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Phenotypic integration between antipredator behavior and camouflage pattern in juvenile sticklebacks

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Predation is a strong selective force that promotes the evolution of antipredator behaviors and camouflage in prey animals. However, the independent evolution of single traits cannot explain how observed phenotypic variations of these traits are maintained within populations. We studied genetic and phenotypic correlations between antipredator behaviors (shoaling and risk-taking) and morphology traits (pigmentation and size) in juvenile three-spined sticklebacks by using pedigree-based quantitative genetic analysis to test phenotypic integration (or complex phenotype) as an evolutionary response to predation risk. Individuals with strongly melanized (i.e., camouflaged) phenotype and genotype were less sociable to conspecifics, but bolder during foraging under predation risk. Individuals with faster growing phenotype and genotype were bolder, and those with lager eyes were more fearful. These phenotypic integrations were not confounded with correlated plastic responses to predation risk because the phenotypes were measured in naïve fish born in the laboratory, but originated from a natural population with predation pressure. Consistent selection for particular combinations of traits under predation pressure or pleiotropic genes might influence the maintenance of the genetic (co)variations and polymorphism in melanin color, growth trajectory, and behavior patterns.

KEY WORDS: **Genetic correlation, heritability, melanin, personality, prey–predator interaction.**

Understanding how observed phenotypic variation is maintained within populations is a long-standing question in evolutionary biology. In the last decades, evolutionary research has been moving away from the study of a single trait variation toward complex phenotypes, resulting from developmental and genetic covariation among functionally related traits in a given organism ("phenotypic integration"; Pigliucci 2003). Phenotypic integration can evolve when natural selection favors certain phenotypic combinations of different traits (Pigliucci 2003). For example, color polymorphism of male side-blotched lizards (*Uta stansburiana*) is integrated with their territorial behaviors, including orange-dominant males, blue-subordinate males, and yellow-sneaker males, and this integration enhances the functionality of these alternative reproductive strategies (Sinervo and Lively 1996). Recently, consistent individual differences in suites of correlated behaviors (i.e., personality; Sih et al. 2004; Réale et al. 2007; Biro and Stamps 2008) has been documented in a variety of species, and great research effort has been devoted to understand how different personalities coexist within populations (Dingemanse and Wolf 2010; Laskowski and Bell 2013). However, behavioral integration may be only a small part of complex phenotypes, and thus it is necessary to simultaneously explore behaviors and other traits to study broader integration (Sih and Bell 2008).

Predation is an important selective force that promotes multiple types of antipredator adaptations in prey animals, including morphology, behavior and life history, leading to covariation among these traits (Endler 1995). Personality-related behaviors, such as risk-taking, grouping, and exploration, are particularly well documented as antipredator strategies (Bell and Sih 2007; Dingemanse et al. 2009; Luttbeg and Sih 2010). Visual camouflage is an important strategy of prey animals to avoid detection or recognition by predators, frequently involving body coloration (Stevens and Merilaita 2009). Phenotypic and genetic correlations between camouflage traits and antipredator behaviors are

expected from covariation among different sources of natural selection and interactions among the traits' functions (Endler 1995). Indeed, a number of studies have shown that different sets of antipredator traits coevolved and gave rise to dramatic variations in complex phenotypes among populations or closely related species (Garcia and Sih 2003; Pröhl and Ostrowski 2011; Santos and Cannatella 2011; Willink et al. 2013). However, few studies have explored how different combinations of antipredator strategies coexist within prey populations (Brodie 1989, 1992; Forsman and Appelqvist 1998).

In the simplest scenario, if an individual has cryptic color that matches the background in its natural habitat, then it can yield more food resource by increasing foraging activity and exploration while suffering less from predation risks than conspicuous individuals that behave in the same way (Sih 1992; Lima 1998). On the other hand, a conspicuous individual should be more careful to avoid detection by predators, but in this case being sociable to conspecifics may be beneficial because grouping dilutes individual risk of predation (Krause and Ruxton 2002). Similarly, a larger individual can be bolder because it is less likely to be eaten or a bolder individual may grow faster by obtaining more food if it survives (Sih 1992; Lima 1998).

Here, we studied phenotypic integration between antipredator behaviors and camouflage patterns in juvenile three-spined sticklebacks (*Gasterosteus aculeatus*). Sticklebacks fall prey to a wide variety of predators, such as aquatic bird, insect, and larger fish species, due to their small size, and thus they have a suite of morphological and behavioral adaptations that defend themselves against predators (Huntingford and Coyle 2007). Sticklebacks exhibit dark coloration particularly in dorsolateral surfaces, which is determined by melanin level and melanophore number, and interestingly both freshwater and marine sticklebacks show striking among- and within-population variations in the amount and pattern of the dark pigmentation (Miller et al. 2007; Greenwood et al. 2011, 2012). These differences are probably associated with ecological differences, particularly in predation pressure, because melanin coloration (brown, gray, and black) against dark background color of river bed or aquatic plants provides crypsis (Price et al. 2008). Predation pressure varies as a function of growth-trajectory or body size because smaller fish are subject to higher predation risks than larger fish (Krause et al. 1998). Another interesting characteristic of sticklebacks are their relatively large eyes (Schluter and McPhail 1992) that turns blue during reproduction in mature males and possibly plays a role in mate discrimination, but make them more conspicuous to predators (Cott 1940; Wickler 1968).

By definition, phenotypic integration should comprise both phenotypic and genetic covariances between integrated traits. Thus, we examined genetic and phenotypic correlations between antipredator behaviors (i.e., shoaling and risk-taking behaviors) and different camouflage strategies (i.e., melanin-based coloration, body size, and eye size) by using pedigree-based quantitative genetic analysis to test phenotypic integration of these traits. Furthermore, we discuss how these covariation patterns will lead further evolution of antipredator behaviors and camouflage. We subjected naïve fish, which were bred and raised in predator-free conditions but originated from a natural freshwater population with predation pressure, to distinguish phenotypic integration from correlated plasticity responses to predation risk (Schlichting 1989; Pigliucci 2003).

Materials and Methods

STUDY POPULATION AND BREEDING DESIGN

In February 2013, a total of 70 immature three-spined sticklebacks were captured with hand nets from a population at Rio Ulla, Galicia, Spain. Captured fish were housed in two 100 L holding aquaria, each containing a water filter, an air stone, and several artificial plants at natural range of seasonal water temperature and photoperiod. Fish were fed daily ad libitum with bloodworms. Among these fish, 16 males and 16 females were used for breeding during April–May. Sexually mature males and females were allocated in individual aquaria (33 × 18 × 19 cm) that contained a sponge filter, an artificial plant, and a Petri dish filled with sand for nesting. One hundred five centimeter long strands of green polyester thread were provided as nesting materials to each male (following Pike et al. 2007). Each fish bred twice with two different mates, producing a total of 32 full-sib families of the F1 generation. Within 3 h after fertilization, the whole clutch was collected from the nest then incubated in an incubation tank (100 L), following standard egg husbandry protocol (Barber and Arnott 2000). Prior to hatching, each full-sib clutch was isolated in a hatching tank (the same housing conditions as the breeding aquaria) then at hatching (eight days after fertilization) fry were counted (mean ± SE number of fry: 57.8 ± 3.0, $n = 32$).

At age 40 days, fish in each full-sib family were divided among two ($n = 7$ families) or four ($n = 25$ families) 24 × 16.5 × 17.5 cm "growth tanks" ($n = 114$ tanks), each housing 11 or 12 juvenile fish, for use in two independent experiments. The rest of the juvenile fish were raised in the laboratory until age two months, and then retained in an artificial pond with suitable living conditions as breeding stock for future studies (Kim and Velando 2014). Juvenile fish were fed to satiation daily (twice up to age five months then once a day) on a progressive diet of newly hatched *Artemia* (from hatching to age three months) and a customized diet (from age two months onwards; Gemma Micro, Skretting, Stavanger, Norway). Throughout this study, water temperature in growth tanks was maintained at natural range in the sampling sites of parent fish (16°C in May∼20°C

in July ~14°C in November). Natural photoperiod was simulated by programmed light-emitting diodes (LEDs) illumination. To prevent the risk of whitespot infection, the salinity was adjusted with sea salt to approximately 1.5 ppt.

SAMPLING, MARKING, AND MEASUREMENT OF MELANIN PIGMENTATION

This study was carried out during September–November by using a subset of F1 sticklebacks of age five months, precisely 143–160 days ($n = 448$) from 31 full-sib families and 112 different growth tanks (four fish/growth tank). Prior to a weekly study bout (total, seven weeks), we prepared eight "study tanks" ($33 \times 18 \times 19$ cm), each containing eight sticklebacks from four to five different full-sib families. Each individual shared the same study tank with only one full-sib from another growth tank and six unrelated individuals. Before allocating to a study tank, individuals were weighed, photographed, and then permanently marked with color elastomer tags (Northwest Marine Technologies, Shaw Island, WA) under a low dose of benzocaine anesthetic. Each anesthetized fish was placed on a wet unwoven fabric of neutral color alongside a color and scale reference then its lateral side (either left or right to reduce handling time) was photographed using a tripod-mounted digital camera (Nikon D90, Nikon Corp., Tokyo, Japan). The fish was illuminated with a LED lamp from the above while photographing. The positions of photographer, camera, lamp, and fish were always the same. Each individual was marked with one of four colors on either anterior or posterior dorsal of both lateral sides to allow rapid identification among eight different individuals in the same tank. The whole process took less than 90 sec per fish.

To quantify pigmentation of fish from the digital photographs, we developed a method to measure pigmented area and grayscale intensity (see also Greenwood et al. 2011 for similar method) by using image analysis software (analySIS FIVE, Olympus). We determined the whole body area of fish except fins as the region of interest then selected areas that ranged 0–60 in intensity (varying from black at 0 to white at 255) across the whole range of hue (1–359) and saturation (0–255), excluding pupil area. Size of the whole body area, sizes of the pigmented areas, and mean intensity of each pigmented area were recorded. In three-spined sticklebacks from the study population, pigmented skin area is fragmented according to the distribution and concentration of melanophores (see Fig. 1). Therefore, mean intensity of the total pigmented area in each fish was calculated based on relative sizes of the fragmented areas and their intensity values. Relative size of the total pigmented area (proportion of total lateral body area) and mean intensity of the area were used in the statistical analyses. Besides, horizontal eye diameter was also measured from the digital image.

BEHAVIOR OBSERVATIONS

Before behavior observations, marked sticklebacks were allowed to acclimate in the study tank for five days. Only the front wall of the study tanks was transparent to allow observation and the others were opaque to prevent visual contact of fish between different tanks. Each tank had a sponge filter, an artificial plant, and a food cup in which bloodworms were provided as food once a day. By the time that behavior observations began, fish were accustomed to feed on bloodworms in the food cup. Shoaling and risk-taking behaviors were measured twice during two consecutive days in all individual sticklebacks ($n = 443$, except five that died during the study).

Shoaling behavior was tested individually in an observation tank that was partitioned in three compartments (acclimatization, middle, and stimulus fish zones) with an opaque barrier and a transparent barrier. The distance between the two barriers was 16 cm. The stimulus fish zone contained three unfamiliar conspecifics (nonstudy fish) of the similar size to the focal fish. In the morning (9–12 h), each individual was netted carefully from its study tank, transferred to an observation tank, and left for 180 sec in the acclimatization zone. The opaque barrier between the acclimatization and middle zones was then removed and the time taken to reach the transparent barrier between the middle and conspecific zones was measured up to 180 sec. Therefore, this test allowed us to assess the individual's willingness to join to the conspecific group. Immediately after the test, individuals were returned to their study tanks.

Risk-taking behavior was measured in individuals' own study tanks in the afternoon (15–17 h). An observer was sat closely to the transparent front of a study tank, and then assessed individual willingness to forage under predation risk by a simulated avian predator (Bell 2005; Bell and Sih 2007). These sticklebacks, born and raised in the laboratory, were accustomed to the presence of an observer. We attached a model head of the gray heron (*Ardea cinerea*) over the experimental tank, and then added 0.4 g of bloodworms to the food cup. When at least one fish took a bite of food from the food cup, the predator's head was quickly released, simulating an attack. Following the simulated attack, we observed individual behaviors for 300 sec, and recorded the time taken to give the first bite of food from the food cup in each individual. Risk-taking behavior was measured simultaneously in all individuals from the same study tank, and thus dependence of samples, for example, by interference, cue, and behavior of other individuals, is an important environmental component of variations in this measure (see Statistical Analyses).

Shoaling and risk-taking behaviors were significantly repeatable within individuals across two observation days (shoaling: permanent environment effect, $pe^2 \pm \text{SE} = 0.141 \pm 0.057$, $P = 0.029$; risk-taking: $pe^2 = 0.398 \pm 0.062$, $P < 0.001$).

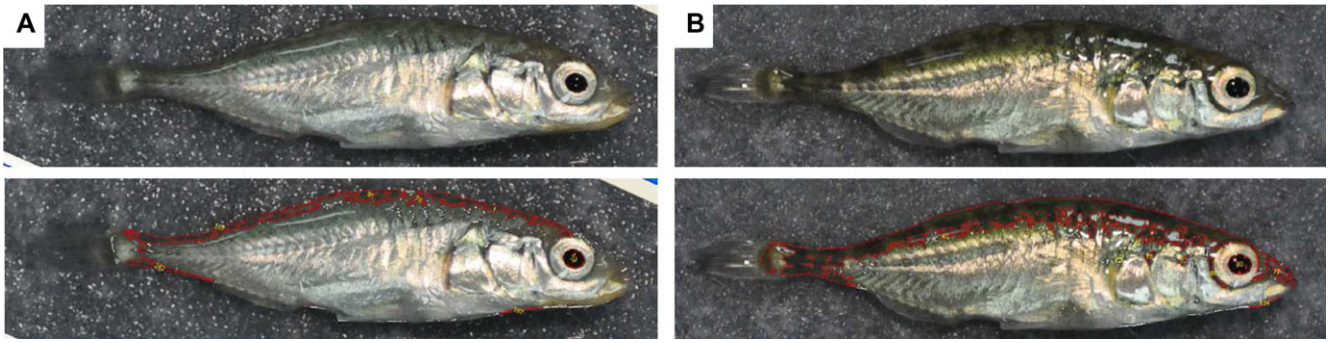

Figure 1. Size-standardized photographs of five-month-old juvenile sticklebacks. In images below the original photos, melanized areas, where color intensity ranged between 0 are 60, are outlined in red. (A) An individual with little melanization (proportion of melanized body area: 0.08; mean intensity of melanized area: 53.48), and (B) a strongly melanized individual (area: 0.24; mean intensity: 44.49).

Therefore, the average behaviors of two repeated measurements were used in the statistical analyses to facilitate exploration of genetic and phenotypic correlations between the behavior traits and other traits that were measured once. After this study, all the fish were retained for another experiment.

STATISTICAL ANALYSES

The animal model is a kind of mixed effect model that estimates quantitative genetic parameters by assessing the phenotypic covariance between all pairs of relatives in the pedigree (Kruuk 2004). Here, we fitted univariate and multivariate animal models with a restricted maximum likelihood (REML) by using ASReml version 3.0 (Gilmour et al. 2008). The estimation of the additive genetic (co)variances in this study was based on full- and half-sib relationships of 443 individuals and 32 parental identities. Significance of (co)variance components were assessed by calculating the log likelihood ratio and testing against a chi-square distribution with degrees of freedom equal to the difference in degrees of freedom between the two models compared (Pinheiro and Bates 2000).

We first used univariate animal models with a REML to estimate (co)variance components for behaviors (i.e., shoaling and risk-taking) and morphological traits (i.e., size and color intensity of pigmented area, body mass, and eye diameter). In each univariate model, a single trait (t) of an individual i is specified as

$$t_i = \mu + age + a_i + gt_i(+st_i) + \varepsilon_i.$$

Fixed effects included in this model were μ, the overall fixed effect mean, and age of fish. As random effects, we included the additive genetic (a_i), growth tank-specific common environment effect (gt_i), and the random residual error (gt_i). Although temperature and light were strictly controlled, some microconditions, such as algae growth and water flow, which may influence development of behavioral and morphological characters of juveniles, were a little variable across growth tanks. In the model fitted to risk-taking behavior, study tank-specific common environment effect (st_i) was included as an additional random effect to account for nonindependence of samples in which this behavior was measured simultaneously in the same study tank. Maternal environmental effects can be calculated by including maternal identity as an additional random effect (Kruuk 2004). However, maternal identity did not explain significant variation for any of the traits in the models in which significant additive genetic variances were estimated based on genetic pedigree (shoaling: $P = 0.155$, risk taking: $P = 1$, pigmented area: $P = 0.359$, pigmentation intensity: $P = 0.083$, mass: $P = 1$, eye size: $P = 1$), probably because prelaying environmental conditions in the laboratory were the same for all the mothers. Thus, maternal identity was not included in the analyses presented here to avoid downward bias in the estimation of the additive genetic variance (de Villemereuil et al. 2013). Total phenotypic variance was calculated as the sum of all variance components, and then heritability and common environment effects were calculated as the proportions of additive genetic and common environment variances in the total phenotypic variance.

We also fitted multivariate animal models to test for genetic and phenotypic correlations between behavior and morphology traits. The two behavior traits and a morphology trait (t, either a pigmentation or size trait) were fitted at a time in a trivariate model because larger models often had convergence problems and we were particularly interested in genetic and phenotypic integrations between morphology and behavior. The same fixed and random effects as the univariate model described above were included in the multivariate model as

$$Sociability_i Boldness_i t_i = \mu + age + a_i(+gt_i + st_i) + \varepsilon_i.$$

Common environment parameters (gt_i and st_i) were as included in the trait-specific univariate models (see above). Trait-specific (co)variances of all zero variance components (i.e., V_{GT} and Cov_{GT} of shoaling and risk-taking behaviors and body mass) were dropped from the multivariate models to converge without any falsely estimated (co)variance. Analyzing all the morphology

Table 1. Variance components (V_A, additive genetic variance; V_{GT} and V_{ST}, common environment variances explained by shared growth tank and study tank, respectively; and V_P, total phenotypic variance), heritability (h^2), and common environment effects (gt^2 and st^2) and their standard errors from univariate models.

	Trait	V_A	V_{GT}	V_{ST}	V_P	h^2	gt^2	st^2
Behavior	Shoaling	445.5 ± 232.6 ($P = 0.003$)	0 ($P = 1$)	-	2580.9 ± 189.9	0.173 ± 0.084	0	-
	Risk taking	1403 ± 833 ($P = 0.018$)	0 ($P = 1$)	338 ± 309 ($P = 0.218$)	10152 ± 732	0.138 ± 0.078	0	0.033 ± 0.030
Pigmentation	Area[1]	1.366 ± 0.609 ($P < 0.001$)	0.424 ± 0.190 ($P = 0.007$)	-	4.105 ± 0.368	0.333 ± 0.128	0.103 ± 0.046	-
	Intensity	13.179 ± 4.586 ($P < 0.001$)	0.782 ± 0.500 ($P = 0.081$)	-	16.099 ± 2.311	0.819 ± 0.176	0.049 ± 0.032	-
Size	Body mass[1]	1.401 ± 0.630 ($P < 0.001$)	0 ($P = 1$)	-	5.976 ± 0.462	0.234 ± 0.095	0	-
	Eye diameter[2]	3.373 ± 1.511 ($P < 0.001$)	0.719 ± 0.445 ($P = 0.070$)	-	10.020 ± 0.907	0.331 ± 0.128	0.071 ± 0.043	-

[1]Variance components and their SE × 10^{-3}.
[2]Variance components and their SE × 10^{-1}.

and behavior traits in a single multivariate animal model yielded a convergence problem. However, to visualize a complete landscape of behavior–morphology integrations, risk-taking and shoaling behaviors were graphed against the principal component estimated from a factorial analysis with the correlation matrix of family means of morphological traits (Fig. S1).

Results

Age effect (a fixed term) was significant in the analysis of pigmented body area ($F_{1,507} = 7.55$, $P = 0.008$). Older fish had smaller body surface area with melanin pigmentation. However, effect of age, which varied only between 143 and 160 days, was not significant in shoaling and risk-taking behaviors, mean color intensity of pigmented area, body mass, and eye diameter ($P > 0.277$).

All the behavioral and morphological traits had significant additive genetic variances, showing high levels of heritability in morphological traits (pigmentation, eye diameter, and body size) and relatively low (but significant) levels of heritability in behavior traits (Table 1). Common environment shared by siblings in the same growth tank explained little variance in these traits, except proportion of pigmented body area, which showed a significant component of variance due to growth tank (Table 1). Study tank variance in risk-taking behavior was also not significant (Table 1).

We explored genetic and phenotypic correlations between the two heritable behavior traits and each of the heritable morphology traits by using multivariate analyses (Table 2). Common environment covariances between behaviors and morphology traits were not estimable because growth tank variances of shoaling and risk-taking behaviors were null ($V_{GT} = 0$, Table 1) and study tank variance was calculated only in risk-taking behavior. Therefore, trait-specific environmental variance matrices were included in the multivariate models for traits with non-zero V_{GT} or V_{ST}. Correlations based on full-sib family mean values are presented in Figure 2 to visualize significant phenotypic integration (i.e., significant genetic and phenotypic correlation) patterns between behavior and morphology traits.

Proportion of pigmented body area was significantly correlated with risk-taking behavior at both the phenotypic and genetic levels, showing that more melanized individuals or genetic families tended to behave bolder in a foraging environment under predation risk (i.e., take less time to feed under predation risk; Table 2; Fig. 2A). However, there was no evidence for significant genetic and phenotypic covariance between proportion of pigmented area and shoaling behavior. Mean color intensity of the melanized body area was significantly correlated to both shoaling and risk-taking behaviors at the genetic and phenotypic levels. Darker (i.e., lower value of color intensity) individuals or genetic families were less sociable and bolder (Fig. 2B).

Table 2. Additive genetic and phenotypic correlations from multivariate (trivariate) models of two behavior traits (sociability and boldness) and a morphology trait.

		Shoaling behavior		Risk-taking behavior	
	Trait	r_G	r_P	r_G	r_P
Pigmentation	Area	0.237 ± 0.337 ($P = 0.498$)	0.012 ± 0.055 ($P = 0.462$)	-0.743 ± 0.274 ($P = 0.045$)	-0.032 ± 0.054 ($P = 0.032$)
	Intensity	-0.665 ± 0.220 ($P = 0.038$)	-0.119 ± 0.065 ($P = 0.038$)	0.765 ± 0.163 ($P = 0.006$)	0.135 ± 0.069 ($P = 0.005$)
Size	Body mass	0.546 ± 0.290 ($P = 0.128$)	0.114 ± 0.052 ($P = 0.031$)	-0.669 ± 0.255 ($P = 0.050$)	-0.070 ± 0.054 ($P = 0.047$)
	Eye diameter	-0.316 ± 0.315 ($P = 0.365$)	-0.041 ± 0.057 ($P = 0.359$)	0.731 ± 0.235 ($P = 0.036$)	0.039 ± 0.059 ($P = 0.021$)

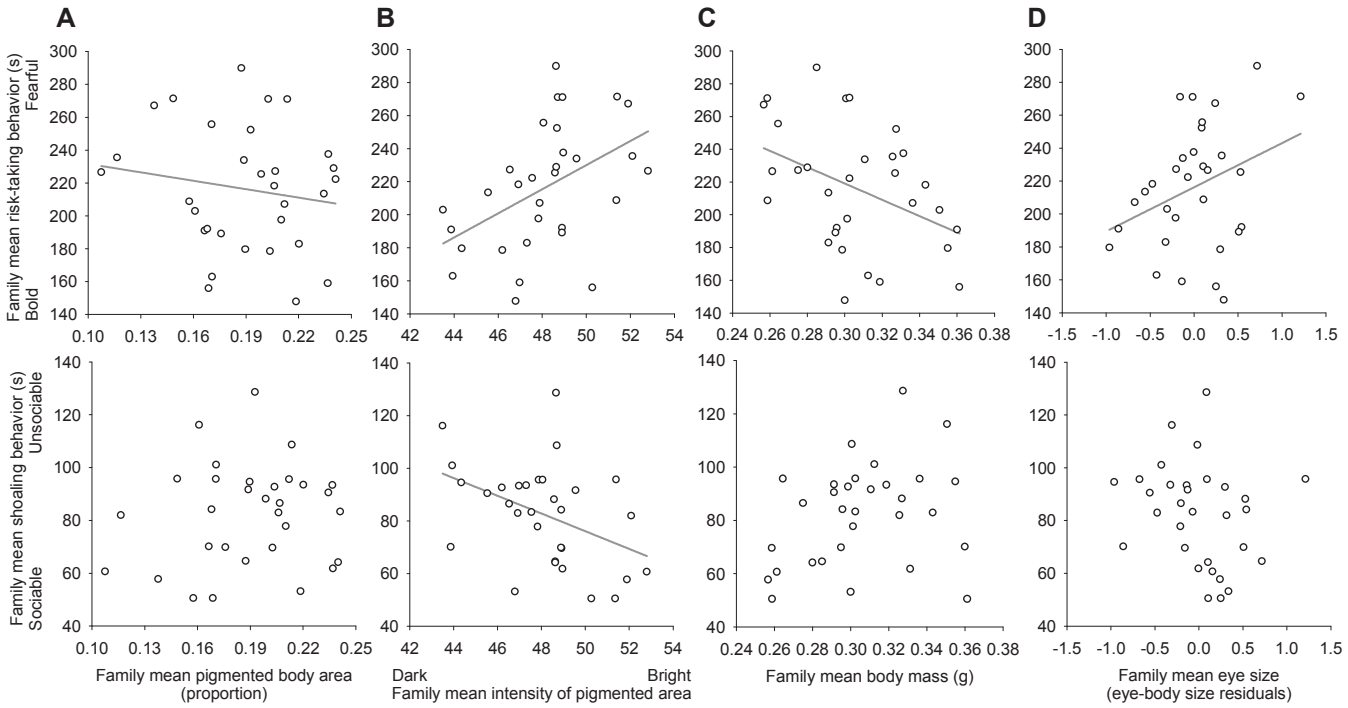

Figure 2. Family mean correlations ($n = 31$ full-sib families, 443 individuals) between behavior traits and different morphology traits, pigmented body area (A), color intensity of pigmented area (B), body mass (C), and eye size (D). Simple linear regression lines are shown for the relationships with a significant genetic correlation.

There were significant genetic and phenotypic covariances between body mass and risk-taking behavior, showing that increased body mass was associated with behaving bolder (Table 2; Fig. 2C). Shoaling behavior was significantly correlated to body mass only at the phenotypic level, with heavier individuals being unsociable to conspecifics (Table 2). Eye diameter (relative size to the lateral body area) was genetically and phenotypically correlated with risk-taking behavior but not with shoaling behavior. Individuals or genetic families with larger eyes were more fearful when foraging under predation risk (Table 2; Fig. 2D).

Shoaling and risk-taking behaviors were phenotypically correlated, with more sociable fish to conspecifics behaving bolder when foraging under predation risk, but there was no significant genetic correlation between the two behaviors in all the multivariate models (r_P, range between 0.193 ± 0.052 and 0.212 ± 0.049, $P < 0.001$; r_G, range between -0.225 ± 0.376 and -0.013 ± 0.413, $P > 0.597$).

Discussion

Recent studies have suggested that animal personality (behavioral integration) is driven by ecological factors such as competition for resource and predation pressure through direct interactions with environments or maternal effects (e.g., Bell and Sih 2007;

Giesing et al. 2011; McGhee et al. 2012; Laskowski and Bell 2013). Our study suggests that behavioral traits may have evolved for integration with camouflage traits to ensure that the individual functions properly to increase survival and reproduction (Brodie 1989, 1992; Forsman and Appelqvist 1998). Individual differences in the integrated phenotype within a population may reflect alternative solutions against predation risk. Here, we provide evidence that morphological traits, camouflage color, and eye and body sizes may be integrated with antipredator behaviors in juvenile three-spined sticklebacks by demonstrating the presence of significant phenotypic and genetic correlations between these heritable traits. Individuals with strongly melanized phenotype and genotype were less sociable to conspecifics, but bolder during foraging under predation risk. Individuals with faster growing phenotype and genotype were bolder, and those with lager eyes were more fearful. The antipredator phenotypes of sticklebacks can be more complex than described here if more than one morphology trait is simultaneously integrated with behaviors. Indeed, our factorial analysis indicates that different morphology traits were integrated across families and correlated with the two behavioral traits, suggesting a broader integration between behavior and morphology (Fig S1). Although it is necessary to explicitly assess the functionality of these integrated phenotypes against predation risk to make robust conclusions, it is possible that consistent selection for particular combinations of traits under predation pressure helped to maintain genetic covariances and polymorphism in melanin color, growth trajectory, and behavior patterns in this population. Thus, phenotypic integration may reflect fitness of several optimal trait-value combinations in an adaptive landscape (Wright 1988; Arnold et al. 2001).

Genes responsible for melanin pigmentation are highly conserved across vertebrates, and melanin-based coloration has a variety of adaptive functions, including mate choice, social conflicts, predation avoidance, and resistance to solar radiation (Hill and McGraw 2006; Hoekstra 2006). Nevertheless, in the present study, juvenile sticklebacks originated from a natural population showed a great within-population variation in melanin-based color traits, which possessed high and significant heritability. Melanization in the dorsal area produces fish countershading, a cryptic coloration in near-surface stream habitats (Johnsen 2002), and thus pigmentation phenotypes of prey can dramatically affect visual detection by predators (Magurran 2005; Maan et al. 2008). The color camouflage may allow the juvenile fish to increase food intake and avoid food competition by behaving bold and unsocial while suffering relatively low risk of detection and predation by aquatic bird, fish, and insect predators. Therefore, strong melanin coloration and bold unsocial behavior types could have integrated by nonrandom selection, leading to linkage disequilibrium, which is one of the principal causes of genetic correlations between different traits (Roff 1997).

Why then have the other genotypes that express bright color patterns been maintained in the population? Bright skin color may have other benefits, for example, by sexual selection on the brightness that stresses nuptial color (i.e., red cheeks and blue eyes) and increases chance of being detected by a female from a distance (McLennan 2007). Although mature males' skin color tends to turn brighter during reproduction, their melanophores developed during the early growth stage seem to maintain throughout the reproductive season (S.-Y. Kim, pers. obs.). Therefore, less melanized male fish may benefit from increased mating success, but this color phenotype should be coupled with fearful and sociable behavior types to increase survival during the vulnerable juvenile stage. Similarly, eye size and risk-taking behavior may be integrated because the relatively large eye size may confer advantages associated with mating (Rowland 1994; McLennan 2007) or detecting small prey (Land and Nilsson 2002), but large eyes can make individuals more conspicuous to predators (Cott 1940; Wickler 1968).

Personality-related behaviors can evolve if life-history trade-offs are linked to consistent behavior patterns (Wolf et al. 2007). In juvenile sticklebacks, individuals with high intrinsic growth rates can benefit from enhanced reproduction later in the life, but they should take more risk for foraging during the juvenile stage because they need more food for growth than slow-growing individuals (Biro et al. 2004). Therefore, between-individual differences in risk-taking behavior may mediate the trade-off between early growth and survival, thereby producing the phenotypic integration between behavior and body size.

An alternative explanation for the phenotypic integration between morphological and behavioral traits is that pleiotropic effects of the genes, for example, those regulating the synthesis of melanin, give rise to the covariance (Ducrest et al. 2008). Besides melanogenesis, the melanocortin receptors are also involved in diverse physiological and behavioral functions, including food intake, aggressiveness, sexual activity, and resistance to stress, in wild vertebrates (Ducrest et al. 2008; Kim et al. 2013; Fargallo et al. 2014). Therefore, the pleiotropic effects could account for at least a part of the covariance between melanin coloration and antipredator behaviors.

Integrated phenotypes probably generate different lifestyles in relation to habitat and life history (Forsman et al. 2008). Different integrated adaptations may explain the persistence of discrete morphs in polymorphic species (e.g., Sinervo and Lively 1996; Shuster and Sassaman 1997; Ahnesjö and Forsman 2003). Our study highlights that the coexistence of different complex phenotypes that adopt different combinations of strategies against predation risks may enable the maintenance of graded phenotypic variations of single traits within populations. The maintenance of within-population genetic (co)variations in complex antipredator strategies may be important when environmental conditions, such

as predator density and availability of food and shelter, change over time. On the other hand, directional selection on certain types of complex phenotypes can result in coupled diversification of different antipredator traits among populations or closely related species. For example, warning signal, chemical defense, and behavior have coevolved as a complex phenotype in poison frogs, resulting in divergence of correlated antipredator traits among populations or species (Pröhl and Ostrowski 2011; Santos and Cannatella 2011; Willink et al. 2013). The evolution of consistent individual differences in a suit of behaviors has been a mystery because we expect natural selection to favor flexibility of behavior to changing environmental conditions (Bell and Sih 2007). However, animal personalities can be given an adaptive explanation based on the insight of phenotypic integration that camouflage patterns and consistent antipredator behaviors are coupled for survival. Evolution may lead not to one winning design, but many combinations of different phenotypes.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Genetic integration between morphological and behavioral traits.