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ORIGINAL ARTICLE

Environmental and genetic effects on exploratory behavior of high- and low-predation guppies (*Poecilia reticulata*)

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Abstract
Exploratory behavior—an individual's response to novel environments, resources, or objects—should vary with the associated benefits, including new sources of food and reduced levels of competition, and the costs, such as predation pressure. Using guppies from multiple streams and rivers in Trinidad, we compared guppies from high- and low-predation populations. We found that wild-caught male and female guppies from low-predation populations were more exploratory than high-predation fish when tested in the field and in controlled laboratory conditions. We did not detect significant evidence for a genetic basis for differences in the behavior of high- and low-predation fish using a common-garden approach, but further study is required before conclusions can be made about the relative contribution of genes to population differences in exploratory behavior of guppies. Theory has assumed that predation risk is a cost that will select against high levels of exploratory behavior; this study is one of the few that has tested this assumption, and we show that exploratory behavior is indeed suppressed in guppies from high-predation localities.

Significance statement
Exploratory behavior is a component of an individual's responses to novel environments, resources, and objects (including potential predators) and thus can affect important decisions of animals in the wild (e.g., whether to sample new, potential sources of food, whether to disperse). We compared exploratory behavior of wild-caught guppies from sites with large, dangerous predators with those from sites with small predators that are only a threat to small individuals. Guppies co-occurring with large predators were less exploratory than those from sites with small predators. Factors contributing to this difference could include exposure to predators directly and to conspecifics' responses to predators. Studies on general behavioral traits (e.g., temperament) combined with knowledge of animals' environment and evolution are expanding our ability to test ideas about the origin and maintenance of intra-specific variation in fascinating and complex traits.

Keywords Temperament · Predation pressure · Dispersal · *Poecilia reticulata*

Introduction

An individual's response to novel environments, resources, or objects can have enormous implications for its fitness. Dispersal to and exploration of novel environments can offer tremendous opportunity for growth and reproduction, especially when the new habitat is under-utilized or the risk of inbreeding is reduced. Investigation of novel sources of food should be especially advantageous when resources are limited, when levels of competition are high and/or in situations where high growth rates are advantageous (e.g., Arendt and Reznick 2005). Indeed, exploratory behavior—an individual's response to novel environments, resources, or objects (Renner

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1990; Réale et al. 2007; Smith and Blumstein 2008)—has been shown to affect reproduction, survival (e.g., Budaev et al. 1999; Dingemanse et al. 2004; Both et al. 2005; Cote et al. 2008; Smith and Blumstein 2008, 2010), and propensity to disperse (Fraser et al. 2001; Dingemanse et al. 2003; Krackow 2003). However, novel situations can be associated with important costs; for example, a high risk of mortality could be associated with sampling unknown materials and moving to unfamiliar areas, which includes the probability of facing new predators. The costs of exploratory behavior should be especially high in high-predation habitats because any novel object or scenario could expose a prey animal to a predator encounter (Greenberg 1984; Réale et al. 2007) and, in a novel environment, individuals would be unfamiliar with refuges and escape routes (Brown 2001). Perhaps surprisingly, there have been few studies to determine whether there is an association between predation level and exploratory behavior and the evidence is mixed in fish (e.g., Bell 2005; Archard and Braithwaite 2011; Herczeg and Valimaki 2011; Archard et al. 2012) although the details of predator communities and the environmental factors associated with them vary across systems and the number of replicate populations was low for some studies.

The Trinidadian guppy (*Poecilia reticulata*) has become a model for studying natural selection in the wild (Houde 1997; Magurran 2005), and there is ample evidence that the traits of guppies, including life histories, coloration, and behavior (e.g., anti-predator behavior), have evolved in response to predation regime and factors associated with it (reviewed by Endler 1995; Houde 1997; Magurran 2005). Populations of guppies in high-predation sites face a community of predators that include large, piscivorous predators, such as the pike cichlid (*Crenicichla alta*) that preys intensively on guppies of all sizes but prefers larger guppies (Mattingly and Butler 1994; Reznick et al. 1996; Johansson et al. 2004). In low-predation populations of guppies, upriver from high-predation sites and separated from them by physical barriers such as waterfalls, the main predator is the gape-limited, omnivorous fish, *Rivulus hartii*, which can only prey on juvenile guppies and small adults (Seghers 1973) and does so relatively rarely compared to *C. alta* (Reznick et al. 1996; Magurran 2005). Therefore, the costs of exploratory behavior should be higher in high-predation sites given the higher mortality rates in those populations.

There are obvious benefits of exploratory behavior for guppies in both high- and low-predation populations. Not only could exploration lead to the discovery of new resources, but the opportunity to establish a new population (Deacon et al. 2011) could confer considerable fitness benefits including the release from intraspecific competition. Males may receive positive reinforcement for exploring new habitats and seeking out novel/unfamiliar females because female guppies are more likely to respond positively to courtship from unfamiliar males (Hughes et al. 1999; Eakley and Houde 2004; Hampton et al. 2009; Graber et al. 2015) and males with locally rare phenotypes have higher mating success (Hughes et al. 2013). The benefits of exploratory behavior should be higher for guppies in low-predation sites for a number of reasons. Population densities tend to be higher (Reznick and Endler 1982) and food availability lower (Grether et al. 2001; Reznick et al. 2001). A propensity to explore and disperse may also be very important for low-predation populations that live in small head-water streams because of the risk of inbreeding depression (Johnson et al. 2010), not only because the tiny pools can become isolated during the dry season (Kaplan 1995) but also because effective population sizes and heterozygosity are lower (Magurran 2005; Neff et al. 2008). Therefore, given the relative costs and benefits of exploratory behavior, we predicted that guppies in low-predation sites would be more exploratory than those in high-predation sites. Additionally, because low-predation populations were founded by guppies moving upstream from high-predation populations on the same river (Alexander et al. 2006), low-predation habitats previously unoccupied by guppies might have been founded by particularly exploratory individuals.

In this study, we asked whether there were differences in the behavioral phenotypes of guppies from high- and low-predation localities. We first tested wild guppies in the field, immediately after capture. This approach has two benefits. One is that it provides an indication of behavior in the wild, allowing a comparison to behavior in more controlled laboratory conditions. If the behavioral patterns are similar, one can have more confidence that an accurate measure has been made of the phenotype as it would occur in the wild. Second, this approach measures behavior before potential convergence of behaviors, among fish from different populations, which can occur due to laboratory acclimation after transferring wild animals to the laboratory (Wilson et al. 1994). We also tested wild-caught fish in the laboratory under more controlled conditions. This testing included activity tests that were intended to represent normal activity when the fish was unstressed. Including activity data in our analyses will allow us to determine whether any differences in exploratory behavior are simply a result of differences in general activity. Finally, to examine whether population-based differences in exploratory behavior were genetically based, we tested second-generation, laboratory-reared descendants of four of the original populations.

Materials and methods

We tested for differences in exploratory behavior between high- and low-predation populations using a classic psychology test of exploratory behavior, the open-field test (reviewed in Burns 2008; De Serrano et al. 2016); we have demonstrated that this a reliable test of exploratory behavior in guppies (Burns 2008). In the open-field test, an individual is placed in a novel environment to gauge its behavior in a mildly stressful situation. We avoided testing guppies with predators or predator models because among-fish variation in predator recognition ability could mask differences in temperament, which was the trait of interest here (Kelley and Magurran 2003a). The open-field test can also easily be standardized and reproduced in other laboratories for replication of experiments and comparison across species.

Populations

In March 2005 and April 2006, we performed open-field tests on adult guppies caught and tested in the field. These fish were from four rivers on the southern slopes of the Northern Range in Trinidad. For three of the rivers, the Arima, Aripo, and Oropuche, we tested paired samples from low- and high-predation populations. For a fourth matched pair of sites in the El Cedro River, we contrasted the behavior of fish from a natural high-predation site with those from an upstream, low-predation site in which, before 1981, there were no guppies, only the gape-limited predator *R. hartii*. In 1981, high-predation guppies from downstream were introduced above a barrier waterfall into the site and they have evolved to low-predation genotypes for life history traits and coloration (Reznick and Bryga 1987; Kemp et al. 2009). The structures of the habitats (e.g., width of stream, canopy openness) of the high- and low-predation sites on the Aripo River are quite similar (Reznick et al. 2001), meaning that differences between the sites are more likely to be due to differences in predation regime than other ecological factors; the same is true of the two El Cedro sites.

Wild-caught fish

Field open-field test

For testing wild-caught fish in the field, an individual fish was gently collected using butterfly nets in its home river and immediately transferred to a 28-cm square, white, plastic tub with 12-cm-high sides filled to a depth of 6 cm with water from the fish's home river. To minimize sampling bias due to habitat-specific preferences of different behavioral types, fish were sampled from throughout the entire pool or stretch of river. The water was replaced for each fish to prevent any possible effects of the release of alarm pheromones or stress hormones by the previous fish. The bottom of the tub was divided into 16 squares, each 7 cm by 7 cm, drawn in black ink. Fish were given 60 s to acclimate to the apparatus and to moderate any differences between fish caused during capture (e.g., differences in time in net). After acclimation, an observer verbally described behavior into a handheld audiocassette recorder for later data transcription. To avoid disturbing the fish with shadows from the observer, a mirror was positioned above the apparatus at a 45° angle to allow the observer to see the fish from above while sitting out of direct sight of the fish and without casting any shadows over the apparatus.

During a 180-s observation period, the observer recorded the amount of time spent immobile and the number of squares through which the fish swam. Time frozen was measured only when a fish remained immobile for more than 1 s; then, all episodes of immobility were summed. The number of squares traversed was divided by the number of seconds that the fish spent swimming (180 s minus time frozen) to give a swimming rate. These two variables are equivalent to "time frozen" and "ambulation" variables measured in analogous experiments on laboratory rodents (Walsh and Cummins 1976). The sample sizes and year of testing for each population are shown in Supplemental Material Table A. All tests (lab and field) were conducted between 10 am and 5 p.m. each day. Whenever possible, we tested fish from paired sites in one river on the same day (randomly assigning the order of testing), but because of the logistics of reaching the field sites, that was not always possible. Nevertheless, tests at every site were done in the shade and in natural water temperatures that vary little between sites or over time (Reznick and Endler 1982).

The values for time frozen and swimming rate were put into a principal components analysis, which we used to reduce open-field behavior to the first principal component (percent variation explained = 72.7 %; eigenvalue = 1.45; loadings, time frozen = −0.707; swimming rate = 0.707). The direction of correlation between the two phenotypes was the same for the high- and low-predation populations. High scores indicate a low amount of time frozen and a high swim rate when not frozen, so we interpret this principal component as exploratory behavior. We recognize that time frozen and swimming rate both incorporate curiosity/motivation to explore as well as fearfulness, and thus, our score represents interplay between exploratory behavior and boldness (Burns 2008). The individual scores for this variable were used as our main dependent variable.

We measured fish standard lengths from photographs, taken after completion of the test, of unanesthetized fish placed into a confined area in a clear container containing water. Sex was identified by body coloration and anal fin morphology.

Laboratory open-field test

In 2006, adult guppies from populations in the Aripo and Oropuche rivers were collected using butterfly nets and transported in sealed plastic bags filled with conditioned water (containing Stresscoat, Amquel, and Novaqua) and highly oxygenated air to our laboratory. Fish were held in 32–80-L tanks, separated by population, at 25 °C on a 12:12-h lighting regime. Fish were fed flake food once every day and also brine shrimp nauplii on weekdays. Before testing commenced, fish were placed in individual 8-L tanks with white gravel lining the bottom. Subjects were tested in open-field, activity level, and novel object tests within 1 month of capture. The order of tests was randomized equally within each population. While open-field behavior of guppies is repeatable over multiple time scales and environments, individual responses to the novel object test were not repeatable (see Burns 2008), so results of the novel object tests are not reported here. No fish tested in the field was tested in the laboratory. Four populations were used for this part of the study: high predation and low predation from both rivers. A fifth population was also tested, a predator-introduction population from the Oropuche drainage, but because of an issue of power with so few populations, it will not be considered in our main analyses (see Appendix A).

After 1 day to acclimatize to the new tank, testing began, with a day of rest between the two tests (open field and activity). The time of day of testing was balanced across high-predation, low-predation, and introduction population fish. Observers were blind to the source of each fish. After testing, fish were anesthetized in buffered MS222 (Holloway et al. 2004; Canadian Council on Animal Care 2005; Barreto et al. 2007) and photographs were taken, with a length standard visible, to determine body length. All fish recovered from anesthesia in 8-L tanks filled with conditioned water before being returned to their home tanks for breeding and future experiments. Sample sizes are shown in Supplemental Material Table B.

The laboratory open-field apparatus was similar to the one used in the field but was a rectangular green tub 33 cm by 28 cm, with 12-cm-high sides. While the open-field apparatuses were of different colors and slightly different sizes, previous work demonstrated that such differences had little impact on guppy behavior (Burns 2008). Conditioned water was filled to a depth of 6 cm and was replaced between trials. The bottom was divided into 24 rectangles, each 5.5 cm by 7 cm, drawn in black ink. To avoid disturbing the fish during the test, the observer viewed a television screen hooked up to a video camera positioned 1.5 m above the apparatus.

As in the field open-field tests, time frozen and swimming rate were observed, but here, we also measured the ratio of outer rectangles (the 16 rectangles lining the sides of the apparatus) to inner rectangles (eight rectangles) traversed. Time frozen was measured differently than in the field tests; we included all periods of immobility including those less than 1 s in duration, rather than only periods greater than 1 s. This method better captured the quick, stuttered stops of some fish (skittering). Data were collected live with a hand counter and stopwatch. The data for the three variables (time frozen, swimming rate, and outer/inner ratio) that were collected for the wild-caught fish and laboratory-reared fish (see below) were used in a principal components analysis that we used to reduce open-field behavior to the first principal component (percent variation explained = 57.0 %; eigenvalue = 1.71; loadings, time frozen −0.514; swimming rate 0.569; outer/inner ratio = 0.641; other components had eigenvalues less than 0.8 and were thus not used in our analyses). The directions of correlations between the three phenotypes were the same for the high- and low-predation populations. As in the analysis of the field experiment data, high scores were associated with short lengths of time frozen and a high swim rate. Thus, we interpreted the first principal component as exploratory behavior. High scores were also associated with more time spent swimming in the outer parts of the open field, as compared to the inner parts. In some species, like lab mice, wall seeking is a common reaction when they are placed in novel environments (e.g., Archer 1973; Boissy 1995; Ramos and Mormede 1998; Choleris et al. 2001; Egan et al. 2009; Ahmed et al. 2011), and this has been assumed to indicate fear (Valle 1970; Kotrschal et al. 2014); however, fish, perhaps especially for wild-caught ones, swimming around the perimeter of the tub may be exploring for a way to escape from the tub (Kotrschal et al. 2014; FHR personal observation).

General activity level test

For comparison with behavior in the novel environment in the open-field test, general activity level tests were conducted in each individual's home tank (8 L) to measure normal activity in a familiar, safe environment. Behavior was videotaped with a camera (Panasonic PV-GS35) 60 cm above the tank, and lighting was provided by two 30-W full-spectrum fluorescent lights. A grid of 5-cm × 4-cm rectangles was marked on the clear plastic tank lid, and we counted the number of rectangles that the fish traversed during a 180-s period to give activity level. The relationships between general activity and exploratory behavior were examined by including the former as a covariate in an analysis of the latter.

Laboratory-reared fish

Exploratory behavior was assessed, using the open-field test, in the second-generation (G_2) laboratory descendants of wild-caught females collected in 2005 from the same four populations and in the same manner as the wild-caught, laboratory-tested individuals (described above). We used this approach to determine whether differences between populations persisted and could be attributed to genetic differences, although we cannot discount any maternal effects that might have been carried forward over two generations in the laboratory. Between 18 and 24 wild-caught females from each population were isolated in 8-L tanks. Because guppies store sperm and are often multiply inseminated (Magurran 2005), their offspring (G_1) should represent a substantial fraction of the naturally occurring genetic variation. Offspring were sexed before maturity using anal fin development (Turner 1941) and then separated by sex. These G_1

fish were crossed with other G_1s from another female of the same population in unique crosses, with each wild-caught mother's lineage contributing equally to these crosses to maintain genetic diversity. The G_2 offspring from these crosses were tested in the same green tub as used for wild-caught fish tested in the laboratory. After testing, fish were anaesthetized and photographs taken to determine body length. Sample sizes are shown in Supplemental Material Table B.

Statistical analyses

The dependent variable (PC1 score) met the assumptions of the analyses and was not transformed. Data for fish tested in the wild were pooled across years because there were no significant differences in exploratory behavior between years for any site individually (all p for year >0.3) or when the eight sites were analyzed together. To account for nonindependence of individuals within populations and populations within river drainages, we used general linear mixed models (GLMM) with random intercepts for drainage and for populations within drainage. Predation regime and sex were used as fixed categorical effects. Population mean body size (centered (e.g., van de Pol and Wright 2009; Schielzeth 2010; Dingemanse and Dochtermann 2013) on the overall mean) and individual body size (centered on population sex-specific means) were used as continuous covariates. We centered the body sizes because we knew from preliminary analyses that our metric of exploratory behavior changed with body size; because populations vary in mean body size (e.g., Reznick and Endler 1982) and because we were interested in differences among populations in behavior that are not due to between-population differences in body size, we centered body size. We initially included all two- and three-way interactions between independent variables in these models, but when interactions were nonsignificant (all $p > 0.10$), they were dropped from the final model. Variance components were estimated using restricted maximum likelihood and degrees of freedom by the Kenward-Roger method (Bell et al. 2014). To assess whether activity level was associated with exploratory behavior, we added log-transformed, centered activity values to the above model. All analyses were performed using SAS Proc Mixed v. 9.3 or 9.4. We also evaluated whether activity level (log-transformed) varied among population regime or sex using models with the same structure as those for PC1 (a generalized linear model assuming a negative binomial distribution for activity level produced equivalent results).

Results

Wild-caught fish tested in the field

In the comparison of natural high-predation versus low-predation populations from the Arima, Aripo, El Cedro and Oropuche Rivers, low-predation guppies were significantly more exploratory than high-predation guppies in the open-field apparatus (Fig. 1, Table 1). Individual body mass (centered on population mean) was significant in this analysis (t value 2.28, $df = 130$, $p = 0.024$), and the estimate was negative (-1.0273 (SE 0.45)) indicating that, across the different populations, smaller fish were more exploratory. Neither sex nor centered population mean body size was significant predictors of exploratory behavior.

Wild-caught fish tested in the laboratory

Open-field tests

When wild fish were tested in the laboratory, there was a significant effect of predation regime on exploratory behavior with low-predation fish again being more exploratory than high-predation ones (males = 67, females = 72) (Fig. 2, Table 2). Note that, in this analysis, the variances associated with the random effects river and population within river were estimated by the analysis as zero (meaning that observations on individuals within populations and populations within streams were uncorrelated); when that happens, the Kenward-Roger degrees-of-freedom method accounts for these variance estimates, so the fixed effects in this analysis are associated with larger denominator degrees of freedom than in other analyses reported here. For this group of fish, females were significantly more exploratory than males, and individual body size was negatively associated with exploratory behavior, but there was not a significant interaction between sex and body size ($F_{1,133} = 1.40$, $p > 0.2$). Population mean body size was also negatively associated with exploratory behavior ($\beta = -0.53$, SE = 0.07, $t = -7.57$, $df = 134$, $p < 0.0001$); again, there was no interaction between sex and population mean body size ($F_{1,133} = 0.0$, $p > 0.98$).

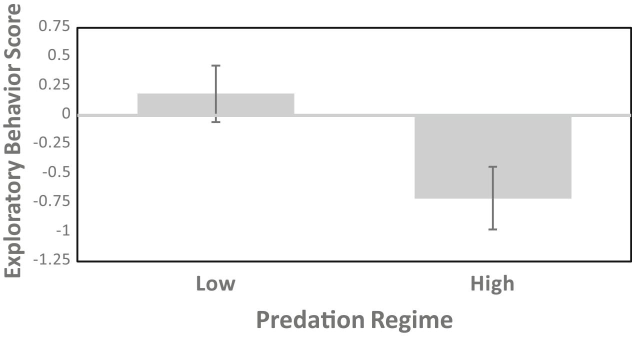

Fig. 1 In open-field tests conducted in the field on wild-caught guppies, low-predation guppies were more exploratory than high-predation ones. Values are least-square means of the first principal component from the principal component analysis of time frozen and swimming rate. High scores indicate a low amount of time frozen and a high swim rate when not frozen, which we interpret as exploratory behavior. *Error bars* represent one standard error

Table 1 Results of GLMM on exploratory behavior of wild-caught guppies from eight high- and low-predation populations, tested in the wild

Type 3 tests of fixed effects

Effect	*df*[a]	*F*	*P*
Predation regime	1,3	14.53	0.032
Sex	1,14	0.11	0.742
cBS	1,128	5.21	0.024
CpmBS	1,11	0.05	0.831

cBS centered individual body size (centered on population mean), *CpmBS* population mean body size (centered on grand mean)

[a] Denominator degrees of freedom was rounded to the nearest whole number

Activity level test

There was not a significant effect of any factor, including predation regime, sex, and body size, or any of the interactions among them, on activity level (Table 3).

Also, there was no evidence of a strong relationship between activity and exploration. When activity was included (population mean activity, centered on overall mean, and individual activity, centered on population mean) in the model for the analysis of exploration, individual activity level was not a significant predictor of exploratory behavior ($p = 0.1$, Table 4) and the trend for the association was negative but not significant ($\beta = -0.23$, SE $= 0.14$, $t = -1.64$, $df = 132$, $p = 0.1034$).

Laboratory-reared fish tested in the laboratory

For the second-generation, lab-reared fish, none of the factors or their interactions had a significant effect on exploratory behavior (Table 5) (males = 59, females = 60). Unfortunately, power was low with just two populations from each of two rivers. Plots of the data indicated that the trend was in the same direction as for wild-caught fish and that, for females, but not males, genetics may play a role in the difference between high- and low-predation populations for the Oropuche River but not the Aripo River (Supplemental Materials Fig. S1; details of the analyses are in Burns (2007)). Study is required of more populations from other rivers before conclusions can be reached about whether predation regime can select for a genetically based difference in exploratory behavior or whether, in the Oropuche River, some other site-specific effect might be involved.

Discussion

As predicted, wild-caught guppies from low-predation populations showed greater exploratory behavior than those from high-predation populations. This pattern was consistent for fish tested in the field and for wild-caught fish that had been held in the laboratory for at least a week before testing. However, we did not detect evidence of a genetic basis for this difference in behavior, although low power may have contributed to this result.

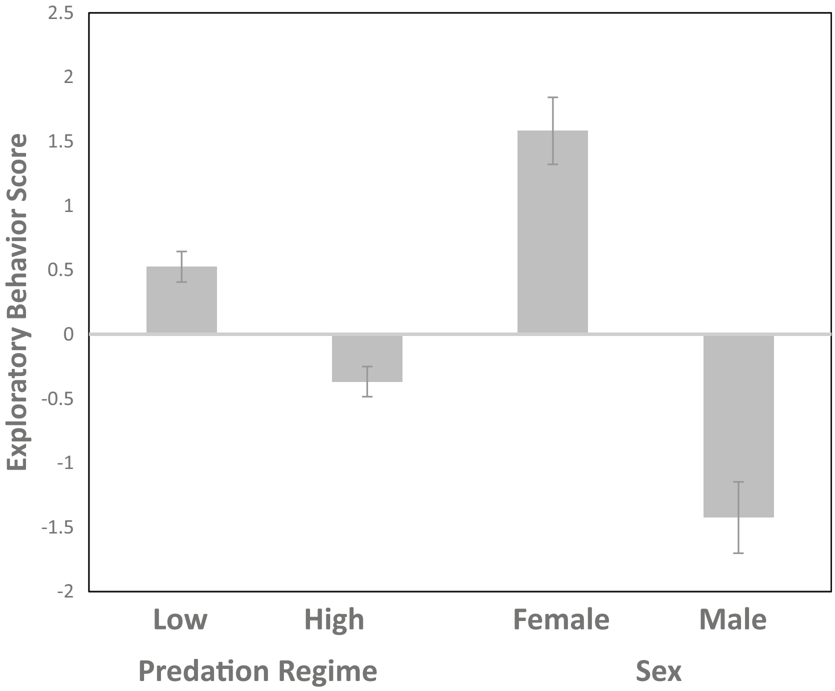

Fig. 2 In open-field tests conducted in the laboratory, on wild-caught guppies, low-predation individuals were more exploratory than high-predation ones. Females were more exploratory than males, and individual body size was negatively associated with exploratory behavior, but there was not a significant interaction between sex and body size. Values are least-square means of the first principal component from the principal component analysis of time frozen and swimming rate. High scores indicate a low amount of time frozen and a high swim rate when not frozen, which we interpret as exploratory behavior. *Error bars* represent one standard error

Table 2 Results of GLMM on exploratory behavior of wild-caught guppies tested in the laboratory

Type 3 tests of fixed effects

Effect	*df*[a]	*F*	*P*
Predation regime	1,134	29.78	<0.0001
Sex	1,134	34.23	<0.0001
cBS	1,134	4.00	0.048
CpmBS	1,134	57.26	<0.0001

cBS centered individual body size (centered on population mean), *CpmBS* population mean body size (centered on grand mean)

[a] Denominator degrees of freedom was rounded to the nearest whole number

We have several lines of evidence suggesting that the observed differences in phenotype that we observed reflect real differences in exploratory behavior in wild-caught fish. First, the difference between high- and low-predation regime guppies was not simply a result of differences in general activity because we did not detect a significant difference in the activity levels of wild-caught fish tested in a familiar environment in the laboratory. This might seem surprising since reducing general activity level can reduce predation risk when active prey are more likely to be caught by predators than less active prey (Werner and Anholt 1993; Lima 1998); however, predation pressure may not be translated into differences in activity level where the costs of reducing activity level, including foraging, at all times can be high (Werner and Anholt 1993). Second, we do not think that the difference in exploratory behavior was motivated primarily by hunger. The most obvious reason is that wild-caught, low-predation guppies were more exploratory than high-predation ones even in the laboratory, where all fish were fed ad lib food levels. In fact, even though high-predation guppies grow faster in the laboratory (Arendt and Reznick 2005) and thus might be more motivated to find food, high-predation guppies were less, not more, exploratory than low-predation guppies. Third, although cues from predators, including Schreckstoff from wounded fish and digested fish in predator feces, can elicit anti-predator behavior including reduced exploratory behavior, this could not have been the only factor contributing to the difference between predator regimes because we observed similar results for both field-tested (where predator cues could have been present in the testing water) and lab-tested fish (where predator cues were not present).

We were unable to detect evidence for a genetic basis for predation regime on exploratory behavior. With only four populations from two rivers, firm conclusions cannot be made about the role of genes in between-locality behavior without further study. A genetic basis for variation in exploratory behavior in guppies was detected in a selection experiment for brain size where larger-brained individuals were more exploratory (Kotrschal et al. 2014). A number of factors may have contributed to this pattern including reduced levels of stress in the large-brained fish when they were in a stressful situation and faster habituation by those fish to the open-field test. Nevertheless, it is clear from our study that exploratory behavior in guppies is plastic, with the environment playing a substantial role. It seems very likely that learning and/or

Table 3 Results for activity behavior for wild-caught, lab-tested guppies based on an analysis with a generalized model with a negative binomial distribution (KR method)

Effect	*df*[a]	*F*	*P* value
Predation regime	1,1	0.29	0.68
Sex	1,1	0.03	0.89
Centered body size[b]	1,133	1.02	0.31
Centered population mean body size[c]	1,1	0.09	0.80

[a] Denominator *df* rounded to the nearest whole number

[b] Centered individual body size (cBS, centered on population mean)

[c] Population mean body size (CpmBS, centered on grand mean)

Table 4 Analysis of exploratory behavior using the KR degrees of freedom method, with activity level included in the model, of wild-caught fish tested in the laboratory, to determine whether there was a relationship between these variables

Type 3 tests of fixed effects

Effect	*df*	*F*	*P*
Predation regime	1,132	25.72	<0.0001
Sex	1,132	34.06	<0.0001
cBS	1,132	3.44	0.06
CpmBS	1,132	54.08	<0.0001
CpmAct	1,132	0.09	0.76
cAct	1,132	2.69	0.10

cBS centered individual body size (centered on population mean), *CpmBS* population mean body size (centered on grand mean), *CpmAct* centered population mean activity, *cAct* centered activity

Table 5 GLMM of exploratory behavior of lab-reared guppies tested in the laboratory

Type 3 tests of fixed effects

Effect	*df*[a]	*F*	*P*
Predation regime	1,2	2.88	0.27
Sex	1,23	0.06	0.81
cBS	1,112	1.18	0.28
CpmBS	1,22	0.04	0.84

cBS centered individual body size (centered on population mean), *CpmBS* population mean body size (centered on grand mean)

[a] Denominator degrees of freedom rounded to the nearest whole number

experience contributes to the pattern that we observed. Guppies, like other fish species, can learn behavior by observing conspecifics (e.g., Reader et al. 2003) and/or by exposure to predators and their cues. Exploratory behavior might be suppressed to a greater degree in high-predation fish than in low-predation ones because the former have more frequent and/or intense interactions with predators and/or because they are exposed to more extreme responses to predators and their cues by conspecifics. It is also possible that the role of learning or "activation" by early experience with predators, causing a reduction in exploratory behavior, is larger in high-predation guppies (i.e., there is a genotype-by-environment interaction). Magurran (1990) found evidence for a genetic basis for differences in anti-predator behavior of high- and low-predation populations of minnows (*Phoxinus phoxinus*) and also showed that an early experience with a predator model affected the behavior of the high-predation fish to a greater degree than with low-predation fish. Similarly, comparisons of laboratory-reared and wild-caught guppies (Kelley and Magurran 2003b) and sticklebacks (Tulley and Huntingford 1987) indicate a greater role of experience in anti-predator behavior in high-predation fish compared to low-predation fish.

Other environmental factors that have been shown to influence behavior, even into adulthood, and that could have affected the behavior of our wild-caught fish include food availability and maternal effects (e.g., Roche et al. 2012). For example, low food ability during development increased exploratory behavior in fruit flies (Burns et al. 2012; but Edenbrow and Croft (2013) observed the opposite effect in mangrove killifish). Maternal stress during development rendered mice more fearful in unfamiliar environments and reduced exploratory behavior (Poltyrev et al. 1996).

Conclusion

Our results for guppies from natural populations are consistent with the idea that exploratory behavior is a balance between the costs of predation risk and the benefits of one or more of dispersing to new habitats, sampling new sources of food, and avoiding inbreeding. One next step, to evaluate the mechanistic basis for this variation, would be to compare gene expression of high- and low-exploratory fish from within and between populations to identify candidate genes contributing to these differences (e.g., the dopamine receptor genes (Fidler et al. 2007)). We have shown that the exploratory behavior of a guppy depends on its experiences in the field. Future studies could study the contributions of direct contact with predators, observations of conspecifics, etc. in producing the field phenotype. It will also be important to examine how correlations with other traits (e.g., responsiveness to stressors (Archard et al. 2012), learning ability) might explain some of the patterns observed here.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. The experiments complied with the current laws of Canada and Trinidad and Tobago. All experiments were approved by the University of Toronto Animal Care Committee (protocol no. 20006260).

Informed consent There were no human participants in this study, but informed consent was obtained from all of those involved in the study.

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