_in_male_aggression_is_influenced_by_genotype_of_prior_social_partners_in_D_melanogaster/185917; Hutchins et al. 2023). Separate files include README (<https://doi.org/10.6084/m9.figshare.24582453.v1>), developmental IGEs R script (<https://doi.org/10.6084/m9.figshare.24578914.v1>), perm_day1_all dataset (<https://doi.org/10.6084/m9.figshare.24578839.v1>), perm_day2_all dataset (<https://doi.org/10.6084/m9.figshare.24578881.v1>), developmental IGE dataset (<https://doi.org/10.6084/m9.figshare.24565858.v1>), and developmental IGE interaction plot dataset (<https://doi.org/10.6084/m9.figshare.24566416.v1>).

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