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Structural consistency of behavioural syndromes: does predator training lead to multi-contextual behavioural change?

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Abstract

Behavioural syndromes are suites of behaviours that are correlated across multiple contexts. Syndromes may occur in populations because behaviours are tightly linked by underlying mechanisms, such as genetics or physiology, which constrain flexibility and preclude multi-contextual plasticity. Alternatively, correlated behaviours may not share a common mechanism and may be able to change independently, allowing for potentially maladaptive combinations of traits to be broken apart. We tested these two hypotheses by training Trinidadian guppies (*Poecilia reticulata*) which possessed a behavioural syndrome encompassing three contexts, to avoid a potential predator. While we found no difference in magnitude of behavioural change between the trained and control groups, we did find that all subjects generally became shyer toward a potential predator following training and hypothesise that this resulted from sensitization and a predisposition to quickly recognize and adjust behaviour to predator-like stimuli. Importantly, behavioural changes in response to a potential predator did not generate changes in 'general activity' or 'exploration', and a tri-contextual syndrome broke apart. Our results suggest that in this population of guppies, individuals differ in behavioural plasticity in terms of their response to experience with predation risk and behaviours across contexts are domain specific and are able to change independently of each other. Future research should focus on populations that evolved in high predation environments and, therefore, may possess more rigid syndromes, to determine whether behavioural flexibility is limited.

Keywords

activity, behavioural syndrome, behavioural type, boldness, exploration, personality, *Poecilia reticulata*, predator training.

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1. Introduction

Behavioural syndromes are suites of behaviours, correlated across multiple contexts (Sih et al., 2004a,b), that influence the response of organisms in a wide-range of ecological situations (Riechert & Hedrick, 1993; Budaev et al., 1999; Marchetti & Drent, 2000; Fraser et al., 2001). Behavioural correlations appear to be adaptive in 'risky' environments and syndromes have been identified in populations of three-spined sticklebacks (*Gasterosteus aculeatus*) under significant predation pressure, but not in those where predation was reduced or absent (Bell & Stamps, 2004; Bell, 2005; Dingemanse et al., 2007, 2010). Syndromes, therefore, may not be present in all populations of the same species, but may instead be the result of adaptive evolution in response to local selection pressures. What is less understood is how experience and learning events that shape behavioural development affect the stability and structure of syndromes ontogenetically (Stamps & Groothuis, 2010a,b; Conrad et al., 2011).

Two possible explanations for the maintenance and flexibility of behavioural syndromes are the adaptive hypothesis and the constraint hypothesis. The adaptive hypothesis states that behaviour is domain specific and predicts that changing behaviour in one context would not lead to correlated changes that are potentially maladaptive in a different context (Wilson, 1998). Alternatively, the constraint hypothesis states that correlated behaviours are tightly linked, perhaps due to underlying physiological (Ketterson & Nolan Jr., 1999) or genetic (van Oers et al., 2004; Bell, 2005) mechanisms, and cannot change independently of each other. Thus, in populations possessing behavioural syndromes, learning and experience may not only change behaviours within single contexts, but may also lead to broader behavioural changes that impose a limit on optimal behaviour in certain situations.

Evidence supporting both of these hypotheses has been gathered from a few longitudinal studies that have examined how behavioural correlations change through ontogeny. Juvenile fishing spiders (*Dolomedes triton*) do not exhibit a multi-contextual syndrome for boldness, but adults do, indicating that syndromes are not a fixed trait within this species (Johnson & Sih, 2007). Dumpling squid (*Euprymna tasmanica*) are also not constrained in expressing context-specific shy/bold behaviour throughout development (Sinn et al., 2008). In contrast to these studies, Bell & Stamps (2004) studied a population of three-spined sticklebacks that evolved under strong predation pressure and found that behaviour fluctuated during development, but correlations remained stable, indicating behavioural types encompassing a syndrome rigidly changed together. Only a few studies (Carere et al., 2005; Brown et al., 2007; Frost et al., 2007) have examined how specific experiences or learning events alter behavioural types, and even fewer have examined how experience changes across-context correlations. One of the most notable was conducted by Bell & Sih (2007) who found that exposure to predation generated the previously absent bold-aggression syndrome within sticklebacks. We know of no studies, however, that have examined how experience or learning changes the structure of syndromes already present in a population prior to these specific events.

In an applied context, tightly linked behaviours that are constrained to change with each other could have significant implications for translocation and reintroduction programmes of endangered species that employ predator training (Griffin et al., 2000). Reintroductions often fail due to high mortality of captive-bred individuals exhibiting inappropriate anti-predator behaviours (Short et al., 1992; Miller et al., 1994; Banks et al., 2002). Predator training has, therefore, been discussed as a potential solution to this problem (Griffin et al., 2000). Predator-naïve animals often exhibit more fearful responses to predator stimuli after only a few training sessions (Miller et al., 1990; Maloney & McLean, 1995; Brown & Smith, 1998) and evidence suggests that the survival prospects of released animals are increased (Berejikian et al., 1999; van Heezik et al., 1999; Mirza & Chivers, 2000; Shier & Owings, 2006). Training animals to be more fearful or shy toward predators could, however, lead to unintended reductions in aggression or general activity levels in populations possessing stringent behavioural syndromes encompassing these traits. Such changes could lead to a reduction in foraging and growth rates (Huntingford et al., 1990; Mangel & Stamps, 2001; Höjesjö et al., 2002; Stamps, 2007) of reintroduced individuals and detrimentally impact long-term reproductive success (Smith & Blumstein, 2008).

In this study, we quantified the behaviour of Trinidadian guppies (*Poecilia reticulata*) in four different contexts to identify behavioural syndromes and then studied how predator training affected within-context behavioural consistency and the structural consistency (Stamps & Groothuis, 2010a) of multi-contextual syndromes. Behavioural changes in multiple contexts following training would support the constraint hypothesis that syndromes are tightly linked, while changes in only a predatory situation would support the adaptive hypothesis that syndromes can break apart through experience.

Predator training often involves pairing the sight of a predator with an aversive experience (Griffin et al., 2000), and our training protocol paired the site of a model predator with a simulated capture. This technique capitalizes on Pavlovian conditioning in which an unconditioned stimulus (US), which elicits a response without any prior training, is paired with a conditioned stimulus (CS), which elicits little response initially (Shettleworth, 1998; Domjan, 2005), leading to a new or an enhanced response to the CS through associative learning. Recent studies have used simulated capture as an aversive experience for conditioning in fish (Brown et al., 2007; Mesquita & Young, 2007). However, the one study we know of that paired a model predator with a simulated capture (Mesquita & Young, 2007) did not test whether changes in behaviour were due, specifically, to associative learning of the predictive relationship between the model predator and the simulated capture. Behaviour may change following a particular experience through other mechanisms independent of associative learning. For example, organisms may be primed to be attentive to and respond quickly to stimuli possessing characteristics representing a survival threat in their evolutionary past (Öhman & Mineka, 2001). Repeated exposure to such evolutionarily primed stimuli, particularly when experiencing a pre-existing state of fear, may then lead to heightened levels of wariness and sensitization toward similar stimuli (Shettleworth, 1998; Öhman & Mineka, 2001; Blumstein, 2006).

Therefore, to determine whether any observed changes in behaviour were due specifically to learning the predictive relationship between a model predator and simulated capture, we exposed a control group to both a model predator and simulated capture in an explicitly unpaired manner (e.g., Griffin et al., 2001). We then compared changes in behaviour to an experimental group in which a model predator and simulated capture were presented together such that the appearance of the model predator predicted an imminent simulated capture.

2. Materials and methods

2.1. Subjects and husbandry

Guppies are small (Max Standard Length (SL): males 3.5 cm, females 5.0 cm), live bearing, teleost fish native to the forest streams of South America and neighbouring islands (Endler, 1978). Variability in numerous phenotypic traits have been documented (Magurran & Seghers, 1994; Endler & Houde, 1995; Reznick et al., 2001) and guppies are, therefore, ideal for studies of intra-individual behavioural differences (Godin & Dugatkin, 1996; Budaev, 1997; Dugatkin & Alfieri, 2003). Thirty-seven subjects used in the current study were captive-bred for several generations and were descendents of individuals wild-caught in the Aripo River in northern Trinidad (G. Grether, personal communication). We tested only adult males because the behaviour of female guppies fluctuates drastically in relation to their ovarian cycle (Warren & Callaghan, 1975). Fish were maintained in 38-l mixed-sex tanks containing 20–30 individuals. They were fed twice a day with commercial flake food (TetraMin; TetraMin Pro, Tetra, Blacksburg, PA, USA), reared under an approximately 12:12 photoperiod, and the temperature of conditioned water (Start Right, Jungle Laboratories, Cibolo, TX, USA; proper pH 7.5, Aquarium Pharmaceuticals, Chalfont, PA, USA) was maintained at 23–26°C. This study received prior approval by the Institutional Animal Care and Use Committee (IACUC) at the University of California and husbandry protocols were reviewed annually to ensure proper compliance with the United States Department of Agriculture (USDA) Animal Welfare Act.

2.2. Experimental protocol

2.2.1. General design

Each subject was first exposed to four experimental treatments (pre-training), one per day between 0830 h and 1830 h over four consecutive days. Although there is some debate about whether it is better to test subjects in different contexts in a fixed or randomized order (e.g., Dingemanse et al., 2007), we chose to present experimental treatments in a randomized order because presenting them in the same order to all subjects could create an unavoidable confound. Each of the behavioural measurements would be influenced by the presentation order, not the experimental situation, and these carryover effects could heighten or diminish potential behavioural correlations (Logue et al., 2009; Dochtermann, 2010). Previous research we conducted with this population of guppies, in which we randomized the order of these same four treatments, indeed found that behavioural outcomes were influenced by the experimental situation, but not the order in which treatments were presented to subjects (Smith & Blumstein, 2010).

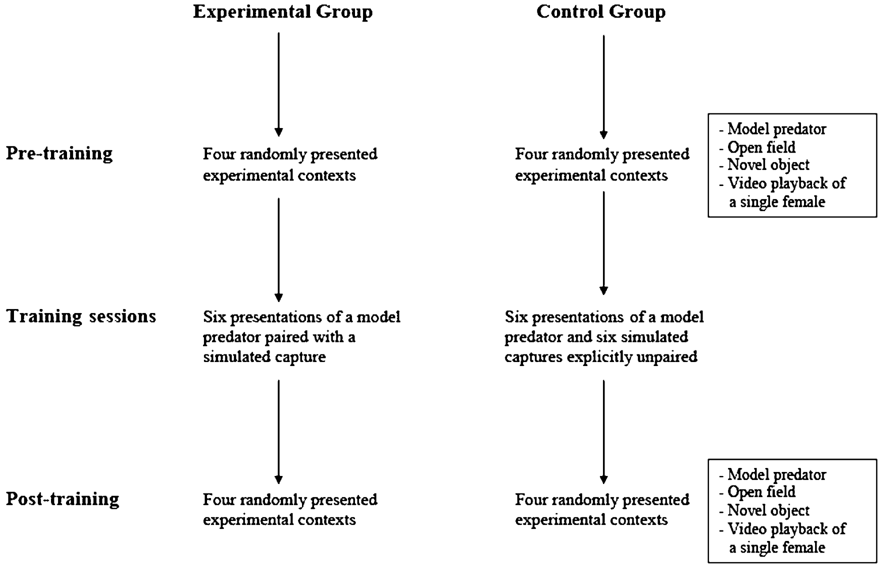

Figure 1. Schematic of the general design followed for the experimental and control groups.

We randomly assigned all subjects to either the paired experimental group or unpaired control group (Shettleworth, 1998) and then conducted training sessions during which a predator model was either paired or explicitly unpaired with a simulated capture. Previous studies that have used simulated capture have conditioned fish for 2 weeks or more (Brown et al., 2007; Mesquita & Young, 2007). Animals may, however, be predisposed to quickly learn predator-like stimuli and adjust their behaviour accordingly. Studies with other vertebrates have successfully enhanced anti-predator behaviour after only a few trials (Miller et al., 1990; van Heezik et al., 1999; Griffin et al., 2001). We, therefore, limited training to six sessions over three days to minimize the risk of habituation (Griffin et al., 2000). Following training, all subjects were exposed to the same four experimental contexts a second time (post-training) to quantify changes in behaviour and to determine whether associative learning had taken place. Figure 1 provides a schematic of the experimental design.

2.2.2. Pre- and post-training experimental contexts

One day prior to starting experiments, we selected males from communal tanks and moved them to 7-l holding tanks (Exo Terra plastic terrarium, Hagen, Mansfield, MA, USA) where they were housed individually throughout the study. Dividers between tanks blocked visual access to conspecifics. We conducted behavioural experiments in a 38-l tank measuring 51 × 27 × 32 cm (L × W × H) with a white-coloured gravel substrate and a grid (5 × 5 cm cells) for quantifying locomotion and location marked on the broad side of the tank. We used a video camera (Digital Viewcam, Sharp, Mahwah, NJ, USA) to record experiments, and an observer monitored each experiment from behind a black curtain. An opaque divider separated the experimental tank into two equal halves, with one half serving as the 'acclimation half', and the other, the 'experimental half', serving as the presentation platform for each treatment. Subjects were randomly chosen and placed individually into the 'acclimation half' of the tank for approximately 13 min. Following acclimation, the divider was remotely raised, exposing subjects to the treatment within the 'experimental half' of the tank, and their behaviour was recorded for 5 min. When each experiment was completed, the subject was removed from the experimental tank, 1/3 of the tank water was replaced with fresh conditioned water, and a mechanical filter (Whisper Power Filter, Tetra) with activated carbon was run for at least 20 min to remove latent chemical cues (B. Wisenden, personal communication) before using the experimental tank again. No more than eight experiments were conducted in a single day.

The four experimental treatments were designed to alter ecological context (e.g., Johnson & Sih, 2007), or perceived risk, and we interpreted each following the framework developed by Réale et al. (2007). A life-size model (10.5 cm SL, 13.5 cm total length) of a blue acara cichlid (*Aequidens pulcher*), a native guppy predator (Magurran et al., 1992; Brown & Godin, 1999; Coleman & Kutty, 2001), was used to measure 'boldness'. The model was constructed from a photographic print of *A. pulcher* in Axelrod et al. (1991, Plate 518), which was glued to a clear, acrylic glass backing, covered with epoxy resin (Ultra-Glo, Environmental Technology, Field Landing, CA, USA), and suspended in the water column by 0.15 mm monofilament line (see Coleman et al., 1985; Galvani & Coleman, 1998). An open field (empty tank) was used to measure 'general activity'. A novel object was used to measure 'exploration'. By definition a novel object is novel to an individual only once, so we varied this stimulus between pre- and post-training experiments. A bright-pink soap dish was used in pre-training and a plastic baseball in post-training. For the final context, we used video playback of a single female guppy recorded against a black background to measure 'sociability'.

We chose video playback rather than a live fish in order to standardize stimulus presentation across subjects (Kodric-Brown & Nicoletto, 1997). Fish respond similarly to recorded video and live conspecifics (Kodric-Brown & Nicoletto, 1997; Clark & Stephenson, 1999) and we used a liquid-crystal display (LCD) monitor (Envision, Fremont, CA, USA) to reduce the likelihood of subjects perceiving flickering images (D'Eath, 1998; Baldauf et al., 2008). Post-training experiments began the day following the last training session for each subject.

All pre- and post-training experiments were quantified from videotapes using the event recorder JWatcher (Blumstein & Daniel, 2007). We chose behavioural variables based on those that have been previously used to measure behavioural types in fishes (Huntingford, 1976; Budaev, 1997; Bell & Stamps, 2004), and based on a pilot study using 56 different guppies in which we identified correlated behavioural variables that varied among subjects. We measured boldness in the presence of a model predator by quantifying the latency to approach to within close proximity (defined as three body-lengths throughout this study) of the model and by counting the number of different areas within the entire grid (50 maximum) used by a subject. We measured general activity in an open field by counting the number of different areas a subject used throughout the entire tank, the number of different areas used in the 'experimental half' of the tank (25 maximum), and the total time spent moving in the 5-min trial. We measured novel object exploration by quantifying the number of different areas used throughout the entire tank, the number of approaches to within close proximity of, and the total time moving toward the object. Lastly, we measured the social response to the video of a female guppy by quantifying the latency to approach to within close proximity and total time spent within close proximity of the video screen.

2.2.3. Training sessions

All training sessions, for both the experimental and control groups, occurred within the 7-l holding tanks in which subjects were housed individually throughout the study. The experimental group was trained twice a day for three consecutive days with at least 270 min separating the sessions within a single day. Training began when the tank lid was removed and a subject was allowed to acclimate for 1-min. A model representing *A. pulcher*, suspended from above by monofilament line, was then immersed in the tank for 3 s, after which we simulated capture by chasing the subject with an aquarium dip-net for 30 s. The appearance of the conditioned stimulus (predator model), therefore, reliably predicted the appearance of the unconditioned stimulus (simulated capture) (Shettleworth, 1998). To minimize risk of habituation, the location of the model presentation within the tank was systematically varied from session-to-session (Griffin et al., 2000), and training order was randomized across subjects.

For three consecutive days, the control group also underwent two simulated captures per day and were exposed to a model predator twice a day, but they did not experience the same predictive relationship between the conditioned and unconditioned stimuli (e.g., Griffin & Evans, 2003). By doing so, we created a design that permits us to focus on the effects (if any) of associative learning (Shettleworth, 1998). In our control sessions, the two stimuli were presented at least 90 min apart with half of the pairings presenting the model first in a systematically varied fashion, and the other half simulating a capture first. Exposure to the model predator began when the tank lid was removed and a subject was allowed 1 min to acclimate. The model was then immersed in the tank for 33 s, thus providing the same exposure time as in the experimental group. A simulated capture followed a 1-min and 3-s acclimation period after the tank lid was removed. Thus, the 30-s simulated capture began after an equivalent time interval as that in the experimental group.

Our control group protocol required us to remove the tank lid four times per day, while training of the experimental group required removal of the lid only twice per day. To control for this additional exposure, the experimental group also underwent two blank sessions per day in which the lid of their tanks were removed for 1-min and 33-s. These blank sessions took place in between the two training sessions each day and were separated by at least 90 min. Thus, tank lids for both the experimental and control groups were removed four times per day during each of the 3 days of training.

2.3. Statistical analysis

2.3.1. Identifying pre-training behavioural syndromes

We used separate principal component analysis (PCA) for each context and extracted components with eigenvalues greater than 1. We then used estimated PCA loadings of quantified variables to calculate an individual's component score. We calculated two-tailed Pearson correlation coefficients between component scores across experimental contexts with our alpha set to 0.05. Significant correlations across contexts were interpreted as defining a behavioural syndrome (Sih et al., 2004b). We also calculated the intraclass correlation coefficient of component scores that comprised a syndrome to estimate the consistency of scores across contexts.

2.3.2. Question 1: Does predator conditioning occur through associative learning?

To test whether guppies learned to associate a model predator with an aversive experience we calculated the change in behaviours quantified in response to a model predator between pre- and post-training (e.g., pre-/ post-response difference, Griffin et al., 2001). We then compared the mean pre-/post-response difference of the experimental group to that of the control group using independent samples t-tests and Cohen's d (Cohen, 1988) to estimate effect size. This allowed us to determine whether changes in behaviours following training sessions were specifically due to associative learning. Because our a priori prediction was that the experimental group would behave differently than the control group in response to a model predator following associative learning, we used one-tailed comparisons (also see Griffin et al., 2001).

We then examined how behaviours measured in correlated contexts changed as a function of associative learning. We compared mean pre-/post-response differences of behaviours between the experimental and control groups using t-tests (two-tailed) and Cohen's d.

2.3.3. Question 2: Does multi-contextual behavioural change occur after predator training?

To determine whether aversive experiences (e.g., simulated capture), independent of associative learning, changed individual behaviour, we pooled together data from all 37 subjects and calculated Spearman rank order correlation coefficients between pre- and post-training behaviours quantified during each of the experimental contexts. We also compared mean behaviours between pre-and post-training using paired t-tests and Cohen's d. To determine whether syndromes identified during pre-training remained intact, we again used separate PCA for each context and calculated two-tailed Pearson correlation coefficients between component scores across experimental contexts. To facilitate comparison of pre- and post-training component scores, we transformed post-training scores to the same scale as that of the pre-training scores by calculating post-training scores based on principal components from the pre-training experiments. We accomplished this by standardizing post-training behavioural variables using the mean and standard deviation of pre-training behavioural variables. We then multiplied each standardized variable by the respective loading from the pre-training PCA, added these variables together, and divided the total by that component's pre-training eigenvalue. We calculated Spearman rank order correlation coefficients between pre-training and transformed post-training component scores to determine whether individual component scores estimated for each context were consistent following simulated capture events. We then subtracted pre-training component scores from the transformed post-training scores to obtain pre-/post-response difference scores and compared the mean pre-/post-response difference scores for each experimental context using one-way ANOVA.

3. Results

3.1. Identifying pre-training behavioural syndromes

For each of the four contexts, the behavioural variables loaded onto a single component that explained 57–85% of the variance in observed data (Table 1, Pre-training). For the model predator context, the number of different areas used throughout the entire tank loaded positively, while latency to approach the model loaded negatively. Subjects that scored highly on this 'boldness' component approached the model predator more quickly and used several different areas while in the presence of the model. For the open field test, the number of different areas used in the 'experimental half', the number of different areas used throughout the entire tank, and the total time moving all loaded positively on a 'general activity' component. Subjects that scored highly on this component spent much of the time moving and used several different areas of the tank. For the novel object context, the number of different areas used throughout the entire tank, the number of approaches, and the total time moving toward the object all loaded positively. Subjects that scored highly on this 'exploration' component approached the novel object more often, spent more time moving toward it, and used many different areas of the tank. For the response to a video of a female, time spent within close proximity of the monitor loaded positively while latency to approach loaded negatively. Subjects that scored highly on this 'sociability' component approached the video monitor more quickly and spent more time near it.

Table 1.
Component loadings for quantified variables and total variance explained by each component that resulted from separate PCAs on experimental contexts during pre-training and post-training.

Behaviour		Loading
Pre-training		
‘Boldness’ component		
Total area used		0.923
Latency to approach		−0.923
Cumulative variance explained	85.2%	
‘General activity’ component		
Experimental area used		0.948
Total area used		0.943
Time moving		0.700
Cumulative variance explained	75.9%	
‘Exploration’ component		
Total area used		0.873
Number of approaches		0.869
Time moving toward object		0.452
Cumulative variance explained	57.3%	
‘Sociability’ component		
Time spent within close proximity		0.876
Latency to approach		−0.876
Cumulative variance explained	76.7%	
Post-training		
‘Boldness’ component		
Total area used		0.922
Latency to approach		−0.922
Cumulative variance explained	85.0%	
‘General activity’ component		
Experimental area used		0.953
Total area used		0.953
Time moving		0.335
Cumulative variance explained	64.3%	
‘Exploration’ component		
Number of approaches		0.888
Total area used		0.815
Time moving toward object		0.587
Cumulative variance explained	59.9%	

The ‘boldness’, ‘general activity’ and ‘exploration’ components all correlated with each other and, therefore, comprised a ‘bold/active/explore’ syndrome (Table 2, Pre-training). The intraclass correlation coefficient for the

Table 2.
Pearson correlation coefficients between experimental context component scores for pre-training and post-training.

	‘General activity’	‘Exploration’	‘Sociability’
Pre-training			
‘Boldness’	0.383 ($p = 0.019$)	0.363 ($p = 0.027$)	0.239 ($p = 0.155$)
‘General activity’	–	0.505 ($p = 0.001$)	0.149 ($p = 0.379$)
‘Exploration’	–	–	0.059 ($p = 0.727$)
Post-training			
‘Boldness’	0.080 ($p = 0.637$)	0.131 ($p = 0.440$)	–
‘General activity’	–	0.460 ($p = 0.004$)	–

three components encompassing this syndrome was significant ($r = 0.424$, $p < 0.001$), indicating that individuals behaved consistently across these three contexts. The ‘sociability’ component did not correlate with any other component (Table 2, Pre-training). Because the purpose of our study was to measure the effects of predator training on behaviours in correlated contexts, the ‘sociability’ component and behaviours quantified in response to the video of a female guppy were excluded from post-training analyses.

3.2. Question 1: Does predator conditioning occur through associative learning?

We found little evidence of behavioural changes following predator training that were the result of associative learning. Although Cohen’s *d* indicated a potentially medium effect (Cohen, 1988) of training on total area of the tank used when subjects were exposed to a model predator (Table 3), the mean pre-/post-response difference of quantified behaviours toward the model predator did not differ between the experimental and control groups (Table 3, Model predator), indicating that an associative training method did not systematically change behaviour. In addition, there were medium effects (Cohen, 1988) of training on total time moving in the open field test and on time moving toward the novel object (Table 3). In general, however, we found no difference between the experimental and control groups in quantified behavioural changes in response to the open field and the novel object (Table 3).

Table 3.
Mean ± SE of experimental (Mean_{exp}) and control (Mean_{con}) groups, Cohen's d, and independent samples t-value for pre-/post-response difference in quantified behaviours during model predator, open field test and novel object.

Behaviour	Mean_{exp}	Mean_{con}	d	t	p
Model predator					
Total area used	-2.79 ± 2.46	-6.39 ± 2.54	0.33	1.018	$0.158^{\dagger}$
Latency to approach (s)	68.50 ± 26.70	56.98 ± 32.87	0.09	0.273	$0.393^{\dagger}$
Open field test					
Experimental area used	2.00 ± 1.78	3.06 ± 2.42	0.12	0.354	0.725
Total area used	2.00 ± 2.16	3.39 ± 3.37	0.11	0.350	0.728
Total time moving (s)	-3.31 ± 9.15	13.67 ± 10.47	0.40	1.225	0.229
Novel object					
Total area used	1.26 ± 2.54	-1.78 ± 3.36	0.24	0.726	0.473
Number of approaches	0.21 ± 0.45	0.56 ± 0.47	0.18	0.533	0.597
Time moving toward object (s)	-6.10 ± 5.88	6.44 ± 5.65	0.51	1.535	0.134

† One-tailed comparison, $N = 19$ Experimental group, $N = 18$ Control group.

3.3. Question 2: Does multi-contextual behavioural change occur after predator training?

When we pooled data from both the training and control groups, we found a significant difference between pre- and post-training experiments in behavioural responses toward the model predator. Cohen's d values indicated medium effects (Cohen, 1988) of training on behaviour as subjects generally took longer to approach the model predator and used fewer areas of the tank while in the presence of the model (Table 4, Model predator). However, the rank order of individuals' latency to approach a model predator was unrelated between pre- and post-training (Table 4), indicating that individual differences in behaviour were not consistent in this context. By contrast, behaviours quantified in an open field and in response to a novel object (Table 4) were unchanged and Cohen's d values suggest little to no effects of training in these contexts. Spearman correlation coefficients also indicated that rank-order of all quantified behaviours was consistent for these two contexts between pre-and post-training (Table 4).

These behavioural changes following training altered the structure of behavioural syndromes compared to pre-training in that the bold/active and bold/explore syndromes broke apart. Quantified behavioural variables for each of the post-training contexts again loaded onto a single component that

Table 4.
Mean ± SE of quantified variables from all subjects ($N = 37$) for pre- ($\mathrm{Mean}_{\mathrm{pre}}$) and post- ($\mathrm{Mean}_{\mathrm{post}}$) training experiments, Cohen's d, paired t-value, and Spearman rank order correlation coefficient (r_{s}) between pre- and post-training behaviours during model predator, open field test and novel object.

Behaviour	$\mathrm{Mean}_{\mathrm{pre}}$	$\mathrm{Mean}_{\mathrm{post}}$	d	t	p_t	r_{s}	p_{r}
Model predator							
Total area used	27.05 ± 1.72	22.51 ± 1.59	0.45	2.568	0.015	0.354	0.032
Latency to approach (s)	134.36 ± 17.86	197.26 ± 18.65	0.57	3.026	0.005	0.269	0.107
Open field test							
Experimental area used	12.89 ± 1.34	15.41 ± 1.26	0.32	1.710	0.096	0.418	0.010
Total area used	27.95 ± 1.97	30.62 ± 1.86	0.23	1.367	0.180	0.488	0.002
Total time moving (s)	240.14 ± 21.47	254.99 ± 17.53	0.12	0.710	0.483	0.409	0.012
Novel object							
Total area used	31.43 ± 1.64	31.22 ± 1.89	0.02	0.104	0.918	0.328	0.048
Number of approaches	2.49 ± 0.26	2.86 ± 0.32	0.21	1.181	0.245	0.367	0.026
Time moving toward object (s)	48.05 ± 3.82	48.06 ± 4.44	0.0001	0.001	0.999	0.466	0.004

explained 60–85% of the variance in observed data (Table 1, Post-training). Estimated component scores for each context followed the same pattern from pre-training experiments in that higher scores represented individuals that were bolder toward a potential predator, more active in an open field, and more exploratory toward a novel object. The correlation matrix of component scores, however, was different from pre-training experiments. Estimated component scores for 'boldness' did not correlate with any other component, while the 'general activity' and 'exploration' components correlated with each other (Table 2, Post-training). The intraclass correlation coefficient was significant ($r = 0.467$, $p = 0.002$), further indicating that individuals behaved consistently across these two correlated contexts.

In addition, after transforming post-training component scores so that they measured on the same scale as pre-training scores, we found that rank order for estimated 'boldness' scores was not significantly consistent between pre- and post-training experiments ($r_\mathrm{s} = 0.322$, $p = 0.052$; Figure 2, top), while estimated scores for both 'general activity' ($r_\mathrm{s} = 0.467$, $p = 0.004$; Figure 2, middle) and 'exploration' ($r_\mathrm{s} = 0.336$, $p = 0.042$; Figure 2, bottom) were significantly consistent. Mean pre-/post-response difference of estimated component scores also differed from each other ($F_{2,108} = 5.48$, $p = 0.005$; Figure 3). Tukey post-hoc tests further indicated that the change of 'boldness' component scores was significantly different from the change of 'general activity' ($p = 0.007$) and 'exploration' ($p = 0.033$) scores, but the change of 'general activity' and 'exploration' scores were not significantly different from each other ($p = 0.841$).

4. Discussion

In adult males from a population of Trinidadian guppies possessing an active/bold/explore syndrome, we found that experiences that increased shyness in response to a model predator did not change guppy activity in an open field or in how they explored a novel object. Furthermore, this tri-contextual syndrome broke apart following training. These results support the adaptive hypothesis that behaviours in this population are domain specific (Wilson, 1998; Bell, 2005). Such domain specificity allows for potentially maladaptive combinations of traits to be broken apart ontogenetically. Interestingly, we found no evidence that behavioural changes following predator training were the result of associative learning, in spite of an experiment explicitly

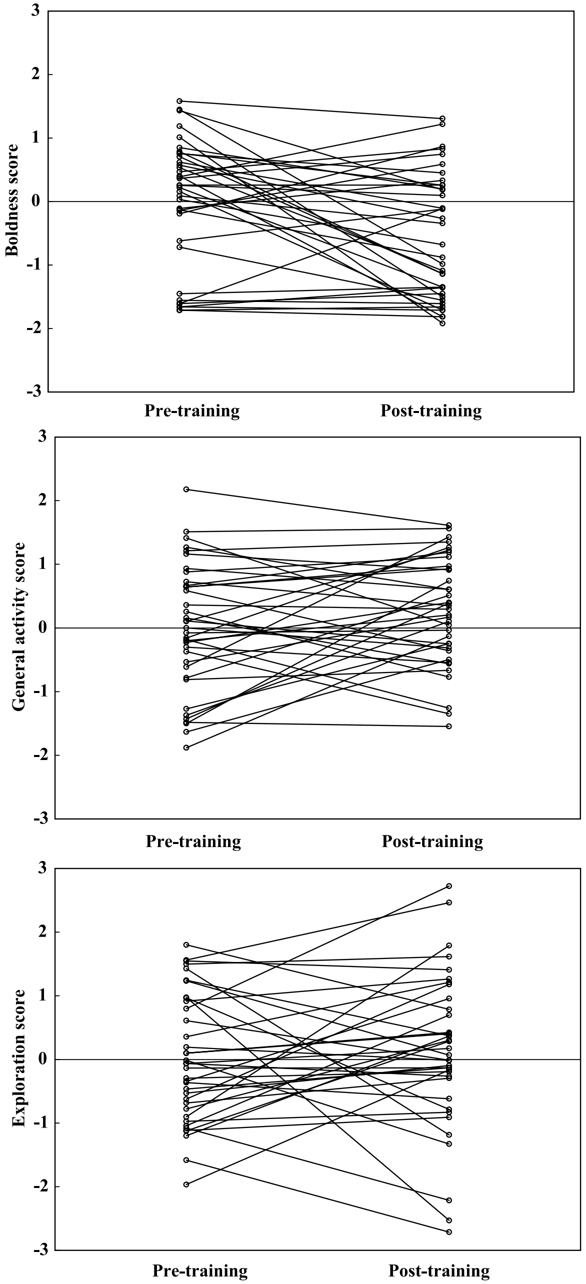

Figure 2. Estimated component scores from pre-training and transformed component scores from post-training for all subjects ($N = 37$) for (top) ‘boldness’, (middle) ‘general activity’ and (bottom) ‘exploration’.

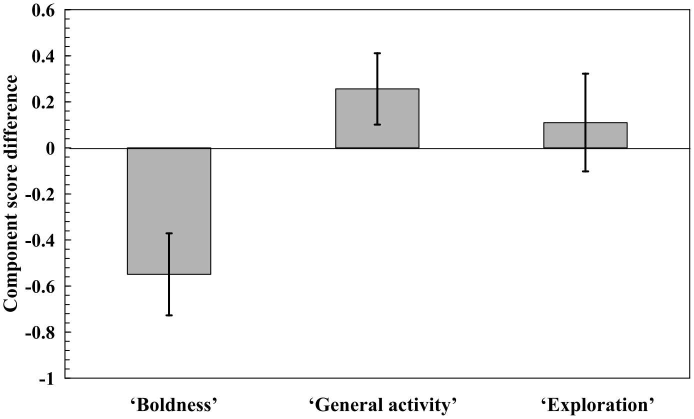

Figure 3. Mean ± SE of pre-/post-response difference in estimated component scores following predator training.

designed to test this idea. Here, we discuss other mechanisms that may have led to behavioural changes.

4.1. Question 1: Does predator conditioning occur through associative learning?

Our results indicate a potentially medium effect (Cohen, 1988) of predator training on total area used when exposed to a model predator. However, there was no effect of training on the latency to approach a model predator and we did not find any significant difference in magnitude of behavioural change in response to a model predator between our experimental and control groups. Thus, we cannot conclude that behavioural changes following our training paradigm were caused by subjects learning a predictive relationship between a CS (model predator) and US (simulated capture). One possible explanation for the lack of behavioural differences between the experimental and control groups is that other stimuli experienced by both groups prior to training may have been predictive of a capture event and, therefore, may have blocked subsequent learning of a model predator predicting a simulated capture (Rescorla, 1988; Hollis, 1997; Shettleworth, 1998). Prior to each of the four pre-training experiments, all subjects had to be transferred from their holding tanks to the experimental tank. Removing the lid of the holding tank during this transfer process always preceded capture with a dip net, and lid removal also reliably predicted a simulated capture by a dip net during six training trials for both the experimental and control groups. Thus, the presence of an already powerful signal (lid removal) that predicted the US (simulated capture) may have blocked the CS (model predator) from becoming associated with the US.

However, subjects still responded more shyly to a model predator following training. One possible explanation for this pattern is second-order conditioning in which an organism learns to associate a CS with a US, and then becomes conditioned to new neutral stimuli associated with the original CS (Rescorla, 1980; Shettleworth, 1998). Thus, if subjects were conditioned to respond to lid removal during pre-training, they then may have learned to associate a model predator with lid removal when these two stimuli were paired during training. Presentation of the model predator occurred after lid removal, however, so association of these two stimuli would have occurred through backward conditioning (Shettleworth, 1998). Although this process has been implicated in the social learning of predatory cues, its relevance in traditional classical conditioning paradigms is less clear (Shettleworth, 1998; Griffin & Galef Jr., 2005; Griffin, 2008).

Another mechanism through which subjects may have increased their responsiveness to a model predator is sensitization. Animals are predisposed to quickly recognize predator-like stimuli and adjust their behaviour accordingly (McLean et al., 1996; Griffin et al., 2000, 2001; Öhman & Mineka, 2003). To explain this phenomenon, Öhman & Mineka (2001) proposed a behavioural, mental, and neural system which they termed a ‘fear module’ — an evolved adaptation that is selectively sensitive to and automatically activated by stimuli representing recurring survival threats in evolutionary history (also Mineka & Öhman, 2002). Individuals that rapidly responded to threats would have had a selective advantage (Mineka & Oehlberg, 2008) and, therefore, evolutionarily primed stimuli would be sufficient to activate the fear module in mildly aversive situations (Öhman & Mineka, 2001). Thus, repeated exposure to a life-sized model of a native guppy predator may have sensitized our subjects and increased the magnitude of their behavioural responses to the model predator.

A third mechanism that could have led to an increase in responsiveness to a model predator is a generalized increase in arousal to any kind of stimulus. Because we did not find behavioural differences between the experimental and control groups, but we did find that subjects, overall, became shyer toward the model predator, we pooled subjects together to examine whether behavioural changes in one context affected behaviour in other contexts.

Pooling subjects in this manner precluded our experimental design from including a control group that was not exposed to a model predator during our training protocols. Without a control, measured behavioural changes following training may be related to training protocols, or other confounding variables such as the passage of time or habituation to experimental tanks and conditions.

In lieu of a control group, the open field test and novel object experiment acted as controls and highlighted that subjects did not exhibit a generalized increase in arousal to any type of stimulus. Measured behavioural responses and PCA scores changed only in response to the model predator following training. There was no difference in measured responses or PCA scores in either the open field test or novel object experiment. These results, thus, preclude habituation or a time effect as valid explanations for why subjects changed their behaviour only in response to the model predator.

4.2. Question 2: Does multi-contextual behavioural change occur after predator training?

After pooling subjects, we found that not only did guppies become shyer toward a model predator following training, but that individual differences in 'boldness' were not consistent between pre- and post-training experiments. Behaviour changes in response to an open field and a novel object were minimal, however, and individual differences between pre- and post-training experiments were consistent in these two contexts. Furthermore, only 'general activity' and 'exploration' correlated with each other following training. This syndrome was stable because rank order consistency in behavioural response to an open field and a novel object were maintained between pre- and post-training. 'Boldness' was not correlated with any other component following training because individual differences in 'boldness' were not consistent. This lack of consistency was driven particularly by an inconsistent rank order between pre- and post-training in the latency to approach and inspect the model predator. These findings suggest that individuals differed in behavioural plasticity in terms of their response to experience with predation risk, which led to a shuffling of rank-order and a breakdown in behavioural correlations involving 'boldness'.

We chose to study the effects of predator training on the structure of behavioural syndromes because unintended behavioural changes in multiple contexts could have detrimental effects on the fitness of individuals trained to be shyer. A meta-analysis of published studies examining the effect of behavioural types on fitness found that shyer individuals survived longer, but they also suffered reduced reproductive success (Smith & Blumstein, 2008), perhaps resulting from correlations with behaviours in other contexts. Shyer individuals in populations possessing behavioural syndromes may also be less active or aggressive and, therefore, experience lower foraging and growth rates (Huntingford et al., 1990; Mangel & Stamps, 2001; Höjesjö et al., 2002; Stamps, 2007). Thus, in populations possessing behavioural syndromes, training individuals to be more shy and fearful around predators may increase survival (Miller et al., 1990; McLean et al., 1996), but could also lead to behavioural changes in other ecological contexts, such as less aggression toward conspecifics when competing for food, that conceivably could offset any fitness benefits.

Our results suggest that in this population of Trinidadian guppies, correlated behaviours are able to change independently of each other. This pattern may not, however, be ubiquitous. In three-spined sticklebacks, syndromes appear to be selected for and develop in populations from high predation environments (Bell, 2005; Bell & Sih, 2007; Dingemanse et al., 2007, 2010). Behavioural types from such populations are tightly correlated genetically (Bell, 2005) and syndromes remain stable throughout ontogeny even though single behavioural types change (Bell & Stamps, 2004). In a population from a low predation area, however, behavioural types are not as tightly related genetically, allowing syndromes that appear early in life to break apart during ontogeny (Bell & Stamps, 2004). Predator-naïve populations may also possess more genetic variation for behavioural plasticity than predator-sympatric populations (Dingemanse et al., 2009). Thus, populations under strong predation pressure may evolve genetically-linked behaviours that limit behavioural plasticity.

Our subjects were descended from a wild guppy population that evolved under low predation pressure (G. Grether, personal communication). Therefore, they may not have possessed behavioural types tightly correlated genetically and their levels of behavioural plasticity allowed predator training to decouple syndromes by shuffling levels of ‘boldness’, but not ‘general activity’ or ‘exploration’.

In captive breeding and reintroduction programmes, decoupling syndromes could create increased behavioural flexibility in multiple contexts, which may increase the success of individuals released into novel environments (Sol & Lefebvre, 2000; Sol et al., 2002; Duncan et al., 2003). Individuals from populations that evolved under strong predation pressure may not, however, exhibit such flexibility and rigid syndromes could to lead to suboptimal behaviour in certain contexts following training. Future research should, therefore, identify guppy populations from high predation areas that possess behavioural syndromes and test whether similar levels of behavioural plasticity exist and if predator training leads to multi-contextual behavioural changes.

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