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Cascading indirect genetic effects in a clonal vertebrate

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Understanding how individual differences arise and how their effects propagate through groups are fundamental issues in biology. Individual differences can arise from indirect genetic effects (IGE): genetically based variation in the conspecifics with which an individual interacts. Using a clonal species, the Amazon molly (*Poecilia formosa*), we test the hypothesis that IGE can propagate to influence phenotypes of the individuals that do not experience them firsthand. We tested this by exposing genetically identical Amazon mollies to conspecific social partners of different clonal lineages, and then moving these focal individuals to new social groups in which they were the only member to have experienced the IGE. We found that genetically different social environments resulted in the focal animals experiencing different levels of aggression, and that these IGE carried over into new social groups to influence the behaviour of naive individuals. These data reveal that IGE can cascade beyond the individuals that experience them. Opportunity for cascading IGE is ubiquitous, especially in species with long-distance dispersal or fission–fusion group dynamics. Cascades could amplify (or mitigate) the effects of IGE on trait variation and on evolutionary trajectories. Expansion of the IGE framework to include cascading and other types of carry-over effects will therefore improve understanding of individual variation and social evolution and allow more accurate prediction of population response to changing environments.

1. Introduction

Understanding how individual differences arise and their consequences for group dynamics are fundamental questions in biology [1–4]. Indirect genetic effects (IGE) are one cause of both individual variation and propagation of effects of individual variation in groups [5–8]. IGE arise when individuals' phenotypes are influenced by genetic variation in their social partners. IGE have been documented to cause behavioural, life history and morphological variation in a wide variety of taxa (e.g. [9–19]). For example, behaviour and body condition of mosquitofish are influenced by genetically based differences in their social partners [20,21], and behavioural, physiological and morphological traits in laboratory mice are influenced by the genotypes of their cage mates [22]. Terms such as 'social genetic effect' and 'genetic nurture' have been used for the same concept in different disciplines [7].

Most of the empirical IGE literature focuses on dyadic interactions: how genetic variation among individuals influences phenotypes of their immediate social partners. Theoretical and empirical work has shown that these dyadic IGE can profoundly influence phenotypes, fitness, and the rate and direction of evolution [23–25]. Much less is known, however, about IGE on group-level

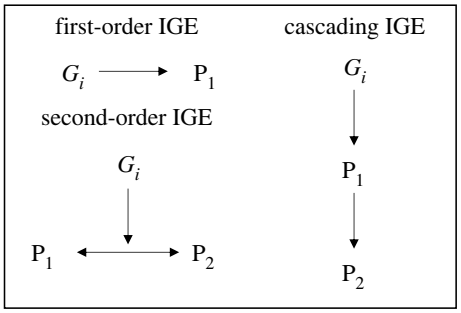

Figure 1. Indirect genetic effects can be divided into three distinct types. First-order IGE capture the effects of the genotype of the individual (G_i) on the phenotype of another individual with which it interacts directly (P_1). Second-order IGE reflect the effects of the genotype of one individual (G_i) on the interactions between other individuals (P_1 and P_2). Cascading IGE capture the effects of the genotype of one individual (G_i) on the phenotype of its direct social partner (P_1), which subsequently affects the phenotype of an individual (P_2) that has never interacted with individual G_i.

characteristics or the degree to which IGE can propagate to affect phenotypes of individuals that do not experience them firsthand.

A few studies have investigated IGE beyond those caused by dyadic interactions, including work showing that genetic variation in a social partner can influence social interactions between other members of the group (so-called 'second-order IGE' [26–28]). Nevertheless, it remains unknown whether IGE previously experienced by one or a few group members can subsequently influence phenotypes of new social partners that never themselves experienced IGE firsthand. Here, we test the hypothesis that IGE 'cascade' beyond individuals that experience them firsthand (figure 1). This hypothesis is motivated by previous work indicating that individual animals can strongly influence group behaviour [1,2,29–32]. However, that literature has generally not focused on prior social experience as a factor that generates differences among influential group members (but see [33–35]), and we know of no studies that implicate IGE as a cause of such differences.

Cascading IGE are distinct from 'cascading maternal effects' [36,37], which occur when traits mediating (genetic or non-genetic) maternal effects are themselves influenced by maternal effects in the previous generation. The two types of cascading effect share the feature that they propagate to individuals that never experienced the originating variation in social environment. Cascading IGE encompass a much broader range of social interactions; however, since they do not depend on the causal interactions occurring between parents and offspring (nor between any kind of relative), they are also explicitly genetic in origin. In this sense 'cascading *genetic* maternal effects' (but not non-genetic maternal effects) are a special case of cascading IGE.

Cascading IGE, if they occur, dramatically increase the scope and potential effects of genetic variation in the social environment. For example, migration among locally adapted populations can influence population-genetic structure and introduce adaptive or non-adaptive alleles into recipient populations. With cascading IGE, however, immigrants can immediately change phenotypes of their social partners in adaptive or non-adaptive ways that are independent of the introduction of novel alleles. Even if cascading IGE are not strongly adaptive or maladaptive at the outset, behavioural change that they induce can modify the selective environment for the entire group, thereby feeding back to influence evolution across generations [38]. Many species exhibit either dispersal or fission–fusion social structure, so understanding IGE caused by prior social environments is critical to understanding the evolution in social organisms.

Despite its potential importance, assessing whether IGE can cascade is an empirical challenge. In sexually reproducing species, it is difficult to replicate genetically based differences in the social environment. Clonally reproducing species provide an opportunity to replicate and investigate the effects of genetic variation in the social environment, allowing effects like cascading IGE to be assessed without using inbred lines or complex breeding designs.

The Amazon molly (*Poecilia formosa*) is a gynogenetic, all-female species [39] that arose from a single hybridization event between a male sailfin molly (*Poecilia latipinna*) and a female Atlantic molly (*Poecilia mexicana*) about 100 000 generations ago [40–42]. Although reproduction is clonal, females require sperm from a male of one of the ancestral species (sailfin or Atlantic molly) to initiate embryogenesis of unreduced ova [42]. Many distinct clonal lineages arose from the original hybrid lineage through mutation or rare incorporation of paternal genetic material [42,43]. This accumulation of genetic diversity in a gynogenetic species produces groups in which social interactions occur on multiple levels: within-clone interactions, among-clone interactions and interspecies interactions between Amazons and their sexual hosts.

In natural populations, the number of clonal lineages that co-occur can vary dramatically from a single lineage to more than a dozen [44–46]. Consequently, the degree of competition and the frequency with which females encounter conspecifics of different lineages can vary greatly across time and space. One of the first studies to investigate social behaviours among different clones reported that females could distinguish between lineages, associate preferentially with fish of their own lineage, and were more aggressive toward unrelated clones [47]. Other research has shown that features of the social environment such as dominance [35] and the degree of familiarity among individuals [48,49] can influence interactions within and among clonal lineages. These data suggest that individual behaviour depends in part on the clonal composition of the social environment; that is, IGE likely influence phenotypic variation and social dynamics in natural populations of Amazon mollies.

We therefore used clonal variation in Amazon mollies to test the hypothesis that IGE propagate beyond individuals that experience them firsthand. We did this by manipulating the genetic makeup of the social environment of focal individuals, then moving focals into groups of naive individuals that were all genetically identical and had the same previous environmental and social experience (figure 2). This experimental design simulates the fission–fusion dynamics often observed in poeciliid fishes in natural environments [50–52]. If IGE occur only through 'traditional' (non-cascading) effects, then naive individuals should not differ systematically in behaviour because they all have the same genotype and the same previous environmental conditions. If IGE cascade, however, the previous social experience of focals will influence the behaviour of the naive individuals. We also predicted that cascading IGE would influence group-emergent behaviour in the naive fish, based on the literature indicating that individual

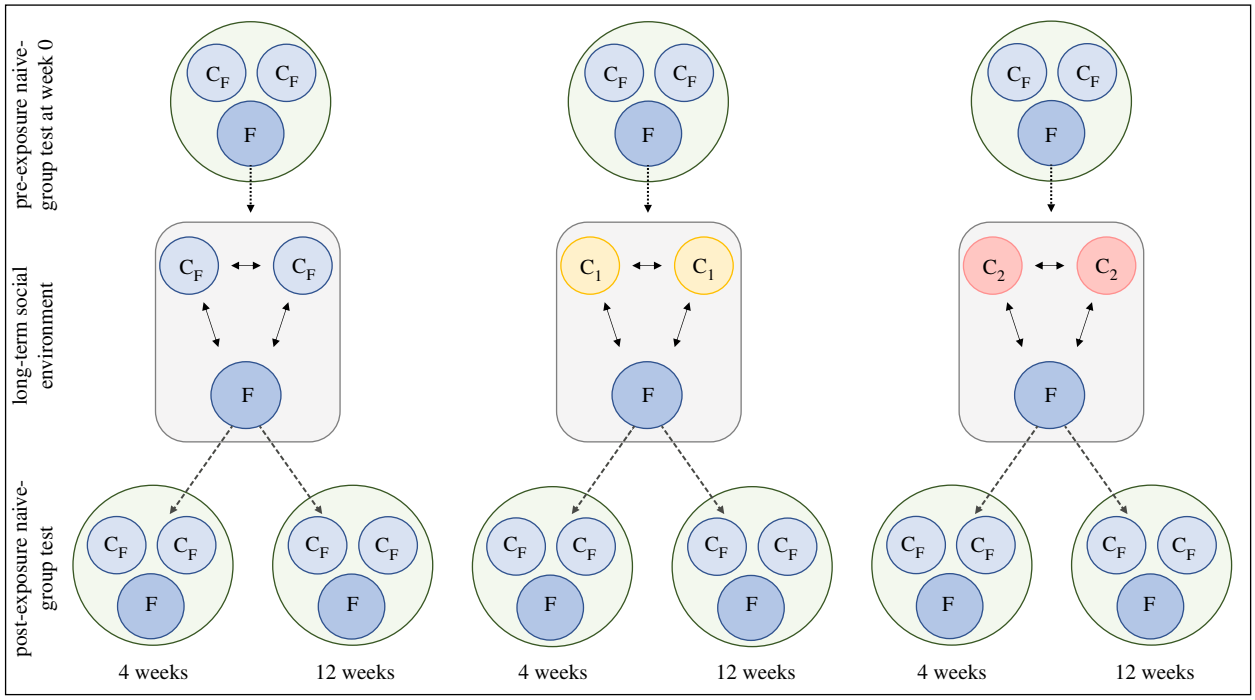

Figure 2. Schematic of experimental design illustrating that the focal females (F) were placed into one of the three different long-term social environments: Monoclonal (F + 2 C_F); Clone 1 (F + 2 C_1); or Clone 2 (F + 2 C_2). After 4 weeks of exposure to these long-term social environments, the exploratory behaviours of the focal females were tested in the naive-group tests with novel C_F individuals. Each F was then replaced into her previous long-term social environment until 12 weeks of exposure, when she was tested again in the naive-group tests. Note that these C_F partners in the naive-group trials were different individuals at each time period. That is, each individual C_F was included in only one trial. Solid black arrows indicate first-order IGE and dashed arrows indicate the possible cascading IGE. (Online version in colour.)

differences in behaviour affect group-emergent phenotypes such as shoaling (reviewed in [1]). Finally, we determined if time spent in social environments influences if and how IGE cascade by using a time-course experimental design.

2. Material and methods

(a) Study specimens

Three distinct clonal lineages were used in this study, each descended from individuals collected from the Río Purificacíon in Nuevo Padilla, Mexico (24°4′42.85″ N, 99°7′21.76″ W) and maintained in a greenhouse at the Mission Road Research Facility of Florida State University. Both Clone 1 (Schartl) and Clone 2 (AMM#11) are diploid with microchromosomes, although the microchromosomes are distinctly different between the two lineages [47,53]. The focal clone (3N) is a triploid without any microchromosomes; this clonal lineage was chosen at random to be the focal clone. See electronic supplementary material, Methods, for details concerning fish husbandry.

(b) Long-term social environments

Focal females were placed into 18.9 l aquaria in one of three different long-term social environments: (i) 1 focal female + 2 sister clones; (ii) 1 focal female + 2 females from Clone 1; and (iii) 1 focal female + 2 females from Clone 2. That is, each aquarium contained 1 focal fish and 2 'social partner' fish. Females placed into the same aquarium were unfamiliar with each other (electronic supplementary material, Methods). Thus, partner fish genotypes, but not the genotype of focal fish, differed among treatments. Each social-environment treatment was replicated 12 times (a total of 36 experimental tanks) using a randomized complete block design (one replicate per treatment per block). Six blocks were set up per week until all 12 blocks were complete. All females used in this study were adults and ranged from 27 to 38 mm in body length, with 4 mm as the maximum size difference among females within each group to reduce the influence of body size on aggression [48,49]. We nevertheless tested for body size effects in initial statistical models.

To characterize differences in the social environment induced by the three different treatments, we measured social interactions nine different times over the 12-week experiment: 10 min after placing the focal fish in the social environment (week 0), weekly for the first four weeks thereafter (weeks 1–4) and then biweekly (weeks 6, 8, 10 and 12). This time-course design allowed us to determine if the social environments varied over time and if and how that variation associated with cascading IGE. Behaviour at week 0 represented a baseline because females had not yet been exposed to experimental social environments beyond the first 10 min acclimation period, and social dominance hierarchies had yet to be established. In each assay, behaviour was recorded for 10 min by a live observer blind to the treatments. In these observations, social behaviour consisted mainly of aggressive interactions (bites, tail beats and chasing); few affiliative or neutral behaviours (e.g. swimming in the same direction or foraging simultaneously within 2 body lengths) were observed. We counted the number of bites and tail beats performed and the total time spent performing these behaviours and chasing other females. Tail beats were rarer than bites, but frequency and time spent in these two behaviours were correlated (Spearman's $\rho = 0.136$, $p = 0.014$). We therefore summed the total number of direct contacts (bites and tail beats), and separately summed the total time spent in all aggressive behaviours to produce two overall measures: total number of aggressive contacts and total time spent in aggression. Individual identification was not possible during trials while fish were in motion and visible

only from one side. We therefore used the total number and duration of behaviours across all fish in the trial as measures of the social environment within the tank.

(c) Naive-group tests

Three times over the course of the experiment (at 0, 4 and 12 weeks), each focal female was introduced to a pair of novel (naive) social partners of the same clonal lineage as the focal (figure 2). Week 0 represented a baseline, measured before any exposure to long-term social environments. Focal females were removed from a holding tank (at week 0) or their long-term social environment tank (at weeks 4 and 12) and placed in a 'naive-group' test chamber with two unfamiliar fish, size matched to the focal fish (±4 mm), and in the same reproductive state. These naive fish were drawn from single-clone, non-breeding tanks and were, therefore, not exposed to the experimental long-term social environments experienced by the focal fish. After introducing the focal fish into the naive-group test chamber, we video recorded all three fish for 10 min, after which the focal female was removed and placed back into her long-term social environment (figure 2). Focal females were tested with a different pair of naive social partners at each test period, and those partner fish were not reused in any other trials.

The test chamber for this assay was an open field, circular tank (55.9 cm diameter), with half the bottom and corresponding sides painted white and the other half grey. A camera (JVC Everio 1920 × 1080 HD video camcorder) was suspended 1.1 m above the tank. Videos were analysed by a blind observer using EthoVision XT (Noldus, v. 14). More information regarding recording and editing is provided in electronic supplementary material, Methods.

Aggressive behaviour was almost never observed in these short-term trials; we therefore assessed movement and shoaling behaviour as the predominant behaviours. Open-field assay of these behaviours provides a validated [54,55] test of consistent individual behavioural differences in poeciliid fish, including Amazon mollies [56], and it is expected to affect fitness-related traits such as dispersal, competition and response to predators [55]. In this assay, stressed individuals tend to be less active, travel shorter distances at lower velocity, spend more time frozen and in the grey zone (negative phototaxis), and be closer together; less stressed individuals tend to be more exploratory and cover more distance, move at higher velocity, enter zones more frequently, spend more time in the white zone and less time frozen, and have more distance between individuals [57,58]. Although fish could be individually tracked, the focal individual could not be distinguished from the other fish on the videos; therefore, we did not calculate separate metrics for focal and novel partner fish.

(d) Analyses

(i) Long-term social environment groups

The two measures of aggression (number of direct-contact aggressive acts and time spent in all aggressive behaviour) were highly correlated ($R^2 = 0.803$, $p < 0.0001$), with the first PC explaining 96.9% of the total variation. Both measures of aggression were log-transformed before principal component analysis (PCA), after adding 1 to account for zero values, after which data were approximately bivariate normal. We therefore used this PC1 score as the dependent variable in linear mixed models to determine effects of long-term social environments on aggressive behaviour. In addition to the social-environment treatment group, initial models included fixed effects of exposure time (weeks), treatment-by-time interaction, baseline (week 0) aggression (PC1), focal female standard length (log-transformed), average standard length of the social-partner females (log-transformed) and a random block effect. We used a random group ID effect to account for repeated measures on the groups. Baseline aggression and size of social partners were never significant predictors in initial models (electronic supplemental material, table S2A) and the random block effect was consistently near zero and never significant. These terms were dropped from the final models. Treatment group, exposure time and treatment-by-time interaction were retained in all final models, since these were the critical terms for testing our hypotheses. See electronic supplementary material, Methods for additional details.

(ii) Naive-group tests

To determine if the presence of the focal individual influenced behaviour in the naive groups, and thus to measure cascading IGE, we calculated two kinds of metrics: those that described average behaviour of the three members of the group, and those that described individual behaviour of fish within the group. For both analyses, we measured distance travelled (cm), velocity (cm s^{-2}), frequency entering white zone (count), duration in white zone (s), latency to enter white zone (s), time spent immobile (s; freezing behaviour) and average shoaling distance between individuals (mm).

(iii) Average behaviour of naive groups

We first averaged each of the variables described above for the three fish within a given trial, and then assessed the correlation structure of the seven group-average behaviours to determine if they could be adequately represented by principal components. The six behaviours that described movement or physical position in the enclosure were all moderately to highly correlated with one another ($0.4 < |r| < 1.0$), but they were uncorrelated with the average shoaling distance between fish (all $|r|$ less than 0.2; electronic supplementary material, figure S1A), indicating that a PCA should include the six movement/position variables, but that shoaling distance should be analysed separately. PC1 explained 75.8% of the total variation in movement/position variables, and it was the only PC with an eigenvalue greater than 1. Behaviours associated with exploration loaded positively on PC1 (distance, velocity and duration in the white zone, and frequency entering white zones), while behaviours associated with stress loaded negatively (freezing, latency to enter white zone; electronic supplementary material, table S3). We therefore considered positive values of PC1 to indicate a tendency to explore and negative values to indicate lack of exploration or stress-related behaviour; we refer to this measure as 'exploratory PC1' for conciseness. We considered the log-transformed average shoaling distance to be a measure of group cohesion, since it arises from the relative positions of all three members of the group.

To determine if these two measures of average group behaviour were affected by the long-term social environment experienced by a single member of the group, we used the values at weeks 4 and 12 as the dependent variable in linear mixed models. Neither baseline behaviour nor size-related covariates were significantly associated with behaviour in initial models (see electronic supplemental material, Methods, tables S2B and C), so final models included only treatment and exposure time (and their interaction) as fixed effects and a random effect of focal female ID to account for the repeated trials (4- and 12-week) in which focal females were used.

Because long-term social environments varied in aggression (see §3), any overall association between aggression experienced in the long-term social environment and the behaviour of naive groups could have been obscured by the treatment effect in the models described above. To assess the overall relationship between aggression in the long-term environment and naive-

group behaviour, we fit models for exploratory PC1 and shoaling identical to those described above, except that the only predictor variable was PC1 of the cumulative aggression experienced in the long-term environment up to the time of the relevant naive-group assay (see electronic supplemental material, Methods).

To assess the consistency of behaviour of the naive groups that contained the same focal female after 4 and 12 weeks of exposure to long-term social environments, we calculated Pearson's correlation between exploratory PC1 scores (or shoaling) at the two time periods [59]. We calculated 95% confidence limits of the correlation using the *z*-transformation method.

(iv) Behaviour of individuals in naive groups

The main purpose of this analysis was to determine if differences in the average behaviour of naive groups was attributable to all members of a group behaving similarly or to specific individuals within the group. For example, if the behaviour of the three females within a group was very similar, then average differences among groups reflect the behaviour of all group members. Alternatively, if individuals within groups behaved differently from one another, then between-group differences could have been driven by the divergent behaviour of a single group member. The former, but not the latter, would support cascading IGE because it would indicate that non-focal behaviour was influenced by the prior social experience of the focal fish. Our primary measure of similarity of the behaviour of individuals within naive groups was the intraclass correlation coefficient (ICC).

We first investigated the correlation structure of the same movement/position behaviours described above, but measured on individuals rather than the group mean. As in the group-average data, these variables were moderately to highly correlated with each other, but not with shoaling distance (electronic supplementary material, figure S1B). We therefore summarized the movement/position behaviour of individual fish using the first PC (electronic supplementary material, table S3). The ICC of this PC score was determined using a linear mixed model with a random effect corresponding to group ID. The ICC is the ratio of among-group variance to the total variance, with confidence intervals determined using parametric bootstrap [59]. We did not calculate ICC for shoaling distance because of the inherent non-independence of these measures within a trial.

3. Results

(a) No differences between treatment groups at baseline

There were no differences among treatment groups in behaviour at baseline (aggression PC1: $F_{2,22} = 1.15$, $p = 0.336$; exploratory PC1: $F_{2,22} = 2.53$, $p = 0.103$; shoaling: $F_{2,22} = 0.37$, $p = 0.695$, see electronic supplemental material, Methods for details). There were no size differences among focal females in different social treatment groups, nor treatment-associated differences in size among the social partner fish used in the long-term and naive-group trials (electronic supplemental material, table S1).

(b) Long-term social environments differ in aggressive behaviour

Long-term social groups in which the focal fish was housed with two females of her own clonal lineage exhibited more aggression than groups where the social partners were Clone 1 or Clone 2 fish; however, time in the social environments did not significantly affect amount of aggression (table 1*a*; effect estimates provided in electronic supplementary material, table S4, figures S2 and S3). *Post hoc* tests indicated that the Monoclonal social environment elicited significantly more aggression than the Clone 1 environment ($t_{256.1} = 3.93$, $p < 0.001$), but no other contrasts were significant after adjustment for multiple tests (Monoclonal versus Clone 2: $t_{248.8} = 2.15$, $p = 0.087$; Clonal 1 versus Clone 2: $t_{247.1} = -2.04$, $p = 0.114$). On average, fish in the Monoclonal environment performed 60% more direct-contact aggressive acts than fish in the Clone 1 environment (14.90 ± 1.41 versus 9.52 ± 1.14 acts per 10 min observation bout, respectively; fish in the Clone 2 environment performed 11.41 ± 1.13 aggressive acts per bout, on average).

(c) Cascading IGE: genetic differences in prior social experience for one group member affected average behaviour of the group

Genetic variation in the long-term social environment experienced by the focal fish affected the average exploratory behaviour of the group when that fish was paired with naive individuals (table 1*b* and figure 3*a*; electronic supplementary material, figure S4, and table S5); however, duration of exposure (4 versus 12 weeks) did not significantly affect exploratory PC1.

Indeed, the treatment group of the focal fish explained 43.1% of the total variation in the exploratory PC1 scores. Naive groups in which the focal individual experienced the high-aggression Monoclonal long-term environment exhibited more stress-related behaviour (negative values on PC1) than groups in which the focal individual experienced Clone 1 or Clone 2 social environments (*post hoc* tests: Monoclonal versus Clone 1, $t_{17.58} = -2.59$, $p = 0.044$; Monoclonal versus Clone 2, $t_{21.88} = -3.12$, $p = 0.015$). Naive groups in which the focal fish had experienced Clone 1 and Clone 2 environments did not differ significantly from each other after correction for multiple tests ($t_{16.81} = -1.31$, $p = 0.405$).

The long-term social environment of focal animals also affected the variance in behaviour among naive groups (i.e. the variance structure differed significantly between treatments; electronic supplementary material, table S6). Groups containing focal fish from Monoclonal long-term environments exhibited less variance in exploratory behaviour than groups containing focals from Clone 1 and Clone 2 ($\chi^2 = 18.4$, d.f. = 4, $p = 0.001$).

Consistency of behaviour of groups that contained the same focal female was also influenced by the long-term social environment (electronic supplementary material, table S7 and figure S5). Naive groups containing the same focal females showed high consistency in behaviour when the focal female experienced the Monoclonal long-term environment ($r = 0.73$, 95% confidence interval (CI) = [0.27, 0.92], $p = 0.007$) but not when the focal female experienced the Clone 2 social environment ($r = 0.280$, 95% CI = [−0.35, 0.74], $p = 0.38$). When the focal female was from the Clone 1 long-term environment, their naive groups exhibited a negative correlation across time periods ($r = -0.759$, 95% CI = [−0.93, −0.33], $p = 0.004$).

At the individual level, cumulative aggression experienced in the long-term social environment was related to

Table 1. (*a*) Aggressive behaviour (PC1) in long-term social environments and (*b*) exploratory behaviour (PC1) in naive-group tests varied significantly among the three treatment groups (Monoclonal, Clone 1 and Clone 2). (*c*) Cumulative aggression experienced within the long-term social environments was significantly associated with exploratory/stress behaviours (PC1) in the naive-group tests. (*d*) Shoaling behaviour was unaffected by long-term social environment and exposure time. (*e*) Shoaling behaviour was not associated with cumulative aggression in the long-term social environments. Predictors with *p*-values below 0.05 are indicated in bold type.

model	effect	proportion of variance explained	statistic	*p*-value
(a) Aggressive behaviour in long-term social treatments (PC1)				
	focal female standard length	$R^2 = 0.015$	$F_{1,208.1} = 3.26$	0.072
	social environment	$R^2 = 0.058$	$\mathbf{F_{2,250.3} = 7.70}$	**<0.001**
	exposure time	$R^2 = 0.039$	$F_{7,221.2} = 1.30$	0.253
	social environment × time	$R^2 = 0.040$	$F_{14,232.6} = 0.69$	0.788
(b) Exploratory/stress behaviour in naive-group trials (PC1)				
	social environment	$R^2 = 0.431$	$\mathbf{F_{2,19.46} = 5.13}$	**0.016**
	exposure time	$R^2 = 0.023$	$F_{1,19.4\ s} = 1.11$	0.306
	social environment × time	$R^2 = 0.089$	$F_{2,18.13} = 0.58$	0.568
(c) Cumulative aggression on exploratory/stress behaviours in naive-group tests (PC1)			$\mathbf{F_{1,19.66} = 5.42}$	**0.031**
(d) Shoaling distance in naive-group tests				
	social environment	$R^2 = 0.056$	$F_{2,33} = 0.36$	0.704
	exposure time	$R^2 = 0.003$	$F_{1,33} = 0.06$	0.816
	social environment × time	$R^2 = 0.242$	$F_{2,33} = 2.63$	0.087
(e) Cumulative aggression on shoaling distance in naive-group trials			$F_{1,26.3} = 1.19$	0.285

exploratory behaviour in the naive-group trials (table 1*c*). The more aggression a focal female experienced in her long-term social environment, the lower her exploratory PC1 score ($\beta = -0.401 \pm 0.172$). This result suggests that the difference in naive-group behaviour between treatment groups could have resulted from genetically based differences in aggression experienced by the focal females in the long-term social environments.

Mean shoaling distance in the naive-groups was unaffected by the long-term social environment of the focal fish or by the duration of exposure (table 1*d*, effect estimates for fixed effects in electronic supplementary material, table S8, electronic supplementary material figure S6). There was a trend for different treatment groups to behave differently over time, but the interaction term did not reach significance (table 1*d*; electronic supplementary material, figure S7). Among-group variance was unaffected by treatment or exposure time ($\chi^2 = 6.28$, d.f. = 4, $p = 0.18$, electronic supplementary material, table S6) and group-level consistency was low for shoaling behaviour ($r = 0.142$; electronic supplementary material, table S7). Cumulative aggression did not significantly predict shoaling distance in the naive-group trials (table 1*e*; $\beta = -0.003 \pm 0.047$).

(d) Individuals within naive groups behave very similarly

Focal and stimulus fish within the naive groups were unfamiliar with one another and had different social experiences prior to the naive-group trials. Nevertheless, the three individuals in each naive group behaved in a remarkably similar manner (figure 3*b* shows representative tracking data for three different trios from naive-group trials; see electronic supplementary material, figure S8 for additional representations). The striking visual similarity of tracking patterns is reflected in high ICC estimates for individual exploratory behaviour (ICC = 0.831, 95% CI = [0.781, 0.882] overall; treatment-group specific values in figure 3*b*; see electronic supplementary material, table S9 for variance estimates used in calculations). That is, less than 17% of the total variation in behaviour occurred among the three females within a given naive-group trial, despite the substantial differences in behaviour among trials that is evident in figure 3*b* and electronic supplementary material, figure S8. This result indicates that all three individuals within a given trial exhibited highly similar behaviour, despite their different prior experience.

4. Discussion

Elucidating heritable causes of individual and group-emergent phenotypes is necessary to understand the evolution of social traits and other interacting phenotypes. Here, we show that the phenotypic effects of genetically different social environments (IGE) can cascade to influence individuals that never experienced IGE. These results indicate that the phenotypic and evolutionary consequences of IGE may be much more pervasive than previously known. Given the prevalence of dispersal and fission–fusion group dynamics, there is substantial opportunity for cascading IGE in nature.

The cascading IGE we observed were associated with different levels of aggression that focal fish experienced in their long-term social environments. Somewhat surprisingly, it was the social environment containing fish of the same clone as the focals that exhibited the most aggression (and the naive groups containing these focal fish exhibited the most stress-related behaviour). Previous studies reported

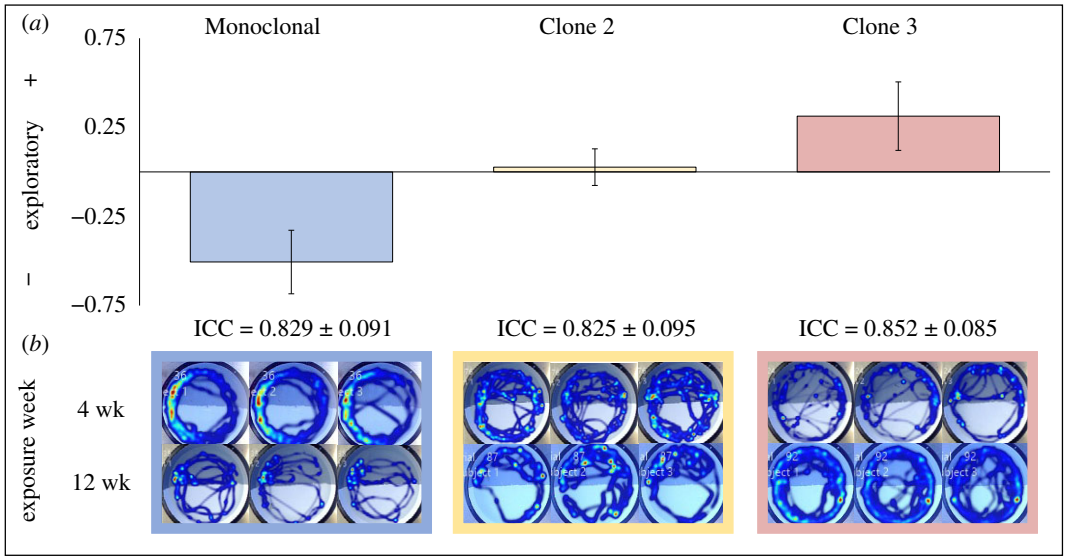

Figure 3. Genetic differences in prior social experience for one group member affected average behaviour of the group. (*a*) Least square means ± standard error for exploratory/stress behaviour PC1 in the naive-group tests. Least-square means reflect the average over both time periods since time was not a significant factor in the analysis. Group-averaged exploratory behaviours with positive values indicating more exploratory behaviours and negative values indicate less exploratory and more stress behaviours. (*b*) Consistency of behaviour among individuals in the same naive-group tests is assessed quantitatively by ICC values and can be visually represented in the similarity of the movement tracks for each individual. High ICC values and representative movement maps for each treatment group illustrate that the three individuals within a naive group behaved very similarly after 4 or 12 weeks of exposure. The three tracks in the top row of each block represent movement of the focal fish and two naive partners after four weeks' exposure to the long-term social environment (4 weeks). The three tracks in the bottom row of each block represent movement of fish after 12 weeks of exposure to the long-term social environment (12 weeks). For a given treatment, the same focal female was present at each time point, but her two social partners were different individuals across time points. (Online version in colour.)

that Amazon mollies exhibited less aggression towards sister clones compared to non-sister clones [47,60]. However, a different focal clonal lineage was used in those studies, suggesting that responses to sister and non-sister clones can vary across genotypes.

We detected no effects of 4 versus 12 weeks of exposure to genetically different social environments on aggression in those environments or on cascading IGE in the naive groups. Time-course effects on first-order IGE have been found in a related poeciliid, the eastern mosquitofish (*Gambusia holbrooki*) [20,21], and increased exposure time led to higher aggression in previous studies of Amazon mollies [49,61]. However, the time-course in mosquitofish occurred during maturation, whereas the fish in our experiment were fully mature at the start of the experiment. The studies that reported exposure-time effects on aggression in Amazon mollies maintained the animals at considerably higher density than that used in our experiment ([53] : 1.9 L/fish; [47] : 4 L/fish; the present study: 6.3 L/fish), suggesting that exposure-time effects could be density-dependent.

Density effects might also account for lack cascading IGE for shoaling cohesion in our study, despite strong cascading IGE for exploratory behaviour. IGE affecting group-emergent phenotypes have been reported for social cohesion in *Drosophila melanogaster* [27] and for larval cooperation in burying beetles (*Nicrophorus vespilloides*) [28]. Given extensive literature indicating that individual differences can substantially influence group-emergent behaviour ([62], reviewed in [1]), we expected that cascading IGE would influence shoaling cohesion [62–64]. In our study, the small enclosure size used in open-field trials might have limited the variation in shoaling distance that could be expressed. Future investigations using larger enclosures could determine if cascading IGE influence group-emergent phenotypes under different conditions. We also note that traditional IGE involving the same traits in focal and interacting individuals tend to be stronger than those involving different traits [65]. We measured different behaviours in the two stages of our experiment, so estimates of cascading IGE involving the same traits might be of even greater magnitude.

The cascading IGE we did observe could have arisen because focal females in Clone 1 and Clone 2 treatment groups experienced a genetic change in their social environment when they moved into the naive-groups, but focal fish in the Monoclonal treatment did not. If this were the primary cause of cascading IGE, we would expect a significant difference in cascading IGE between the Monoclonal treatment and both Clone 1 and Clone 2 (which we did find), but not between Clone 1 and Clone 2 (for which we found only a non-significant trend). Clone 1 and Clone 2 treatments did differ significantly in variance among naive groups and in the consistency of behaviour at 4 and 12 weeks, however. These differences between Clone 1 and Clone 2 treatments suggest that sister-clone recognition was not the only cause of cascading IGE, and point to differences in phenotypic variance as an under-explored consequence of IGE. Nonetheless, our data support the hypothesis that genetically identical fish (the naive partners) behave differently depending on genetic differences in the prior social environment experienced by another member of the group (the focal female). Whether cascading IGE depend on the degree of genetic similarity between past and current social partners is an intriguing question for future studies. Movement among groups that differ in genetic similarity from the migrating individual is likely to occur in species such as Amazon mollies that exhibit strong population-genetic structure [41].

Given the potential for cascading IGE in many species, their adaptive consequences are likely to be context-dependent. For

example, cascading IGE are most likely to be adaptive in populations with an evolutionary history of frequent mixing between groups. The fission–fusion dynamics of Amazon mollies (and other species) is one scenario in which we envision that IGE (cascading and otherwise) might evolve to become adaptive. By contrast, maladaptive effects should be more likely when migration is rare and when subpopulations exhibit strong local adaptation.

Theoretical models incorporating cascading IGE could produce more precise predictions for their evolutionary consequences. Recent work on cascading maternal effects suggests these could be profound. For example, McGlothlin *et al*. [36] and Pick *et al*. [37] report that cascading genetic maternal effects change the expected evolutionary trajectories for both maternal and offspring traits when compared to maternal effects that do not cascade. Models incorporating cascading IGE such as those found in our experiment could illuminate how substantially these can change evolutionary dynamics compared to traditional IGE that do not cascade. Embedding both traditional and cascading IGE into network models of social interaction is one avenue ripe for exploration.

In summary, we found that IGE propagate beyond individuals that directly experience them. These cascading IGE are a potentially important cause of individual differences in behaviour and other ecologically important phenotypes. We expect cascading IGE to either amplify the effects of traditional IGE on the heritability and expected evolutionary trajectory of the target traits, or to diminish those effects, depending on the direction of the cascading IGE. Expansion of the quantitative genetic framework of IGE to include cascading and other types of carry-over effects will facilitate understanding of individual variation and social evolution and allow more accurate prediction of population response to changing environments.

Ethics. This research was approved by the Institutional Animal Care and Use Committee of Florida State University (1704 and 201900038).

Data accessibility. All data are archived on figshare (doi:10.6084/m9.figshare.19615062). Electronic supplemental material is available online [66].

Authors' contributions. A.M.M.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, writing—original draft, writing—review and editing; D.B.: formal analysis, software, writing—review and editing; C.R.: data curation, investigation, methodology; K.A.H.: conceptualization, formal analysis, methodology, project administration, software, supervision, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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References

1. del Mar Delgado M, Miranda M, Alvarez SJ, Gurarie E, Fagan WF, Penteriani V, di Virgilio A, Morales JM. 2018 The importance of individual variation in the dynamics of animal collective movements. *Phil. Trans. R. Soc. B* **373**, 20170008. (doi:10.1098/rstb.2017.0008)
2. Jolles JW, King AJ, Killen SS. 2020 The role of individual heterogeneity in collective animal behaviour. *Trends Ecol. Evol.* **35**, 278–291. (doi:10.1016/j.tree.2019.11.001)
3. Liu J, Lewis G, Evans L. 2012 Understanding aggressive behaviour across the lifespan. *J. Psychiatr. Ment. Health Nurs.* **20**, 156–168. (doi:10.1111/j.1365-2850.2012.01902.x)
4. Peake TM, McGregor PK. 2004 Information and aggression in fishes. *Learn. Behav.* **32**, 114–121. (doi:10.3758/BF03196012)
5. Wolf JB, Brodie III ED, Cheverund JM, Moore AJ, Wade MJ. 1998 Evolutionary consequences of indirect genetic effects. *Trends Ecol. Evol.* **13**, 64–69. (doi:10.1016/S0169-5347(97)01233-0)
6. Bijma P, Wade MJ. 2008 The joint effects of kin, multilevel selection and indirect genetic effects on response to genetic selection. *J. Evol. Biol.* **21**, 1175–1188. (doi:10.1111/j.1420-9101.2008.01550.x)
7. Baud A, McPeek S, Chen N, Hughes KA. 2022 Indirect genetic effects: a cross-disciplinary perspective on empirical studies. *J. Hered.* **113**, 1–15. (doi:10.1093/jhered/esab059)
8. Moore AJ, Brodie III ED, Wolf JB. 1997 Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution* **51**, 1352–1362. (doi:10.1111/j.1558-5646.1997.tb01458.x)
9. Mutic JJ, Wolf JB. 2007 Indirect genetic effects from ecological interactions in *Arabidopsis thaliana*. *Mol. Ecol.* **16**, 2371–2381. (doi:10.1111/j.1365-294X.2007.03259.x)
10. Hunt J, Simmons LW. 2002 The genetics of maternal care: direct and indirect genetic effects on phenotype in the dung beetle *Onthophagus taurus*. *Proc. Natl Acad. Sci. USA* **99**, 6828–6832. (doi:10.1073/pnas.092676199)
11. Wilson AJ, Gelin U, Perron M-C, Réale D. 2009 Indirect genetic effects and the evolution of aggression in a vertebrate system. *Proc. R. Soc. B* **276**, 533–541. (doi:10.1098/rspb.2008.1193)
12. Bleaker BH, Brodie III ED. 2008 Indirect genetic effects influence antipredator behavior in guppies: estimates of the coefficient of interaction *Psi* and the inheritance of reciprocity. *Evolution* **63**, 1796–1806. (doi:10.1111/j.1558-5646.2009.00672.x)
13. McFarlane SE, Gorrell JC, Coltman DW, Humphries MM, Boutin S, McAdam AG. 2015 The nature of nurture in a wild mammal's fitness. *Proc. R. Soc. B* **282**, 20142422. (doi:10.1098/rspb.2014.2422)
14. Santostefano F, Wilson AJ, Niemela PT, Dingemanse NJ. 2017 Indirect genetic effects: a key component of the genetic architecture of behaviour. *Sci. Rep.* **7**, 10235. (doi:10.1038/s41598-017-08258-6)
15. Alemu SW, Bijma P, Møller SH, Janss L, Berg P. 2014 Indirect genetic effects contribute substantially to heritable variation in aggression-related traits in group-housed mink (*Neovison vison*). *Genet. Sel. Evol.* **46**, 30. (doi:10.1186/1297-9686-46-30)
16. Camerlink I, Turner SP, Bijma P, Bolhuis JE. 2013 Indirect genetic effects and housing conditions in relation to aggressive behaviour in pigs. *PLoS ONE* **8**, e65136. (doi:10.1371/journal.pone.0065136)
17. Baud A, Casale FP, Nicod J, Stegle O. 2020 Dissecting the mechanisms underlying indirect genetic effects on biomedical phenotypes: a study on 170 behavioural, physiological and morphological phenotypes measured in adult laboratory mice. *bioRxiv* 302349. (doi:10.1101/302349)
18. Fisher DN, Wilson AJ, Boutin S, Dantzer B, Lane JE, Coltman DW, Gorrell JC, McAdam AG. 2019 Social effects of territorial neighbours on the timing of spring breeding in North American red squirrels. *J. Evol. Biol.* **32**, 559–571. (doi:10.1111/jeb.13437)
19. Peeters K, Eppink TT, Ellen ED, Visscher J, Bijma P. 2012 Indirect genetic effects for survival in domestic

chickens (*Gallus gallus*) are magnified in crossbred genotypes and show a parent-of-origin effect. *Genetics* **192**, 705–713. (doi:10.1534/genetics.112.142554)

20. Kraft B, Lemakos VA, Travis J, Hughes KA. 2018 Pervasive indirect genetic effects on behavioral development in polymorphic eastern mosquitofish. *Behav. Ecol.* **29**, 289–300. (doi:10.1093/beheco/arx180)
21. Culumber ZW, Kraft B, Lemakos V, Hoffner E, Travis J, Hughes KA. 2018 GxG epistasis in growth and condition and the maintenance of genetic polymorphism in *Gambusia holbrooki*. *Evolution* **72**, 1146–1154. (doi:10.1111/evo.13474)
22. Baud A *et al.* 2017 Genetic variation in the social environment contributes to health and disease. *PLoS Genet.* **13**, e1006498. (doi:10.1371/journal.pgen.1006498)
23. Wolf JB, Moore AJ. 2010 Interacting phenotypes and indirect genetic effects: a genetic perspective on the evolution of social behavior. In *Evolutionary behavioral ecology*, pp. 225–245. New York, NY: Oxford University Press.
24. Bailey NW, Marie-Orleach L, Moore AJ, Simmons L. 2018 Indirect genetic effects in behavioral ecology: does behavior play a special role in evolution? *Behav. Ecol.* **29**, 1–11. (doi:10.1093/beheco/arx127)
25. Bailey NW, Kolliker M. 2019 Social runaway: Fisherian elaboration (or reduction) of socially selected traits via indirect genetic effects. *Evolution* **73**, 1549–1563. (doi:10.1111/evo.13791)
26. Saltz JB. 2013 Genetic composition of social groups influences male aggressive behaviour and fitness in natural genotypes of *Drosophila melanogaster*. *Proc. R. Soc. B* **280**, 20131926. (doi:10.1098/rspb.2013.1926)
27. Anderson BB, Scott A, Dukas R. 2017 Indirect genetic effects on the sociability of several group members. *An. Behav.* **123**, 101–106. (doi:10.1016/j.anbehav.2016.10.028)
28. Rebar D, Bailey NW, Jarrett BJM, Kilner RM. 2020 An evolutionary switch from sibling rivalry to sibling cooperation, caused by a sustained loss of parental care. *Proc. Natl Acad. Sci. USA* **117**, 2544–2550. (doi:10.1073/pnas.1911677117)
29. Jolles JW, Boogert NJ, Sridhar VH, Couzin ID, Manica A. 2017 Consistent individual differences drive collective behavior and group functioning of schooling fish. *Curr. Biol.* **27**, 2862–2868. (doi:10.1016/j.cub.2017.08.004)
30. Ancillotto L, Allegrini C, Serangeli MT, Jones G, Russo D. 2014 Sociality across species: spatial proximity of newborn bats promotes heterospecific social bonding. *Behav. Ecol.* **26**, 293–299. (doi:10.1093/beheco/aru193)
31. Ruploh T, Henning M, Bischof HJ, von Engelhardt N. 2015 Effects of social conditions during adolescence on courtship and aggressive behavior are not abolished by adult social experience. *Dev. Psychobiol.* **57**, 73–82. (doi:10.1002/dev.21262)
32. Cox JA, Cusick JA, DuVal EH. 2019 Manipulated sex ratios alter group structure and cooperation in the brown-headed nuthatch. *Behav. Ecol.* **30**, 883–893. (doi:10.1093/beheco/arz030)
33. Jolles JW, Fleetwood-Wilson A, Nakayama S, Stumpe M, Johnstone RA, Manica A. 2014 The role of previous social experience on risk-taking and leadership in three-spine sticklebacks. *Behav. Ecol.* **25**, 1395–1401. (doi:10.1093/beheco/aru146)
34. Jolles JW, Taylor BA, Manica A. 2016 Recent social conditions affect boldness repeatability in individual sticklebacks. *An. Behav.* **112**, 139–145. (doi:10.1016/j.anbehav.2015.12.010)
35. Laskowski KL, Wolf M, Bierbach D. 2016 The making of winners (and losers): how early dominance interactions determine adult social structure in a clonal fish. *Proc. R. Soc. B* **283**, 20160183. (doi:10.1098/rspb.2016.0183)
36. McGlothlin JW, Galloway LF. 2014 The contribution of maternal effects to selection response: an empirical test of competing models. *Evolution* **68**, 549–558. (doi:10.1111/evo.12235)
37. Pick JL, Postma E, Tschirren B. 2019 The more you get, the more you give: positive cascading effects shape the evolutionary potential of prenatal maternal investment. *Evol. Lett.* **3**, 412–423. (doi:10.1002/evl3.125)
38. Roulin A, Ducrest AL. 2011 Association between melanism, physiology and behaviour: a role for the melanocortin system. *Eur. J. Pharmacol.* **660**, 226–233. (doi:10.1016/j.ejphar.2011.01.036)
39. Hubbs CL, Hubbs LC. 1932 Apparent parthenogenesis in nature in a form of fish of hybrid origin. *Science* **76**, 628e630. (doi:10.1126/science.76.1983.628)
40. Avise JC, Trexler JC, Travis J, Nelson WS. 1991 *Poecilia mexicana* is the recent female parent of the unisexual fish *P. formosa*. *Evolution* **45**, 1530–1533. (doi:10.1111/j.1558-5646.1991.tb02657.x)
41. Stöck M, Lampert KP, Möller D, Schlupp I, Schartl M. 2010 Monophyletic origin of clonal lineages in an asexual fish (*Poecilia formosa*). *Mol. Ecol.* **19**, 5204–5215. (doi:10.1111/j.1365-294X.2010.04869.x)
42. Schartl M, Nanda I, Schlupp I, Wilde B, Eppens JT, Schmid M, Parzefall J. 1995 Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. *Nature* **373**, 68–71. (doi:10.1038/373068a0)
43. Schlupp I, Riesch R. 2011 Evolution of unisexual reproduction. In *Ecology and evolution of poeciliid fishes* (eds J Evans, A Pilastro, I Schlupp), pp. 50–58. Chicago, IL: University of Chicago Press.
44. Kallamn KD. 1962 Population genetics of the gynogenetic teleost, *Mollienesia formosa* (Girard). *Evolution* **16**, 497–504. (doi:10.1111/j.1558-5646.1962.tb03239.x)
45. Darnell RM, Lamb E, Abramoff P. 1965 Matroclinous inheritance and clonal structure of a Mexican population of the gynogenetic fish, *Poecilia formosa*. *Evolution* **21**, 168–173. (doi:10.1111/j.1558-5646.1967.tb00140.x)
46. Lamatsch DK, Nanda I, Schlupp I, Epplen JT, Schmid M, Schartl M. 2004 Distribution and stability of supernumerary microchromosomes in natural populations of the Amazon molly, *Poecilia formosa*. *Cytogenet. Genome Res.* **106**, 189–194. (doi:10.1159/000079286)
47. Makowicz AM, Tiedemann R, Steele RN, Schlupp I. 2016 Kin recognition in a clonal fish, *Poecilia formosa*. *PLoS ONE* **11**, e0158442. (doi:10.1371/journal.pone.0158442)
48. Makowicz AM, Murray L, Schlupp I. 2020 Size, species, and audience type influence heterospecific female–female competition. *An. Behav.* **159**, 47–58. (doi:10.1016/j.anbehav.2019.11.001)
49. Makowicz AM, Schlupp I. 2015 Effects of female–female aggression in a sexual/unisexual species complex. *Ethology* **121**, 904–914. (doi:10.1111/eth.12406)
50. Croft DP, Arrowsmith BJ, Bielby J, Skinner K, White E, Couzin ID, Magurran AE, Ramnarine I, Krause J. 2003 Mechanisms underlying shoal composition in the Trinidadian guppy, *Poecilia reticulata*. *OIKOS* **100**, 429–438. (doi:10.1034/j.1600-0706.2003.12023.x)
51. Flood CE, Wong MYL. 2017 Social stability in times of change: effects of group fusion and water depth on sociality in a globally invasive fish. *Anim. Behav.* **129**, 71–79. (doi:10.1016/j.anbehav.2017.05.003)
52. Bierbach D, Krause S, Romanczuk P, Lukas J, Arias-Rodriguez L, Krause J. 2020 An interaction mechanism for the maintenance of fission–fusion dynamics under different individual densities. *PeerJ* **8**, e8974. (doi:10.7717/peerj.8974)
53. Warren WC *et al.* 2018 Clonal polymorphism and high heterozygosity in the celibate genome of the Amazon molly. *Nat. Ecol. Evol.* **2**, 669–679. (doi:10.1038/s41559-018-0473-y)
54. Bierbach D, Laskowski KL, Wolf M. 2017 Behavioural individuality in clonal fish arises despite near-identical rearing conditions. *Nat. Commun.* **8**, 15361. (doi:10.1038/ncomms15361)
55. Burns JG. 2008 The validity of three tests of temperament in guppies (*Poecilia reticulata*). *J. Comp. Psychol.* **122**, 344–356. (doi:10.1037/0735-7036.122.4.344)
56. Muraco, Jr. JJ, Monroe DJ, Aspbury AS, Gabor CR. 2021 Do females in a unisexual-bisexual species complex differ in their behavioral syndromes and cortisol production? *Biology* **10**, 186. (doi:10.3390/biology10030186)
57. Cachat JM *et al.* 2011 Video-aided analysis of zebrafish locomotion and anxiety-related behavioral responses. In *Zebrafish neurobehavioral protocols* (eds AV Kalueff, JM Cachat), pp. 1–14. New York, NY: Humana Press.
58. Baker MR, Goodman AC, Santo JB, Wong RY. 2018 Repeatability and reliability of exploratory behavior in proactive and reactive zebrafish, *Danio rerio*. *Sci. Rep.* **8**, 12114. (doi:10.1038/s41598-018-30630-3)
59. Nakagawa S, Schielzeth H. 2010 Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol. Rev.* **85**, 935–956. (doi:10.1111/j.1469-185x.2010.00141.x)
60. Makowicz AM, Moore T, Schlupp I. 2018 Clonal fish are more aggressive to distant relatives

in a low resource environment. *Behaviour* **155**, 351–367. (doi:10.1163/1568539X-00003494)

61. Doran C, Bierbach D, Laskowski K. 2019 Familiarity increases aggressiveness among clonal fish. *An. Behav.* **148**, 153–159. (doi:10.1016/j.anbehav.2018.12.013)
62. Jolles JW, Weimar N, Landgraf T, Romanczuk P, Krause J, Bierbach D. 2020 Group-level patterns emerge from individual speed as revealed by an extremely social robotic fish. *Bio. Lett.* **16**, 20200436. (doi:10.1098/rsbl.2020.0436)
63. Bierbach D, Langraf T, Romanczuk P, Lukas J, Nyguyen H, Wolf M, Krause J. 2018 Using a robotic fish to investigate individual differences in social responsiveness in the guppy. *R. Soc. Open Sci.* **5**, 181026. (doi:10.1098/rsos.181026)
64. Herbert-Read JE, Perna A, Mann RP, Schaef TM, Slumpter DJT, Ward AJW. 2011 Inferring the rules of interaction of shoaling fish. *Proc. Natl Acad. Sci. USA* **108**, 18 726–18 731. (doi:10.1073/pnas.1109355108)
65. Bailey NW, Desjonquères C. 2021 The indirect genetic effect interaction coefficient ψ: theoretically essential and empirically neglected. *J. Hered.* **113**, 79–90. (doi:10.1093/jhered/esab056)
66. Makowicz AM, Bierbach D, Richardson C, Hughes KA. 2022 Cascading indirect genetic effects in a clonal vertebrate. Figshare. (doi:10.6084/m9.figshare.c.6060604)