Title: Evolutionary feedbacks for *Drosophila* aggression revealed through experimental evolution

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Abstract: Evolutionary feedbacks occur when evolution in one generation alters the environment experienced by subsequent generations. Despite longstanding hypotheses that feedbacks should be nearly ubiquitous for social behaviors, we still know little about how feedbacks influence evolution. Using experimental evolution, we manipulated the social environment in which aggression was expressed and selected in fruit fly (*Drosophila melanogaster*) populations to allow or limit feedbacks. We selected for increased male-male aggression while allowing either positive, negative, or no feedbacks, alongside unselected controls. Populations undergoing negative feedbacks showed the weakest responses to selection, while populations undergoing positive evolutionary feedbacks evolved supernormal aggression. Further, the underlying social dynamics evolved only in the negative feedbacks treatment. Our results demonstrate that evolutionary feedbacks can alter the rate and pattern of behavioral evolution.

Main Text: Evolutionary feedbacks occur when trait evolution in one generation alters selection pressures in subsequent generations (*1–3*); Figure 1). Traditional behavioral biology focused on how the environment affects behavior, often ignoring reciprocal effects whereby behaviors shape the environment (*4*). Yet, organisms typically—perhaps universally—alter their environments (*5–7*), e.g., by excreting waste, eating other organisms, and behaving socially. This interplay has important implications for evolution, because the environment mediates the genetic basis and fitness consequences of variation in traits. Consequently, the potential for evolutionary feedbacks between organisms and their environments has come into focus as a major, but unexplored, force driving phenotypic evolution (*1*, *8*, *9*), even on short timescales (*1*, *10*). Hypotheses abound for the role of feedbacks in climate change (*11*, *12*), community dynamics (*13*), population persistence (*10*), social interactions (*14*), the genetic basis of evolution (*15–17*), and more.

For social behaviors, the "environment" in which behaviors are expressed is formed by the behaviors of interacting conspecifics. Indirect genetic effects (IGEs) explicitly consider how genes expressed in these social partners may influence a focal individual's behavior (*18–21*). Note that "indirect" refers here to the effect of one individual's genes on another individual's behaviors, and is distinct from other "indirect effects" in ecology (*12*). IGEs stand in contrast to "direct" genetic effects, DGEs, whereby an individual's own genotype affects its own behavior. For example, guppies (*Poecilia reticulata*) are more willing to inspect a predator if they are paired with a partner who is also willing to inspect; and these behaviors are heritable (*22*). This example illustrates how social interactions may be determined by the genotypes of the participants. Indirect genetic effects are widespread, and have been identified for a wide range of traits and taxa (*23–26*).

Under indirect genetic effects, the environment itself can evolve: evolutionary changes in population-mean behavior also represent changes in the environment in which the behavior is expressed (*18*, *27*). Indeed, many familiar evolutionary feedbacks are examples of evolutionary outcomes of indirect genetic effects for social behaviors, such as Fisherian runaway selection and arms races (*8*). The existence of these feedbacks highlights the challenge of predicting behavioral evolution over more than one generation (*28–31*). Yet, empirical demonstrations of such feedbacks are scarce (*29*, *32–34*), likely because isolating the effects of evolutionary feedbacks – if any – is nearly impossible for wild populations (*35*).

To directly identify the role(s) of feedbacks in behavioral evolution, we founded 12 replicate *Drosophila melanogaster* populations from the same wild base population and subjected them to 24 generations of selection for increased aggression, while allowing or limiting the opportunity for different evolutionary feedbacks. Flies are a model system allowing us to use experimental selection to rigorously test hypotheses about evolutionary processes.

We focused on aggressive behavior because IGEs have been well-established for aggressive behavior in flies (*36*, *37*) and other species (*38*). Aggression is inherently a highly interactive trait, because animals plastically adjust their fighting behavior based on the responses of their opponents. Thus, the aggressive behavior of one individual is an environment that influences the aggressiveness of opponents. This plasticity occurs on two distinct timescales. On short timescales, i.e., during fights, aggression by one individual typically provokes retaliatory aggression by its opponent (in flies: (*37*, *39*) across taxa: (*40*)). Such **retaliation** represents a social environment that augments aggression: the more aggressive your opponent is, the more aggressive you are in return. Over longer timescales, i.e., across fights, flies who have previously experienced aggressive attacks show reduced aggressiveness in subsequent encounters (*37*, *41–44*). These **prior experience effects** thus represent a social environment that reduces aggression: the more aggressive your first opponent is towards you, the *less* aggressive you will be in your next encounter.

These social dynamics -- retaliation and prior experience effects -- are predicted to produce potent and opposite evolutionary feedbacks. Selection for increased aggressiveness means that future generations will experience a highly-aggressive social environment—an environment in which retaliation will augment aggression even further. This process represents a positive feedback because the direct effect of selection and the indirect effects of changing the environment in which the behavior is expressed act in the same direction—i.e., to increase aggression, augmenting the expected evolutionary response (Figure 1).

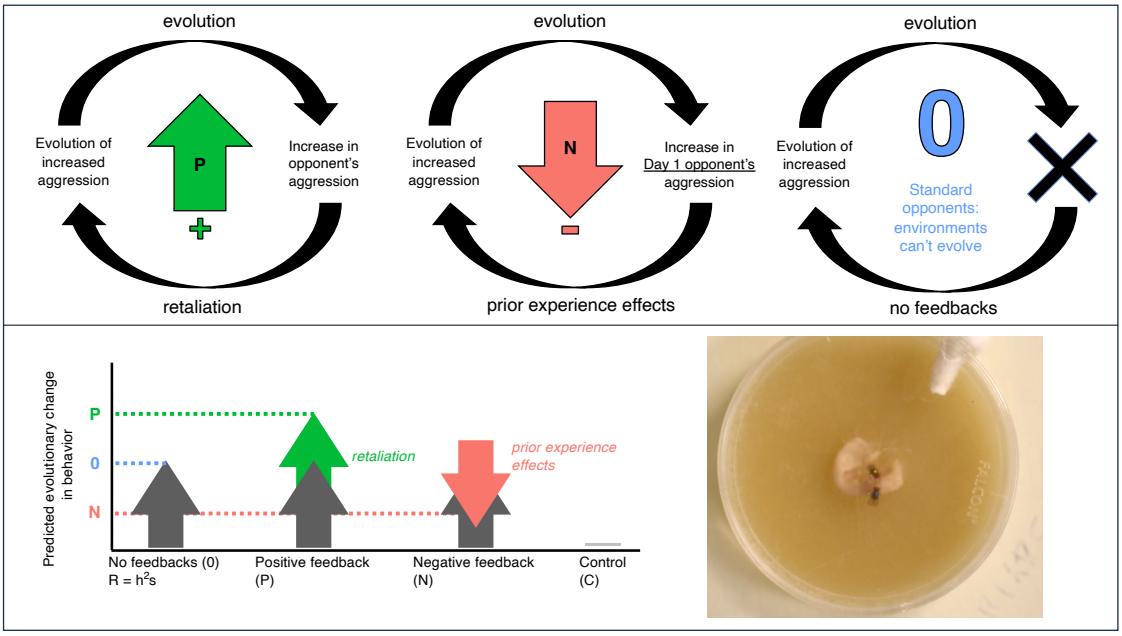

Figure 1. Study design and predictions. Top: evolutionary feedbacks arise for evolutionary changes in the environment in which behavior is expressed and selected. Our selection treatments manipulated the opportunity for different feedbacks, revealing the evolutionary consequences of feedbacks. Bottom left: positive feedbacks are predicted to enhance the observed phenotypic response to selection, while negative feedbacks are predicted to dampen selection response. Bottom right: marked flies fighting in an aggression arena.

To test this prediction, we had a **Positive Feedbacks (P) treatment.** To permit positive feedbacks, two naïve males from the same experimental population were paired each generation, for a total of 100 males in 50 pairs. Total aggression of each male was measured, and the 50 most aggressive individuals were selected. This treatment is expected to produce positive feedbacks because, as aggression evolves (i.e., the population responds to selection), males will face increasingly-aggressive opponents that will stimulate increased retaliatory responses, exaggerating the population's response to selection (Figure 1).

In contrast, with prior experience effects, as the population-mean aggressiveness increases (i.e., the population responds to selection), more individuals will experience aggressive opponents in their first aggressive encounter, resulting in increasingly suppressed aggressiveness in subsequent encounters. This process represents a negative feedback because the direct effect of selection and the indirect effect of changing the environment in which the behavior is expressed are in opposite directions—selection acts to increase aggression, provoking more potent prior experience effects that reduce aggression, dampening the expected evolutionary response (Figure 1).

To test this prediction, we had a **Negative Feedbacks (N) treatment.** In this treatment, males were subjected to 2 days of behavioral analysis. On Day 1, two naïve males from the same experimental population were paired, for a total of 100 males in 50 pairs; this experience

provides the opportunity for prior experience. The next day, Day 2, the same males were then paired with a standard opponent and aggressiveness was again measured. These standard opponents were males from the DGRP-208 inbred genotype, an inbred line whose progenitors were wild-collected and then highly inbred over many generations. Importantly, the inbred nature of these opponents mean that every male standard opponent was genetically identical to every other standard opponent in every generation. Selection was based only on males' day 2 aggressiveness: males from the experimental population were compared for total aggression on Day 2, and the 50 most aggressive males were selected.

It may seem intuitive that "negative" feedbacks should involve selection for reduced aggression—however, this is not the case. Negative feedbacks occur when behavioral plasticity and evolutionary changes in behavior act in **opposite** directions, i.e., there is a negative covariance between behavior of opponents (*18*). Here, this occurs because selection for increased aggression should result in behavioral plasticity (prior experience effects) that reduces aggression.

To quantitatively identify the effects of feedbacks, we compared the **P** and **N** treatments to a **No feedbacks (0) treatment.** To limit opportunities for feedbacks, 100 males from the experimental population were paired with standard opponents – the same genotype of standard opponents as in the **N** treatment on day 2 (i.e., DGRP-208 inbred genotype). Males from the experimental population were compared for total aggression, and the 50 most aggressive males were selected. This treatment abrogates the opportunity for IGE-mediated evolutionary feedbacks because the standard genotype was genetically identical in every generation, meaning the social environment was not allowed to evolve. Selection response should proceed approximately as $R=h^2s$, as expected under typical artificial selection (Figure 1).

Finally, we had **Controls (C)** populations that did not undergo selection. As expected, unselected control populations did not evolve increased aggression (table S3.S2).

Each replicate was founded by collecting 4 samples of the base population, with each sample consisting of 100 males and 100 females. These 4 samples became the 4 populations from this replicate, and each one was subjected to a different experimental treatment: one population from each of the 3 selection treatments and one unselected control population. In generation 0, 100 males from each population were paired together and measured for baseline aggression to ensure similar starting points. In generations 1-24, each population was subjected to evolution for increased aggression (except unselected control populations). Each population was measured for aggression every generation as part of the selection protocol (see below), except control populations, which were measured at generation 0 and again at the midway point and at the end of the experiment. Populations within each replicate were carefully matched demographically (see Materials and Methods and Table S1) to rule out any confounding effects of population history on selection response. In the final generation (generation 25 for replicates 1 and 2, generation 26 for replicate 3), samples from all populations were measured for prior experience effects so that we could test whether prior experience effects evolved, and the experiment was concluded.

By measuring 39,018 males over 25 generations in 12 initially-identical populations, our design allowed us to directly identify how feedbacks shape changes in aggressive behavior.

RESULTS

In every generation, 100 males from each population (except for unselected controls) were scan-sampled (*45*) for aggression 24 times in one day during peak activity periods. For each observation, trained observers recorded the presence or absence of 3 components of aggressive behavior: fencing - both flies extending and engaging with each other's legs by tapping and/or pushing; lunging - one fly rearing on his back legs and slamming his foretarsi onto his opponent; and boxing - both flies rearing on their hindlegs, "kangaroo style," and engaging each other with their front legs by pushing and/or hitting (*39*). When fencing and lunging occurred, observers noted which individual was the aggressor. Boxing can only occur when both individuals are engaged. Finally, observers noted whether either of the flies appeared to be temporarily stuck on the food patch, as occasionally occurred. We quantified total aggression as the unweighted sum of these behaviors; in the selection treatments (**P, N,** and **0**), the 50 males with the highest total aggression (in their treatment-specific social environment) were chosen to become fathers to the next generation alongside randomly-chosen, unmated females from their population.

During the experiment, it was apparent even through casual observation that the **P** populations showed extreme levels of boxing compared to the other treatments (Figure 2). Thus, we used a trait covariance approach to analyze how total aggression and each component of aggression evolved, i.e., lunging, fencing, and boxing we treated as repeated measures of each individual male. The resulting Generalized Linear Mixed Model (GLMM) describing evolution in the selected populations (Model 1) revealed a 3-way interaction between generation, treatment, and component (Table 1 - model 1; Table 2; Figure 1), meaning that treatment differences in evolutionary change differed between fencing, lunging, and boxing. For fencing, we found that all three selection treatments evolved increased fencing; P and 0 had indistinguishable slopes, and both P and 0 were faster than N (Table 2). For lunging, only the **0** treatment showed significant evolutionary changes in lunging (Table 2). For boxing, we found that P and N showed significant evolution of boxing, but not 0 (Table 2). The **P** treatment in particular showed dramatically faster evolution of boxing compared to the other treatments; indeed, on the scale of the original data (i.e., not the latent scale), the estimated slope for the P treatment was more than 35 times greater than the corresponding estimate for the N treatment (Table 2). As unselected control populations did not evolve increased aggression, we can be confident that these evolutionary changes are due to experimental selection. Overall, these results demonstrate that evolutionary feedbacks alter populations' phenotypic response to selection.

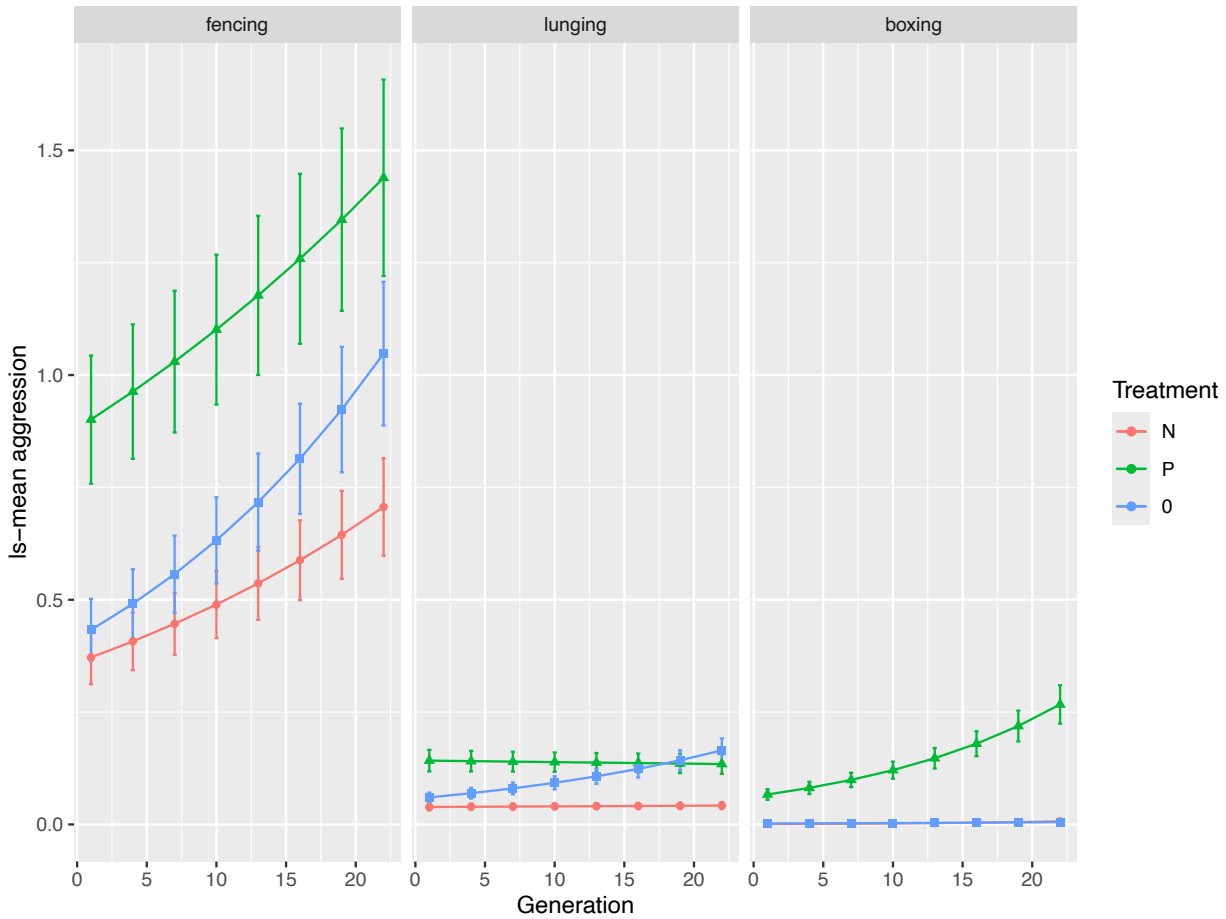

Figure 2. Evolutionary response to selection over 24 generations for each component of aggression. Y axis is least-squares means, adjusted for other terms in the model, estimated every 3 generations from model 1. N = 3 populations per treatment. Unselected control populations (not shown) did not evolve increased aggression.

model	key results				
	term	**parameter estimate (SE)**	**chisq**	**df**	**corrected p**
model 1: did feedbacks influence the evolution of aggression?	Generation	0.09289 (0.03473)	7.155	1	**0.02**
	Treatment	.	97.003	2	**0.00**
	component	.	355.479	2	**0.00**
	focal.stuck	-0.07609 (0.01464)	27.001	1	**0.00**
	Generation:Treatment	.	1.238	2	1.00
	Generation:component	.	11.414	2	**0.01**
	Treatment:component	.	65.049	4	**0.00**
	Generation:Treatment:component	.	19.196	4	**0.00**

	non-linear term	**edf**	**F**	**reference df**	**corrected p**
model 2: did prior experience effects evolve over generations?	day 1 opponent aggression	4.61099	873.724	5.489	**0.00**
	day 2 opponent aggression	2.78316	261.97	3.392	**0.00**
	Generation:day 1 opponent aggression	2.24636	14.948	2.638	**0.01**
	Generation:day 2 opponent aggression	1.01328	0.159	1.026	1.00

	non-linear term	**edf**	**F**	**reference df**	**p**
model 3: did prior experience effects differ across treatments at the end of the experiment?	effect of day 1 opponent behavior in control populations	1.00E+00	1	12.844	**0.00**
	effect of day 2 opponent behavior in control populations	1.00E+00	1	2.289	0.13
	difference in effect of day 1 opponent behavior between control and 0 treatment	1.00E+00	1	1.059	0.30
	difference in effect of day 1 opponent behavior between control and P treatment	1.00E+00	1	0.027	0.87
	difference in effect of day 1 opponent behavior between control and N treatment	1.00E+00	1	4.014	**0.05**
	difference in effect of day 2 opponent behavior between control and 0 treatment	1.00E+00	1	0.041	0.84
	difference in effect of day 2 opponent behavior between control and P treatment	1.00E+00	1	0.002	0.96
	difference in effect of day 2 opponent behavior between control and N treatment	1.00E+00	1	0.514	0.47

Table 1. Evolutionary feedbacks influenced the evolution of aggression and prior experience effects. Important results from each model are presented. Model 1 was a Generalized Linear Mixed Model (GLMM) describing the evolution of each component of aggressive behavior in selected populations during selection (generations 1-24). We used a trait covariance approach with each individual represented by three measurements corresponding to each component of aggression: lunging, fencing, and boxing. Thus, main effects indicate evolutionary changes to total aggression, independent of which component is considered, while interactions between predictors the variable "component" indicates component-specific evolutionary changes. We present parameter estimates for linear terms; p-values are corrected for the 3 different models run on the data (models 1, 2, and S3). Model 2 was a Generalized Additive Mixed Model (GAMM) using only data from the N treatment, which is the only treatment for

which prior experience effects were measured during generations 1-24. We tested whether changes in aggressive behavior over days varied across generations and due to opponent aggression on days 1 and 2 (i.e., prior experience effects). We present results from non-linear terms, which were fit as thin-plate splines. EDF describes the degree of non-linearity estimated for each term. In model 3, we tested whether prior experience effects differed across treatments at the end of the experiment. We fitted a GAMM describing the effects of opponent aggression on each day in the Control treatment as the "reference smooth;" the other terms represent "difference smooths" that estimate differences between each treatment's prior experience effects and those of the Control populations. For modeling details please see Materials and Methods; for full model results please see Supplemental Tables.

aggression component	selection treatment	estimated trend over generations (SE)	is the slope greater than 0?	results from planned contrasts
fencing	0	0.0291 (0.00491)	**yes** (z ratio = 5.9, p < 0.001)	a
	N	0.0160 (0.00307)	**yes** (z ratio = 5.2, p < 0.001)	b
	P	0.0257 (0.00535)	**yes** (z ratio = 4.8, p < 0.001)	a
lunging	0	0.00492 (0.000952)	**yes** (z ratio = 5.2, p < 0.001)	a
	N	0.000146 (0.000388)	**no** (z ratio = 0.377, p = 0.7)	b
	P	-0.000358 (0.000792)	**no** (z ratio = -0.5, p = 0.7)	b
boxing	0	0.000142 (0.000103)	**no** (z ratio = 1.4, p = 0.2)	b
	N	0.000256 (0.0000894)	**yes** (z ratio = 2.9 p = 0.004)	b
	P	0.00917 (0.00158)	**yes** (z ratio = 5.8, p < 0.001)	a

Table 2. Evolutionary effects of feedbacks for each component of aggression. Estimated marginal means of linear trends, representing change in behavior over generations, extracted from Model 1. Slopes are presented on the scale of the original data. We tested whether each slope differed from 0, indicating no detectable evolution, using t-tests, and we performed planned contrasts to test for slope differences between treatments. Comparisons between slopes were adjusted for multiple testing using the tukey method.

Feedbacks for social behavior depend on the underlying social dynamics – here, retaliation and prior experience effects – which may themselves evolve. While retaliation was present in all populations tested, none of the treatments showed evidence that the magnitude or form of retaliation changed over generations. Thus, we did not find evidence that retaliation evolved in our experiment (Table S3.S3). In contrast, we observed that prior experience effects evolved over generations in the Negative feedbacks **(N)** treatment (table 1 – model 2). To test whether this evolutionary change in prior experience effects was unique to the **N** treatment, we compared prior experience effects between the **P, N, 0,** and **C** treatments at the end of each replicate. Our generalized additive mixed model (GAMM) analysis (table 1 – model 3) revealed that prior

experience effects differed significantly from the unselected control **(C)** populations only in the **N** treatment, not in **P** or **0**.

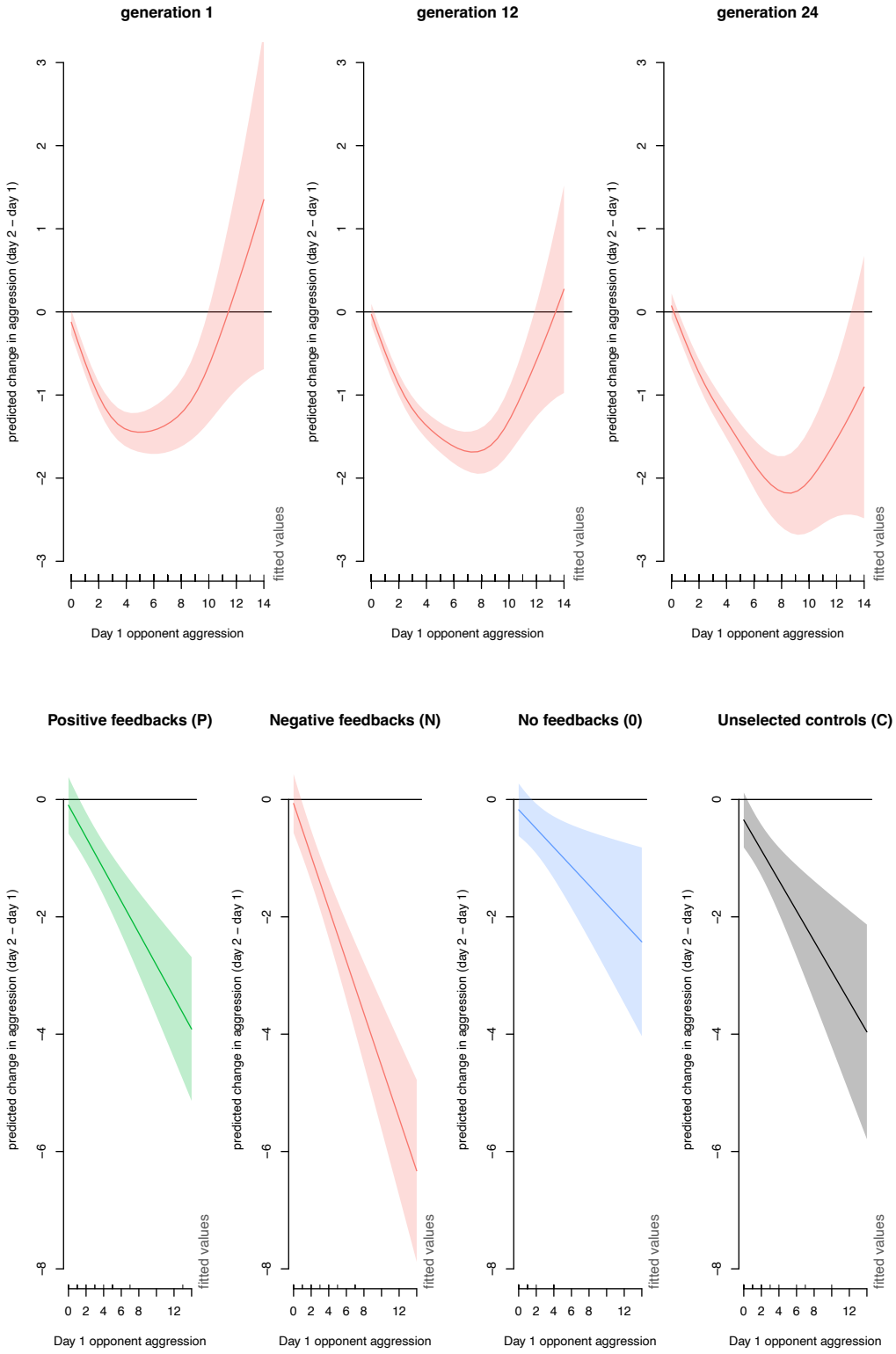

Figure 3. Prior experience effects evolved in the Negative Feedbacks treatment. Top: prior experience effects over 24 generations in the negative feedbacks treatment. Y axis is the change in behavior over days, where positive values mean that focal male aggression was enhanced after day 1 social experience, negative values mean that focal male aggression was suppressed after day 1 social experience, and zero indicates no effect of prior experience. The x-axis is the aggression of the focal males' day 1 opponent, and the line represents the cubic spline from Model 2 with associated standard error. Prior experience effects evolved to be more pronounced at higher values of day 1 opponent aggression. Bottom: comparison of prior experience effects at the end of the experiment for each treatment. The x-axis is the aggression of the focal males' day 1 opponent, and the line represents the cubic spline from Model 3 with associated standard error. Only the N treatment showed evolutionary changes in prior experience effects.

DISCUSSION

Evolutionary feedbacks are like the "dark matter" of evolution—we know they must be there, and we know they must be important, but they are nearly impossible to see directly. Here, we manipulated the social environment in which aggression was expressed and selected to provide the opportunity for IGE-mediated feedbacks via retaliation, prior experience effects, or neither of these to contribute to evolution (Figure 1). We tracked the resulting evolutionary dynamics over 24 generations in 12 populations, requiring behavioral measurement of tens of thousands of males. Our results broadly support predictions from IGE theory (Figure 2, Table 2), i.e., that positive feedbacks should accelerate phenotypic evolution while negative feedbacks should slow evolution (*18*, *19*, *27*, *31*). As unselected control populations did not evolve increased aggression, we can be confident that these evolutionary changes are due to experimental selection. Thus, these results demonstrate that evolutionary feedbacks alter populations' phenotypic response to selection.

Unexpectedly, we saw that not every component of aggression responded to feedback-mediated selection the same way; in particular, the evolutionary response in the Positive feedbacks (**P**) treatment was dominated by the very striking evolution of supernormal boxing behavior (Figure 2, Tables 1, 2). Boxing is the only component of aggression measured here in which active participation for both males is required. Thus, small increases in aggressiveness over generations may have been magnified in the Positive feedbacks (**P**) treatment through feedbacks, making mutually-expressed, high-intensity fights substantially more common.

Predictions about the evolutionary consequences of feedbacks hinge on the assumptions that the dynamics underlying feedbacks – here, retaliation and prior experience effects -- are constant over generations. While this was true for retaliation, prior experience effects evolved to be more negative at high levels of Day 1 opponent aggression (Figure 3). These findings suggest that negative feedbacks and their evolutionary consequences would be exacerbated under continued selection. Predicting and testing the evolution of social dynamics as indirect targets of selection is a critical future direction for understanding evolutionary feedbacks (*48*, *49*).

CONCLUSIONS

Predicting how interactive behaviors will evolve is an exciting challenge for modern biology (*50*). Our results provide a powerful proof-of-concept that largely validates longstanding

hypotheses, supporting the deployment of IGE and related theory in diverse applications (*51*), such as conservation (*52*) and animal welfare (*38*). The existence and potency of IGE-mediated evolutionary feedbacks highlight how simple behavioral choices – such as responding to an aggressive attack – can scale up to impact the entire evolutionary process.

MATERIALS AND METHODS

Experimental Overview: *Replicate populations were selected for increased aggression under different social conditions, alongside unselected controls*

We had 3 selection treatments representing selection for increased aggression under positive, negative, or no evolutionary feedbacks, as described below. In addition, we had control populations that did not undergo selection.

In generation 0, 100 males from each population were paired together and measured for baseline aggression to ensure similar starting points. In generations 1-24, each population was subjected to evolution for increased aggression, or not, corresponding to their assigned treatments. Each population was measured for aggression every generation as part of the selection protocol (see below), except control populations, which were measured at generation 0 and again at the midway point (generation 12 or 13). In the final generation (generation 25 for replicates 1 and 2, generation 26 for replicate 3), samples from all populations were measured for prior experience effects so that we could test whether prior experience effects evolved, and the experiment was concluded.

In total, this experiment measured phenotypic changes over 25 generations for 12 populations, requiring measurements of 39,018 males. By manipulating feedbacks and tracking the resulting changes in aggression, retaliation and prior experience effects, across replicates, we were able to directly identify the evolutionary consequences of feedbacks for aggressive behavior.

Replication structure

Each replicate was founded by collecting 4 samples of the base population, with each sample consisting of 100 males and 100 females. These 4 samples became the 4 populations from this replicate, and each one was subjected to a different experimental treatment: one population from each of the 3 selection treatments (see below) and one unselected control population. Replicate 1 was founded in July 2021, Replicate 2 was founded in September 2021, and Replicate 3 was founded in February 2022.

Experimental details: flies and rearing conditions.

Base population: As our base population, we used an outbred population of flies originally collected from Coral Gables, FL, by the de Bivort lab. Mated females were collected from the same wild population over a period of June-November 2018 and their descendants were kept at large population sizes in the de Bivort lab (*53*).

Standard opponents. We used standard opponents in several of the treatments as explained below. These were males from the DGRP-208 inbred genotype, an inbred line whose progenitors were wild-collected and then highly inbred over many generations. DGRP-208 was chosen because it showed intermediate aggression in prior studies (Hutchins et al 2024). Importantly, the inbred nature of these opponents mean that every male standard opponent was genetically identical to every other standard opponent in every generation.

Standard opponents were reared for trials by adding 10 unmated DGRP-208 females and 10 DGRP-208 males to a vial containing standard fly food. This approach minimizes variation in rearing conditions across vials. Male progeny were collected within 12 hours of eclosion and housed with other standard opponent males for 5-7 days prior to testing.

Rearing and preparation for aggression experiments

Experimental populations were reared on high-protein food (consisting of standard fly food supplemented with additional dead yeast to produce a 4:1 protein:carbohydrate ratio; (*54*)) in 6oz plastic bottles. Each bottle was founded by 25 males and 25 unmated females. The high protein food ensured that sufficiently large numbers of males eclosed around the same time, allowing us to conduct the experiment. Newly-eclosed unmated females were housed in same-sex groups. Newly-eclosed experimental males were marked with a small dot of yellow or blue acrylic paint under anesthesia so that we could distinguish individuals during behavioral trials. After painting, males were isolated for 5-7 days. This approach ensured that males were recovered from anesthesia, but had no adult social experience prior to behavioral trials.

Selection on aggressive behavior

Aggression arenas

Arenas were composed of two small Petri dishes (3 cm x 1 cm) taped together. The floor of the arena was filled with standard fly food medium and a dot of active yeast. This is a typical arena design for measuring aggression in flies (e.g., (*37*)).

Measuring aggression

Because of the large number of males that needed to be measured simultaneously, we used an instantaneous scan sampling approach to measure aggression (*45*). Flies were added to arenas in the morning, at approximately 9:00am. Every 5 minutes during the following 60 minutes, we noted whether flies in each arena were engaged in any of 3 aggressive behaviors. A second 60-minute observation period occurred from 3:00-4:00 pm. Thus, we collected 24 total observations of each fly.

For each observation, trained observers recorded the presence or absence of 3 components of aggressive behavior: fencing - both flies extending and engaging with each other's legs by tapping and/or pushing; lunging - one fly rearing on his back legs and slamming his foretarsi onto his opponent; and boxing - both flies rearing on their hindlegs, "kangaroo style," and engaging each other with their front legs by pushing and/or hitting (*39*, *46*). When lunging occurred, observers noted which individual was the aggressor. Fencing and boxing are mutually performed behaviors so both individuals were counted as engaging in each instance of these. Finally, observers noted whether either of the flies appeared to be temporarily stuck on the food patch, as occasionally occurred. Observers did not know which flies were from which treatments while measuring aggression. To choose flies to become fathers of the next generation (in the selection treatments), we computed total aggression, which was the unweighted sum of fencing, lunging, and boxing instances observed for each individual male. For each selected population, the 50 males with the highest total aggression were selected each generation. If fewer than 50 males showed non-zero aggression, then all the males that showed any aggression were selected alongside randomly-chosen additional males until we reached 50 total males. For example, if only 45 males showed aggression, 5 males that did not show aggression (during our sampling periods) would be randomly chosen, resulting in 50 fathers for the next generation.

Founding the next generation. After selection, the 50 chosen males were placed into food bottles with 50 randomly chosen unmated females from their own population. The progeny of these flies became the next generation.

Deviations from the protocol. Due to unavoidable variability in fly eclosion rates, there were some generations in which fewer than 100 males were available from one or more of the populations. In these cases, the other populations in that replicate were subsampled to produce equal numbers of males considered for selection. For example, if one population only had 70 males available for testing, we measured those 70 males alongside 100 males from the *other* populations in that replicate. Before selection, we randomly discarded data from 30 males from the other populations, and chose the 50 most aggressive males from the remaining 70 data points.

For replicate 3 only, we had 3 generations where no selection was possible. In Generation 15, flies eclosed almost a week later than usual, possibly due to unusual weather in Houston TX. Since we were unsure of whether these flies were healthy and representative of the evolving population, we chose to allow random parents to found the next generation rather than conducting selection. Later, the standard opponent colonies became contaminated with bacteria resulting in insufficient standard opponents in generations 22, 23, and 25 (for Replicate 3 only). To ensure identical demographic histories among Replicate 3 populations, we therefore chose random flies to propagate all the populations during these "lost" generations. We concluded the experiment in Generation 26, in which we measured prior experience effects. Note that all results are adjusted for these and any other differences between Replicates.

In sum, this approach ensured identical demographic histories among populations from the same replicate. A complete record of the number of males measured for each population in each replicate is available in Table S1 in supplementary materials.

Final generation: test for prior experience effects. In the final generation, males from selected and control populations were reared as normal. But instead of their normal testing protocol, males from all populations were tested for prior experience effects: males from each population were paired together on Day 1 and aggression was measured, then those same males were paired with a standard opponent the next day and their aggression was measured again (i.e., as in (*37*, *44*)).

Analysis Methods

Overview. We fit a series of models using data from generations 1-24 to test for evolutionary changes in aggression, retaliation, and prior experience effects, and differences in these slopes among treatments. Finally, we analyzed prior experience effect differences in the final generation of each replicate. In a supplementary analysis, we confirmed that unselected control populations did not evolve increased aggression (Table S3.S2). All models included a random effect of Replicate to account for differences among replicates. A full summary of each model, the question that it answers, and its structure, is available in table S2.

In initial models, we found that the relationship between focal male aggression and opponent aggression was highly non-linear. Thus, analyses that included opponent aggression as a predictor were fit as generalized additive mixed models (GAMMs) in the package mcgv, enabling us to capture these nonlinear effects (*55*). Opponent aggression and its interactions were

fit using thin-plate splines, which include penalties for overfitting (*55*). We allowed the model to fit up to 10 "knots" for each spline. Models not including the effects of opponent behavior were fit as generalized linear mixed models (GLMMs) using the package glmmTMB, enabling us to include zero-inflation terms as needed (*56*).

Our measure of prior experience effects focused on changes in aggression across days and was calculated as: focal male Day 2 aggression – focal male Day 1 aggression, for each male (*57*, *58*). For these models, we used the scaled t error distribution, which is appropriate for errors that have a heavy-tailed gaussian-type distribution. For models where aggression itself was the response variable, we used a negative binomial error distribution, which is appropriate for overdispersed count data.

Preliminary models showed that fly age (i.e., whether the flies were 5, 6, or 7 days post-eclosion) was never significant, so we did not include this term in final models.

Model fitting and inference. Residual plots from each model were examined to ensure adequate model fit. For GLMMs, residual plots were produced by simulation using the package DHARMa (*59*); for GAMMs, we used the gam.check function in mcgv. For GLMMs, we also used AIC analysis to decide what zero-inflation terms to include, if any.

In the final models, we used Wald type III tests (implemented in the 'car' package for GLMMs (*60*), and F tests in the "summary.gam" function in mcgv for GAMMs) to test the significance of fixed effects. We did not conduct inference on random effects.

Data from generations 1-24 of the selected populations were used in models 1, 3, and S3, so we implemented the Bonferroni correction to reduce the false positive rate. We report corrected p-values.

To further interpret significant effects in model 1, we estimated slopes, i.e., estimated marginal means of linear trends, representing change in behavior over generations. Slopes were de-transformed to the scale of the original data, which is appropriate for interpreting evolutionary patterns (*61*). We tested whether each slope differed from 0, indicating no detectable evolution, using t-tests, and we performed planned contrasts to test for slope differences between treatments. Comparisons between slopes were adjusted for multiple testing using the tukey method. These computations were implemented in the emmeans package (*62*).

Model details

<u>Model 1: how did each component of aggression evolve?</u> We used a trait covariance approach to analyze how total aggression and each component of aggression evolved in our experiments. We modeled "focal male aggression" as our response variable, and each individual was represented by three measurements –lunging, fencing, and boxing-- with a variable, "component," indicating which observation corresponded to which component of aggression. Therefore, any predictor variable influencing "focal male aggression" independent of component indicates evolutionary (or other) changes to total aggression, independent of which component is considered. Any predictor variable whose effect on focal aggression depends on the variable "component" indicates component-specific evolutionary changes. This dependence manifests as a statistical interaction between the relevant predictor variable and the variable "component."

Thus, the model included fixed effects of generation, treatment, component, and all possible interactions among these variables (for full model structures Table S2). We also included a fixed covariate indicating the number of times the fly appeared stuck on the food patch. We included terms for replicate, the date the fly was measured, the identities of the trained observers, and individual ID as random effects. The random effect of individual ID accounted for the non-independence of the 3 measurements from each individual.

Model 2: did prior experience effects evolve in the N treatment? In this model, we used only data from the N treatment, which is the only treatment for which prior experience effects were measured during generations 1-24. We tested whether changes in aggressive behavior over the 2 days of trials (measured as Day 2 aggression –Day 1 aggression for each male, see above) varied over generations and due to opponent aggression on days 1 and 2. Generation, opponent aggression on each day, and 2-way interactions between generation and opponent aggression on each day were included as fixed effects, with other terms in the model as above (table X). In this model, an effect of a generation x opponent aggression interaction (on either day) would indicate an evolutionary change in how males responded to opponent behavior. We included terms for replicate, the date the fly was measured, and the identities of the trained observers as random effects.

Model 3: did prior experience effects evolve in any of the treatments? While total aggression and retaliation were measured for every pair of males, prior experience effects were only measured in the N treatment populations. To test whether prior experience effects may have evolved in the other treatments, we measured prior experience effects for every population (including unselected controls) at the end of the experiment (generation 25). To compare the magnitude of prior experience effects across treatments, we tested whether changes in aggressive behavior across days (measured as Day 2 aggression – Day 1 aggression for each male, see main text) differed across treatments and due to opponent aggression on days 1 and 2. Using GAMMs, we fitted the effects of opponent aggression on each day in the Control treatment as the "reference smooth," and fitted effects that estimated differences between Control and each of the other treatments for these relationships as "difference smooths" (*63*). Significant effects of each difference smooth would indicate significant differences between this treatment's prior experience effects and those of the control populations. We included terms for replicate, the date the fly was measured, and the identities of the trained observers as random effects.

Model S1 & S2: checking assumptions. In these models we confirmed that (a) populations had indistinguishable levels of aggression at the beginning of the experiment (generation 0) and (b) unselected control populations did not show directional changes in aggression. See Table S3.S2.

Models S3.0, S3.N, and S3.P: did retaliation evolve? In this series of models, we tested whether retaliation changed over generations in each selection treatment. See Table S3.S3

References and Notes

1. D. M. Post, E. P. Palkovacs, Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 1629–1640 (2009).

2. K. N. Laland, F. J. Olding-Smee, M. W. Feldman, Evolutionary consequences of niche construction and their implications for ecology. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 10242–10247 (1999).
3. J. N. Thompson, Rapid evolution as an ecological process. *Trends in Ecology & Evolution* **13**, 329–32 (1998).
4. F. Odling-Smee, K. Laland, M. Feldman, Niche construction. *American Naturalist* **147**, 641–648 (1996).
5. T. G. Whitham, J. K. Bailey, J. A. Schweitzer, S. M. Shuster, R. K. Bangert, C. J. LeRoy, E. V Lonsdorf, G. J. Allan, S. P. DiFazio, B. M. Potts, D. G. Fischer, C. A. Gehring, R. L. Lindroth, J. C. Marks, S. C. Hart, G. M. Wimp, S. C. Wooley, A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics* **7**, 510–23 (2006).
6. K. Donohue, L. Dorn, C. Griffith, E. Kim, A. Aguilera, C. R. Polisetty, J. Schmitt, Niche construction through germination cueing: life-history responses to timing of germination in Arabidopsis thaliana. *Evolution* **59**, 771–85 (2005).
7. J. B. Saltz, A. P. Geiger, R. Anderson, B. Johnson, R. Marren, What, if anything, is a social niche? *Evolutionary Ecology* **30**, 349–364 (2016).
8. B. J. Crespi, Vicious circles: Positive feedback in major evolutionary and ecological transitions. *Trends in Ecology & Evolution* **19**, 627–633 (2004).
9. F. Pelletier, D. Garant, A. P. Hendry, Eco-evolutionary dynamics. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 1483–1489 (2009).
10. M. T. Kinnison, N. G. Hairston, Eco-evolutionary conservation biology: Contemporary evolution and the dynamics of persistence. *Functional Ecology* **21**, 444–454 (2007).
11. R. Bonduriansky, A. J. Crean, T. Day, The implications of nongenetic inheritance for evolution in changing environments. *Evolutionary Applications* **5**, 192–201 (2012).
12. J. K. Bailey, M. A. Genung, I. Ware, C. Gorman, M. E. Van Nuland, H. Long, J. A. Schweitzer, Indirect genetic effects: an evolutionary mechanism linking feedbacks, genotypic diversity and coadaptation in a climate change context. *Functional Ecology* **28**, 87–95 (2014).
13. G. F. Fussmann, M. Loreau, P. A. Abrams, Eco-evolutionary dynamics of communities and ecosystems. *Functional Ecology* **21**, 465–477 (2007).
14. J. B. Saltz, B. R. Foley, Natural Genetic Variation in Social Niche Construction: Social Effects of Aggression Drive Disruptive Sexual Selection in Drosophila melanogaster. *The American Naturalist* **177**, 645–54 (2011).
15. L. M. Meffert, S. K. Hicks, J. L. Regan, Nonadditive genetic effects in animal behavior. *The American Naturalist* **160 Suppl**, S198-213 (2002).
16. G. C. K. Chiang, D. Barua, E. Dittmar, E. M. Kramer, R. R. De Casas, K. Donohue, PLEIOTROPY IN THE WILD: THE DORMANCY GENE DOG1 EXERTS CASCADING CONTROL ON LIFE CYCLES. *Evolution* **67**, 883–893 (2012).
17. J. B. Saltz, S. V. Nuzhdin, Genetic variation in niche construction: Implications for development and evolutionary genetics. *Trends in Ecology and Evolution* **29** (2014).
18. J. Wolf, E. D. Brodie III, J. J. M. Cheverud, A. J. Moore, M. J. Wade, Evolutionary consequences of indirect genetic effects. *Trends in Ecology & Evolution* **13**, 64–69 (1998).
19. A. J. Moore, E. D. Brodie III, J. B. Wolf, Interacting Phenotypes and the Evolutionary Process: I. Direct and Indirect Genetic Effects of Social Interactions. *Evolution* **51**, 1352–1362 (1997).
20. P. Bijma, Multilevel selection 4: modeling the relationship of indirect genetic effects and group size. *Genetics* **186**, 1029–31 (2010).
21. K. Donohue, Setting the stage: phenotypic plasticity as habitat selection. *International Journal of Plant Sciences* **164**, S79–S92 (2003).

22. B. H. Bleakley, E. D. Brodie III, Indirect genetic effects influence antipredator behavior in guppies: estimates of the coefficient of interaction psi and the inheritance of reciprocity. *Evolution* **63**, 1796–806 (2009).
23. J. J. Mutic, J. B. Wolf, Indirect genetic effects from ecological interactions in Arabidopsis thaliana. *Molecular Ecology* **16**, 2371–81 (2007).
24. B. H. Bleakley, D. J. Parker, E. D. Brodie III, Nonadditive effects of group membership can lead to additive group phenotypes for anti-predator behaviour of guppies, Poecilia reticulata. *Journal of Evolutionary Biology* **20**, 1375–1384 (2007).
25. T. A. Linksvayer, Direct, maternal, and sibsocial genetic effects on individual and colony traits in an ant. *Evolution* **60**, 2552–61 (2006).
26. D. Petfield, S. F. Chenoweth, H. D. Rundle, M. W. Blows, Genetic variance in female condition predicts indirect genetic variance in male sexual display traits. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 6045–50 (2005).
27. J. W. McGlothlin, A. J. Moore, J. B. Wolf, E. D. Brodie III, Interacting Phenotypes and the Evolutionary Process. III. Social Evolution. *Evolution* **64–9**, 2558–2574 (2010).
28. E. Kazancıoğlu, H. Klug, S. H. Alonzo, The evolution of social interactions changes predictions about interacting phenotypes. *Evolution* **66**, 2056–64 (2012).
29. M. D. Greenfield, S. Alem, D. Limousin, N. W. Bailey, The dilemma of Fisherian sexual selection: Mate choice for indirect benefits despite rarity and overall weakness of trait-preference genetic correlation. *Evolution* **68**, 3524–3536 (2014).
30. A. F. Agrawal, E. D. Brodie III, M. J. Wade, On indirect genetic effects in structured populations. *The American Naturalist* **158**, 308–23 (2001).
31. N. W. Bailey, L. Marie-Orleach, A. J. Moore, Indirect genetic effects in behavioral ecology: does behavior play a special role in evolution? *Behavioral Ecology* **29**, 1–11 (2018).
32. S. H. Alonzo, Social and coevolutionary feedbacks between mating and parental investment. *Trends in Ecology & Evolution* **25**, 99–108 (2010).
33. M. Cantor, A. A. Maldonado-Chaparro, K. B. Beck, H. B. Brandl, G. G. Carter, P. He, F. Hillemann, J. A. Klarevas-Irby, M. Ogino, D. Papageorgiou, L. Prox, D. R. Farine, The importance of individual-to-society feedbacks in animal ecology and evolution. *J Anim Ecol* **90**, 27–44 (2021).
34. Y. G. Araya-Ajoy, D. F. Westneat, J. Wright, Pathways to social evolution and their evolutionary feedbacks. *Evolution* **74**, 1894–1907 (2020).
35. R. A. Duckworth, S. M. Aguillon, Eco-evolutionary dynamics: investigating multiple causal pathways linking changes in behavior, population density and natural selection. *Journal of Ornithology* **156**, 115–124 (2015).
36. J. B. Saltz, Genetic composition of social groups influences male aggressive behaviour and fitness in natural genotypes of Drosophila melanogaster. *Proceedings of the Royal Society B: Biological Sciences* **280**, 31926 (2013).
37. M. Hutchins, T. Douglas, L. Pollack, J. B. Saltz, Genetic Variation in Male Aggression Is Influenced by Genotype of Prior Social Partners in *Drosophila melanogaster*. *The American Naturalist* **203**, 551–561 (2024).
38. I. Camerlink, W. W. Ursinus, P. Bijma, B. Kemp, J. E. Bolhuis, Indirect Genetic Effects for Growth Rate in Domestic Pigs Alter Aggressive and Manipulative Biting Behaviour. *Behav Genet* **45**, 117–126 (2015).
39. S. P. Nilsen, Y.-B. Chan, R. Huber, E. A. Kravitz, Gender-selective patterns of aggressive behavior in Drosophila melanogaster. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 12342–7 (2004).

40. C. Rutte, M. Taborsky, M. W. G. Brinkhof, What sets the odds of winning and losing? *Trends in Ecology & Evolution* **21**, 16–21 (2006).
41. A. Yurkovic, O. Wang, A. C. Basu, E. A. Kravitz, Learning and memory associated with aggression in Drosophila melanogaster. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 17519–24 (2006).
42. J. K. M. Penn, M. F. Zito, E. A. Kravitz, A single social defeat reduces aggression in a highly aggressive strain of Drosophila. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 12682–12686 (2010).
43. S. Trannoy, J. Penn, K. Lucey, D. Popovic, E. A. Kravitz, Short and long-lasting behavioral consequences of agonistic encounters between male Drosophila melanogaster. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 4818–4823 (2016).
44. J. B. Saltz, Genetic variation in social environment construction influences the development of aggressive behavior in Drosophila melanogaster. *Heredity (Edinb)* **118**, 340–347 (2017).
45. J. Altmann, Observational Study of Behavior: Sampling Methods. *Behav* **49**, 227–266 (1974).
46. S. Chen, A. Y. Lee, N. M. Bowens, R. Huber, E. A. Kravitz, Fighting fruit flies: A model system for the study of aggression. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 5664–5668 (2002).
47. H. A. Dierick, R. J. Greenspan, Molecular analysis of flies selected for aggressive behavior. *Nature Genetics* **38**, 1023–1031 (2006).
48. S. F. Chenoweth, H. D. Rundle, M. W. Blows, EXPERIMENTAL EVIDENCE FOR THE EVOLUTION OF INDIRECT GENETIC EFFECTS: CHANGES IN THE INTERACTION EFFECT COEFFICIENT, PSI (Ψ), DUE TO SEXUAL SELECTION. *Evolution* **64**, 1849–1856 (2010).
49. N. W. Bailey, M. Zuk, Socially flexible female choice differs among populations of the Pacific field cricket: geographical variation in the interaction coefficient psi ( ). *Proceedings of the Royal Society B: Biological Sciences* **279**, 3589–3596 (2012).
50. D. N. Fisher, R. J. Kilgour, E. R. Siracusa, J. R. Foote, E. A. Hobson, P. Montiglio, J. B. Saltz, T. W. Wey, E. W. Wice, Anticipated effects of abiotic environmental change on intraspecific social interactions. *Biol Rev* **96**, 2661–2693 (2021).
51. A. Baud, S. McPeek, N. Chen, K. A. Hughes, Indirect Genetic Effects: A Cross-disciplinary Perspective on Empirical Studies. *Journal of Heredity*, esab059 (2021).
52. M. Murray, J. Wright, Y. G. Araya-Ajoy, Evolutionary rescue from climate change: male indirect genetic effects on lay-dates and their consequences for population persistence. *Evolution Letters* **8**, 137–148 (2024).
53. J. Akhund-Zade, D. Yoon, A. Bangerter, N. Polizos, M. Campbell, A. Soloshenko, T. Zhang, E. Wice, A. Albright, A. Narayanan, P. Schmidt, J. Saltz, J. Ayroles, M. Klein, A. Bergland, B. de Bivort, "Wild flies hedge their thermal preference bets in response to seasonal fluctuations" (preprint, Evolutionary Biology, 2020); https://doi.org/10.1101/2020.09.16.300731.
54. E. W. Wice, J. B. Saltz, Selection on heritable social network positions is context-dependent in Drosophila melanogaster. *Nat Commun* **12**, 3357 (2021).
55. S. N. Wood, *Generalized Additive Models: An Introduction with R* (Chapman and Hall/CRC, ed. 2, 2017; https://www.taylorfrancis.com/books/9781498728348).
56. M. E. Brooks, K. Kristensen, K. J. van Benthem, A. Magnusson, C. Berg, A. Nielsen, H. J. Skaug, M. Maechler, B. M. Bolker, glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal* **9**, 378–400 (2017).

57. J. A. Stamps, P. A. Biro, D. J. Mitchell, J. B. Saltz, Bayesian updating during development predicts genotypic differences in plasticity: PREDICTING GENOTYPIC DIFFERENCES IN PLASTICITY. *Evolution*, doi: 10.1111/evo.13585 (2018).
58. J. B. Saltz, S. Lymer, J. Gabrielian, S. V. Nuzhdin, Genetic Correlations among Developmental and Contextual Behavioral Plasticity in Drosophila melanogaster. *Am. Nat.* **190**, 61–72 (2017).
59. F. Hartig, DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models., version 0.3.3.0 (2020); https://CRAN.R-project.org/package=DHARMa.
60. J. Fox, S. Weisberg, *An {R} Companion to Applied Regression, Second Edition* (Sage, Thousand Oaks CA, Second., 2011; http://socserv.socsci.mcmaster.ca/jfox/Books/Companion).
61. P. De Villemereuil, H. Schielzeth, S. Nakagawa, M. Morrissey, General Methods for Evolutionary Quantitative Genetic Inference from Generalized Mixed Models. *Genetics* **204**, 1281–1294 (2016).
62. R. Lenth, emmeans: Estimated Marginal Means, aka Least-Squares Means, version 1.3.5 (2019); https://CRAN.R-project.org/package=emmeans.
63. M. Sóskuthy, Generalised additive mixed models for dynamic analysis in linguistics: a practical introduction. arXiv [Preprint] (2017). https://doi.org/10.48550/ARXIV.1703.05339.

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Supplementary Materials

Materials and Methods

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Data files

Readme for data files

R Code

SUPPLEMENTAL TABLES

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SECTION 1.

Table S1. Number of males measured for aggression every generation for every population. In cases where less than 100 were measured, we matched the demographic histories of all populations in that replicate. See Materials and Methods for details.

Replicate	Treatment	Generation	n.males
1	C	0	98
1	N	0	100
1	P	0	99
1	Z	0	100
1	N	1	95
1	P	1	100
1	Z	1	99
1	N	2	101
1	P	2	139
1	Z	2	142
1	N	3	105
1	P	3	90
1	Z	3	105
1	N	4	65
1	P	4	62
1	Z	4	67
1	N	5	97
1	P	5	100
1	Z	5	98
1	N	6	99
1	P	6	100
1	Z	6	100
1	N	7	95
1	P	7	100
1	Z	7	99

1	N	8	96
1	P	8	100
1	Z	8	99
1	N	9	90
1	P	9	100
1	Z	9	99
1	N	10	98
1	P	10	100
1	Z	10	100
1	N	11	98
1	P	11	94
1	Z	11	97
1	C	12	87
1	N	12	99
1	P	12	100
1	Z	12	99
1	N	13	98
1	P	13	100
1	Z	13	99
1	N	14	95
1	P	14	100
1	Z	14	99
1	N	15	93
1	P	15	100
1	Z	15	98
1	N	16	93
1	P	16	100
1	Z	16	98
1	N	17	127
1	P	17	132
1	Z	17	130
1	N	18	67
1	P	18	68
1	Z	18	66
1	N	19	97
1	P	19	100
1	Z	19	84
1	N	20	100
1	P	20	100
1	Z	20	100
1	N	21	99

1	P	21	100
1	Z	21	100
1	N	22	97
1	P	22	100
1	Z	22	100
1	N	23	97
1	P	23	97
1	Z	23	100
1	N	24	96
1	P	24	100
1	Z	24	97
1	C	25	70
1	N	25	72
1	P	25	122
1	Z	25	72
2	C	0	95
2	N	0	96
2	P	0	94
2	Z	0	96
2	N	1	98
2	P	1	100
2	Z	1	100
2	N	2	97
2	P	2	100
2	Z	2	100
2	N	3	95
2	P	3	100
2	Z	3	100
2	N	4	96
2	P	4	100
2	Z	4	100
2	N	5	96
2	P	5	100
2	Z	5	99
2	N	6	100
2	P	6	100
2	Z	6	100
2	N	7	96
2	P	7	100
2	Z	7	100
2	N	8	96

2	P	8	100
2	Z	8	100
2	N	9	91
2	P	9	100
2	Z	9	96
2	N	10	95
2	P	10	98
2	Z	10	99
2	N	11	98
2	P	11	100
2	Z	11	98
2	C	12	41
2	N	12	98
2	P	12	100
2	Z	12	99
2	N	13	91
2	P	13	97
2	Z	13	98
2	N	14	93
2	P	14	100
2	Z	14	100
2	N	15	97
2	P	15	100
2	Z	15	98
2	N	16	98
2	P	16	100
2	Z	16	100
2	N	17	100
2	P	17	100
2	Z	17	100
2	N	18	98
2	P	18	100
2	Z	18	100
2	N	19	99
2	P	19	100
2	Z	19	99
2	N	20	98
2	P	20	100
2	Z	20	98
2	N	21	97
2	P	21	100

2	Z	21	95
2	N	22	98
2	P	22	100
2	Z	22	99
2	N	23	99
2	P	23	100
2	Z	23	100
2	N	24	79
2	P	24	100
2	Z	24	100
2	C	25	124
2	N	25	126
2	P	25	131
2	Z	25	130
3	C	0	100
3	N	0	99
3	P	0	94
3	Z	0	99
3	N	1	96
3	P	1	98
3	Z	1	100
3	N	2	94
3	P	2	100
3	Z	2	100
3	N	3	99
3	P	3	100
3	Z	3	99
3	N	4	97
3	P	4	100
3	Z	4	99
3	N	5	94
3	P	5	100
3	Z	5	96
3	N	6	92
3	P	6	100
3	Z	6	99
3	N	7	93
3	P	7	98
3	Z	7	97
3	N	8	97
3	P	8	90

3	Z	8	98
3	N	9	96
3	P	9	100
3	Z	9	100
3	N	10	96
3	P	10	100
3	Z	10	99
3	N	11	97
3	P	11	98
3	Z	11	100
3	N	12	96
3	P	12	98
3	Z	12	99
3	C	13	95
3	N	13	91
3	P	13	94
3	Z	13	97
3	N	14	97
3	P	14	100
3	Z	14	98
3	N	15	0
3	P	15	0
3	Z	15	0
3	N	16	93
3	P	16	100
3	Z	16	99
3	N	17	96
3	P	17	100
3	Z	17	92
3	N	18	97
3	P	18	98
3	Z	18	99
3	N	19	85
3	P	19	100
3	Z	19	97
3	N	20	75
3	P	20	78
3	Z	20	92
3	N	21	86
3	P	21	96
3	Z	21	93

3	N	22	0
3	P	22	0
3	Z	22	0
3	N	23	0
3	P	23	0
3	Z	23	0
3	N	24	66
3	P	24	85
3	Z	24	73
3	N	25	0
3	P	25	0
3	Z	25	0
3	C	26	66
3	N	26	70
3	P	26	125
3	Z	26	68

SECTION 2. Table S2.
Full model structures for main and supplemental models

model name	question	generations included	treatments included	response variable	fixed effects	random effects	error distribution	model type
model 1	how did each component of aggression evolve?	1 to 24	0, N, P	each component of aggressive behavior	Generation*Treatment*component+ focal.stuck	Replicate + Date + Individual.ID + observer identities	negative binomial	GLMM
model 2	did prior experience effects evolve?	1 to 24	N	change in aggression: total aggression on day 2 - total aggression on day 1	Generation + day1 opponent aggression*Generation + day2 opponent aggression*Generation + focal.stuck	Replicate + Date + observer identities	scaled *t*	GAMM -- day1 and day2 opponent aggression and their interactions fit as thin plate splines
model 3	did prior experience effects differ across treatments at the end of the experiment?	25	C, 0, N, P	change in aggression: total aggression on day 2 - total aggression on day 1	Treatment + day1 opponent aggression*Treatment + day2 opponent aggression*Treatment + focal.stuck	Replicate + Date	negative binomial	GAMM -- opponent aggression and its interactions fit as thin plate splines
model S1	were populations the same at the beginning?	0	C, 0, N, P	each component of aggressive behavior	Treatment*component + focal.stuck	Replicate + Date + Individual.ID + observer identities	negative binomial	GLMM
model S2	did control populations change their aggression?	0, 12/13, 25	C	each component of aggressive behavior	Generation*component + focal.stuck	Replicate + Date + Individual.ID + observer identities	negative binomial	GLMM
model S3.0, S3.N, S3.P	did retaliation evolve?	1 to 24	0, N, P in separate models	total aggression	total aggression ~ opponent aggression*Generation + focal.stuck	Replicate + Date + observer identities	negative binomial	GAMM -- opponent aggression and its interactions fit as thin plate splines

SECTION 3. Full model Results.

Table S3.1. Model 1.

model 1: did feedbacks influence the evolution of aggression?				
Fixed effects:				
term	parameter estimate (se)	Chisq	Df	nominal p
(Intercept)	-6.39 (0.63)	103.8782	1	< 2.2e-16
Generation	0.092 (0.035)	7.1549	1	0.0074759
Treatment	.	97.0031	2	< 2.2e-16
component	.	355.4775	2	< 2.2e-16
focal.stuck	-0.076 (0.015)	27.0019	1	2.03E-07
Generation:Treatment	.	1.2374	2	0.538651
Generation:component	.	11.4142	2	0.0033223
Treatment:component	.	65.0494	4	2.51E-13
Generation:Treatment: component	.	19.1938	4	0.0007199
Random effects:				
term	Variance	Std.Dev.		
Replicate	0.02202	0.14839		
Date	0.04616	0.21486		
individ.ID	0.02008	0.14172		
AM.Observer	0.06459	0.25415		
PM.Observer	0.00543	0.07369		
Zero-inflation model:				
	Estimate	Std. Error	z value	nominal p
(Intercept)	-0.1565	0.2224	-0.703	0.482
componentfencing	-2.7478	0.3025	-9.085	<2e-16
componentlunging	-14.3528	441.0606	-0.033	0.974

Table S3.2. Model 2.

model 2: did prior experience effects evolve over generations?				
Parametric terms:				
	Estimate	Std. Error	z value	nominal p
(Intercept)	-0.452	0.076	-5.926	3.10E-09
Generation	0.00848	0.0026	3.263	0.0011
focal.stuck	-0.0268	0.0192	-1.392	0.164
Smooth terms -- fixed				
	edf	Ref.df	F	nominal p
s(day1.opp.all)	4.61	5.489	873.724	<2e-16
s(opp.all)	2.78	3.392	261.97	<2e-16
ti(Generation,opp.all)	1.013	1.026	0.159	0.69746
ti(Generation,day1.opp.all)	2.25	2.638	14.948	0.00381
Smooth terms -- random				
	edf	Ref.df	F	nominal p
s(Replicate)	1.73	2	20.763	7.93E-05
s(Date)	15.97	222	18.533	0.07437
s(AM.Observer)	1.21	4	4.904	0.03497
s(PM.Observer)	0.078	6	0.11	0.2339

Table S3.3. Model 3.

model 3: did prior experience effects differ across treatments at the end of the experiment?				
Parametric Terms:				
	Estimate	Std. Error	z value	nominal p
(Intercept)	-0.532	0.15991	-3.326	0.00093
TreatmentZ	0.351	0.1808	1.942	0.05254
TreatmentP	0.223	0.18438	1.208	0.22765
TreatmentN	-0.098	0.19007	-0.517	0.60528
day1.focal.stuck	0.057	0.07659	0.748	0.45492
day2.focal.stuck	-0.021	0.06059	-0.345	0.72988
Smooth terms -- fixed				
	edf	Ref.df	F	nominal p
s(day1.opp.all)	1.00E+00	1	12.844	0.000363
s(day2.opp.all)	1.00E+00	1	2.289	0.130768
s(day1.opp.all):TreatmentZ	1.00E+00	1	1.059	0.303919
s(day1.opp.all):TreatmentP	1.00E+00	1	0.027	0.868465
s(day1.opp.all):TreatmentN	1.00E+00	1	4.014	0.045521
s(day2.opp.all):TreatmentZ	1.00E+00	1	0.041	0.840132
s(day2.opp.all):TreatmentP	1.00E+00	1	0.002	0.961652
s(day2.opp.all):TreatmentN	1.00E+00	1	0.514	0.473546
Smooth terms -- random				
	edf	Ref.df	F	nominal p
s(Replicate)	6.67E-05	2	0	0.523254
s(Date)	7.18E+00	11	2.225	0.000146

Table S3.S1. Model S1.

model S1: were populations the same at the beginning?				
Fixed effects:				
term	parameter estimate (se)	Chisq	Df	nominal p
(Intercept)	-0.13 (0.12)	1.0107	1	0.31474
Treatment	.	0.1267	3	0.98846
focal.stuck	-0.13 (0.078)	2.8529	1	0.09121
Random effects:				
term	Variance	Std.Dev.		
Replicate	1.02E-02	1.01E-01		
PM.Observer	1.24E-02	1.11E-01		
Date	8.29E-09	9.10E-05		

Table S3.S2. Model S2.

model s2: did control populations change their aggression?

Fixed effects:				
term	parameter estimate (se)	Chisq	Df	nominal p
(Intercept)	-0.17 (0.17)	0.9731	1	0.32392
generation (modeled as a factor)	.	6.79	2	0.03354
focal.stuck	-0.26 (0.19)	1.8238	1	0.17686
Random effects:				
term	Variance	Std.Dev.		
Replicate	2.19E-02	1.48E-01		
PM.Observer	2.27E-09	4.76E-05		
Date	1.50E-01	3.87E-01		
least squares means for generation.factor				
generation	ls-mean (se)	z.ratio	p.value	results of planned contrasts
beginning	0.82 (0.14)	-1.124	0.2611	ab
middle	1.03 (0.19)	-2.709	0.0067	b
end	0.49 (0.13)	0.176	0.8601	a

Table S3.S3. Model S3.

model S3.0: did retaliation evolve in the no feedbacks treatment?				
Parametric Terms:				
	Estimate	Std. Error	t value	nominal p
(Intercept)	-1.06	0.187	-5.643	1.74E-08
Generation	0.055	0.0041	13.294	<2e-16
focal.stuck	-0.1	0.0281	-3.565	0.000366
Smooth terms -- fixed				
	edf	Ref.df	F	nominal p
s(opp.all)	1.00E+00	1.00E+00	212.919	<2e-16
ti(Generation,opp.all)	1.11E+00	1.20E+00	3.039	0.0588
Smooth terms -- random				
	edf	Ref.df	F	nominal p
s(Replicate)	1.23E+00	2.00E+00	6.501	0.0755
s(Date)	1.04E+02	2.28E+02	0.996	<2e-16
s(AM.Observer)	3.96E+00	5.00E+00	45.514	<2e-16
s(PM.Observer)	7.85E-03	6.00E+00	0.001	0.5669

model S3.N: did retaliation evolve in the negative feedbacks treatment?				
Parametric Terms:				
	Estimate	Std. Error	t value	nominal p
(Intercept)	-1.24	0.213	-5.816	6.31E-09
Generation	0.031	0.0052	6.117	1.01E-09
focal.stuck	-0.034	0.027	-1.252	0.211
Smooth terms -- fixed				
	edf	Ref.df	F	nominal p
s(opp.all)	3.458	4.16	110.599	<2e-16
ti(Generation,opp.all)	1.006	1.011	0.182	0.671
Smooth terms -- random				
	edf	Ref.df	F	nominal p
s(Replicate)	1.815	2	87.963	4.80E-06
s(Date)	113.66	228	1.291	<2e-16
s(AM.Observer)	3.346	5	54.888	<2e-16
s(PM.Observer)	1.384	6	2.849	0.0887

model S3.P: did retaliation evolve in the positive feedbacks treatment?				
Parametric Terms:				
	Estimate	Std. Error	t value	nominal p
(Intercept)	0.236	0.157	1.505	0.132293
Generation	0.014	0.003	4.674	3.01E-06
focal.stuck	-0.09	0.025	-3.627	0.000289
Smooth terms -- fixed				
	edf	Ref.df	F	nominal p
s(opp.all)	6.304	7.117	111.047	<2e-16
ti(Generation,opp.all)	3.087	4.292	1.504	0.19649
Smooth terms -- random				
	edf	Ref.df	F	nominal p
s(Replicate)	1.949	2	113.909	<2e-16
s(Date)	42.05	224	0.242	0.00905
s(AM.Observer)	3.474	5	30.629	5.63E-06
s(PM.Observer)	3.372	6	4.635	0.13382