INDIRECT GENETIC EFFECTS INFLUENCE ANTIPREDATOR BEHAVIOR IN GUPPIES: ESTIMATES OF THE COEFFICIENT OF INTERACTION *PSI* AND THE INHERITANCE OF RECIPROCITY

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How and why cooperation evolves, particularly among nonrelatives, remains a major paradox for evolutionary biologists and behavioral ecologists. Although much attention has focused on fitness consequences associated with cooperating, relatively little is known about the second component of evolutionary change, the inheritance of cooperation or reciprocity. The genetics of behaviors that can only be expressed in the context of interactions are particularly difficult to describe because the relevant genes reside in multiple social partners. Indirect genetic effects (IGEs) describe the influence of genes carried in social partners on the phenotype of a focal individual and thus provide a novel approach to quantifying the genetics underlying interactions such as reciprocal cooperation. We used inbred lines of guppies and a novel application of IGE theory to describe the dual genetic control of predator inspection and social behavior, both classic models of reciprocity. We identified effects of focal strain, social group strain, and interactions between focal and group strains on variation in focal behavior. We measured ψ, the coefficient of the interaction, which describes the degree to which an individual's phenotype is influenced by the phenotype of its social partners. The genetic identity of social partners substantially influences inspection behavior, measures of threat assessment, and schooling and does so in positively reinforcing manner. We therefore demonstrate strong IGEs for antipredator behavior that represent the genetic variation necessary for the evolution of reciprocity.

KEY WORDS: Cooperation, inbred lines, IGE, ψ, social behavior, guppy, *Poecilia reticulata*.

The inheritance and evolution of social behavior are enigmatic because such behaviors are expressed by individuals, but fundamentally depend upon interactions with others. Genes expressed by social partners may alter the magnitude and form of social behaviors expressed by a focal individual; the sources of genetic variation for social traits such as cooperation, dominance, aggression, and communication remain elusive due to this potential for dual genetic control (West-Eberhard 1979; Moore et al. 1997). Frameworks such as game theory and optimality modeling typically assume that genetic variation exists for social behavior, but do not specify the form or interactions (Maynard Smith 1982;

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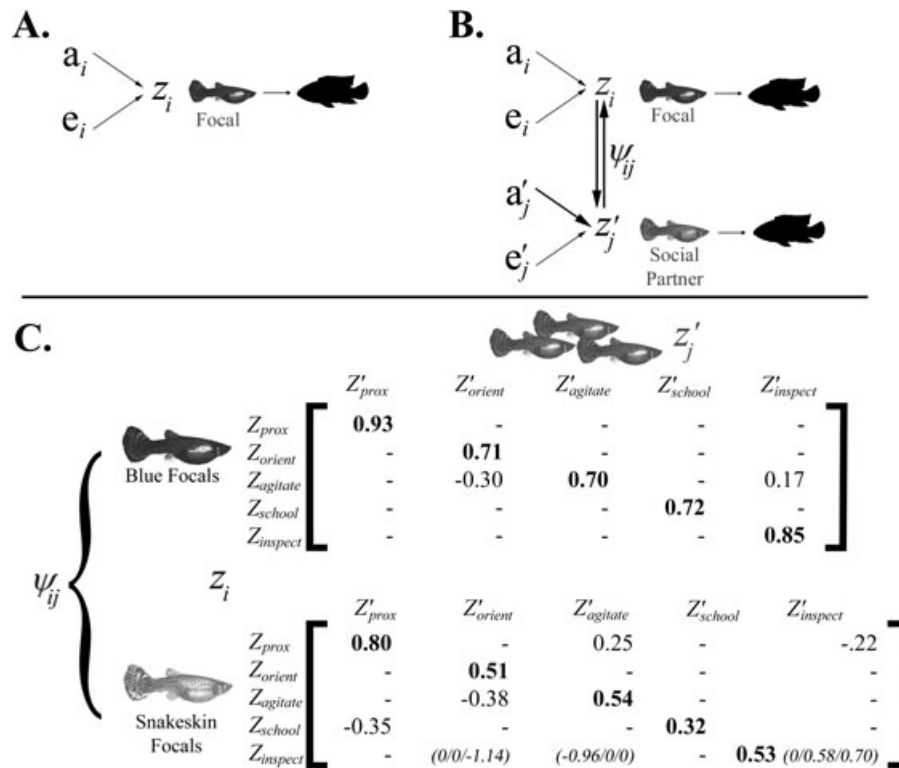
Figure 1. (A) Noninteracting phenotype, where an individual's phenotype (in this case number of inspections) is influenced exclusively by its own genes and environment (Moore et al. 1997). (B) Interacting phenotype, where the focal individual's phenotype is influenced by its own genes and general environment, but also the genes of its social partner through the interaction between them (an additional environmental influence imposed by the social environment). The magnitude of the interaction is scaled by the coefficient of interaction (ψ_{ij}), which is a set of partial regression coefficients obtained from regressing focal phenotype (Z_i) on the phenotype of her social partners (Z'_j). Reciprocal interactions are generated when the same trait in the social partner influences the trait in the focal individual (Moore et al. 1997). (C) The partial regression coefficients (ψ_{ij}) for both focal strains. Values indicate the magnitude of the impact of social context (columns) on focal behavior (rows). A dash indicates that a particular focal behavior is not significantly influenced by a particular aspect of social context. Interactions between the same traits in focal individuals and social context are shown on the diagonal (bold). Parenthetical values were obtained from context strain interactions (Blue / $^1/_2$ Green / $^1/_2$ Yellow).

Dugatkin 2002). Multilevel and kin selection models consider the evolution of interactions. However, both typically require among-group selection and relatedness between interactants to generate a response to selection in a social trait (Bijma and Wade 2008) and focus on the fitness consequences to a given allele, for example for cooperative behavior, possessed by multiple individuals (Hamilton 1964; Trivers 1971). None of these perspectives considers how the inheritance of traits expressed by an individual in a social context might be determined by the genetic network created by interactions among unrelated social partners. Indirect genetic effects (IGEs) describe the influence of genes expressed by conspecifics on a focal individual's phenotype and thereby provide a framework for understanding the inheritance of traits expressed in social contexts (Fig. 1A, B, Moore et al. 1997).

The influence of IGEs can be observed when the behavior of a focal individual responds to changes in the genetic component of the social environment. Here we varied the inbred strain with which focal individuals interacted, directly manipulating

the genetic component of the social environment. Inbred strains minimize behavioral variance among focal individuals from the same strain due to additive genetic variation and provide replicate groups of social partners (i.e., environmental manipulations) that standardize the IGEs to which focal individuals are exposed, allowing us to measure the magnitude of the effect of different aspects of the social environment on focal behavior (Fig. 1B, Moore et al. 1997). The "coefficient of the interaction" in the IGE framework, termed ψ_{ij}, is the partial regression coefficient of the behavior of the focal individual (i) on the behavior of its social partners (j). When ψ_{ij} is measured using standardized traits ($\bar{x} = 0, \sigma = 1$), it ranges from -1 to 1, providing an intuitive scale for describing both the magnitude and direction of influence of the behavior of a social partner on the behavior of a focal individual (Moore et al. 1997). For example, if allogrooming performed by a focal individual (i) is influenced by the amount of grooming performed by a social partner (j), ψ_{ij} is likely to be large and positive. Alternatively, if grooming (i) may only be performed when the

social partner is not acting aggressively (j), ψ_{ij} will be large and negative (Bleakley et al. 2009). By capturing the magnitude and direction of influence of specific traits in social partners on traits of focal individuals, ψ_{ij} delineates explicit predictions about how an interacting trait is likely to evolve within the context of the social environment. By changing the relationship between genotype and phenotype within a population, the existence of IGEs further alter the expected evolutionary responses, rates, and trajectories of traits expressed in social interactions (Moore et al. 1997; Wolf et al. 1999; Agrawal et al. 2001; Bijma and Wade 2008). For example, a trait that is influenced by a large positive ψ is likely to evolve much more rapidly and to a larger degree whereas a trait that is influenced by a large negative ψ may not evolve, or will do so very slowly, even under strong selection pressure.

Reciprocal cooperation, typically defined as individuals taking turns performing a costly behavior to accrue long-term benefits, requires that the behavior of one social partner influences the behavior of another in a positively reinforcing manner (Hamilton 1964; Trivers 1971; Dugatkin 1997). Although reciprocity is often measured in the context of time series, it may evolve in the absence of time structured interactions, such as tit-for-tat, if the behavior of social partners is strongly correlated (Santos and Pacheco 2006; Santos et al. 2006). Positive values of the coefficient of interaction describe reinforcing feedback between traits of social partners. Where positive feedback exists between the same trait expressed in different individuals, such as responding to cooperative behavior with cooperative behavior, IGEs represent a mechanism by which behavioral reciprocity can be inherited. When both the focal individual and its social partners come from inbred lines reared separately to control for effects of common environment, large values of ψ_{ij} for the influence of a social partner's behavior on the same behavior in a focal individual provide evidence for behavioral covariance among individuals generated specifically by IGEs. IGEs therefore describe an explicit genetic mechanism that generates reciprocal interactions among nonrelatives and can be used to predict how such interacting phenotypes may emerge, be inherited, and evolve (Moore et al. 1997; Wolf et al. 1999).

The evolutionary importance of the genetic pathways resulting from social interactions is best examined in ecologically important traits expressed in social contexts, particularly those thought to evolve through reciprocal interactions among individuals. Common guppies, *Poecilia reticulata*, "inspect" when faced with a potential predator, swimming out alone or in a pair to observe the predator at close range (Dugatkin and Godin 1992). Inspections may deter predation or provide information about the activity level and satiation of a potential predator but expose inspecting guppies to increased mortality risk. However, the risk to individuals may be diluted by inspecting in pairs or small groups. As such, predator inspection has served as a model system for understanding the evolution of cooperation (reviewed in Croft et al. 2006).

Individual guppies vary in their tendency to inspect (Dugatkin 1992). Further, different predation regimes appear to select for different levels of inspection with guppies from high-predation populations inspecting more frequently than guppies from low-predation populations (Magurran et al. 1992). Different predation regimes also impact other social behaviors such as courtship (Rodd and Sokolowski 1995). Evolution of inspection behavior in response to predation regime and the conspecific social environment (Dugatkin and Alfieri 1992; Dugatkin and Godin 1992; Magurran et al. 1992; Rodd and Sokolowski 1995) provides evidence that guppy predator inspection behavior is influenced by additive genetic effects (also described as "direct genetic effects" in Fisher 1918). Other aspects of antipredator behavior are also influenced by social environment (Bleakley et al. 2007) and antipredator behavior influences affiliation among individuals, including female mating preferences and association patterns (Dugatkin and Alfieri 1991a; Magurran and Seghers 1994; Croft et al. 2006). As such, there are several behavioral pathways by which guppy behavior may be influenced by the genetic component of the social environment.

Using inbred lines of guppies that differ in their expression of antipredator behavior, we quantified the genetic influences of both social partners and focal individuals on antipredator behavior. Despite being isolated from predatory influences for many generations, inbred guppies retain the ability to recognize and respond appropriately to predatory cues (Bleakley et al. 2006) and respond to sometimes subtle differences in social environment (Bleakley et al. 2007). Inbred lines of "designer" guppies have minimal variance in individual behavior resulting from additive genetic differences within strains and can therefore be used to control the differences in the environment provided by the social group. We predicted that guppy antipredator behavior would reflect both direct genetic effects (effect of the focal female's strain) and IGEs (effect of the social group's strain). In addition, we predicted that the specific behavior of a social group would influence the behavior of the focal individual, providing measurements of the coefficient of the interaction, with the largest effects resulting from the same behavior in the social group on that behavior in the focal individual.

Materials and Methods

We examined the antipredator and social behavior of two inbred focal strains of guppies (Snakeskin and Blue: Bleakley et al. 2006, 2008) that each interacted with three or four sets of inbred "context" strains known to vary in their antipredator behavior in the presence of a model cichlid predator (Bleakley et al. 2006) and that differ substantially from each other genetically ($F_{ST} = 0.293$

to 0.494; Bleakley et al. 2008). Each focal female was paired with a single context group comprised entirely of a strain other than her own, thus eliminating any influence of relatedness. Several antipredator and social behaviors, such as number of predator inspections as well as time spent in close proximity to and orientation toward the model, agitation, and schooling (Bleakley et al. 2006, 2007), were video-taped and scored for both the focal female and all of her social partners. We first identified any influences of direct genetic effects, resulting from the genetic identity (i.e., strain) of the focal female, and IGEs, resulting from the strain of her social partners using the two context strains in common. We then quantified the extent of interactions among traits (denoted by values of ψ_{ij}) for each focal strain separately using all context strains with which they each interacted. A large absolute value for ψ_{ij} relating the focal behavior to the same behavior in the context group specifically identifies a substantial reciprocal interaction resulting from IGEs (i.e., Table 1, Fig. 1C).

BEHAVIORAL TESTING

Five strains of inbred ornamental guppies were used for this study: Snakeskin, $\frac{1}{2}$Green, $\frac{1}{2}$Yellow, Blue, and R-Cobra. All strains have reduced heterozygosity and allelic diversity at neutral markers (Bleakley et al. 2008) and four have been demonstrated to respond appropriately to predatory stimuli (Bleakley et al. 2006). Although the R-Cobra strain has not been previously behaviorally phenotyped, it resembles the Red strain, which has been phenotyped (Bleakley et al. 2006) and originates from the same breeder (Angels Plus, Olean, NY). Strains were maintained in multiple strain-specific community tanks, such that individuals naïve to behavioral experiments were separated from experienced individuals and naïve individuals were split across two to five rearing tanks to minimize the influences of social learning and common environment, respectively. The animals were maintained with water temperature $24 \pm 1°C$ and a 14:10 light:dark cycle. The animals were fed Hikari Fancy Guppy Food^{TM} twice daily, six days per week, except test animals on test days, which were fed a single time after testing. This research was approved by Indiana University, Bloomington IACUC (#05–075) and adhered to national and institutional regulations for animal welfare.

Fifty-two Snakeskin and 30 Blue females were used as focal individuals. A set of guppies was created by pairing each focal animal with a "context" group comprising two male and one female fish from a single strain different than its own. Snakeskin focals were tested with 15 $\frac{1}{2}$Yellow, 14 Blue, 14 $\frac{1}{2}$Green, and 9 R-Cobra context groups. Blue focals were paired with 10 context groups each from the $\frac{1}{2}$Yellow, Snakeskin, and $\frac{1}{2}$Green strains. In most cases, context groups were drawn from multiple rearing tanks such that no more than two individuals in a trial had previous social experience with one another. A set of trials comprised the context group tested in the absence of the focal female, the focal

individual tested alone, and the focal female tested in the presence of her context group. Every fish was used in a single set only, limiting the influence of social learning and prior social experience on behavior. The order of trials within a set was randomized, with approximately half of the focal females tested alone first and half tested first in the presence of her context group. Only results from the combined trial are presented here.

Behavioral trials were conducted in a 10-gallon tank containing an artificial plant for cover on one side; a model of a large *Crenicichla alta*, the principle cichlid predator of wild Trinidadian guppies, on the opposite side; and a 1″ grid across the back of the tank. The water was changed and the tank was thoroughly rinsed before every trial. Focal females or groups were acclimated alone or together, respectively, overnight and then introduced into the test tank near the cover plant. In the case of the combined trials, the context group was always introduced into the tank prior to the focal female. All trials were recorded directly to a DVD for 8 min using a Sony DVD403 digital video recorder at 2.048 effective megapixels (Sony Electronics, Inc., San Diego, CA) and later scored using an event recorder (Eve-Row 1990, J. Ha, Seattle, WA) for the behavior of every individual. This study focuses exclusively on the trials conducted with a focal female paired with a context group; behavior of individuals tested alone or context groups tested without the focal female present were not analyzed. All individuals were scored for five previously described behaviors (Bleakley et al. 2006, 2007): time spent oriented toward the model ("orientation"); time spent in close proximity to the model ("proximity"), defined as time spent within two body lengths of the model; number of inspections; time spent in agitated swimming ("agitation"), extremely fast randomly patterned swimming often including drops to the bottom of the tank; and time spent schooling ("schooling"). To partially control for pseudoreplication, schooling was defined slightly differently for focal individuals versus content individuals. A focal female was defined as schooling when she was within two body lengths of at least two other social partners. However, a context individual was defined as schooling only when it was within two body lengths of both other members of the context group, irrespective of the location of the focal female. Inspections, orientation, proximity, and agitation indicate threat evaluation and response whereas schooling reflects social cohesion

STATISTICAL ANALYSIS

Statistical analysis was performed in JMP (SAS Institute Inc., 1989–2002). Focal behavior was measured in the presence of the social group. Context group behavior was defined as the mean behavior of all individual social partners when paired with the focal fish. Measures of schooling and time spent oriented on the model were normally distributed. The other behaviors were normalized where necessary using natural log or square-root transformations.

Table 1. Estimates of direct and indirect genetic effects. This table shows the influence of specific context group behaviors on focal behavior, broken down by strain where significant context strain × behavior interactions were initially observed. Estimates of ψ were obtained from the partial regression coefficients for each influence. *denotes $P<0.05$, **$P<0.025$, ***$P<0.005$. The proportion of variance explained by each IGE can be estimated as the square of the path coefficient (Price 1998).

Focal strain	Focal behavior	Context group behavior	Estimate of $\psi\pm$SE	P	F_{DF}
Blue	Time in proximity	Time in proximity	0.93 ± 0.12	<0.0001***	$63.81_{1,28}$
		Time oriented	0.01 ± 0.12	0.9091	$0.01_{1,28}$
		Time agitated	-0.07 ± 0.09	0.4894	$0.50_{1,28}$
		Time schooling	-0.07 ± 0.11	0.5281	$0.41_{1,28}$
		Inspections	-0.09 ± 0.08	0.2693	$1.29_{1,28}$
Blue	Time oriented	Time in proximity	0.17 ± 0.18	0.3499	$0.91_{1,28}$
		Time oriented	0.71 ± 0.18	0.0009***	$15.09_{1,28}$
		Time agitated	0.10 ± 0.15	0.5127	$0.44_{1,28}$
		Time schooling	-0.07 ± 0.17	0.6698	$0.19_{1,28}$
		Inspections	0.10 ± 0.12	0.4603	$0.57_{1,28}$
Blue	Time agitated	Time in proximity	-0.04 ± 0.10	0.6908	$0.16_{1,28}$
		Time oriented	-0.30 ± 0.10	0.0064**	$9.17_{1,28}$
		Time agitated	0.70 ± 0.08	<0.0001***	$77.25_{1,28}$
		Time schooling	0.005 ± 0.09	0.9592	$0.003_{1,28}$
		Inspections	0.17 ± 0.07	0.0221**	$6.11_{1,28}$
Blue	Time schooling	Time in proximity	-0.05 ± 0.22	0.8189	$0.05_{1,28}$
		Time oriented	-0.11 ± 0.22	0.6122	$0.26_{1,28}$
		Time agitated	-0.19 ± 0.17	0.2918	$1.17_{1,28}$
		Time schooling	0.72 ± 0.20	0.0020***	$12.40_{1,28}$
		Inspections	0.11 ± 0.15	0.4876	$0.50_{1,28}$
Blue	Inspections	Time in proximity	0.02 ± 0.04	0.6552	$0.21_{1,28}$
		Time oriented	-0.03 ± 0.04	0.4382	$0.62_{1,28}$
		Time agitated	-0.03 ± 0.03	0.2468	$1.42_{1,28}$
		Time schooling	0.03 ± 0.04	0.3630	$0.86_{1,28}$
		Inspections	0.84 ± 0.03	<0.0001***	$996.30_{1,28}$
Snakeskin	Time in proximity	Time in proximity	0.80 ± 0.12	<0.0001***	$43.57_{1,52}$
		Time oriented	0.20 ± 0.10	0.0466*	$4.20_{1,52}$
		Time agitated	0.25 ± 0.10	0.01558**	$6.34_{1,52}$
		Time schooling	0.06 ± 0.10	0.5619	$0.34_{1,52}$
		Inspections	-0.22 ± 0.10	0.0258*	$5.33_{1,52}$
Snakeskin	Time oriented	Time in proximity	-0.16 ± 0.17	0.3247	$0.99_{1,52}$
		Time oriented	0.51 ± 0.13	0.0004***	$14.45_{1,52}$
		Time agitated	-0.24 ± 0.14	0.0860	$3.09_{1,52}$
		Time schooling	-0.01 ± 0.14	0.9695	$0.001_{1,52}$
		Inspections	0.14 ± 0.13	0.2950	$1.12_{1,52}$
Snakeskin	Time agitated	Time in proximity	0.10 ± 0.18	0.5940	$0.29_{1,52}$
		Time oriented	-0.23 ± 0.14	0.1219	$2.49_{1,52}$
		Time agitated	0.54 ± 0.15	0.0006***	$13.69_{1,52}$
		Time schooling	-0.05 ± 0.15	0.7237	$0.13_{1,52}$
		Inspections	-0.05 ± 0.14	0.7183	$0.13_{1,52}$
Snakeskin	Time schooling	Time in proximity	-0.35 ± 0.17	0.0383*	$4.57_{1,52}$
		Time oriented	-0.20 ± 0.13	0.1436	$2.22_{1,52}$
		Time agitated	-0.03 ± 0.14	0.8357	$0.04_{1,52}$
		Time schooling	0.32 ± 0.14	0.0250**	$5.38_{1,52}$
		Inspections	-0.01 ± 0.13	0.9310	$0.01_{1,52}$

Continued

Table 1. Continued.

Focal strain	Focal behavior	Context group behavior	Estimate of $\psi\pm$SE	P	F_{DF}
Snakeskin	Inspections				
	w/ Blue	Time in proximity	-0.21 ± 0.49	0.6788	$0.18_{1,14}$
		Time oriented	-0.23 ± 0.24	0.3752	$0.87_{1,14}$
		Time agitated	0.04 ± 0.27	0.8861	$0.02_{1,14}$
		Time schooling	-0.49 ± 0.23	0.0676	$4.31_{1,14}$
		Inspections	0.58 ± 0.23	0.0299*	$6.63_{1,14}$
	w/ $^1/_2$Green	Time in proximity	0.20 ± 0.34	0.5768	$0.34_{1,12}$
		Time oriented	0.09 ± 0.28	0.7401	$0.12_{1,12}$
		Time agitated	-0.96 ± 0.26	0.0078**	$13.55_{1,12}$
		Time schooling	-0.08 ± 0.30	0.8019	$0.07_{1,12}$
		Inspections	0.56 ± 0.28	0.0776	$4.27_{1,12}$
	w/ $^1/_2$Yellow	Time in proximity	0.21 ± 0.41	0.6186	$0.27_{1,14}$
		Time oriented	-1.14 ± 0.36	0.0123**	$9.74_{1,14}$
		Time agitated	-0.43 ± 0.28	0.1607	$2.33_{1,14}$
		Time schooling	0.74 ± 0.40	0.0939	$3.51_{1,14}$
		Inspections	0.70 ± 0.27	0.0319*	$6.43_{1,14}$
	w/ R-Cobra	Time in proximity	-0.10 ± 0.27	0.7391	$0.13_{1,8}$
		Time oriented	0.13 ± 0.33	0.7143	$0.16_{1,8}$
		Time agitated	0.14 ± 0.29	0.6572	$0.24_{1,8}$
		Time schooling	0.58 ± 0.44	0.2740	$1.78_{1,8}$
		Inspections	0.26 ± 0.30	0.4486	$0.75_{1,8}$

All measures of any behavior were transformed in the same manner (e.g., number of inspections performed by Snakeskin-focals, Blue-focals, and all groups were all natural log transformed).

A first analysis comprising both the Snakeskin and Blue focal females paired with their two context strains in common, $^1/_2$Green and $^1/_2$Yellow, was conducted to determine (1) if focal strains differed in the behavior they expressed in common context environments, (2) if context groups differed in their mean effect on focal behavior, and (3) if nonadditive phenotypic effects of social context were evident among strains (e.g., focal females respond differently to the same behavior depending on the context strain with which they are paired). These measures qualitatively assess the relative influence of direct phenotypic effects of focal strain and the indirect phenotypic effects of social context on focal behavior, rather than providing quantitative measurements of the impact of context group (i.e., ψ). A general linear model was constructed for each focal behavior with focal strain, context strain, and a focal strain $\times$ context strain interaction terms (Fig. 2).

Behaviors of the Snakeskin and Blue focals were then analyzed separately with respect to all tested context groups to quantitatively assess the strength of interactions among interacting traits (i.e., generate measures of ψ). All behavioral measures were standardized by setting the mean to zero and the variance to one, removing differences in scale and allowing direct comparisons of the magnitude of effects of different context group behaviors. The influence of the context strain, the measured behavior of the context groups, and interactions between context strain and context behavior on each focal behavior were assessed using multiple regression. An initial model was constructed to assess the relative importance of all above effects. A second more specific (reduced) model was then constructed for each focal behavior keeping all main effects and any interactions identified as contributing significantly to variation in the initial model. Interaction terms were found to explain variance only in inspections performed by Snakeskin focals and were thus dropped from the analysis for all other behaviors. A general linear model with all of the main effects was completed for each context strain separately for inspections in Snakeskins (Table 1). The coefficient of the interaction is derived as the partial regression coefficient of focal behavior on context behavior. Values of ψ are summarized in a ψ matrix (Fig. 1C).

Results

Direct genetic effects (identified by the strain to which the focal female belonged) significantly influenced several behaviors. Irrespective of the context strain with which a focal female was paired, Blue focals spent less time oriented toward the model, more time in close proximity to the model, and more time in
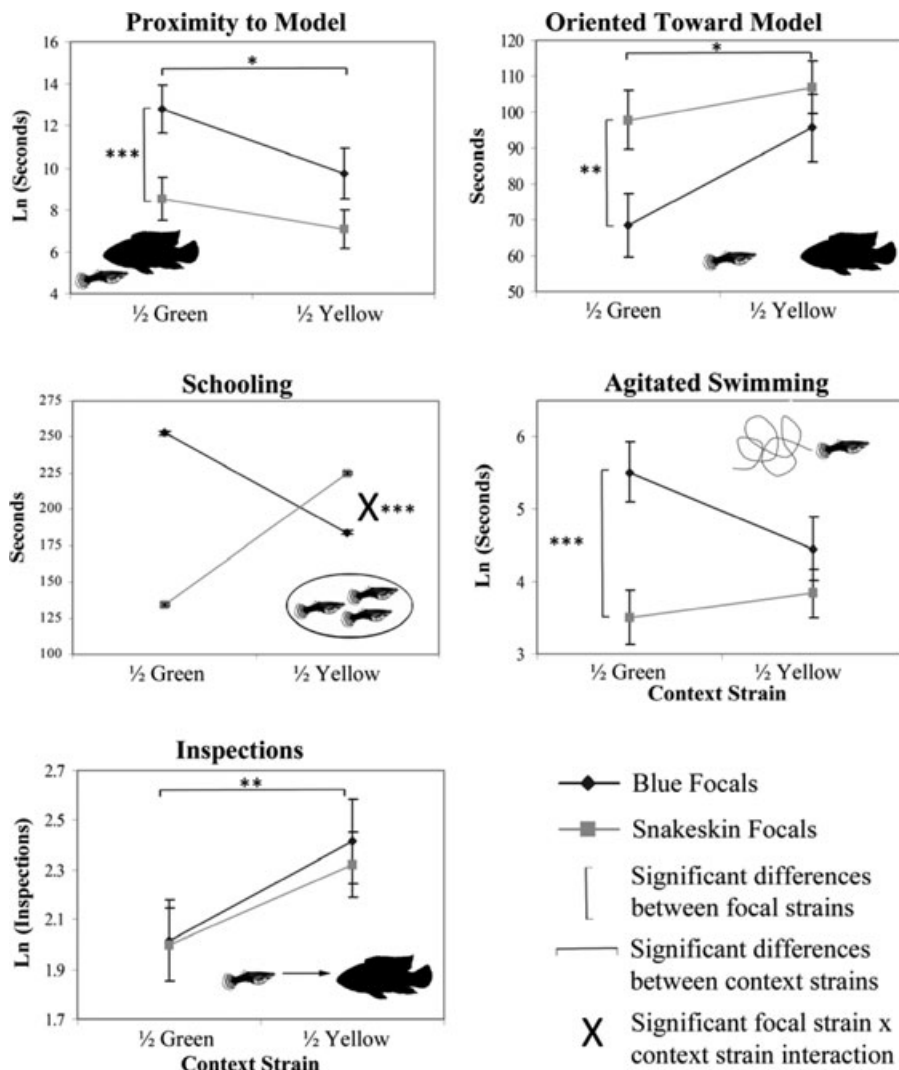
Figure 2. The influence of direct genetic effects (focal strain), indirect genetic effects (context strain), and focal strain × context strain interactions for proximity to the predator model (A), time spent oriented on the model (B), time spent schooling (C), time spent in agitated swimming (D), and number of inspections (E). Error bars indicate standard error around least squares means. *denotes $P < 0.05$, **$P < 0.025$, ***$P < 0.005$

agitated swimming than did Snakeskin focals (Fig. 2). These results are qualitatively consistent with previous work demonstrating that these strains vary in their responses to predatory cues (Bleakley et al. 2006) and with studies that have demonstrated the heritability of social behavior (Rodd and Sokolowski 1995). The behavior of Snakeskin focals was more variable than that of the Blue focals. The Snakeskin trials were conducted over a longer period of time and used focal females drawn from several cohorts representing successive generations, whereas Blue focals were tested over a much shorter period of time and were drawn from only two cohorts of fish. As such, the general environmental effects on behavior were likely greater on Snakeskin females, increasing the phenotypic variance in observed behavior.

Context strain-specific effects identify the influence of IGEs resulting from unspecified differences in the entire multivariate phenotype and identify patterns of covariance between focal females and their social partners that result from behaviors not measured in this study. Focals inspect more when paired with $\frac{1}{2}$Green context groups, irrespective of their own strain identity. Context strain also influenced the time focals spent in close proximity to the model (greater when paired with $\frac{1}{2}$Green) and time spent oriented toward the model (greater when paired with $\frac{1}{2}$Yellow; Fig. 2). A focal strain × context strain interaction was identified for schooling, to the extent that a full-scale reversal in focal behavior was observed. Blue focals spend more time schooling with $\frac{1}{2}$Green groups than with $\frac{1}{2}$Yellow groups and spend more time schooling with $\frac{1}{2}$Green groups than do Snakeskin females. The

reverse was true for Snakeskin focals (Fig. 2). Individuals from the Blue and $\frac{1}{2}$Green strain are generally more active than the Snakeskin and $\frac{1}{2}$Yellow strains, which may impact their affinity for interacting with each other.

Positive reciprocal interactions between the same trait in a focal individual and its social partners generate a pattern of strong positive covariance between social partners, the most important prerequisite for the emergence of cooperative behavior (Santos and Pacheco 2006; Santos et al. 2006). The strongest IGE on the behavior of a focal (i.e., largest values of ψ_{ij}; Fig. 1C and Table 1) was in fact the same behavior expressed by her context group for all behaviors. Influences on inspections performed by Snakeskin focals were nonadditive, with focals responding differently to the behavior of the context group depending on the strain of the context group. Across all context strains and accounting for context strain interactions, inspections performed by the context group had the greatest influence on focal Snakeskin behavior ($\psi = 0.52 \pm 0.14$, $F_{1,52} = 14.77$, $P = 0.0006$). When paired with $\frac{1}{2}$Green or $\frac{1}{2}$Yellow context groups, agitation and orientation become the most influential context group behaviors on Snakeskin focal inspections, respectively (Fig. 1C, Table 1). Focal behavior influenced by other context group behavior besides the matching behavior may reflect a weaker influence of the same behavior in the context group. However, in virtually all cases the ψ_{ij} associated with the same behavior in the context group is two to three times as large as those for other context behaviors on focal phenotype, again emphasizing the importance of reciprocal trait interactions for guppy social behavior (Fig. 1C, Table 1).

Discussion

Guppies respond to sometimes subtle differences in social environment, with the net effect that the behavior of an individual fish coalesces toward the mean phenotype of its shoal (Day et al. 2001; Bleakley et al. 2007), a phenomenon that is predicted by IGE theory (Moore et al. 1997; Wolf et al. 1999). As such, it is not unexpected that the behavior of the focal female was impacted by the identity of the context strain with which she was paired. These effects of context strain reflect an impact of the overall phenotype of the context strain, irrespective of specific trait interactions. In addition, because focal phenotype responds to the environment, which is provided by the social partners that have their own genes, these interactions reflect a G × G interaction (analogous to a G × E interaction), which may greatly increase rates of divergence among subdivided populations (Wade 2000). More importantly however, they provide examples of ψ that vary in magnitude depending on the context strain with which focal females were paired and between the two focal strains but are additive (i.e., predictable) in nature. That is, all focal females are impacted in the same way by interacting with context groups

from a particular strain. Selection on one level of organization (i.e., multilevel selection acting on individuals and interactions among individuals; reviewed in Bijma and Wade 2008) or on one social partner might therefore generate a similar response to selection at another level of organization or in the other social partner, generating positive feedback across the system (Wolf et al. 1999; Agrawal et al. 2001). Guppy behavior, including cooperation during predator inspection; morphology; and life history are known to evolve extremely rapidly in the wild in response to predation pressure (Reznick et al. 1997). However, predation pressure alone is not sufficient to fully predict patterns of guppy evolution in the wild (Karim et al. 2007). Selection at multiple levels and G × G interactions may contribute to the rapid divergence observed in natural guppy populations (Moore et al. 1997; Wolf et al. 1999; Wade 2000). Nonadditivity, wherein individuals responded differently depending on the strain they were paired with, was observed in two instances: when schooling was nonadditive due to strain-specific interactions (presence of multivariate IGEs, Fig. 2) and when ψ_{ij} was nonadditive with respect to context strain for inspections in Snakeskin focals (Fig. 1C). For traits that covary nonadditively, the outcome of selection depends on the specific combination of social partners (Lande and Arnold 1983; Brodie 2000). Such evolutionary dynamics can result in responses to selection opposite to those predicted for additively related traits, in part because selection at one level of organization may oppose selection operating at another level of organization (Agrawal et al. 2001).

In the wild, guppy populations are potentially strongly genetically subdivided, even under similar predation regimes and in close geographical proximity (Ludlow and Magurran 2006; Russell and Magurran 2006). A guppy may not stay within the population in which it develops. Reznick et al. (2001) estimated the emigration rate of guppies from any of their marked pools over the course of 12 observation days at < 5%. As such, individuals may find themselves outside their natal pool and interacting with social partners who did not develop under the same predation regime or social conditions, as well as those that differ genetically from the immigrant's natal population. Any individual emigrating to another population that displays differences in behavior and life history may experience selection differently based on the details of its new social environment and its own responsiveness to the social environment. Guppies prefer to school with cooperative conspecifics that inspect (Dugatkin and Alfieri 1991b) but individuals that inspect more often suffer higher risk of predation, potentially because of differences in "boldness" (Dugatkin 1992). As such, an individual guppy's fitness is likely to be determined in large part by the interactions in which it engages in its new social context and the ability to respond to different social environments may be an important component of an individual's fitness. In addition, the balance between natural, sexual, and

Table 2. Phenotypic correlation matrixes. This table shows phenotypic correlation matrixes for focal female behaviors, which include the influence of social partners. Shaded cells above the diagonal give trait correlations for Blue focal females and white cells below the diagonal give trait correlations for Snakeskin focal females.

	Proximity	Orientation	Agitation	Schooling	Inspection
Proximity		−0.37	0.31	−0.08	−0.24
Orientation	−0.49		−0.52	−0.34	−0.01
Agitation	0.29	−0.46		0.20	0.38
Schooling	−0.21	0.15	0.12		0.22
Inspection	−0.28	0.08	0.32	0.23	

social selection may be shifted by the presence of IGEs, altering the way an individual experiences selection and potentially shifting adaptive peaks in populations with different social landscapes. For example, if a male guppy inspects more frequently as a result of interacting with a particular social group, he may obtain better access to mates (Godin and Dugatkin 1996) but may also have a shorter life expectancy (Dugatkin 1992), both aspects of life history that impact the fitness of guppies and vary with predation pressure (Reznick and Endler 1982; Reznick 1996). The specific selective regime a guppy experiences, including selection on non-interacting traits, thus emerges as a consequence of the specific genetics of and interactions among individuals.

The specific combination of additive and nonadditive effects of social environment on focal behavior will directly impact how and under what conditions social behavior evolves. Because the social environment contains genes, it too may evolve (Moore et al. 1997; Wolf et al. 1999; Agrawal et al. 2001). Selection acting directly on an individual interacts with selection acting on social partners, generating positive feedback between individuals and their social environment, further altering the selective landscape in which individuals interact. In addition, traits that are correlated within an individual (e.g., Table 2) may experience indirect selection (Lande and Arnold 1983). Trait-based IGE models assume for simplicity that the strength of ψ_{ij} is constant within a population, but predict that ψ_{ij} should vary and evolve if it is an individual trait with a genetic basis (Moore et al. 1997). Performance-based models of IGEs implicitly include variation in individual response to the complete interaction with a social partner (e.g., Bijma et al. 2007). The individual measurements of ψ_{ij} presented here differ between focal strains. In addition, the measurements of ψ_{ij} vary within the Snakeskin focal strain depending on the context strain with which they are paired for inspections. These strain differences in ψ_{ij} provide evidence for genetic variation in ψ_{ij}. Individual guppies are known to respond differently to similar group contexts in the wild, as well. For example, high predation guppies are much more likely to shoal with conspecifics and when they do shoal, are more cohesive, than fish from low predation environments given similar opportunities to shoal (Magurran and Seghers 1991). Differences in respon-

siveness to social environment provide the substrate by which ψ_{ij} might evolve in both inbred guppies, through inbreeding and drift, and in natural populations of guppies, perhaps due to differences in predation pressure or competition.

Within the environment of any particular behavioral interaction, the strength of ψ_{ij} is influenced both by variation in the behavior of the social group and how responsive the focal individual is. Differences in responsiveness to social environment (i.e., an individual's reaction norm; reviewed in Roff 1997) establish the phenotypic variation for generating differences in reciprocal interactions. Populations that are more socially responsive (displaying greater values of ψ_{ij}) are thus primed to exhibit greater coordination (Croft et al. 2005, 2006) of behavior among individuals that may then lead to greater degrees of cooperation. Although our results do not directly demonstrate cooperation through time-series measurements such as tit-for-tat, reciprocal interactions among traits were evident, demonstrating strong behavioral covariance among social partners resulting from genes carried in both focal individuals and their social partners. IGEs for antipredator behavior thus provide a set of genetic interactions for generating reciprocity, even in the absence of additive genetic variation for cooperation. In other words, cooperation can emerge and be inherited through the reciprocal influence of inspection behavior generating covariance among social partners. Cooperation emerges in networks of interacting individuals that generate strong positive behavioral covariances among individuals (Santos and Pacheco 2006), such as those quantified by large numerical values of ψ_{ij} (Moore et al. 1997), irrespective of adherence to game rules such as tit-for-tat (Santos and Pacheco 2006). The presence and consistently large influence of IGEs thus illuminates a potential genetic mechanism for generating observed patterns of reciprocity in wild guppies and provides insight into the emergence, inheritance, and evolution of cooperative social behavior.

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