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Behavioural syndromes differ predictably between 12 populations of three-spined stickleback

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Summary

1. Animals often differ in suites of correlated behaviours, comparable with how humans differ in personality. Constraints on the architecture of behaviour have been invoked to explain why such 'behavioural syndromes' exist. From an adaptationist viewpoint, however, behavioural syndromes should evolve only in those populations where natural selection has favoured such trait covariance, and they should therefore exist only in particular types of population.

2. A comparative approach was used to examine this prediction of the adaptive hypothesis. We measured behavioural correlations in 12 different populations of three-spined stickleback (*Gasterosteus aculeatus*) and assessed whether they indeed varied consistently according to the selective environment, where population was unit of analysis.

3. For a sample of fry from each population, we measured five different behaviours within the categories of (i) aggression (towards conspecifics); (ii) general activity; and (iii) exploration–avoidance (of novel foods, novel environments and altered environments).

4. We show that behavioural syndromes are not always the same in different types of stickleback population: the often-documented syndrome between aggressiveness, activity and exploratory behaviour existed only in large ponds where piscivorous predators were present. In small ponds where predators were absent, these behaviours were not (or only weakly) associated.

5. Our findings imply that population variation in behavioural syndromes does not result from stochastic evolutionary processes, but may result instead from adaptive evolution of behaviour favouring what should prove to be optimal trait combinations.

Key-words: adaptation, correlational selection, genetic constraints, personality

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Introduction

Many animal populations exhibit behavioural syndromes, individual differences in suites of correlated behaviours (Sih *et al*. 2004a), also referred to commonly as temperament (Réale *et al*. 2007) or animal personality (Gosling 2001). A behavioural syndrome is a characteristic of a population (Bell 2007) and exists whenever the same behaviour is correlated across different contexts, and/or when one type of behaviour is correlated with another of type behaviour. Examples of behavioural syndromes include positive correlations between aggressiveness in the context of territory defence and foraging in spiders (Riechert & Hedrick 1993), positive correlations between exploration and dispersal in birds (Dingemanse *et al*. 2003) and positive correlations between aggressiveness and boldness towards predators in fish (Huntingford 1976).

Behavioural syndromes may exist because of absolute constraints (*sensu* Hallgrímsson & Hall 2005) on the

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independent evolution of behaviours (Lande 1979; Loeschke 1987; Arnold 1992; Bell 2005) or because selection has favoured suites of correlated behaviours (Lande 1986; Price & Langen 1992; Dall, Houston & McNamara 2004; Dingemanse & Réale 2005; McElreath & Strimling 2006; Stamps 2007; Wolf *et al*. 2007). Despite rapidly accumulating evidence for the existence of behavioural syndromes in a wide variety of taxa (Réale *et al*. 2007), few empirical studies exist that have considered explicitly these evolutionary hypotheses (but see Riechert & Hedrick 1993; Bell 2005; Moretz *et al*. 2007; Bell & Sih, in press). Recently, however, Bell (2005) was able to reject the constraint hypothesis for the existence of a behavioural syndrome in three-spined stickleback *Gasterosteus aculeatus* by showing that the syndrome existed in a population with high numbers of predators, but not in a population with low numbers of predators. This finding implies that trait associations can, in principle, be altered in the course of evolution.

The population variation in behavioural syndromes of stickleback may exist either because selection favoured correlated behaviours only in particular types of selective environment (hereafter called the adaptive hypothesis), as hypothesized by Bell (2005), or because of stochastic processes such as mutation, drift, founder effects or gene flow (Lande 1992; Armbruster & Schwaegerle 1996; Whitlock, Phillips & Fowler 2002). Hence, rejection of the constraint hypothesis does not necessarily equate to acceptance of the adaptive hypothesis (contra Bell 2005). Of these explanations, fortunately, the adaptive hypothesis is the only one that generally predicts an association between a behavioural correlation and the environmental characteristics of a population. This unique prediction can be evaluated by means of a comparison, using replicates of different types of population. Therefore, this study is aimed specifically at evaluating whether behavioural syndromes exist only in particular types of population, where population is the unit of analysis.

Table 1. Time-table of experimental procedures (see Methods for more detail). The main behavioural tests are highlighted in bold type

Day	Action/behavioural test type
0	Capture and transfer to laboratory
1	Size measurements, individual housing (09.00–10.00 h)
1–4	Settling period, feeding training (16.00 h)
5	Transfer to experimental room (09.00–10.00 h)
5–7	Settling period, feeding training (16.00 h)
8	1. Activity and **aggression** test (09.00–12.00 h)
	2. Activity and familiar food feeding (16.00–18.00 h)
9	1. Activity and **novel environment** test (09.00–15.30 h)
	2. Activity and familiar food feeding (16.00–18.00 h)
10	1. Activity and **control for predator** test (09.00–12.00 h)
	2. Activity and **novel food** test (16.00–18.00 h)
11	1. Activity and **predator** test (09.00–12.00 h)
	2. Activity and familiar food feeding (16.00– 18.00 h)
12	Release into the wild

We examined whether behavioural traits were indeed structured more tightly into syndromes in populations that live sympatrically with predators (hereafter called predator-sympatric) compared with populations where predators were absent (hereafter called predator-naïve). For stickleback fry from each of six predator-sympatric and six predator-naive populations, we measured five behaviours indicative of (i) intraspecific aggressiveness; (ii) general activity; and (iii) exploration–avoidance (of novel foods, novel environments, and altered environments). We then analysed whether predation regime explained between-population variation in the within-population correlations between these behaviours.

Methods

STUDY POPULATIONS

The 12 study populations were all ponds on the island of Anglesey, North Wales, UK, about 20–100 years old and situated between 1·8 and 28·2 km apart. In six ponds, piscivorous predators (perch *Perca perca*, pike *Esox lucius* and/or rainbow trout *Oncorhynchus mykiss*) had been introduced (directly after the ponds were created) and maintained at high densities, and in a further six ponds piscivorous predators had not been introduced nor observed during any ecological surveys (Countryside Council for Wales, personal communication). The presence of piscivorous predators was correlated highly with pond size: piscivorous predators were present in the six largest ponds, but were absent in the smallest six ponds. Therefore, we do not attempt to differentiate between the effects of pond size and predation regime. We nevertheless consider predators to be the most likely cause of any possible selection pressure on behavioural syndromes (see Introduction and Discussion), and therefore use predator presence/absence as a label to distinguish small ponds lacking piscivorous predators from large ponds containing such predators.

Gene flow between these 12 ponds is largely restricted, and presence of piscivorous predators is independent of ancestry, as shown by phylogenetic analyses (Dingemanse *et al*., unpublished). Any evolutionary change in response to the presence of piscivorous predators will have been independent of ancestry, and population can therefore be treated as the independent unit of analysis.

EXPERIMENTAL PROTOCOL

Stickleback fry were collected in six batches over a period of 6 successive weeks in July/August 2003, several populations being sampled within each batch. Fish were transported to the laboratory, kept overnight in holding tanks, and then subjected to a series of experiments for a period of 12 days (see Table 1). The day following capture (day 1), size (standard length) of each fish was measured. Each individual was then

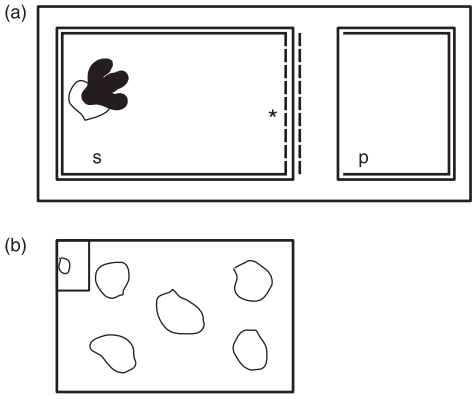

Fig. 1. Diagram of the experimental set-up. (a) Arrangement of the subject tank ('s') and experimental tank ('p') contained within a larger water bath (for dimensions see text). The subject tank contained a refuge (illustrated), and both tanks were lined internally on three sides with opaque white polythene. The asterisk (*) denotes the position where the intruder fish was introduced (aggression test) and where food was provided (during familiar food feeding and novel food test). The dotted line inside the subject tank indicates position of the opaque barrier that was removed at onset of the control for the predator test; the dotted line outside the subject tank shows position of same removable barrier in the 15-min period prior to onset of the predator test (for further details see Methods). (b) Set-up of the novel environment tank, showing the area in which the subject was confined directly after capture from its home tank (upper left corner), and the arrangement of stones during the subsequent test.

assigned to one of three size categories within batch and population: (i) 'small' (lower quartile); (ii) 'medium' (middle two quartiles combined); and (iii) 'large' (upper quartile). Only 'large' fish were used as experimental subjects ($n = 7$ per population; mean ± SE size = 20·81 ± 0·16 mm), while 'small' fish were used as stimuli in the aggression test described below ($n = 7$ per population; size = 17·18 ± 0·20 mm). This allowed easy identification of the subject fish vs. the intruder during interactions, and avoided issues connected with marking individuals. This selection procedure based on size did not bias the data, as size did not correlate with any of the assayed behaviours (see Results). Notably, the lack of correlation between standard length and behaviour has been confirmed in a recent breeding experiment (Dingemanse *et al*. in preparation).

Subjects were housed in isolation in tanks 30 × 20 × 22 cm (L × W × H), containing 10 cm depth of water, a filter with aeration and a clump of artificial weed attached to a stone (used as a refuge). Subjects were allowed to settle for 4 days (days 1–4), and were trained to feed on a familiar food item (one frozen bloodworm), provided daily at 16·00 h from a fixed location (see Fig. 1a). They were then transferred, in water, to individual tanks in an experimental room (day 5) that were lined internally with opaque white polythene to minimize external influences during the experiments. Each tank was positioned on top of a transparent sheet containing 126 uniquely numbered 2 × 2 cm squares to facilitate recording of subject location, and was placed 3 cm from an experimental tank (half the size of the home tank) within a large water-bath, to minimize potential visual interference created by air–glass–water interfaces (Fig. 1a). Subjects settled and were fed familiar food items for another three days (days 5–7), and were then subjected to a range of behavioural tests during the following 4 days (days 8–11). Fish were maintained at 19 ± 1 °C throughout, under a 15 h light/9 h dark illumination cycle.

BEHAVIOURAL TESTS

We measured three categories of behaviour (following ecological terminology outlined by Réale *et al*. 2007) that have often been found to constitute a syndrome, namely: *aggressiveness* (an individual's agonistic reaction towards a conspecific intruder); *activity* (the general level of activity in a non-novel and non-risky environment) and *exploration–avoidance* (an individual's reaction to a new situation). A different behavioural trial was carried out daily on each fish from day 8 to day 11. Each trial started by first scoring general activity of the subject in its own tank, followed by either an experimental test or feeding of the subject (see Table 1). Various behavioural measures were taken during each trial (listed in Table 2). Each trial was run by one of three observers, with trials being randomized between observers within batches. A randomized block design (Sokal & Rohlf 1995) was used for trials with respect to time of day and population, and all individuals received the tests in the same order to facilitate comparison between individuals.

ACTIVITY

Activity was measured in a non-risky and non-novel environment for eight different trials (two per day, days 8–11; Table 1), by observing movements of each subject within its home tank for a 60-s period. Position of the subject was noted every 5 s using point-time sampling.

AGGRESSIVENESS

Aggressiveness was measured as the subject's agonistic response towards a smaller stimulus intruder fish, introduced into the subject's home tank (day 8; Table 1; see location in Fig. 1a) for a period of 120 s, and then removed. This provides a more realistic stimulus than an intruder confined behind glass, in that the subject receives appropriate sensory feedback from both its own and the opponent's actions. Importantly, a glass container might have been perceived as a novel object, thus potentially confounding responses to the object with those to the conspecific in the behavioural measure obtained. While subject behaviour in both types of live interaction may depend in part upon that of the stimulus fish, in our case each stimulus fish was used only once to avoid pseudoreplication (Hurlbert 1984) – hence,

any variation in the behaviour of different intruders can simply be regarded as adding (unbiased) noise.

Stimuli fish were assigned to subjects according to their respective rank positions within their own size category (i.e. larger subjects paired with the larger of the small stimuli fish). All subjects acted aggressively towards the intruder, as manifested by chasing and attempted biting (rather than attempting to maintain proximity, i.e. shoaling), whereas the stimulus fish was generally submissive. The minimal distance between subject and stimulus and the number of times the subject attempted to bite the latter within each 10-s interval were measured.

EXPLORATION–AVOIDANCE OF A NOVEL ENVIRONMENT

On day 9 (Table 1), subjects were captured from their home tanks, transferred to a confined area (4 × 6 cm, six squares) in the upper-left corner of a similar-sized tank (Fig. 1b) and left to acclimatize for a period of 300 s. The barrier was then removed, allowing the subject to explore the remainder of this novel environment for a further 300 s. The tank contained five large stones, each covering an area of approximately 4 × 5 cm (see Fig. 1b). The tank was filled with only 5 cm of water to prevent the subject from swimming above the stones: information about the environment could thus only be gained by moving around the stones. Following release from the confined area, position of the subject was noted every 5 s using point-time sampling.

EXPLORATION–AVOIDANCE OF A NOVEL FOOD

On day 10 (Table 1) each fish received a piece of commercial fish flake (about 0·5 × 0·5 cm) instead of the food it was trained to receive during the preceding 9 days. The flake constituted a novel food type, and accordingly the fish showed inspection behaviour (as defined by Huntingford 1976) prior to biting the flake. The subject's latency until first inspection and latency until first bite were recorded, and the position of both the subject and the flake noted every 10 s for 2 min using point-time sampling.

EXPLORATION–AVOIDANCE OF AN ALTERED ENVIRONMENT

The initial aim of the behavioural test described here was to measure *shyness–boldness* (an individual's reaction to any risky, but not new, situation; Réale *et al*. 2007). This behaviour was measured by visually exposing the subject to a predator for 5 min. On day 11 (see Table 1), a live perch *P. fluviatilis* was introduced into the tank, the end of which was placed 5 cm from the end of the subject's tank (Fig. 1a). Fifteen minutes later, the opaque barrier visually isolating the two tanks was removed to reveal the predator. After barrier removal, position and angle of the subject's body (in steps of 45°) were noted every 5 s using point-time sampling. We further noted the total number of inspection bouts. Inspection bout was defined as starting when the subject faced the experimental tank while swimming towards the stimulus tank, and terminated when the subject turned away from the stimulus (Walling *et al*. 2004). Seventy individual perch (mean ± SE total length = 13·335 ± 0·162 cm, $n = 45$ measured) were used as stimuli.

To ascertain whether the elicited behaviour reflected boldness towards the predator, rather than exploration of the altered environment of the tank, we first ran the testing procedure without the predator. The day preceding the predator test (day 10, Table 1), the subject was shown an empty tank. This arrangement was left in place for 24 h to familiarize the subject with its altered environment. The barrier was replaced only 15 min prior to the onset of the predator trial, and then raised to commence the trial. Analyses revealed a relatively high repeatability (for a behavioural trait; Stirling, Réale & Roff 2002) at the individual level of the subject's behaviour (0·455; see Results; Table 3), with the effect of trial type being negligible (experimental day in Table 3; see Results). Behaviour in both trials therefore appeared to measure primarily how subjects reacted to an alteration of their physical environment (i.e. exploration, not boldness). We therefore regarded the predator and control trials as repeated measures of the same behaviour, hereafter referred to as exploration of an altered environment.

ETHICAL NOTE

Behavioural testing was conducted in accordance with UK regulations governing care and use of laboratory animals and had prior approval of the local Home Office. During the aggression tests, the small stimulus fish was always able to evade the subject easily, and no signs of injury were observed during this brief experience. Upon the completion of all tests within each batch, stickleback were released into a private pond in north-west Wales (day 12; Table 1), and perch were released back into ponds of the fishery from which they were obtained.

STATISTICAL ANALYSES

Extraction of behavioural axes

Principal component analysis (PCA) followed by varimax rotation (Tabachnick & Fidell 2001) was used to summarize the behavioural measures for each type of test (Table 2). Individuals were usually represented once in these analyses, but eight times for activity tests and twice for altered environment tests. The resulting PCA scores per individual were then used in further analyses.

We included repeated measures of the same individual within these PCAs for the activity and altered environment

Table 2. Results of principal component analyses on behavioural measures recorded, for each of five behavioural tests; (a) activity in a familiar environment; (b) intraspecific aggressiveness; and exploration of (c) a novel environment; (d) a novel food (fish flake); and (e) an altered environment (visual contact with predator or new adjacent place). Loadings, eigenvalues and explained variance are given for the emerging axes. Sample sizes are (a) $n = 8$ observations for each of 84 individuals (total $n = 672$); (b–d) $n = 1$ observation for each of 84 individuals (total $n = 84$); (e) $n = 2$ observations for each of 84 individuals (total $n = 168$)

(a) Locomotor activity	Component 1 (A1)	
Behaviour		
No. of unique squares visited	**0·98**	
Distance travelled	**0·96**	
Number of square changes	**0·99**	
Eigenvalue	2·85	
% variance explained	95·08	
(b) Aggression test	Component 1 (B1)	
Behaviour		
Mean minimal distance from intruder	**–0·71**	
Total number of bites	**0·90**	
Prop. of 10-s interval with biting	**0·94**	
Eigenvalue	2·19	
% variance explained	73·02	
(c) Novel environment test	Component 1 (C1)	
Behaviour		
No. of unique squares visited	**0·96**	
Distance travelled	**0·94**	
Number of square changes	**0·99**	
Eigenvalue	2·78	
% variance explained	92·70	
(d) Novel food test	Component 1 (D1)	
Behaviour		
Distance from food at start	0·39	
Latency to first inspection	**0·87**	
Latency to first bite	**0·87**	
Minimum distance from food	**0·89**	
Eigenvalue	2·45	
% variance explained	61·30	
(e) Altered environment test (predator test and control)	Component 1 (E1)	Component 2 (E2)
Number of square changes	**0·94**	–0·09
Mean angle of orientation	–0·04	**0·97**
SD angle of orientation	**0·70**	–0·54
Minimum distance from glass	**–0·76**	0·12
No. of inspections	**0·93**	–0·04
Eigenvalue	2·83	1·26
% variance explained	56·65	25·28

tests, thereby violating the assumption of independent observations (Tabachnick & Fidell 2001). Therefore, to confirm the validity of these analyses, we also ran PCAs for each trail separately (activity: eight PCAs; altered environment: two PCAs), which produced reassuringly very similar components (results not shown), thereby justifying combining these data from multiple trials in one analysis.

ESTIMATION OF INDIVIDUAL PHENOTYPES

Generalized linear mixed models (GLMM) were used to: (i) estimate effects of fixed effects that could bias the data (in our case, seasonal or time of day effects; Table 3); and (ii) subtract phenotypic values at the level of individual (so-called best linear unbiased predictors – BLUPS; Henderson 1975) while controlling for significant fixed effects. Population ($n = 12$) was fitted as a random effect; individual (nested within population; $n = 84$) was also fitted as a random effect for those tests where repeated measures of the same individual were taken (data points per individual, $n = 8$ in activity tests, and $n = 2$ in altered environment tests). The following continuous (co) and categorical (ca) fixed effects were fitted in the initial model (Table 3): week (co; to model effects of season), time of day (co), observers (c), mean population value of body size ('size population level'; co), and individual body size expressed as deviations from the population mean ('size individual level'; co). For activity, trial type (feeding vs. non-feeding trials; c) and experimental day (co) were also fitted as fixed effects to evaluate whether activity differed between the two trial periods and whether activity changed over days, respectively. Similarly, in the case of the altered environment test, experimental day (c) was fitted as a fixed effect to test for differences between the control and the predator trials. Backward elimination of non-significant fixed effects ($P > 0·05$) was used as a model selection criterion (Crawley 1993). To check whether lack of significance of certain fixed effects (see Results; Table 3)

Table 3. Sources of variation in activity in a familiar environment (A1 in Table 2a), intraspecific aggressiveness (B1 in Table 2b), and exploration of a novel environment (C1 in Table 2c), a novel food (D1 in Table 2d) and an altered environment (E1 in Table 2e). The results are from GLMMs with normal errors, with population (all tests) and individual nested within population (activity and altered environment tests) entered as random effects, using backward elimination of non-significant fixed effects. Population and individual repeatabilities (calculated after Rasbash *et al*. 2004) are given in parentheses; significant *P*-values are highlighted in bold type. For sample sizes see Table 2

					Exploration of:					
	Activity		Aggressiveness		Novel environment		Novel food		Altered environment	
Behaviour variable	$\chi^2_{d.f.}$	*P*	$\chi^2_{d.f.}$	*P*	$\chi^2_{d.f.}$	*P*	$\chi^2_{d.f.}$	*P*	$\chi^2_{d.f.}$	*P*
Fixed effects										
Final model*	$8{\cdot}966_2$	**0·011**§	NA	NA	$3{\cdot}827_1$	**0·050**	$11{\cdot}960_2$	**0·002**§	$10{\cdot}618_2$	**0·004**§
Week	$5{\cdot}134_1$	**0·023**	$1{\cdot}408_1$	0·235	$3{\cdot}827_1$	**0·050**	$9{\cdot}243_1$	**0·002**	$5{\cdot}914_1$	**0·015**
Observer	$1{\cdot}664_2$	0·435	$3{\cdot}733_2$	0·155	$3{\cdot}846_1$	0·146	$1{\cdot}885_2$	0·390	$1{\cdot}090_2$	0·580
Time of day	$0{\cdot}090_1$	0·764	$0{\cdot}204_1$	0·652	$0{\cdot}428_1$	0·512	$6{\cdot}953_1$	**0·008**	$0{\cdot}380_1$	0·538
Size (population level)	$0{\cdot}056_1$	0·813	$0{\cdot}040_1$	0·841	$1{\cdot}562_1$	0·453	$0{\cdot}250_1$	0·617	$0{\cdot}101_1$	0·751
Size (individual level)	$0{\cdot}000_1$	1·000	$0{\cdot}003_1$	0·956	$0{\cdot}271_1$	0·602	$0{\cdot}050_1$	0·823	$0{\cdot}016_1$	0·899
Experimental day	$3{\cdot}833_1$	**0·050**	–	–	–	–	–	–	$4{\cdot}704_1$	**0·030**
Trial	$1{\cdot}078_1$	0·299	–	–	–	–	–	–	–	–
Random effects										
Population (repeatability)	$1{\cdot}569_1$ (0·155)	0·210	$0{\cdot}070_1$ (0·018)	0·791	$3{\cdot}618_1$ (0·336)	0·057	$2{\cdot}837_1$ (0·244)	0·092	$1{\cdot}453_1$ (0·187)	0·228
Individual (repeatability)	$26{\cdot}231_1$ (0·424)	**< 0·0001** –	– –	– –	– –	– –	– –	– (0·455)	$12{\cdot}552_1$	**0·0003**

*Combined effect of all fixed effects kept in final model; NA: all fixed effects were non-significant. §Final model is significant after sequential Bonferroni adjustment of error rates.

was caused by over-parameterization of the models, we also re-ran each model including only one fixed effect at the time. These analyses gave the same outcomes (results not shown), implying that over-parameterization was not an issue here. We controlled statistically for significant fixed effects (see above) only in cases where the final model was significant after Bonferroni adjustment of experimental error rates (see below). We used the Wald statistic to evaluate statistical significance. BLUPS were expressed in SD units for further analyses.

SOURCES OF VARIATION IN BEHAVIOURAL SYNDROMES

For each population, we calculated Spearman's rank correlations between the BLUPS (see above) of each of the five behaviours. One-way analyses of variance (ANOVAS) were then used to assess whether behavioural correlations differed between predator-sympatric vs. predator-naïve populations, where population was unit of analysis. Correlation coefficients were Fisher's *Z* transformed prior to analysis (Zar 1999). Mann–Whitney *U*-tests were used to evaluate whether variances differed between the two types of population.

Sequential Bonferroni adjustment of experimental error rates was applied to account for multiple testing (Sokal & Rohlf 1995) in cases where 'families of tests' were conducted to evaluate the same hypothesis (Chandler 1995). Because such type I error control is over-conservative and may lead to type II errors (Moran 2003), we also present an overall test of each of the main hypotheses (Table 4), by counting the number of significant results and calculating the probability of finding that particular number by chance alone (where the value of *P* was calculated using a Bernoulli process, following Moran 2003).

The data were analysed using MLwiN version 2·0 (mixed model analyses; Rasbash *et al*. 2004) and SPSS version 13·0 (all other analyses; SPSS Inc. 1988). All tests were two-tailed and a critical value of $P = 0{\cdot}05$ was applied throughout.

Results

AXES OF BEHAVIOUR

In each of the five PCAs, the first principal component described the behaviour of interest (A1–E1 in Table 2). For all subsequent analyses, we therefore used the PCA scores derived from these first components.

A1 described 'activity in a familiar environment', with high positive loadings for all measures of activity.

B1 described 'aggressiveness', with positive loadings for both the number of attempted bites and the proportion of intervals with attempted biting, and negative loadings for the distance between the subject and the stimulus fish. Aggressive subjects actively chased the intruder.

C1 described activity in the novel tank, with high positive loadings for all measures of activity. Subjects actively inspected the environment, whereas they did not show such behaviour during the activity trials in

Table 4. Sources of variation in Spearman's rank correlations (r) between five behavioural traits. For each pair of behaviours, we present the average within-population $r \pm$ SE, separately for predator-naïve populations ($n = 6$) and predator-sympatric populations ($n = 6$), and test whether distributions of within-population correlations differ from zero. We further test (using one-way ANOVAs) whether population type explains variation in r ($n = 12$). Prior to analyses, correlations were transformed using Fisher's Z transformation for correlation coefficients (Zar 1999). Population is the unit of analysis, with significant P-values highlighted in bold type

	Small ponds without predators			Large ponds with predators			Population type		
Behaviour pairs	$r \pm$ SE	t_5	P	$r \pm$ SE	t_5	P	$F_{1,10}$	P	r^2
Activity–aggressiveness	–0·089 ± 0·106	–0·828	0·445	0·411 ± 0·056	6·456	**0·001***	17·036	**0·002***	0·593
Activity–novel environment	0·244 ± 0·115	1·912	0·114	0·754 ± 0·051	8·809	**< 0·001***	15·651	**0·003***	0·571
Activity–novel food	–0·363 ± 0·120	–2·437	0·059	–0·143 ± 0·103	–1·379	0·226	2·001	0·188	0·083
Activity–altered environment	0·286 ± 0·171	1·723	0·146	0·667 ± 0·050	8·061	**< 0·001***	4·578	0·058	0·245
Aggressiveness–novel environment	–0·202 ± 0·202	–1·053	0·340	0·436 ± 0·136	2·788	**0·039**	6·635	**0·028**	0·339
Aggressiveness–novel food	0·048 ± 0·183	0·368	0·728	0·232 ± 0·435	1·552	0·181	0·405	0·539	0·039
Aggressiveness–altered environment	–0·101 ± 0·096	–1·064	0·336	0·435 ± 0·135	2·659	**0·045**	8·169	**0·017**	0·395
Novel environment–novel food	–0·470 ± 0·135	–2·904	**0·034**	0·090 ± 0·115	0·799	0·461	8·471	**0·016**	0·404
Novel environment–altered environment	0·196 ± 0·081	2·352	0·065	0·623 ± 0·115	4·425	**0·007***	9·140	**0·013**	0·425
Novel food–altered environment	–0·202 ± 0·145	–1·303	0·249	–0·036 ± 0·193	–0·223	0·832	0·525	0·486	0·050
Number of significant tests			1 of 10			6 of 10		6 of 10	
P§			0·315			**< 0·001**		**< 0·001**	

*Significant after sequential Bonferroni correction.
§Probability of finding this number of statistically significant tests due to chance alone; value of P is calculated using a Bernoulli process (following Moran 2003).

their home tank. While swimming through the novel tank, the number of unique squares visited per minute was over 1·9 times higher than during the activity trial in the home tank prior to onset of the test (mean ± SE = 4·860 ± 2·400 vs. 2·496 ± 0·228; paired t-test: $t_{83} = -8{\cdot}874$, $P < 0{\cdot}0001$, $n = 84$ individuals). Activity in the familiar vs. the novel environment was not simply a repeated measure of the same trait, because the behaviours were not correlated tightly in all populations (see Results, section 'Sources of variation in behavioural syndromes'). We therefore considered the behaviour in the novel tank as a measure of 'exploration of novel environments' not activity, following Réale *et al.* (2007).

D1 described 'avoidance of novel food', with high positive loadings for time until first approach or biting, and high positive loadings for the subject's minimum distance from the food during the test. D1 did not simply reflect differences in hunger levels, because the percentage of fish biting the novel food within 30 s was relatively low (63·1%) compared with the percentage biting the familiar food on the preceding day (98·8%; logistic regression, $\chi^2_1 = 34{\cdot}743$, $P < 0{\cdot}0001$, $n = 84$ individuals) and the day after the novel food trial (97·6%; $\chi^2_1 = 31{\cdot}714$, $P < 0{\cdot}0001$, $n = 84$ individuals). D1 was multiplied by –1, so that in further analyses it indicated exploration – rather than avoidance – of the novel food.

E1 described 'exploration of the altered environment', with high positive loadings for variables describing movement, number of inspections and variation in the angle of orientation: inspecting fish moved towards the stimulus, then turned around, returned to cover and initiated a new inspection, whereas non-inspecting fish remained still and did not come near the glass interface. A second component (E2) described 'absence of attention to the stimulus', with high positive loadings for the mean angle of orientation.

SOURCES OF VARIATION IN SINGLE BEHAVIOURS

None of the behavioural traits were biased due to observer effects or subject size (Table 3). Two fixed effects that could potentially bias the data did explain significant variation in some of the behaviours (Table 3). First, activity (A1), exploration of novel food (D1) and exploration of altered environments (E1) decreased with week within populations. Secondly, exploration of novel food increased with time of day. To avoid potential bias, these effects were controlled for statistically in all further analyses (see Methods).

Although significant, exploration of altered environments was only 0·288 ± 0·105 SD units higher during the predator test than during the control test (experimental day in Table 3). Subjects showed only slightly more inspection behaviour when confronted with the predator than with an empty tank. This effect of test type did not differ between populations (GLMM with random slopes; interaction experimental day × population; variance in within-population slopes ± SE = 0·030 ± 0·068, $\chi^2_1 = 0{\cdot}193$, $P = 0{\cdot}660$, $n = 2$ observations for each of seven individuals within each of 12 populations), implying that predators did not elicit stronger additional responses from subjects that originated from predator-sympatric populations. Individuals were repeatable over the two trials (Table 3) whereas trial type explained only 5·3% of the within-individual variance. These two trials were therefore regarded as repeated measures of the same exploration behaviour of altered environments.

SOURCES OF VARIATION IN BEHAVIOURAL SYNDROMES

Predator-sympatric populations were characterized predictably by the presence of a behavioural syndrome: the average correlation was positive and significant for six of 10 behaviour pairs (Table 4). Activity, aggressiveness, exploration of novel environments and exploration of altered environments were all correlated within this type of pond: animals that were relatively aggressive were also relatively active in a familiar environment and were relatively explorative in novel and altered environments, compared to less aggressive animals. However, for two of these six cases, the average phenotypic correlation was not significant after accounting for multiple testing (Table 4), implying that these particular findings should be considered less than definitive. Nevertheless, the probability of finding six of 10 significant tests was significantly greater than expected by chance (Table 4), implying that the existence of a behavioural syndrome was supported with high confidence. Exploration of novel foods was consistently not part of the behavioural syndrome (Table 4; Fig. 2).

Predator-naïve populations were characterized predictably by the absence of a behavioural syndrome (Table 4; Fig. 2). The average correlation was significant for only one of 10 behaviour pairs: exploration of novel environments and novel foods were correlated negatively. This single significant correlation was, notably, not significant after accounting for multiple testing (Table 4). Moreover, the probability of finding one of 10 significant tests by chance was high (Table 4), suggesting that this result was likely to represent a type I error.

Consistent with the findings outlined above, behavioural correlations differed predictably between the two types of population: for six of 10 behaviour pairs, the average correlation was significantly more positive for predator-sympatric populations (Table 4). However, for four of these six cases, the average phenotypic correlation was not significant after accounting for multiple testing (Table 4), implying that these particular findings should be considered less than definitive. Nevertheless, the probability of finding this number of significant tests was significantly greater than expected by chance (Table 4), implying that the existence of predictable population differences in behavioural correlations was supported with high confidence.

The lack of correlation between behaviours in predator-naïve populations may have been caused either by a lack of between-individual variation in single behaviours (i.e. all individuals were of the same behavioural type), or by a true lack of association between behaviours (i.e. individuals did differ in single behaviours but at the same time different behaviours were simply not associated; see Coleman & Wilson 1998). We therefore tested for each trait whether the amount of between-individual variation differed between the two types of population, which was not the case (Table 5). Moreover, for two traits where we had multiple records per individual, significant and comparable individual repeatabilities were found within both types of population. Average within-population repeatability of activity in the familiar environment was 0·426 ($\chi^2_1 = 13{\cdot}166$, $P < 0{\cdot}0001$; hierarchical GLMM with observation, individual and population as random effects) for predator-naïve ponds, and 0·417 ($\chi^2_1 = 15{\cdot}186$, $P < 0{\cdot}0001$) for predator-sympatric ponds ($n = 8$ observations for each of seven individuals within each of six populations (total $n = 336$) in both cases); repeatability of exploration of altered environments was 0·552 ($\chi^2_1 = 8{\cdot}521$, $P = 0{\cdot}003$) for predator-naïve ponds, and 0·339 ($\chi^2_1 = 3{\cdot}832$,

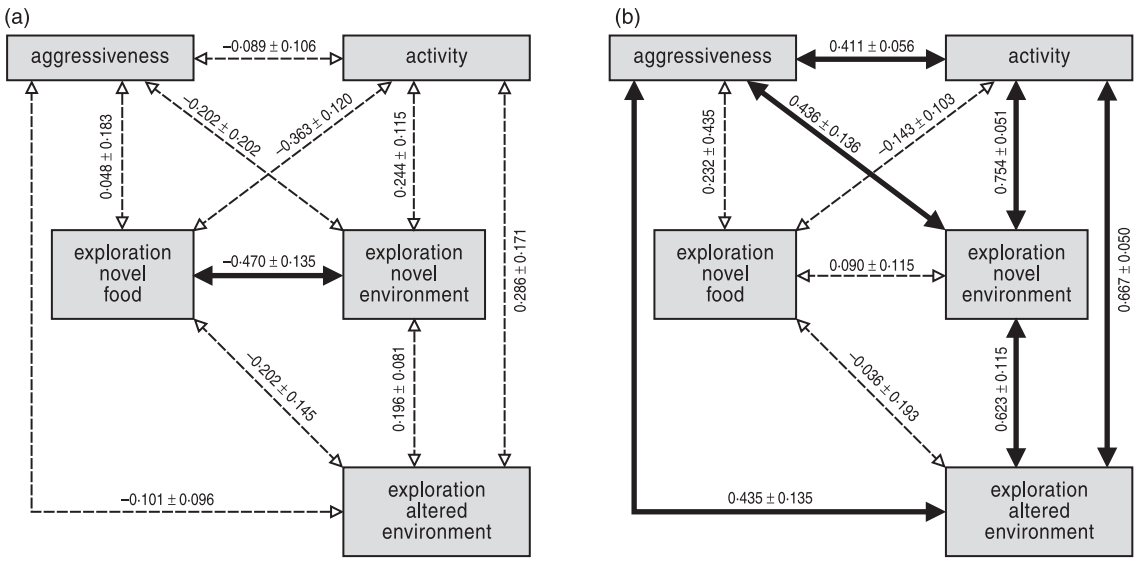

Fig. 2. Behavioural syndromes for two types of populations: (a) predator-naïve ($n = 6$); and (b) predator-sympatric ($n = 6$). For each pair of behaviours, we present the average within-population $r \pm$ SE. Solid lines indicate significant correlations ($P < 0{\cdot}05$; for statistical analyses see Table 4).

Table 5. Between-individual variances for five behavioural traits for each of 12 populations; we tested whether variances differed between predator-sympatric vs. predator-naïve populations

Behaviour			Exploration of:		
Population	Activity	Aggressiveness	Novel environment	Novel food	Altered environment
Predator-naïve					
Bengyl	3·211	0·589	0·760	0·313	1·308
Bryngwyn Bach	0·205	0·674	0·278	0·977	0·774
Cae Mawr	1·018	0·858	0·584	0·110	0·658
Cors Bordeilio	0·551	0·751	0·777	1·995	0·829
Llyn Grigyll	0·091	0·698	0·152	0·578	0·610
Rhos-y-Gad	0·067	1·206	0·439	0·867	0·753
Predator-sympatric					
Llyn Alaw	0·511	2·409	1·251	1·789	0·786
Llyn Coron	1·018	0·782	0·954	0·724	0·630
Llyn Llywenan	0·200	0·885	1·343	1·391	0·419
Llyn Pen-y-parc	1·165	1·087	0·234	1·210	0·978
Llyn Traffwll	1·123	0·639	0·956	1·308	0·510
Tacan	0·631	1·065	0·147	0·590	0·573
Mann–Whitney U	12	10	11	10	10
P	0·337	0·200	0·262	0·200	0·200

$P = 0{\cdot}050$) for predator-sympatric ponds ($n = 2$ observations for each of seven individuals within each of six populations (total $n = 84$) in both cases). The general lack of correlation between traits in predator-naïve populations was therefore not caused by a lack of individual variation. In contrast, it appears to be a genuine lack of association between behaviours in this particular environment.

Discussion

POPULATION VARIATION IN BEHAVIOURAL SYNDROMES

The adaptive hypothesis for the existence of behavioural syndromes predicts that they should evolve only in populations where they are favoured by natural selection. If so, then population characteristics should explain between-population variation in behavioural correlations that constitute syndromes. The results presented here confirm this prediction: behavioural correlations between aggressiveness, activity in the familiar environment and exploration of novel and altered environments were significantly tighter, or tended to be so, in predator-sympatric populations, when compared with predator-naïve populations (Table 4; Fig. 2). These findings imply that population variation in behavioural syndromes does not simply result from stochastic evolutionary processes (random effects of mutation, drift or founder effects). Our findings thus show that behavioural syndromes within a species are not ubiquitous, and that when they do exist they may well result from natural selection favouring optimal trait combinations.

The effect of population type (Table 4; Fig. 2) was significant not because the two types of populations differed in amount of phenotypic variation in behavioural traits: phenotypic variances (Table 5) and individual repeatabilities were similar between the two types of populations. Thus, the lack of correlations between behaviours in predator-naïve populations (Fig. 2a) was not because all individuals had the same 'behavioural type' (*sensu* Sih, Bell & Johnson 2004b): individuals did differ in their behaviour but the behavioural traits completely lacked, or showed very weak, covariance.

ENVIRONMENTAL CORRELATES OF BEHAVIOURAL SYNDROMES

Bell (2005) hypothesized that predators might shape behavioural syndromes in stickleback: aggressiveness, boldness and exploration were correlated tightly in one population with presumed high predation risk, whereas those traits were not correlated in another population with presumed low predation risk. Due to a lack of replication, she was unable to evaluate statistically the occurrence of a correlation between predation regime and behavioural syndromes. Using replicates of different population types, our study confirms that behavioural syndromes are indeed correlated with the presence of piscivorous predators.

It is important to note, however, that piscivorous predators are most likely to occur only in certain types of pond habitats and other correlated factors (e.g. competitive regimes, pond sizes, temperature regimes, habitat heterogeneity) may instead be the cause of between population variation in behavioural syndromes. We have therefore recently conducted an experiment to test whether piscivorous predators do tighten behavioural correlations (Dingemanse *et al.* in preparation). Experience with predators during ontogeny tightened the genetic correlation between activity and exploration of novel environments in stickleback fry originating from one of the predator-naïve populations. Therefore, we feel confident that piscivorous predators are the probable causal affect leading to the expression of behavioural correlations, as suggested by the results of the present study.

The obvious next step is to confirm the adaptive nature of behavioural syndromes in predator-sympatric populations, by testing experimentally whether predators do indeed induce correlational selection gradients (Dingemanse & Réale 2005), and the behavioural and selective mechanisms by which this effect comes about. Presence of predators may, for instance, induce spatial variation in predation risk within ponds and consequently favour the evolution of alternative behavioural strategies. As an effective antipredator strategy individuals might, for instance, either adopt a solitary strategy and monopolize a patch of habitat that is relatively poor foraging but safe, or adopt a shoaling strategy and roam patches of habitat that are relatively dangerous but likely to contain more food (Krause & Ruxton 2002). Such alternative strategies would have associated costs and benefits that are both density and frequency-

dependent (Wilson *et al*. 1994). They might also have different optimal levels of both aggressiveness and exploratory behaviour, resulting in a correlational selection gradient within predator-sympatric populations. Individuals adopting a solitary strategy might have to be relatively aggressive, because competition for safe patches would be intense. Individuals adopting a shoaling strategy, in contrast, might have to be relatively non-aggressive, because high levels of fighting might offset any benefits of shoaling. Individuals adopting a solitary strategy might also have to be relatively explorative compared with individuals adopting a shoaling strategy, in order to offset the lack of socially acquired information (Sih 1997). In freshwater fish, individuals with solitary tendencies are indeed relatively explorative and relatively aggressive (e.g. Ward *et al*. 2004). Importantly, predatory fish have recently been shown to induce correlational selection gradients in artificial laboratory environments (Bell & Sih, in press), but we do not yet know whether natural selection also favours the evolution of behavioural correlation in the wild. However, it is important to note here that various other scenarios are equally conceivable (Sinervo & Svensson 2002; Stamps 2007; Wolf *et al.* 2007), and more work is clearly required to elucidate why particular combinations of behavioural types coexist and certain others do not. Furthermore, future work must address why particular types of explorative behaviour are structured into syndromes whereas others, in our case exploration of novel foods (Fig. 2), are not.

Conclusions

In agreement with the predictions of the adaptive hypothesis for population variation in behavioural syndromes, we have shown that behavioural correlations covary consistently with aspects of the selective environment. These findings imply that behavioural syndromes are not fixed according to physiological and/or genetic constraints, and indicate that natural selection may have favoured the evolution of behavioural syndromes only in certain types of environment.

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