Behavioral types as predictors of survival in Trinidadian guppies (*Poecilia reticulata*)

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Studies of the fitness consequences of behavioral types often focus on isolated behaviors and ignore potential across-context correlations that may affect fitness. This approach leads to heterogeneous results across studies because correlations themselves may be adaptive in populations under significant predation pressure. We quantified suites of behaviors in 4 different contexts and identified a consistent behavioral syndrome in a population of Trinidadian guppies (*Poecilia reticulata*). We then measured fitness effects of the correlated behaviors that made up this syndrome and found that more active, bold, and exploratory individuals survived longer when exposed to a predator. Behavioral syndromes may, therefore, be advantageous in populations under significant predation risk if an individual's behavior in the presence of a predator is an honest signal of escape abilities. Interestingly, we also found a significant effect of the individual cichlids (*Aequidens pulcher*) used as predators in our experiments. We suggest that future studies should test whether interactions between predator behavior and prey behavioral types maintain behavioral variation. *Key words:* activity, behavioral syndrome, behavioral type, boldness, fitness, personality. *[Behav Ecol 21:919–926 (2010)]*

Individuals exhibit consistent and varying behavioral types, often referred to as "personality" (e.g., Gosling 2001), "coping style" (Koolhaas et al. 1997), "emotional reactivity" (Boissy 1995), and "temperament" (Réale et al. 2007). Hypotheses to explain the evolution and maintenance of this variation include fluctuating resource availability (Dingemanse et al. 2004; Boon et al. 2007), predation levels (Réale and Festa-Bianchet 2003), and social conditions (Both et al. 2005) which alter selection pressures. Microhabitat adaptation (Buchholz and Clemmons 1997; Wilson 1998) and frequency-dependent selection (Wilson et al. 1994; Dall et al. 2004) also may lead to balancing selection (Futuyma 1998) on alternative phenotypes within and between populations. Behavioral type can affect fitness (for reviews, see: Dingemanse and Réale 2005; Smith and Blumstein 2008), and fitness trade-offs across contexts may maintain behavioral diversity within populations (Stamps 2007; Wolf et al. 2007; Smith and Blumstein 2008).

Boldness, for example, which is defined as the behavioral response in a potentially risky situation (Réale et al. 2007), has been found to correlate with measures of reproductive success (Sarno and Franklin 1999; Wielebnowski 1999) and survival (Réale and Festa-Bianchet 2003; Bremner-Harrison et al. 2004). Variation in boldness is often quantified by assaying the response of individuals to a potential predator or simulated predatory event (van Oers et al. 2004; Blumstein et al. 2006). A shortcoming, however, of studies that quantify the fitness of shy versus bold individuals is that they often focus on isolated behavior within a single context and ignore potential correlations with behaviors across contexts.

Behavioral syndromes are correlations in behavioral patterns across multiple observations or contexts (Sih, Bell, and Johnson 2004; Sih, Bell, Johnson, and Ziemba 2004). An often identified syndrome, for example, is the correlation between boldness toward predators and aggressiveness toward conspecifics. This relationship has been found in several species and individuals within populations exhibiting such a syndrome differ consistently from each other in both their boldness and aggression intensity levels (Huntingford 1976; Riechert and Hedrick 1993; Johnson and Sih 2005). Previous studies of syndrome variation, however, have focused on population-level questions, such as the presence or absence of syndromes (Bell 2005; Dingemanse et al. 2007), whereas fitness consequences are often studied in single-context behavioral types (Bremner-Harrison et al. 2004; Dingemanse et al. 2004). We, therefore, know little about the effects of selection on correlated behaviors, and this knowledge gap may explain heterogeneous results found within and between species (Smith and Blumstein 2008).

Comparing the fitness consequences of just one axis of behavioral variation may not tell the whole story (e.g., Lind and Cresswell 2006) if that behavior is correlated with other traits that compensate for predation risk. For example, individuals that are in better condition and possess greater ability to evade a predatory attack may be more likely to be bolder and inspect potential predators than individuals that are in worse condition and have reduced fleeing ability (Godin and Davis 1995; Milinski and Boltshauser 1995). Several lines of evidence suggest that more active individuals forage and grow at higher rates (for reviews, see: Mangel and Stamps 2001; Stamps 2007) and, therefore, may be in better overall condition than less active individuals. If general activity is also correlated with boldness in the presence of a predator, very active and bold individuals may, therefore, be better able to evade predators than less active and shy ones (FitzGibbon and Fanshawe 1988; Christensen 1996) and bold behavior could act as a deterrent to potential predators (Hasson 1991). Thus, the fitness consequences of boldness, as an isolated trait, may vary depending on the presence or absence of correlations with other traits and behaviors (i.e., syndromes).

To understand the ecological and evolutionary consequences of behavioral variation, it is important to study the fitness consequences of correlated behaviors (i.e., behavioral

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syndromes). We know of only 2 studies (Sih and Watters 2005; Logue et al. 2009) that reported the fitness consequence of correlated behaviors and both of these measured effects only on reproductive success. There is, however, evidence to suggest that predation is also a selective force that generates syndromes within populations (Bell 2005; Bell and Sih 2007).

We applied a method that has been used in human personality research for quantifying risks associated with the "Big Five" personality traits (Kerby 2003) to examine the extent to which variation in correlated behaviors within Trinidadian guppies (*Poecilia reticulata*) is predictive of survival. We first quantified behavior in 4 different contexts: general activity in an open field, sociability toward a conspecific, boldness in the presence of a potential predator, and exploration of a novel object. In each context, we measured a number of behaviors and used principal component analysis (PCA) to combine these nonindependent measures. Previous studies of behavioral syndromes in fish have also used PCA for identifying syndromes (Bell and Stamps 2004; Bell 2005). This method avoids the problems of multiple comparisons because statistical tests can be done on a few component scores rather than many different variables. We then looked for correlations in component scores across contexts and summed correlated scores to create a syndrome score and to test whether variability in correlated behaviors, or personality traits, affected survival time when individuals were placed in a tank with a guppy predator. By doing so, we generated 2 important insights: boldness may be a predator deterrent and thus be an honest signal to predators, and within-species variation in individual predators may be one mechanism maintaining behavioral variation in prey populations.

MATERIALS AND METHODS

Subjects

Trinidadian guppies are small (max standard length (SL)—males: 3.5 cm; females: 5.0 cm), live bearing, teleost fishes native to the forest streams of South America and neighboring islands (Endler 1978). They exhibit variability in a wide range of traits (Endler and Houde 1995; Reznick et al. 2001) and have been used in several studies examining interindividual differences in behavioral patterns (Godin and Dugatkin 1996; Budaev 1997; Dugatkin and Alfieri 2003). Only male guppies were used in this study because the behavior of female guppies fluctuates drastically in relation to their ovarian cycle (Warren and Callaghan 1975). Sixty subjects were captive bred for several generations and were descendants of individuals wild caught in the Aripo River in northern Trinidad (Grether G, personal communication).

Fish were maintained in 38-l mixed-sex tanks containing 20–30 individuals. They were fed twice a day with commercial flake food (TetraMin; TetraMin Pro, Tetra, Blacksburg, PA), reared under an approximately 12:12 photoperiod, and the temperature of conditioned water (Start Right, Jungle Laboratories Corp., Cibolo, TX; proper pH 7.5, Aquarium Pharmaceuticals, Inc., Chalfont, PA) was maintained at 23–26 °C. Two days prior to initiating an experiment, males were selected from communal holding tanks and moved to 4-l containers (Aquatic Gardens aquarium tote, International Pet Supplies and Distribution, Inc., San Diego, CA) where they were housed individually for the duration of behavioral experiments. Dividers between containers blocked visual access to conspecifics. After completion of all behavioral experiments, we lightly anesthetized subjects with ethyl-3 aminobenzoate methanesulfonate salt (MS-222), photographed both sides of their bodies, and used ImageJ (http://rsb.info.nih.gov/ij/) to measure the SL (cm), total body area (cm^2), and amount of orange coloration

from digital photos (Cyber-shot DSC-P100, Sony Electronics, San Diego, CA). The proportion of orange coloration on each subject was then found by dividing the total amount of orange coloration by total body area.

Identifying syndromes

We conducted all experiments in a 38-l tank measuring $51 \times 27 \times 32$ cm^3 (L $\times$ W $\times$ H) with a white colored gravel substrate and a grid (5×5 cm^2 cells) for quantifying locomotion and location marked on the broad side of the tank. A video camera (Digital Viewcam, Sharp, Mahwah, NJ) recorded and an observer monitored each experiment from behind a black curtain to minimize disturbance. An opaque divider separated the tank into 2 equal halves, and subjects were randomly chosen and placed individually into the experimental apparatus and allowed to acclimate for approximately 13 min. After acclimation, the divider was remotely raised, exposing subjects to 1 of 4 stimuli on the opposite side of the tank, and behavior was recorded for 5 min. When an experiment was completed, the subject was removed, one-third of the tank water was replaced with fresh conditioned water, and a mechanical filter (Whisper Power Filter, Tetra, Blacksburg, VA) with activated carbon was run for at least 20 min to remove possible latent chemical cues (Wisenden B, personal communication) before using the tank again. No more than 8 experiments were run in a single day.

Each subject was exposed to 4 experimental treatments, once per day, between 08:30 and 18:30 h over 4 consecutive days. We presented experimental treatments in a randomized order so that all subjects did not see the 4 treatments in the same order, and each subject was exposed to the 4 treatments in 1 of 24 possible combinations. We chose this method because presenting treatments in the same order to all subjects would create an unavoidable confound. Each of the behavioral measurements would be influenced by the presentation order, not the experimental situation, and these carryover effects could heighten or diminish potential behavioral correlations (Logue et al. 2009; Dochtermann 2010). We, therefore, randomized the order of treatments and used general linear models to test whether order of the 4 experiments affected behavioral outcomes.

The 4 experimental treatments were designed to alter ecological context (e.g., (Johnson and Sih 2007)), or perceived risk, and we interpreted each following the framework developed by Réale et al. (2007). An open field (empty tank) was used to measure "general activity." Video playback of a single female guppy recorded against a black background was used to measure "sociability." We chose video playback, which was broadcast on a liquid crystal display monitor (Envision, Fremont, CA) to reduce the likelihood of subjects perceiving flickering images (Baldauf et al. 2008), in order to standardize stimulus presentation across subjects (Kodric-Brown and Nicoletto 1997). A life-size model (10.5 cm SL; 13.5 cm total length) of a blue acara cichlid (*Aequidens pulcher*), a native guppy predator (Magurran et al. 1992), was used to measure "boldness." The model was constructed from a photographic print of *A. pulcher* in Axelrod et al. (1991, Plate 518), which was glued to a clear acrylic glass backing, covered with epoxy resin (Ultra-Glo, Environmental Technology, Inc., Field Landing, CA), and suspended in the water column by 0.15 mm monofilament line (see Coleman et al. 1985; Galvani and Coleman 1998). The final context was a novel object (bright-pink soap dish) used to measure "exploration."

Experiments were quantified from videotapes using the event recorder JWatcher (Blumstein and Daniel 2007). We chose behavioral variables based on those that have been previously used to measure behavioral types in fishes (Huntingford 1976; Budaev 1997; Bell and Stamps 2004) and based on a pilot study using 56 guppies in which we

identified correlated behavioral variables that varied among subjects. We measured general activity in an open field by counting the number of different areas within the entire grid (50 maximum) a fish used, the number of different areas used in the novel half of the tank (25 maximum), and the total time spent moving in the 5-min trial. We measured the social response to the video of a female guppy by quantifying the latency to approach to within close proximity (defined as 3 body lengths throughout) of the video screen and the total time moving toward the monitor. We measured boldness toward a predator by quantifying the latency to approach to within close proximity of the model and number of different areas used throughout the entire tank. Lastly, we measured novel object exploration by quantifying the latency to approach to within close proximity, the number of times a fish approached to within one body length, and the distance of the closest approach to the object.

Quantifying fitness consequences of correlated behaviors

Ten groups of 6 randomly chosen subjects underwent a staged predation experiment with a live piscivorous predator for a maximum of 5 days. We selected this group size because it has been used previously to assess predation on guppies (Dugatkin 1992) and is similar to shoal sizes found in the wild (Dugatkin and Godin 1992a). We used a 110-l tank that measured $77 \times 32 \times 48$ cm^3 (L $\times$ W $\times$ H) and had a natural colored gravel substrate, 4 sections of PVC pipe (diameter range, 3–7 cm), 2 plastic aquarium plants, conditioned water held constant at 25–28 °C, and a 12:12 photoperiod. One of 3 commercially acquired blue acara cichlids (~8–11 cm SL) was resident in the tank for 3 days prior to the experiment's initiation, during which it was fed once a day with a small amount of flake food (TetraCichlid, Tetra, Blacksburg, PA) and one guppy (to acclimate it to guppies). Two of the cichlids were used for 3 predation experiments and one was used for 4, for a total of 10 predation experiments.

On the first day of a predation experiment, at least 4 days after subjects had completed all behavioral experiments, 6 guppies were placed into the predation tank behind an opaque divider that separated one-third of the tank and allowed to acclimate for approximately 10 min. After acclimation, the divider was raised allowing the predator and guppies to interact. Experiments began at precisely the same time of day for each of the 10 groups. The divider was raised at 13:06 h and we quantified survival every 15 min for the first 2 h. After the 2 h had elapsed, we checked survival every hour for the remainder of day one until lights out, every 2 h on day 2 starting 30 min after lights were turned on, every 3 h on day 3, and twice a day at 12:00 and 18:00 h on days 4 and 5. Each observation period lasted approximately 5 min and disturbance to fish was minimized as observations were made through the tank wall using natural body markings to distinguish guppies. The experiment ended at 12:00 h on day 6. Thus, a full-term experiment encompassed 28 observation intervals over 7134 min. Only 2 experiments had any guppies remaining beyond day 2, and only 1 went the full 5 days. At the end of each experiment, we emptied and cleaned the tank.

Ethical note

Following accepted ethical standards for predation experiments, we limited the number of subjects in this experiment (Huntingford 1984; ABS/ASAB 2003). Allowing predators to interact with prey was essential to quantify fitness, but numerous objects, similar to those found in our community tanks, were placed in experimental tanks to provide guppies refuge. This study received prior approval by the Institutional Animal Care and Use Committee at the University of California, Los Angeles (UCLA), and protocols were reviewed annually to ensure proper compliance with the United States Department of Agriculture Animal Welfare Act.

Statistical analysis

We used separate PCAs to estimate PCA loadings and then used these to estimate an individual's component score. For each experiment, we extracted components with eigenvalues greater than 1 and reported component loadings (the correlation between each variable and the component), and the percent of variance within variables explained by each component (Table 1). We calculated nonparametric Spearman rank correlations between component scores across contexts because the distributions of component scores were not all normally distributed. All tests were 2-tailed, and significant correlations ($P < 0.05$) across contexts were interpreted as representing behavioral syndromes (Sih, Bell, Johnson, and Ziemba 2004). The reliability of identified syndromes was also determined by calculating the intraclass correlation coefficient of the estimated component scores that comprised a syndrome. Standardized PCA scores for each of the components within an identified syndrome were summed to produce overall syndrome scores that were subsequently used in survival analyses.

An alternative approach for testing the effects of correlated behaviors on survival is to develop a model that includes behavioral measurements from each context as separate independent variables to test for main effects, as well as interactions between these variables. The problem with such a method of analysis is that the additional independent variables included in the model equates to a reduction in power to detect significant effects, particularly when dealing with smaller sample sizes. The method we used of summing standardized PCA scores, termed unit weighting, was first proposed by Wilks

Table 1

Component loadings for quantified variables and total variance explained by each component that resulted from PCAs for open field test, video of a female conspecific, model predator, and novel object

Behavior	Loading
Open field test	
General activity component	
Total area used	0.950
Novel area used	0.925
Time moving	0.728
Cumulative variance explained	76.3%
Video of a female conspecific	
Sociability component	
Time moving toward monitor	0.774
Latency to approach	−0.774
Cumulative variance explained	59.9%
Model predator	
Boldness component	
Total area used	0.912
Latency to approach	−0.912
Cumulative variance explained	83.2%
Novel object	
Exploration component	
Latency to approach	0.953
Closest approach	0.941
Number of approaches	−0.790
Cumulative variance explained	80.6%

Table 2

Spearman rank correlations between context component scores and syndrome scores

	Sociability	Boldness	Exploration	Active/bold/explore syndrome
General activity	0.174	0.263[*]	0.439[***]	0.735[***]
Sociability	—	0.255[*]	0.183	0.309[*]
Boldness	—	—	0.270[*]	0.683[***]
Exploration	—	—	—	0.695[***]

[*]$P < 0.05$, [***]$P < 0.001$.

(1938), who confirmed that unit-weighted regression correlates highly with multiple regression. This result was further confirmed by Schmidt (1971). Unit weighting has also been used in studies of human personality to assess risks related to personality scores. Kerby (2003) utilized unit weighting to determine whether scores on the "Big Five" traits predict potential suicide risk, with effect sizes calculated from this method nearly identical to those calculated from multiple regression. Thus, unit weighting of standardized PCA scores is a valid method and justified in the current study because, as Table 2 suggests, individual component scores and composite scores for the correlated behaviors were related, and using a single behavioral variable in our models allowed for adequate statistical power to detect an effect.

We used Cox proportional hazards models (Cox 1972) to test for the effect of syndrome scores on survival time in the presence of a predator. This model has been used extensively in medical research to model survival times (Collett 2003) and has been used in behavioral ecology to model habitat selection (Freitas et al. 2008) and foraging decisions (Wajnberg et al. 2006). The model is written as:

$$h(t) = \exp(\beta_1 X_1 + \ldots + \beta_p X_p)h_0(t) \qquad (1)$$

where $h(t)$ is the hazard function at elapsed time t, X_p are the covariates in the model, β_p are the regression coefficients that give the relative contributions of the covariates, and $h_0(t)$ is the baseline hazard function.

In our study, the hazard function is the risk of an individual being preyed on at a given time. Along with the syndrome scores, we also included SL and proportion of orange coloration as covariates in the model because larger or more colorful individuals may be more susceptible to predation (Külling and Milinski 1992; Godin and McDonough 2003). We also tested the interaction of the individual predator and syndrome scores to determine whether risk factors varied between the predators. Because predation groups were formed by randomly choosing 6 subjects and did not control for behavioral variation between groups (e.g., Dugatkin 1992), we clustered robust standard errors of syndrome scores within each predation group to account for between-group variance in scores (Lee et al. 1992). The assumption of proportional hazards was verified for all fitted Cox models using chi-squared goodness of fit tests of Schoenfeld residuals (Schoenfeld 1980) and by plotting scaled residuals (Therneau and Grambsch 2000). Finally, the use of clustering terms in our models precluded a stepwise approach that utilized likelihood ratio tests to identify the best model. We still, however, developed multiple models that we varied a priori in included predictors and used Wald chi-square tests to evaluate model significance.

RESULTS

Identifying syndromes

For each of the 4 contexts, the behavioral variables loaded onto a single component that explained 60–83% of the variance in observed data. For the open field test, the number of different areas used in the novel half, the number of different areas used throughout the entire tank, and the total time moving all loaded positively on a general activity component (Table 1). Subjects that scored highly on this component spent much of the time moving and used several different areas of the tank. For the response to a female, time spent moving toward the monitor loaded positively, whereas latency to approach loaded negatively (Table 1). Subjects that scored highly on this sociability component approached the video monitor more quickly and spent more time moving toward it. For the model predator context, the number of different areas used throughout the entire tank loaded positively, whereas latency to approach the model loaded negatively (Table 1). Subjects that scored highly on this boldness component approached the model predator more quickly and used several different areas while in the presence of the model. For the novel object context, latency to move within close proximity and closest approach loaded positively onto the exploration component, whereas the number of times approaching to within one body length loaded negatively (Table 1). To make this component more intuitive and consistent with the other 3 contexts, component scores were multiplied by −1 so that high scores indicated fish that approached the object more quickly, closely, and often.

General activity, boldness, and exploration correlated with each other and, therefore, encompassed an "active/bold/explore" syndrome (Table 2). The intraclass correlation coefficient for the 3 components encompassing this syndrome was significant ($r = 0.26$, $P < 0.001$), indicating that individuals behaved consistently in these 3 contexts. We, therefore, summed the 3 sets of component scores for each subject to create an overall syndrome score. Individuals that scored highly on this active/bold/explore syndrome were generally more active, bolder in a potentially risky situation, and more exploratory toward a novel object. Composite syndrome scores were highly correlated with each other and with the general activity, boldness, and exploration component scores (Table 2) and occurred independently of morphological measurements (SL: $r_s = 0.024$, $P = 0.857$; proportion orange: $r_s = -0.120$, $P = 0.361$).

The sociability component only correlated with the boldness component and made up a "social/bold" syndrome (Table 2). However, the intraclass correlation for the 2 components was smaller and not significant ($r = 0.19$, $P = 0.07$). Because individuals did not behave consistently in response to the video of the female conspecific and model predator, we excluded the social/bold relationship from further analyses.

Order effects

For all 4 experiments, there was no significant effect of presentation order on principal components scores extracted from each treatment (open field: adjusted $R^2 = -0.02$, $F = 0.62$, $P = 0.61$; video of female guppy: adjusted $R^2 = 0.07$, $F = 2.45$, $P = 0.07$; model predator: adjusted $R^2 = -0.02$, $F = 0.64$, $P = 0.59$; and novel object: adjusted $R^2 = 0.05$, $F = 2.11$, $P = 0.11$). Thus, the order in which treatments were presented had no effect on the measurement of behavioral traits.

Quantifying fitness consequences of correlated behaviors

All fitted Cox models included censored observations of 4 subjects that were not preyed on after 5 days. Neither SL nor the

Table 3

Estimated regression coefficients (β), standard errors (SE), 95% confidence intervals (CI), hazard ratios (e^{β}), Z-scores, and P values for Cox proportional hazards models with active/bold/explore syndrome scores and morphological covariates, active/bold/explore syndrome scores alone, and interaction between active/bold/explore syndrome scores and predator identification

Covariates	β	SE	CI	e^{β}	Z	P
Active/bold/explore syndrome scores and morphological covariates						
Syndrome scores	−0.123	0.059	−0.239 to −0.007	0.884	−2.08	0.037
SL (cm)	−0.106	0.093	−0.289 to 0.077	0.899	−1.14	0.254
Proportion orange	−0.675	2.652	−5.872 to 4.523	0.509	−0.25	0.799
Overall significance of model: Wald $\chi_3^2 = 7.80$; $P = 0.0504$						
Active/bold/explore syndrome scores alone						
Syndrome scores	−0.128	0.063	−0.251 to −0.005	0.880	−2.03	0.042
Overall significance of model: Wald $\chi_1^2 = 4.13$; $P = 0.042$						
Interaction between active/bold/explore syndrome scores and predator identification						
Syndrome scores	−0.253	0.087	−0.423 to −0.083	0.776	−2.92	0.004
Predator 2 X syndrome	0.229	0.086	0.061 to 0.397	1.257	2.67	0.008
Predator 3 X syndrome	0.288	0.107	0.078 to 0.499	1.334	2.68	0.007
Overall significance of model: Wald $\chi_5^2 = 17.01$; $P = 0.005$						

proportion of orange coloration predicted survival, and the model that included these covariates was not significant (Table 3). Regression coefficients and hazard ratios for the active/bold/explore syndrome were similar with and without the morphological covariates included. We, therefore, interpreted relative risk based on the model that did not include the morphological covariates (Table 3).

Of the 4 single-context behavioral types we measured, only general activity significantly predicted survival with more active individuals less at risk ($e^{\beta} = 0.831$, $P = 0.004$). The hazard ratio for boldness alone was nearly identical but not significant ($e^{\beta} = 0.830$, $P = 0.053$). However, we found correlations between behavioral types were important as more active, bold, and exploratory individuals survived longer. The relative risk of subjects with higher active/bold/explore syndrome scores was 0.88 that of individuals with lower scores (i.e., they had a 12% decrease in predation risk; Table 3). However, there were significant predator effects. Individuals generally survived longer when exposed to predator 1 (Figure 1), and there was a significant interaction between syndrome score and predator ($\chi_2^2 = 7.94$; $P = 0.02$). The relative risk of higher syndrome scores was 0.78 that of lower scores during exposure to predator 1 (Table 3), translating to a 22% decrease in the risk of predation for more active, bold, and exploratory individuals.
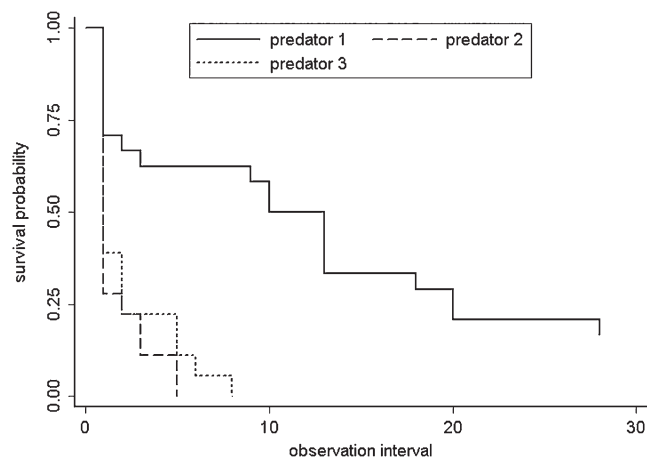

Figure 1
Kaplan–Meier curves of guppy survival probability during exposure to each of the blue acara cichlid predators for the 28 observation intervals.

viduals. Relative risks when exposed to predator 2 and predator 3 were significantly different than risk with predator 1 (Table 3) with only a 2.4% decrease ($e^{\beta} = 0.976$, $P = 0.048$) in relative risk for higher syndrome scores during exposure to predator 2, and active/bold/explore syndrome score was not a significant predictor of predation risk ($e^{\beta} = 1.036$, $P = 0.555$) during exposure to predator 3.

DISCUSSION

Taken together, we found that more active, bold, and exploratory guppies survived longer when exposed to a piscivorous predator. An alternative explanation for our results is that boldness did not affect predation risk. When survival for each of the individual behavioral types was examined, only general activity was a significant predictor. The hazard ratio for boldness alone was, however, nearly identical to that of general activity alone. Given the correlation between these 2 behavioral types, our results, therefore, indicate that, at the very least, higher boldness levels were indirectly favored.

To our knowledge, this is the first study in which PCA scores were summed across contexts to generate a syndrome scale for testing the fitness consequences of correlated behaviors, although similar methods have been used in human personality research (Kerby 2003). Individual behavior across the open field, model predator, and novel object contexts were consistent, and composite syndrome scores encompassing these 3 contexts were highly correlated with the components from which they were generated. Furthermore, our reliability analysis found that subjects maintained the same rank order (Sih, Bell, Johnson, and Ziemba 2004) within component scores across these 3 contexts. Thus, calculated syndrome scores represent an accurate summation of interindividual behavioral differences in each of these 3 contexts.

Hypotheses for the presence of behavioral syndromes

Previous studies have found that predation is an important selective pressure in generating behavioral syndromes, but it is unclear as to why syndromes might be advantageous in risky environments. Syndromes have been found in populations of 3-spined sticklebacks (*Gasterosteus aculeatus*) under significant predation pressure, yet absent in those where predation is reduced or absent (Bell and Stamps 2004; Bell 2005; Dingemanse et al. 2007). Furthermore, Bell and Sih (2007) found that exposure to predation generated the previously

absent bold–aggression syndrome within sticklebacks. Based on our results, which indicate that bolder and more active individuals are favored when under predation risk, we discuss 3 alternative hypotheses that may explain why syndromes develop in populations.

The first hypothesis is that boldness in the presence of a predator is a behavioral carryover of being generally more active or aggressive (e.g., Sih et al. 2003). Active and aggressive individuals forage more and grow at higher rates (Huntingford et al. 1990; Mangel and Stamps 2001; Höjesjö et al. 2002; Stamps 2007) and, therefore, the benefits of these behaviors may override the risks of increased boldness (Sih et al. 2003). In our study, we quantified 2 variables to measure boldness: the number of different areas a subject used and the latency to inspect a predator model, both of which are common measures of boldness in fish (Budaev 1997; Bell and Stamps 2004). A number of fishes engage in predator inspection, which is defined as a directed approach toward predators (Dugatkin and Godin 1992b; Fishman 1999), and "bolder" individuals are those who exhibit earlier and more frequent inspection than "shyer" ones (Huntingford 1976; Magurran 1986; Godin and Dugatkin 1996). We found that individuals that were generally more active also had shorter latency times in approaching a predator model. Thus, according to the carryover hypothesis, active individuals may have had shorter inspection times solely because of their high activity levels, and boldness has no adaptive function. Our results, however, found a significant fitness effect of active/bold/explore behavioral types. Furthermore, the relationship between predation pressure and syndrome presence in natural populations (Bell 2005; Dingemanse et al. 2007) suggest that syndromes are adaptive and not merely behavioral carryovers.

The second hypothesis is that active and aggressive individuals are at a higher risk of predation and, therefore, behave more boldly to assess risk. Activity and aggression are related to foraging and growth rates and predators may prefer larger individuals (Külling and Milinski 1992) because of the increased resource benefits relative to cost (Stephens and Krebs 1986). Predators may prey on unwary individuals (FitzGibbon 1989; Krause and Godin 1996), and prey can accurately assess risk by exploring and inspecting their environment (Licht 1989). Body size had no effect on boldness in both ours and in a previous study (Walling et al. 2004). SL was also not a predictor of predation risk in our study, suggesting that predators had no preference for larger individuals.

The third hypothesis is that behavioral syndromes develop in populations under a significant predation risk as part of an honest signal between prey and predator. For such communication to evolve, signals must be closely related to an individual's physical condition and ability to escape, and predators should select prey on the basis of the prey's behavior (FitzGibbon and Fanshawe 1988; Leal 1999; Laiolo et al. 2004). An individual's position in the shy–bold continuum may reflect an ability to evade predators if boldness is condition dependent (López et al. 2005). In guppies, for example, bolder individuals tend to be more brightly colored (Godin and Dugatkin 1996). Carotenoid-based colors are an indicator of quality as brightly colored males are better foragers (Endler 1978), more resistant to parasites (Houde and Torio 1992), and exhibit enhanced predator escape behaviors (Godin and Dugatkin 1996) as compared with drab males. When given the choice, however, females prefer bold males as potential mates independent of coloration, suggesting that boldness may be a more reliable indicator of quality than coloration (Godin and Dugatkin 1996).

The active/bold/explore syndrome we found may indicate that more active and exploratory individuals foraged more and were in better condition (Mangel and Stamps 2001; Stamps 2007), thus, allowing them to behave more boldly in "risky"

situations. We did not observe predation behavior of the cichlids in our study, so it is unclear whether individuals with higher active/bold/explore syndrome scores survived longer because of a greater ability to escape, fewer predation attempts than on individuals with lower syndrome scores, or a combination of these 2 factors. What is needed to demonstrate honest signaling in future studies are independent measures of individual quality or escape ability.

Individual predators may shape variability

Notably, we found that the fitness advantage of being active, bold, and exploratory was not generalized across all 3 of the blue acara cichlids we used as predators. Guppies exposed to 1 of the 3 cichlids survived much longer than those exposed to the other 2. Higher syndrome scores equated to a reduction in predation risk when exposed to predator 1, whereas active, bold, and exploratory individuals experienced only a slight decrease in risk during exposure to predator 2 and behavior did not predict risk during exposure to predator 3. These results seem to suggest that variability in the cichlids themselves may maintain variability in prey behavioral types. Given the limited number of individual predators used in the current research, however, additional studies are needed to provide further support for this hypothesis.

Behavioral diversity is often viewed as resulting from fitness trade-offs across contexts (Sih, Bell, and Johnson 2004; Stamps 2007; Smith and Blumstein 2008). Selection pressure can, however, vary within a single context when individual predators also possess varying phenotypes, including behavioral types. Predation studies often assume a generalized risk between all individuals of the same predatory species and ignore behavioral interactions between predator and prey that may shape observed patterns of risk (Lima 2002). We should expect that, like prey, predators also vary in their boldness and aggression levels, which may affect how they respond to prey behavior and signals. Within-species variability in predators could then lead to varying boldness levels of prey, and the presence of predators alone may not predict the presence or absence of behavioral syndromes. Future studies of the fitness effects of correlated behaviors should, therefore, account for the behavior of both predator and prey.

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