

Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*)

A.M. BELL

Center for Population Biology, University of California, Davis, CA, USA

Keywords:

aggression;
antipredator behaviour;
behavioural syndromes;
correlated traits;
personality;
stickleback.

Abstract

Behavioural syndromes are correlations between behaviours in different functional contexts. Behavioural syndromes are attracting the attention of evolutionary biologists because they mean that different behaviours might not be free to evolve independently of one another. In a landmark study, Huntingford (1976) showed that individual stickleback which were bold toward predators were also aggressive toward conspecifics and active in an unfamiliar environment. Here, I revisited the activity-aggression-boldness syndrome in stickleback and tested the hypothesis that correlations between behaviours might act as evolutionary constraints. I measured a suite of behaviours on wild-caught individuals and their offspring from two different populations and calculated heritabilities and genetic correlations between the different behaviours. I found that these behaviours were phenotypically and genetically correlated in one population but not another. On average, boldness and aggression were negatively related to each other across the populations. These results suggest that behavioural syndromes don't always act as evolutionary constraints.

Introduction

Observers of animal behaviour have long recognized that individuals often differ in behaviour (Clark & Ehlinger, 1987; Coleman & Wilson, 1998; Wilson, 1998). Upon detecting a predator, for example, some individuals might boldly approach the predator, whereas others might be more shy and immediately seek shelter (Magurran, 1993; Wilson *et al.*, 1994; Wilson, 1998). A number of studies have shown that some individual differences in behaviour extend to different functional contexts so that the individuals that are, for example, very bold, might also be relatively voracious (Riechert & Hedrick, 1993), or less conspicuous in courtship (Hedrick, 2000), or very aggressive (Huntingford, 1976). In other words, there is accumulating evidence for correlations between individual behaviours in different functional

contexts, also known as behavioural syndromes (Gosling, 2001; Sih *et al.*, 2004).

Interest in behavioural syndromes is gaining momentum because of their evolutionary significance (Wilson, 1998; Sih *et al.*, 2004). An important evolutionary consequence of behavioural syndromes is that correlated behavioural traits might not be free to evolve independently so that selection favouring one type of behaviour, e.g. boldness, could lead to changes in a completely different behaviour, e.g. aggression (Stamps, 1991). This hypothesis, hereafter referred to as 'the constraint hypothesis', assumes that behavioural syndromes stem from a shared proximal link between behaviours, e.g. a hormone with effects at more than one target tissue (Ketterson & Nolan, 1999) or the pleiotropic effects of genes. The constraint hypothesis assumes that the mechanisms underlying correlated traits do not evolve easily because they would require genetic changes or the evolution of different hormonal machinery, for example. As a result, the constraint hypothesis predicts that evolutionary changes in one behaviour result in changes in a correlated behaviour. In other words, the correlation between

Correspondence: A. M. Bell, Division of Evolutionary and Environmental Biology, Graham Kerr Building, University of Glasgow, Glasgow, Scotland G12 8QQ, UK.
Tel.: 0141 330 5985; fax: 0141 330 5971;
e-mail: alison.bell@bio.gla.ac.uk

boldness and aggression might act as a constraint on the evolution of either trait because evolution occurs along 'the line of least resistance' (Schluter, 1996).

Another view is that we should expect selection to favour behaviour that is domain-specific, or appropriate for a particular context (Wilson, 1998). In some environments, for instance, a positive correlation between aggression and boldness could be deleterious if ecological conditions favour individuals that behave timidly toward predators and aggressively toward conspecifics. As a result, individuals that are timid around predators and aggressive around conspecifics will outperform individuals that are generally aggressive in all contexts. In other words, selection should favour the ability to adaptively modulate behaviour according to the particular context, and should break apart maladaptive combinations of traits (Wilson, 1998). Following this adaptive reasoning, some researchers have argued that when correlations between behaviours exist, they might do so because a particular combination of traits works well together. That is, a behavioural syndrome might be an integrated suite of behaviours that is the result of selection, not a constraint on it (Cheverud, 1996). For example, individual male field crickets (*Gryllus integer*) which produce long calls, which are attractive to both females and predators, take more time to emerge from a safe refuge compared with short-calling males (Hedrick, 2000). The correlation between courtship and antipredator behaviour is adaptive because males with long calls compensate for this risky behaviour by behaving cautiously. This view, hereafter referred to as the 'adaptive hypothesis' states that a priori, we should not expect behavioural syndromes to exist unless the correlation, itself, is adaptive.

Although it is now generally appreciated that there is often considerable geographic variation in behavioural traits among populations (Foster & Endler, 1999) and there is accumulating evidence for correlations between behaviours (behavioural syndromes) within a population (Sih *et al.*, 2004), we know little about geographic variation in correlated behaviours. The 'constraint' and 'adaptive' hypotheses about the evolution of behavioural syndromes can help to build a framework for studying geographic variation in correlated behaviours because they make different predictions about how correlations between traits might differ from one environment to another, or about the evolutionary fate of behavioural syndromes. If behavioural syndromes are difficult to break apart because the shared mechanism underlying the different behaviours is difficult to modify, then a behavioural syndrome should be a general characteristic of a species, or ubiquitous across different populations (the 'constraint hypothesis'). On the contrary, according to the 'adaptive hypothesis', we should not necessarily expect to see the same correlations between behaviours across individuals in a different selective environment because another combination of traits might be favoured

in that environment or because selection might favour decoupling between the behaviours. These predictions have rarely been tested (Tulley & Huntingford, 1988; Hedrick & Riechert, 1989; Palmer & Dingle, 1989) and represent two extreme views, but they are central in assessing the pervasiveness and evolutionary significance of behavioural syndromes.

Threespined stickleback (*Gasterosteus aculeatus*) are suitable candidates for testing whether correlations between behavioural traits can act as evolutionary constraints because a behavioural syndrome has already been identified in a British population of this species. Relative to others, individual stickleback that actively explored an unfamiliar environment were also very aggressive and behaved boldly under predation risk (Huntingford, 1976). Moreover, stickleback are widely-distributed in the Northern Hemisphere, inhabit different selective environments and show tremendous geographic variation in morphology and behaviour, much of which has been attributed to differences in predation pressure (Reimchen, 1994, 2000).

Here, I compare the behaviour of stickleback from two different freshwater drainages in Northern California separated by over 240 km: the Navarro River (Mendocino County) and Putah Creek (Yolo County). Presumably, stickleback in the Navarro River and Putah Creek are the descendants of marine ancestors that independently colonized the streams over 10 000 years ago (Bell & Foster, 1994), and stickleback from Putah Creek and the Navarro River inhabit very different selective environments where different behaviours might be favoured. The Navarro River is one of the few rivers in California that is not dammed and is characterized by dramatic seasonal changes in flow. Stickleback in the Navarro River are subject to predation by piscivorous birds [e.g. great blue herons (*Ardea herodias*), green herons (*Butorides virescens*), belted kingfishers (*Ceryle alcyon*) and common mergansers (*Mergus merganser*)], fish [e.g. coho salmon (*Oncorhynchus kisutch*), steelhead (*Oncorhynchus mykiss*), coast range sculpin (*Cottus aleuticus*) and prickly sculpin (*Cottus asper*)] as well as garter snakes (*Thamnophis couchii*), and odonate naiad predators.

Putah Creek, on the other hand, is a regulated stream in the Central Valley of California. It is dammed at two locations and subject to less extreme fluctuations in flow and temperature than the Navarro River (Marchetti & Moyle, 2000, 2001). Because Putah Creek is not subject to dramatic seasonal flooding, it has abundant vegetation and habitat structure along its bottom, which provides refuge for stickleback and suitable habitat for their invertebrate prey. There are fewer vertebrate predators native to Putah Creek [chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout (*O. mykiss*), prickly sculpin (*Cottus asper*) and ruffle sculpin (*C. gulosus*)] than to the Navarro River, although Putah Creek is currently inhabited by a variety of introduced predators, such as bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus*

salmoides), most of which were introduced in the last 50 years or so (P. Moyle, personal communication).

In this study, I revisited the boldness-aggression-activity syndrome in threespined stickleback to test whether a behavioural syndrome can act as an evolutionary constraint. For simplicity, just considering the correlation between boldness and aggression, if we imagine that high levels of aggression are favoured in one population but not another population, the constraint hypothesis makes two predictions about evolutionary fate of the boldness-aggression behavioural syndrome (Fig. 1b). First, within the different populations, individual levels of boldness and aggression should be positively correlated with each other. That is, regardless of population of origin, fish that are bolder should also be more aggressive. Secondly, boldness and aggression should be positively related between the two populations so that the population which is, on average, more bold should also be more aggressive. In contrast, according to the 'adaptive' hypothesis, we do not necessarily expect to see the same relationships between behaviours either within or between populations. In fact, according to the adaptive hypothesis, if selection favours different levels of either boldness or aggression in different populations, a number of scenarios are conceivable (Fig. 1c). First, perhaps boldness and aggression are independent of one another within each population, but the average behaviours are positively related to each other between populations, i.e. the population that is more bold is also more aggressive. Alternatively, perhaps boldness and aggression are positively correlated within one population and negatively correlated within the other, and on average they are negatively related to each other between

populations. Finally, the behaviours might be positively related to each other within one population, but independent of each other in another population, and the means are negatively related to each other between populations. The point of going through these different scenarios is to emphasize that comparing the same suite of behaviours on individuals from different populations can tell us whether behavioural syndromes might act as evolutionary constraints. Although two populations are not sufficient to make claims about why traits might be correlated in some populations but not others, if just one population is an exception to the rule that boldness and aggression always go together, that is sufficient to reject the constraint hypothesis that the syndrome is a general characteristic of the species, i.e. one black swan is sufficient to reject the claim that all swans are white (Popper, 1968).

Therefore, to distinguish between these two hypotheses, I compared the relationship between boldness, aggression and activity in an unfamiliar environment within and between the Putah Creek and Navarro River populations. Activity in an unfamiliar environment is increasingly being recognized as an interesting measure of exploratory behaviour (Verbeek *et al.*, 1994) and an important component of 'personality' in other species e.g. (Sih *et al.*, 2003). In order to obtain more information about selective conditions in the different populations, I also measured morphological and life history traits such as the number of lateral plates and size of the egg mass. Finally, to help determine how easily the behaviours and correlations between them might evolve, I obtain estimates of quantitative genetic parameters such as heritabilities and genetic correlations within the two populations.

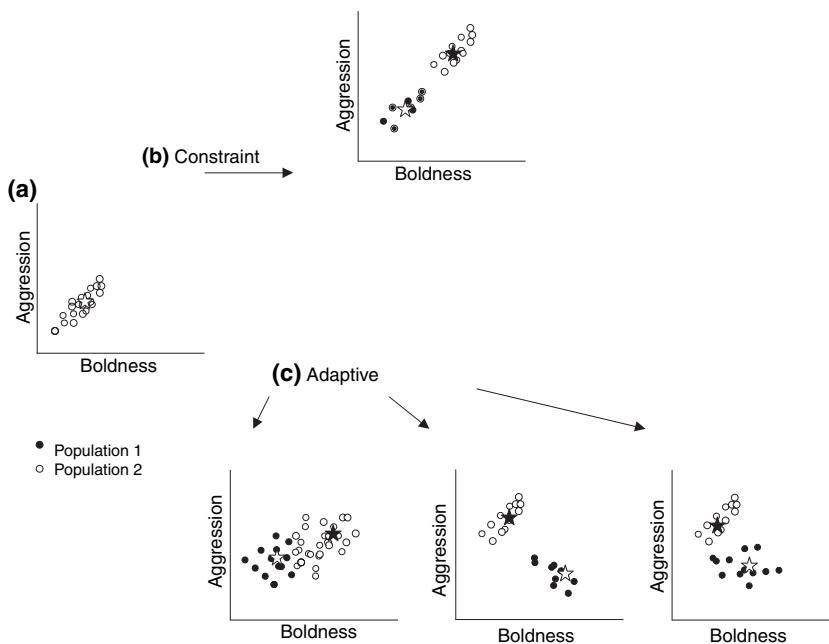


Fig. 1 Contrasting predictions of the 'constraint' and 'adaptive' hypotheses. Each data point represents a different individual, coded by population. Population means are marked by a star. (a) Huntingford, 1976 showed that individual stickleback that were more aggressive were also more bold under predation risk. (b) The constraint hypothesis predicts behaviours should be positively correlated across the populations. Within each population, the behaviours should be positively correlated across individuals. (c) The adaptive hypothesis predicts that we should not necessarily expect to see the same correlations between behaviours in different populations, and no a priori reason to expect the same relationships between traits within and between populations. See text for details.

Methods

This experiment was designed to measure how individual fish from the two populations behaved in three different contexts, and to assess whether there were correlations between individual behaviours in the different contexts. Therefore, for each fish, I measured three different types of behaviour (activity in an unfamiliar environment, aggression and boldness under predation risk). In a separate experiment, I measured the same behaviours on the lab-reared offspring of the fish described in this experiment on three occasions during development (Bell & Stamps, in press). The same behavioural protocol was followed with minor modifications (Bell & Stamps, in press). Specifically, in order to prevent the offspring from habituating to a particular predator, different predators were used at each age. Here, I use the data on the lab-reared offspring to calculate genetic variances and covariances for these behaviours, but concentrate on the data for wild-caught adults for phenotypic means and correlations.

I collected adult threespined stickleback from Putah Creek and the Navarro River between May and June 2000 with seines and minnow traps and brought them to the Institute of Ecology on the University of California, Davis campus. Fish were placed in 379-liter flow-through holding tanks ($18 \pm 1^\circ$ Celsius) and exposed to an ambient (Davis) photoperiod from collection to behavioural observations (2–6 weeks). The fish were fed frozen brine shrimp, live tubifex worms and trout chow (Silver Cup, Nelson and Sons, Murray, UT, USA) once a day. The experiment was conducted between 4 July and 9 August, 2000.

The fish spawned in individual tanks. The day after spawning, I removed the eggs from the male's nest and weighed the egg mass. To control for paternal effects (Tulley & Huntingford, 1987), I removed the eggs from the males' nests and artificially incubated the eggs. I then transferred the male and female singly to one of six 37.9-L observation tanks for behavioural observations. Each observation tank was bisected by a removable green mesh divider, which prevented the fish from crossing to the other side, and the tank had exterior markings, which visually divided the tank into 12 equally sized areas. One side of the tank was relatively 'empty', with few refuges (one terra cotta pot and a 1-L plastic bottle filled with water), whereas the other side of the tank was structurally 'complex' with many refuges (three plastic plants, a sponge filter, another terra cotta pot and a worm dispenser). At the start of the experiment, the fish were always placed in the 'empty' side of the observation tanks.

The fish were deprived of food 1 day prior to behavioural observations, while they were in the observation tanks. Prior to behavioural observations, between 12 and 15 h after the fish were introduced to the observation tanks, I inserted opaque dividers between the observation tanks to prevent visual contact between

the fish. I then observed each individual's ('focal fish') behaviour in three contexts in the following order: activity in an unfamiliar environment, aggression and boldness under risk. All behavioural observations took place between 08.00 and 18.00 hours. I observed the behaviour of the focal fish continuously and recorded the observations into a laptop computer using the event recording program *Observer* (Noldus Technology, 1991).

Behavioural assays

Activity in an unfamiliar environment

I gently lifted the removable mesh divider half-way out of the tank and recorded the number of seconds that elapsed until the fish crossed under the divider to the 'complex' side of the tank ('latency to explore'). I also recorded the number of times the fish froze, or maintained a still position without moving ('number of freezes'), and the number of different areas in the tank used by the fish ('area used') for 180 s. After the fish crossed to the 'complex' area, I removed the divider from the tank to allow the fish to swim freely throughout the tank. If the focal fish did not cross under the divider within 15 min after lifting the divider, I terminated the observation and excluded the focal fish from subsequent analyses (see below).

Aggression

Between 30 and 60 min after I observed the fish's activity in an unfamiliar environment, I placed a fish of the same sex and from the same population ('stimulus fish') into a 1-L transparent bottle located in the observation tank. The focal and stimulus fish were matched for size within 5 mm. After the focal fish first oriented (body facing toward the stimulus fish with binocular fixation) to the stimulus fish, I recorded the number of times the focal fish bit at the stimulus fish ('bites at conspecific') and the total amount of time (in seconds) it spent within one body length of the conspecific ('time with conspecific') for 5 min. If the focal fish did not orient to the conspecific within 10 min after I added the conspecific to the observation tank, I terminated the observation and excluded the focal fish from subsequent analyses (see below). At the end of the aggression observation, I removed the stimulus fish from the observation tank. At least five different fish were used as stimulus fish on a given day, and a particular fish was never used as a stimulus fish twice in a row.

Boldness under risk

Between 30 and 60 min after the stimulus fish was removed, I attached a great egret (*Casmerodius albus*) skull over the observation tank. Ten minutes later, I added 15–20 live tubifex worms to the worm dispenser. When the focal fish approached the food within one body length of the worm dispenser, I released the egret skull twice in quick succession, simulating a strike. The egret

skull was situated directly over the worm dispenser so that when I released the skull via a string and lever, the egret skull 'struck' within 4 cm of the worm dispenser. Following the simulated strikes, I recorded the focal fish's behaviour for 5 min. Specifically, I quantified the total time (in seconds) the fish spent foraging within one body length of the worm dispenser ('time foraging under risk') and the latency (in seconds) to the first bite at the food after the egret strike ('latency to forage under risk'). If the focal fish did not approach the food dispenser within 10 min after adding the food, I terminated the observation and excluded that individual from subsequent analyses (see below).

Following the observation of boldness under risk, I removed the focal fish from the observation tank. I then drained and replaced the water in the observation tank and added another focal fish. At the end of the experiment, the fish were sacrificed and measured for standard length, weight and the number of lateral plates on the left side of the body.

Data analysis

In order to summarize behaviour in each context, I used principal components analysis (PCA). There are two advantages to using component scores rather than single behaviours in analyses below. First, by performing statistical tests on a few component scores rather than many different variables, problems of multiple comparisons can be avoided. Secondly, component scores are standardized (between -3 and 3), regardless of the scale on which the single behaviours were measured. This facilitated the comparison of these data with the data on the lab-reared offspring, which were measured slightly differently (Bell & Stamps, in press). Separate analyses of the behaviours used in the PCA yielded the same results, see *Supplementary material*. I tested for normality of the component scores with the Kolmogorov–Smirnov test. Because the data were not normally distributed, I compared average differences between the populations and sexes with Mann–Whitney U tests. I examined correlations among each of the behavioural variables and body size (length and weight) for the populations separately with Spearman rank correlations. I asked whether populations differed in the relationship between behaviours by comparing the correlation coefficients of the two populations (Zar, 1999). All tests were two-tailed and statistical significance was inferred if $P < 0.05$ after the sequential Bonferroni procedure (Rice, 1989).

To estimate genetic variances and covariances based on eleven full-sib families per population, three individuals per family, I used a mixed model Bayesian strategy. The software used to implement this strategy is the commonly available MTGSAM [Multiple Trait Gibbs Sampling in an Animal Model (Van Tassell, 1995)] and the analysis was based on the resemblance between the wild-caught adults described here and their lab-reared offspring as well as full-sib resemblance, as described in (Bell &

Stamps, in press). An advantage of this approach is that all known relationships, e.g. parent-offspring and full-sib, can be incorporated into the analysis. The offspring of the fish described here were measured in each context on three occasions during development. Therefore, I estimated genetic variances and covariances in the three contexts by treating the offspring's repeated measure in the same context at different ages as an uncorrelated random effect ('permanent environment'). In order to allow for differences in the same context but at different ages, I included 'age' as a fixed effect in the model.

The advantage of using a Bayesian method is that I could obtain point estimates of heritabilities and genetic correlations as well as estimates of their distributions, including standard deviations. The distribution of variance and covariance components was estimated by numeric integration via Gibbs sampling (Geman & Geman, 1984). To choose starting values, I used the known estimates of phenotypic variance from the data, estimated additive and environmental variance assuming different heritabilities, and selected starting values for the final analysis based on their likelihood ratio. This strategy eventually produced solutions that were relatively insensitive to changes in starting values. The algorithm was based on the iterative generation of a sequence of random variables from the known conditional distributions of the parameters, given the likelihood function of the data. The parameter estimates were obtained by analysing this Gibbs sample. In this analysis, for each parameter to be estimated, I generated 20 000 samples of possible parameters. Parameter estimates were calculated as the mean of every 25th iterate, after discarding the first 10 000 samples, for a total of 7600 sample observations, i.e. $(20\ 000 - 10\ 000)/25$. The Gibbs sampling process and the theoretic basis for this analysis have been described elsewhere (Searle *et al.*, 1992). I obtained empirically based confidence intervals by dropping the lowest and highest 190 samples from the Gibbs sample ($0.025 \times 7600 = 190$) and noted the remaining highest and lowest values.

Overall, I observed the behaviour of 83 fish. Of these individuals, seven did not cross to explore the structurally complex side of the tank within 15 min, three did not orient to the conspecific within 10 min, and four did not approach the food within 10 min of adding the food and hence did not experience the simulated egret attack. I excluded these individuals from subsequent analyses. Complete datasets containing observations of individual behaviour in all three contexts were obtained for 71 animals ($n = 29$ Navarro fish, $n = 42$ Putah fish).

Results

Separate PCAs on behaviour in the three contexts compressed the data into a single component for each context (Table 1). For 'activity in an unfamiliar environment', the latency to explore the unfamiliar area loaded

Table 1 Results of principal component analyses on (a) activity in an unfamiliar environment; (b) aggression; (c) boldness under risk

Behaviour	Loading
(a) Activity in an unfamiliar environment	
Number of freezes	0.347
Latency to explore (s)	0.882
Area used	-0.877
Cumulative variance explained	55.6%
(b) Aggression	
Bites at the conspecific	0.910
Time spent with conspecific (s)	0.910
Cumulative variance explained	82.8%
(c) Boldness	
Time foraging under risk (s)	0.894
Latency to forage under risk (s)	-0.894
Cumulative variance explained	79.9%

positively on the component, although the amount of area used loaded negatively (Table 1a). To help make this axis more intuitive, I inverted the scale in statistical analyses, so that high values indicate an active fish, whereas low scores on this component indicate a fish, which took a long time to explore the unfamiliar area. For 'aggression', both the number of bites and the time spent with the conspecific loaded positively on the component; therefore high values on this component are indicative of a very aggressive fish (Table 1b). For the 'boldness under risk' context, the amount of time spent with the food loaded positively on the component, although the latency to forage loaded negatively (Table 1c). Therefore, high values of this factor indicate a bold fish which rapidly began foraging following the egret strike, and which spent a lot of time with the food.

Morphological and life history differences between the populations

There are some striking differences between the two populations in morphological and life history traits. Stickleback from Putah Creek were larger in standard

Table 2 Spearman correlations between body size and behaviours

	Length (cm)	Weight (g)	Activity	Aggression	Boldness
Length (cm)	-	0.838**	0.080	-0.053	0.027
Weight (g)	0.837**	-	0.007	-0.179	-0.097
Activity	0.016	0.085	-	0.167	0.434*
Aggression	-0.176	-0.169	0.094	-	0.520**
Boldness	0.079	0.094	0.149	-0.132	-

Values for Navarro correlations are above the diagonal, values for Putah correlations are below the diagonal. $n = 29$ Navarro fish, $n = 42$ Putah fish.

*Indicates a statistically significant correlation between traits after the sequential Bonferroni procedure.

length (Navarro: $4.50\text{cm} \pm 0.06$ SE, Putah: $4.72\text{cm} \pm 0.05$ SE, $F_{1,82} = 7.54$, $P < 0.01$) and had larger egg masses (Navarro: 64.65 ± 6.70 SE, Putah: 99.00 ± 6.73 SE, $F_{1,82} = 11.20$, $P < 0.01$) than stickleback from the Navarro River. Stickleback from the Navarro River had extensive armouring (lateral plates) along the sides of their bodies whereas Putah fish had fewer lateral plates (Navarro: 25.92 ± 1.27 SE, Putah: 7.10 ± 0.20 SE, $F_{1,45} = 172.75$, $P < 0.0001$). Within each of the populations, an individual's standard length or weight was not statistically related to its behaviour (Table 2).

Average behavioural differences between the populations

There was variation among individuals in their activity in an unfamiliar environment. Some individuals immediately crossed to the other side of the tank, whereas others waited over 5 min to explore the unfamiliar area. There was not a statistically significant difference in activity in an unfamiliar environment between stickleback from the Navarro River and Putah Creek (Fig. 2a, Mann-Whitney $U = 587$, n.s., $n = 71$). Females tended to be more active than males, but this was not a statistically significant difference (Fig. 2a, Mann-Whitney $U = 473$, $P = 0.07$, $n = 71$).

Individuals varied greatly in aggression. Some individuals bit at the conspecific over 640 times in 5 min, whereas others bit fewer than ten times. On average, fish from the Navarro River were much more aggressive toward the intruding conspecific than fish from Putah Creek (Fig. 2b, Mann-Whitney $U = 345$, $P < 0.01$, $n = 71$). There was not a statistically detectable difference in aggression between males and females in either population (Fig. 2b, Mann-Whitney $U = 622$, n.s., $n = 71$).

After the simulated attack by the egret, some individuals quickly resumed foraging on the worms, and continued to forage during the rest of the behavioural observation, whereas others scarcely foraged at all. Following the simulated egret strike, Putah fish were much more active and willing to incur predation risk in order to gain food than Navarro fish (Fig. 2c, Mann-Whitney $U = 286$, $P < 0.0001$, $n = 71$). For both populations, females tended to be more willing to risk exposure to a predator in order to gain food than males (Fig. 2c, Mann-Whitney $U = 443$, $P < 0.05$, $n = 71$).

Behavioural syndromes

Across individuals from the Navarro River, individual behaviour in a given context was frequently related to individual behaviour in a different context (Table 2). Boldness under predation risk was positively correlated with aggression toward a conspecific: individuals which spent a lot of time foraging following the egret strikes also vigorously bit at the conspecific (see Fig. 3). In addition,

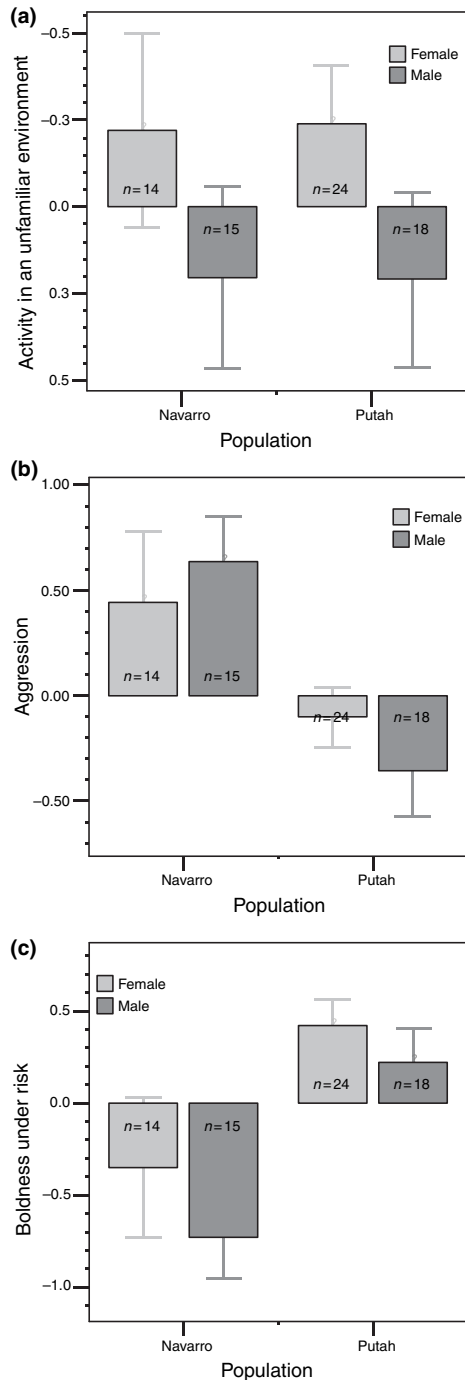


Fig. 2 Mean \pm SE differences in behaviour between the sexes and two populations. (a) Component scores on the 'activity in an unfamiliar environment' component by sex and population. There was no difference between the populations with respect to this behaviour. (b) Component scores on the 'aggression' component by sex and population. Fish from the Navarro River were significantly more aggressive than fish from Putah Creek. (c) Component scores on the 'boldness under risk' component by sex and population. Fish from Putah Creek were more willing to forage under predation risk than fish from the Navarro River.

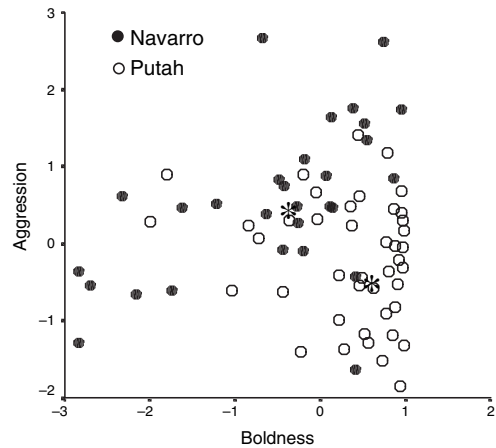


Fig. 3 The relationship between aggression and boldness. Each data point represents a single individual, coded by population. Population mean values are marked by a star. Aggression and boldness are significantly positively correlated among fish from the Navarro River, but not among fish from Putah Creek. The relationship between boldness and aggression is significantly different between the two populations (see text for details). See Table 2 for statistics.

boldness under predation risk was positively associated with active exploration of the unfamiliar environment. Individuals that spent a lot of time foraging under predation risk had a short latency to explore the unfamiliar environment. In other words, active explorers were also bold under predation risk.

In sharp contrast, across individuals from Putah Creek, there were no statistically significant correlations between behaviours in each of the different contexts (Table 2). Compared with Navarro fish there were more combinations of behaviours in the Putah population, or the Putah population inhabited more 'phenotypic space'. For example, although Navarro fish that were bold were also aggressive, some Putah fish were bold and aggressive, whereas others were bold and not aggressive and some were timid and aggressive, etc.

I asked whether the two populations differed in the relationship between behaviours by comparing correlation coefficients for the Putah and Navarro populations (Zar, 1999). The relationship between behaviour under predation risk and aggression was significantly different between the two populations ($Z = 2.79$, $P < 0.01$). There was not a statistically detectable difference between the two populations in the relationship between activity in an unfamiliar environment and behaviour under predation risk ($Z = 1.24$, $P = 0.107$).

Heritabilities and genetic correlations

Comparison of the relative estimates of genetic variances and covariances between the two populations reveals some intriguing differences (Table 3). First, in general, there is suggestive evidence for greater genetic variation

for these traits in the Putah population. For example, in the Navarro population, heritability estimates were 0.048 and 0.011 for activity in an unfamiliar environment and aggression, respectively. In contrast, in the Putah population, the estimates were 0.156 and 0.140, respectively. Secondly, there were significantly tighter positive genetic correlations in the Navarro population compared with the Putah population. For example, the genetic correlation between boldness and aggression in the Navarro population is 0.837, whereas the correlation in the Putah population is only 0.260.

Discussion

The hypothesis that behavioural syndromes can act as an evolutionary constraint predicts that boldness, aggression and activity in an unfamiliar environment should be positively related to each other both within and between different populations of stickleback. The results presented here do not support either of these predictions. Although bold Navarro individuals were also aggressive, there was no correlation between these behaviours within the Putah population. Moreover, the population that was, on average, more aggressive (Navarro) was actually *less* bold (Fig. 3). Although the means of the populations reported here represent just two independent data points, the results are not consistent with the proposition that boldness, aggression and activity in an unfamiliar environment are positively related to each other across stickleback populations generally.

It is worth noting that there was sufficient statistical power to detect a behavioural syndrome in the Putah population. Although Huntingford (Huntingford, 1976) detected strong correlations ($r = 0.48$) between boldness and aggression with only 25 animals, the sample size of fish from Putah Creek was almost twice that ($n = 42$), and my power to detect a similar correlation was >0.9 . Nor was the failure to detect correlations in the Putah population because of low variance in that population. In fact, Putah fish were more variable than Navarro fish with respect to each of the traits measured, but the variation was not aligned with another trait.

These results suggest that 'personality' traits can be readily uncoupled and that correlations between behaviours do not necessarily impose evolutionary constraints, but they beg the question as to why boldness and aggression might be associated in some populations but not others. Unfortunately, comparing the selective environment of just two populations is not sufficient to definitively infer which selective factors might be important. However, this study suggests that predation regime might be a key selective factor. For example, one of the most striking differences between stickleback from Putah Creek and the Navarro River concerns their morphological and behavioural antipredator defences. Navarro fish have extensive armouring in the form of lateral plates along the sides of their bodies, an effective morphological defence against toothed predators (Reimchen, 1994, 2000), whereas Putah fish have few lateral plates. Moreover, Navarro stickleback are much more timid, and show more pronounced antipredator behaviours than Putah stickleback. Therefore, the morphological and behavioural differences between the populations suggest that predation risk may have been higher in the Navarro River than in Putah Creek. Whether differences in predation pressure influences why behaviours are correlated in some populations but not others is a promising area for further investigation.

In further support of the argument that behavioural syndromes can be uncoupled evolutionarily, despite a genetic correlation between boldness and aggression in the Navarro population, boldness and aggression were independent of one another in the Putah population. This result, along with other studies (Weber, 1992), urge us to question the idea that genetic correlations impose constraints on the effectiveness of selection, and suggest that proximal mechanisms underlying correlated traits are not necessarily difficult to uncouple. The failure to detect the same genetic correlations within different populations, or intraspecific variation in syndromes of co-functioning traits, has also been found in other species, such as a migratory syndrome reported for some, but not all, populations of milkweed bugs (Palmer & Dingle, 1989).

A few other results from the heritability analyses are worth noting here. First, for all three behaviours,

Table 3 Heritabilities and genetic correlations for (a) Navarro and (b) Putah

	Activity	Aggression	Boldness
(a) Navarro			
Activity	0.048 (0.008–0.160)	0.699 (–0.134–0.946)	0.753 (0.010–0.980)
Aggression		0.011 (0.001–0.048)	0.837 (0.278–0.990)
Boldness			0.036 (0.006–0.135)
(b) Putah			
Activity	0.156 (0.028–0.442)	0.849 (0.221–0.989)	0.650 (–0.258–0.982)
Aggression		0.140 (0.024–0.358)	0.260 (–0.772–0.926)
Boldness			0.002 (0.002–0.073)

Heritabilities are on the diagonal, genetic correlations are on the off-diagonal. 95% confidence intervals are in parentheses.

heritability estimates were quite low. Low heritabilities are consistent with two hypotheses: they suggest that either strong selection has depleted genetic variation (low V_A) or they suggest that the traits are very sensitive to environmental influences (high V_E). Unfortunately, these two possibilities cannot be distinguished at present. Secondly, although the estimates of heritabilities were quite low, estimates of genetic correlations between traits were quite high. These results should be treated with caution because they were based on estimates from only eleven full sib families per population, have large confidence intervals, and estimates of genetic correlations are notoriously unstable (Falconer & Mackay, 1996). However, they do suggest that although there might be little genetic variation for these behaviours (low heritabilities), the few genes for these traits that are present in the populations affect more than one behaviour (large genetic correlations). Thirdly, estimates of genetic correlations were generally greater than estimates of phenotypic correlations. This trend ($r_G > r_P$) has also been noted for other species and for other traits (Roff, 1996).

It is worth noting that although these observations were conducted during the breeding season, for both populations, there was no difference in levels of aggression between males and females. At first glance, this may be surprising because male stickleback, which defend territories during the breeding season, are typically more aggressive than females during that time (Bakker, 1994). However, although reproductive, the fish had spent just 1 day in the observation tanks prior to behavioural observations, perhaps not long enough for males to become territorial. Perhaps, then, the aggression observed in this experiment was not territorial aggression, but a different form of aggression, such as from competition for limited food or refuges for hiding.

Altogether, these results suggest that behavioural syndromes do not necessarily act as an evolutionary constraint on optimal behaviour. However, the presence of syndromes in some circumstances but not others urges us to ask whether there are selective factors which favour the assembly of a suite of traits in some ecological conditions but not others, and to investigate the genetic and proximal mechanisms that allow such evolutionary flexibility.

Acknowledgments

Thanks to J. Feliciano, P. Crain, P. Moyle, M. Marchetti, M. Johnson, J. Kiernan and J. Steimetz for informative conversations about Putah Creek and the Navarro River, and access to unpublished data. Thanks to G. Nevitt for the use of her space at IOE. R. Cole, S. Ferranto, J. Watters, P. Lutes and B. Bentley provided critical logistical support. J. Stamps played a critical role in advising me throughout this work. Special thanks are due to T. Famula for his careful lessons on MTGSAM and Animal Models. Thanks to J. Stamps, A. Sih, H. Dingle, A. Hedrick, D. Reale,

N. Dingemanse and F. Huntingford for provocative discussions and comments on the manuscript. This research was supported by a dissertation improvement grant from the National Science Foundation, a STAR fellowship from the Environmental Protection Agency and the Center for Population Biology, UC Davis. The animals' care was in accordance with institutional guidelines (University of California, Davis, Animal Care Protocol No. 8938).

Supplementary material

The following material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/jeb/jeb817/jeb817sm.htm>

Correlation analyses of the raw behavioural data rather than PCA scores. The first correlation matrix is for the Navarro population, the second for the Putah population.

Fig. S1. Another version of Fig. 3, plotting the raw data rather than PCA scores.

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Received 15 March 2004; revised 1 July 2004; accepted 12 July 2004