



Development of behavioural differences between individuals and populations of sticklebacks, *Gasterosteus aculeatus*

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Behavioural syndromes are correlations between behaviours in different contexts. For example, an individual's behaviour in response to a predator might be related to the same individual's behaviour towards conspecifics. We examined the developmental stability of single behaviours (activity in an unfamiliar environment, aggressive behaviour and boldness under predation risk) and correlations between these behaviours in two Californian populations of three-spined sticklebacks. Individually marked fish were measured for all three behaviours at three points during development, as juveniles, subadults and adults. Even though single behaviours were unstable through time, some correlations between behaviours were stable. For example, in one population, neither boldness nor aggression was stable but the positive correlation between them was. Certain correlations between behaviours were apparent at some developmental stages but not others, and the pattern of correlations differed between the two populations. These data suggest that behavioural syndromes do not necessarily limit behavioural plasticity, and suggest that ecological and developmental circumstances might favour different suites of traits.

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In a landmark study, [Huntingford \(1976\)](#) found that individual sticklebacks that were active in an unfamiliar environment were also relatively aggressive towards conspecifics and bold towards predators. Since then, there has been accumulating evidence that individuals differ in suites of correlated behavioural traits ([Wilson 1998](#); [Fraser et al. 2001](#); [Iguchi et al. 2001](#); [Dingemanse et al. 2003](#); [Drent et al. 2003](#); [Reale & Festa-Bianchet 2003](#); [Sih et al. 2003](#)). Correlations between behaviours in different contexts have important ecological and evolutionary implications because they might generate trade-offs between different behaviours. For instance, if boldness and aggression are positively correlated with each other, then aggressive individuals might do well in situations where high levels of aggression are favoured, for example during competition for resources, but do poorly when high levels of boldness are deleterious, for example under predation risk ([Sih et al. 2004a, b](#)). Correlations between behaviours

that reflect the persistence of rank order differences between individuals in different functional contexts (e.g. in mating, antipredator, foraging and competitive contexts) have been termed 'behavioural syndromes' ([Sih et al. 2004a, b](#)). The term 'behavioural syndrome' was coined because suites of correlated traits are frequently called 'syndromes' in evolutionary ecology (e.g. the 'migration syndrome' described by [Palmer & Dingle 1989](#)).

Interest in behavioural syndromes is burgeoning because correlations between behaviours might impose a limit on optimal behaviour through either developmental or evolutionary time ([Sih et al. 2004a, b](#)). For instance, if two behaviours are tightly linked because they are both governed by a common, underlying physiological mechanism, then those two behaviours cannot change independently of one another, and the correlation between them might be difficult to uncouple (e.g. [Ketterson & Nolan 1999](#)). In this situation, we might expect to see the same association between behaviours in closely related taxa. To date, the prediction that different populations of the same species would have the same behavioural syndromes has rarely been explicitly tested (but see [Riechert & Hedrick 1993](#)). Contrary to this prediction, in an earlier study, we found the aforementioned

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activity–aggression–boldness behavioural syndrome in one population of three-spined sticklebacks, but not another. Activity, aggression and boldness were positively correlated with each other among wild-caught adults from the Navarro River but not among wild-caught adults from Putah Creek, another population in northern California, U.S.A. (Bell, *in press*). The Navarro River and Putah Creek are separated by over 240 km and are in different drainages which were presumably independently colonized by marine ancestors (Bell & Foster 1994). Although Bell (*in press*) compared only two populations, this result suggests that this behavioural syndrome is evolutionarily labile in sticklebacks, and that it might not impose evolutionary constraints on the evolution of the behaviour of this species.

However, these data were just a ‘snapshot’, or a cross-sectional measurement of the behaviour of adult sticklebacks from the two populations. Another way in which behavioural syndromes might limit optimal behaviour is if the traits are consistently linked together within an individual, such that they must always change in concert with each other through ontogeny. In that situation, the syndrome might impose a developmental constraint on the evolution of different behavioural phenotypes across ontogeny. That is, a tight connection between two behaviours early in ontogeny might mean that an individual cannot change levels of a single behaviour later in ontogeny without changing other ones.

To study ontogenetic changes in behavioural syndromes, we need to measure single behaviour patterns at different ages, and then compute relations between them at each of these ages. Hence, we are concerned not only with the stability of single types of behaviour (e.g. aggression to conspecifics) across ontogeny, but also with the stability of relations between different types of behaviour (e.g. aggression and boldness) across ontogeny. Below, we discuss each type of stability in turn.

When individual differences in a particular single behaviour are correlated through ontogeny, then the behaviour is ‘stable’ (Hinde & Bateson 1984). The stability of a single behaviour refers to the persistence of rank order differences between individuals measured at successive ages: a behaviour is stable if individuals express relatively high levels of the behaviour both at a young age and when older. If a single behaviour is stable, its absolute value might change through time (e.g. on average, aggression might increase through ontogeny), but the rank order of individuals does not (Hinde & Bateson 1984). Examples of stable individual behavioural differences include aggression in sticklebacks (Bakker 1986) and exploratory behaviour in great tits, *Parus major* (Dingemans et al. 2003).

As with single behaviours, behavioural syndromes can also be either stable or unstable. Behavioural syndromes are stable when the same association between different behaviours occurs at different stages of ontogeny. That is, if two behaviours are measured on the same group of individuals at two stages of ontogeny, and if the two behaviours are similarly associated or correlated with each other at both of those stages, then the behavioural syndrome is stable through development. In contrast,

a behavioural syndrome is unstable when behaviours are correlated at some stages of ontogeny but not others. According to this definition, a behavioural syndrome can be stable even if the single behaviours that comprise the behavioural syndrome are not. That is, even if the rank order of individuals with respect to boldness changed through ontogeny, a boldness–aggression syndrome would be stable if, at all ages, bolder individuals also tended to be more aggressive.

When both single behaviours and a behavioural syndrome are stable, the result is similar to conventional definitions of ‘temperament’, as a dispositional, intrinsic, developmentally fixed tendency to behave a certain way in a variety of circumstances (Plomin & Dunn 1986). There is evidence for stable ‘temperamental’ differences between individual rhesus monkeys, *Macaca mulatta* (Stevenson-Hinde et al. 1980; Suomi et al. 1996), dairy goats, *Capra hircus* (Lyons et al. 1988), bighorn sheep, *Ovis canadensis* (Reale et al. 2000) and wolves, *Canis lupus* (MacDonald 1983).

In addition, when both a behavioural syndrome and the single behaviours that constitute it are stable, then selection at one stage of ontogeny might produce unexpected changes in a completely different behaviour at a later stage. This scenario has an important evolutionary implication because it means that selection on a particular behaviour at a young stage of ontogeny might affect the distribution of a correlated behaviour later in life. For example, if boldness, aggression and the relation between them are all stable, and if predators disproportionately consume bold juveniles, then not only will there be a deficit of bold adults, but there will also be fewer aggressive adults in the population.

Given the increasing interest in individual differences (Wilson et al. 1993, 1994; Coleman & Wilson 1998; Wilson 1998), behavioural syndromes (Sih et al. 2003, 2004a, b), temperament (Reale et al. 2000; Reale & Festa-Bianchet 2003), coping styles (Benus et al. 1991; Koolhaas et al. 1999), personality (Gosling 2001) and behavioural strategies (Verbeek et al. 1994; Dingemans et al. 2003; Drent et al. 2003), the time is ripe for studies that consider how single behavioural traits and behavioural syndromes change through ontogeny. Furthermore, the distinction between stable syndromes and stable single behaviours underscores the importance of measuring the same behaviours on the same marked individuals throughout ontogeny when attempting to infer whether behavioural syndromes act as developmental constraints, which was our goal in the current study. If behavioural syndromes act as developmental constraints, we would predict that an individual with a certain combination of behaviours would maintain those correlations through ontogeny.

We examined the developmental stability of a suite of behavioural traits in two freshwater populations of three-spined sticklebacks from northern California (the Navarro River and Putah Creek). At three stages of ontogeny (juveniles, subadults and adults), we measured three behaviours (activity in an unfamiliar environment, aggression towards conspecifics and boldness under predation risk) on individually marked fish. Therefore,

we have a window into each individual's behaviour at different points throughout the life span. For each population, we asked three questions. First, are the three behaviours stable? Second, are there behavioural syndromes at a given stage of ontogeny? Third, if behavioural syndromes do exist, are they stable?

METHODS

Our aim was to measure the same three behaviours on the same individuals at three stages of ontogeny. To this end, we reared fish in the laboratory and followed their behavioural development.

Using seines and minnow traps, we collected adult three-spined sticklebacks from Putah Creek and the Navarro River (75–80 fish from each population) between May and June 2000. The fish were transported by car in insulated coolers with airstones to the Institute of Ecology at the University of California, Davis campus. The fish were fed frozen brine shrimp, live tubifex worms and trout chow (Silver Cup, Nelson and Sons, Murray, Utah, U.S.A.) *ad libitum* and maintained on a 16:8 h light:dark regime at 20°C in 379-litre flow-through tanks with fewer than 80 fish per tank. Twenty-two full-sibling families (11 families per population) were produced by mating each of 22 males to a different female from the same population. Males were housed in 37.9-litre (26 × 29 cm and 51 cm high) aquaria, each of which contained a gravel substrate and string (filamentous) algae for nesting. After a male completed his nest, a gravid female from the same population was placed in the aquarium. To eliminate environmental effects that might arise from differences in paternal care (Tulley & Huntingford 1987), we removed the eggs from the male's nest and artificially incubated them. After hatching, each clutch of fry was placed in a separate aquarium until the fry were big enough to be permanently marked by spine clipping (20 mm, 2–3 months of age). We clipped the spines by cutting the base of either the dorsal or ventral spine with dissecting scissors. Fish quickly recovered from the procedure and showed no signs of distress afterwards. Each family was assigned a unique mark. Excess fry were used as stimuli for the observations of aggressive behaviour (see below) and were reared in separate tanks (one tank per population) until the end of the experiment.

After marking, the fry were transferred to the Putah Creek Aquaculture Facility, University of California, Davis, where they were reared in one of six 106-litre 'home tanks' (43 × 43 cm and 49 cm high) for the duration of the experiment. There were three replicate home tanks for each population. Each home tank contained a sponge filter, a gravel and sand substrate, artificial plants, a cinderblock and a terracotta pot. The home tanks were kept in a water bath and maintained at $16 \pm 2^\circ\text{C}$ and exposed to an ambient (Davis, CA) photoperiod throughout the experiment. Half of the water in the home tanks was changed every month. For each population, one offspring from each full-sibling family was placed in each of the three home tanks (11 fish total in the tank). By rearing the fish in mixed family groups, we reduced the possibility of resemblance between relatives caused by a shared environment.

The fish were fed a limited ration: the amount of food provided was adjusted so that the fish received approximately 10, 5, 3 and 1% of their body mass per day as fry, juveniles, subadults and adults, respectively, as recommended by the manufacturers of the diet. The diet included a variety of food types: as fry, they were fed brine shrimp nauplii and Golden Pearls 'Juvenile Diet' (Brine Shrimp Direct, Ogden, Utah, U.S.A.) twice a day. At approximately 2 months of age, the fish were gradually introduced to a mixed diet, which included trout chow, live and frozen brine shrimp and live and frozen tubifex worms.

Behavioural Observations

At three points in development, we observed three types of behaviour for each individual: (1) activity in an unfamiliar environment; (2) aggression towards a conspecific and (3) boldness under predation risk. We conducted the behavioural observations when the fish could be grouped into broad stages of ontogeny as juveniles (98–171 days of age, mean = 137 days), subadults (176–229 days of age, mean = 210 days) and adults (288–340 days of age, mean = 322 days). Therefore, there were a total of nine observations per fish: activity, aggression and boldness of each fish as a juvenile, subadult and adult. At each age, a fish's activity in an unfamiliar environment was observed first, followed by the measurement of aggression, and then boldness under risk.

The fish in a home tank were deprived of food 1 day before observations. The next day, each of the fish from a particular home tank was placed singly in one of 12 observation tanks (26 × 29 cm and 51 cm high, 37.9 litres). The extra observation tank served as a 'back up' tank in case of technical problems, for example if the divider or heater was not working properly. Each observation tank contained two plastic plants, a terracotta pot, a food dispenser fastened to the side of the aquarium, an airstone, a 1-litre clear plastic cylinder and a heater that maintained the water temperature at $16 \pm 1^\circ\text{C}$. For the observer's reference, lines were drawn on the outside of the observation tanks to separate the tank into 12 equal-sized areas. The fish were allowed to adjust to the observation tanks overnight (ca. 12 h). The next day, each fish ('focal fish') was observed for all three types of behaviour. For each behaviour, we recorded several variables on a laptop computer using the program Observer (Noldus Technology 1991). An assistant placed the stickleback in the observation tank, on the opposite side of the room from the holding tanks, to ensure that observations were conducted blind with respect to the identity of the fish.

Each observation period consisted of 6 days of observation, 1 day for each of the home tanks. For each observation period, the order in which the home tanks were observed was random. All the fish from a particular home tank were observed on the same observation day.

Activity in an unfamiliar environment

To measure the behaviour of fish in an unfamiliar environment, we continuously recorded the fish's behaviour in the observation tank for 180 s. Specifically, we

recorded the number of times the focal fish swam or moved to a different area of the tank (number of movements), the total number of areas used by the focal fish (area used) and the number of times the focal fish moved, not necessarily to a different area of the tank (activity).

Aggression

At least 1 h after we had measured the focal fish's activity level, a conspecific from the same population and of the same approximate (<10% difference in standard length) size was placed in the plastic cylinder in the observation tank. The stimulus fish came from the pool of excess fry that were reared in separate tanks, and did not serve as focal fish themselves. Approximately 10 different fish per population served as stimulus fish. The number of times the focal fish bit at the tube containing the conspecific (number of bites) and the total time the focal fish spent within one body length of the conspecific (time near conspecific) were continuously recorded for 5 min. At the end of this observation, the conspecific was removed from the plastic cylinder and the observation tank.

Boldness under risk

To simulate the different types of predators on different age and size classes, we measured boldness under predation risk in a different way at each stage of ontogeny. Throughout ontogeny, sticklebacks increase more than 10-fold in mass, inhabit different microhabitats and are susceptible to different types of predators (Bell & Foster 1994). Therefore, at each stage of ontogeny, we simulated predation risk by a predator appropriate for that stage. This approach was adopted from developmental psychology, in which there is a tradition of using different types of task to measure the same type of behaviour at different ages, because a particular task may not be meaningful to all age groups (Kagan et al. 1986). For example, inhibition in an infant may be measured as its attachment to its mother, and in a toddler as its reaction to peers (Kagan et al. 1986).

When the sticklebacks were juveniles, we observed their response to a live Coast Range sculpin, *Cottus aleuticus*, predator. When the sticklebacks were subadults, we recorded foraging under predation risk from a model great egret, *Casmerodius albus*. Finally, when the sticklebacks were adults, we recorded foraging under predation risk from a live largemouth bass, *Micropterus salmoides*. The observations of boldness under risk for subadults and adults contained elements of both the motivation to forage and fearfulness towards a predator, whereas juveniles were just confronted by a predator and not presented with food. However, the common motivation underlying each of these observations was to assess how individuals behaved under predation risk, and 'boldness' is considered to be reflected in a range of behaviours that are likely to expose an individual to predation, such as foraging near a predator, high levels of activity or predator inspection behaviour (Huntingford et al. 1994).

The observations of boldness began at least 1 h after the focal fish's aggressive behaviour had been observed.

Juveniles: response to a sculpin predator. One of two adult sculpins (12–14 cm standard length) was placed in a small

transparent (17 × 7 cm and 7 cm high) Plexiglas box with holes and introduced to the far left corner of the observation tank. The sculpins were collected with minnow traps in the Navarro River. The box prevented the sculpin from escaping and confined its movements but allowed visual and olfactory cues to reach the sticklebacks. The box was opaque in some sections and therefore offered some degree of refuge for the sculpin. After the focal fish first oriented to the sculpin, the number of times the fish approached, or actively swam towards, the sculpin (number of approaches), the total area used by the focal fish (area used) and the number of times the focal fish moved (number of movements) were continuously recorded for 5 min. When confined to the Plexiglas box, both sculpins were inactive and behaved the same way. Because confinement might have been stressful for the sculpin, we alternated which sculpin was used for a given focal fish, and each sculpin was never confined for more than 10 min at a time. When not being used in behavioural observations, the sculpin were maintained in a 379-litre flow-through tank at 16°C and exposed to an ambient (Davis, CA) photoperiod. The sculpin were each fed 5–10 live bloodworms daily and were given to a fish laboratory at UC Davis at the end of the experiment.

Subadults: foraging under risk of predation by an egret. An egret skull was fastened over the observation tank and, after 10 min, 10 tubifex worms were added to the food dispenser. When the fish approached within one body length of the food dispenser, the skull was released twice in quick succession via a string attached to the skull, simulating two strikes within 3 cm of the food dispenser. The number of times the fish bit at the food (foraging under risk), the number of times the fish moved (number of movements) and the total area used by the fish were continuously recorded for 5 min.

Adults: foraging under risk of predation by a live bass. For this observation only, fish were transferred to a different observation tank, which was placed inside a 758-litre, flow-through, 1.22-m-diameter circular fibreglass tank. This large tank contained a live largemouth bass and a cinderblock which served as a hiding place for the bass, which was obtained from a local fish farm. The observation tank was located directly opposite the cinderblock, on the other side of the standpipe. The observation tank was surrounded by removable opaque panelling, which prevented visual contact between the bass and the stickleback. The observation tank contained plastic plants, a terracotta pot and a food dispenser that was fastened to the side of the aquarium closest to the standpipe. The tank was arranged so that when the opaque panel was lifted, and when the focal fish was foraging near the food dispenser, the fish had a clear view of the bass.

The focal fish was transferred to the observation tank and allowed to acclimate to the new tank for 5 min. To lure the fish to the side of the tank closest to the bass, the observer added approximately 10 tubifex worms to the food dispenser, and waited for the focal fish to approach the food. When the focal fish was within one body length of the food dispenser, the opaque panel on the side of the

tank closest to the bass was gently raised, allowing the stickleback to have a clear view of the predator. The bass hid in the cinderblock shelter during the observations and remained inactive. On the few occasions when the bass left the shelter during the behavioural observations, we noted its movements. In subsequent analyses, we determined that the bass's movements had no additional effect on the stickleback's behaviour. The following behaviours of the focal fish were continuously recorded for 5 min: the number of times the focal fish moved (number of movements), the total area used by the focal fish (area used), the number of times the focal fish oriented to the bass (body facing the bass, binocular vision) (number of orientations), and the number of times the fish bit at the food (foraging under risk).

When not being used in behavioural observations, the bass was maintained in a 454-litre flow-through tank at 16°C and exposed to an ambient (Davis, CA) photoperiod. The bass was fed 6–10 bloodworms and 'trout chow' daily, and was given to a fish laboratory at UC Davis at the end of the experiment.

At the end of an observation day, we determined the identity of each focal fish by checking its spine clipping mark. All the fish were then removed from their observation tanks and returned to their home tank. The water in the observation tanks was changed, and the fish from a different home tank were transferred to the observation tanks, as before.

At the end of the experiment, the fish were killed by an overdose of anaesthetic (MS 222) as part of a separate experiment (Bell 2004).

Ethical Note

Spine clipping proved to be a relatively risk-free method of marking sticklebacks. The procedure lasted for approximately 30 s per fish: the fish was gently netted from the tank, removed from the net and was gently held between two fingers. Using a metal dissecting prod, we gently lifted the spine and quickly cut at its base with a pair of sharp dissecting scissors. The fish was then gently placed in a bucket with a refuge for recovery (10 min to 1 h). The fish were monitored after the procedure and showed no signs of stress. No signs of fungal infection or adverse health effects of spine clipping were detected. The fish were not sedated while they were being clipped. We chose not to sedate the fish because, in our experience, anaesthetizing the fish can be more traumatic than the actual procedure, especially if the procedure is performed quickly and efficiently.

Although mild, temporary physiological activation may have occurred during behavioural observations, we took a number of steps to minimize stress to the animals. Although frequently both the focal individual and the simulated intruder swam vigorously towards each other, the transparent cylinder prevented contact or injury between the focal individual and the conspecific, and the observation was short (5 min). The observations of boldness under predation risk were short, the sticklebacks were never in physical contact with the predator and they

had access to shelter at all times. Upon detecting the predator, the focal fish frequently either froze in the same position or quickly darted to the nearest shelter. The study was approved by the Animal Use and Care Committee, University of California, Davis. The fish were collected under a permit granted by the State of California Department of Fish and Game.

Data Analysis

For each of the nine observations (e.g. juvenile activity, subadult activity, etc.), we used separate principal components analyses (PCA) to summarize the different variables (e.g. the number of movements, area used) into single component scores (Table 1). This generated a total of nine summary component scores: juvenile activity, juvenile aggression, juvenile boldness, subadult activity, subadult aggression, subadult boldness, adult activity, adult aggression and adult boldness. Each of these component scores is a summary of several variables that were correlated with each other and which we collectively used to estimate 'activity', 'aggression' or 'boldness'.

For each of the three observations of 'activity', the variables loaded heavily on a primary component that explained 74–86% of the variance in the data. Higher scores on this component indicate higher levels of activity (movement, area used) and vice versa. Similarly, for each of the three observations of 'aggression', both the number of bites and the total time spent with the conspecific were positively correlated with each other and loaded heavily on a single component. For juveniles and subadults, this component explained over 80% of the variance in the data, whereas the percentage of variance explained for adults was 68%. At each of the three stages of ontogeny, higher factor scores on this component indicate higher levels of aggressive behaviour, and vice versa.

The principal components analyses of 'boldness' are slightly different because behaviour under predation risk was measured differently at each stage of ontogeny. However, a pattern for each stage was for activity in the presence of predation risk and 'risky' behaviours (predator inspection behaviour or willingness to forage under predation risk) to be positively correlated and to load on the same component. For example, for juveniles, measurements of activity under predation risk (e.g. number of movements and area used) and approaches towards the predator loaded positively on one component, which accounted for 76% of the variance in the data. Similarly, for subadults, the number of movements, area used and bites at the food all loaded positively on a component, which accounted for 82% of the variance in the data. Finally, for adults, activity under predation risk, foraging under predation risk and boldness towards the predator (orientations) were positively correlated and all loaded positively on a component, which accounted for 76% of the variance in the data. As a result, for each stage of ontogeny, higher levels on a 'boldness' component indicate higher levels of behaviours such as activity under predation risk, foraging under predation risk, or predator inspection, and vice versa.

Table 1. Principal components analyses

Behaviour	Loading
Juvenile activity	
Area used	0.932
Number of movements	0.920
Activity	0.710
Cumulative variance explained (%)	73.98
Juvenile aggression	
Number of bites	0.904
Time near conspecific (s)	0.904
Cumulative variance explained (%)	81.76
Juvenile boldness	
Number of movements	0.888
Number of approaches	0.828
Area used	0.899
Cumulative variance explained (%)	76.06
Subadult activity	
Area used	0.946
Number of movements	0.926
Activity	0.787
Cumulative variance explained (%)	79.01
Subadult aggression	
Number of bites	0.921
Time near conspecific (s)	0.921
Cumulative variance explained (%)	84.83
Subadult boldness	
Number of movements	0.909
Foraging under risk (s)	0.855
Area used	0.925
Cumulative variance explained (%)	82.14
Adult activity	
Number of movements	0.928
Activity	0.928
Cumulative variance explained (%)	86.17
Adult aggression	
Number of bites	0.827
Time near conspecific (s)	0.827
Cumulative variance explained (%)	68.41
Adult boldness	
Number of movements	0.934
Area used	0.833
Foraging under risk (s)	0.715
Number of orientations	0.931
Cumulative variance explained (%)	75.73

Values for each behaviour indicate loadings on the component, and total variance explained by that component is indicated.

The PCA component scores were not normally distributed. Therefore, we used the nonparametric Spearman rank correlation procedure to assess relations between behaviours. For each of the behaviours, we tested for differences between the two populations using the nonparametric Mann–Whitney *U* test, which does not assume normality. Since it is possible that an individual's behaviour at one age might not be independent of its behaviour at another age, or that at a given age, an individual's behaviour in one context might be related to its behaviour in another context, we adjusted for multiple comparisons using the sequential Bonferroni procedure (Rice 1989) and have noted significant results according to this procedure in the tables. All statistical tests were two tailed.

Owing to mortality during the experiment, as well as the fact that individuals occasionally failed to respond to the test procedure, the number of individuals measured in each of the nine behavioural observations differs. Mortality was approximately 10%, a value typical for this species

when reared in captivity. For longitudinal analyses of behavioural stability, only individuals with data on all three stages of ontogeny are included (activity: *N* = 30 Navarro, *N* = 14 Putah; aggression: *N* = 31 Navarro, *N* = 14 Putah; boldness: *N* = 29 Navarro, *N* = 14 Putah). For cross-sectional analyses of behavioural syndromes, only individuals with data on all three behaviours at that stage of ontogeny are included (juveniles: *N* = 35 Navarro, *N* = 28 Putah; subadulthood: *N* = 32 Navarro, *N* = 25 Putah; adulthood: *N* = 32 Navarro, *N* = 20 Putah).

RESULTS

Average Differences between Populations

At each stage of ontogeny, fish from Putah Creek and the Navarro River differed in average behaviour (Table 2). In general, Putah fish were more bold towards predators, more aggressive towards a conspecific and more active in an unfamiliar environment than Navarro fish.

The Navarro Population

Among Navarro fish, the single behaviours were not stable over ontogeny. For example, fish that scored high on boldness as juveniles did not necessarily score high on boldness as subadults or adults. The only significant correlation across ontogeny was between juvenile and adult levels of aggression (Table 3).

At each stage of ontogeny, however, some behaviours were correlated with others, that is, there were behavioural syndromes (Table 4). In juveniles, activity, aggression and boldness were positively associated with each other: individuals that were relatively bold were also relatively aggressive and relatively active in an unfamiliar environment. In subadults, boldness and aggression were positively correlated with each other. In adults, the same positive relations between activity, aggression and boldness reappeared: all three behaviours were positively correlated with each other.

Therefore, some behavioural syndromes were stable but others were not (Table 4). The boldness–aggression behavioural syndrome was stable through ontogeny: as juveniles, subadults and adults, individuals that were bold towards a predator were also aggressive towards a conspecific. In contrast, the activity–aggression and boldness–activity syndromes were unstable through ontogeny: activity and aggression were positively correlated in juveniles and adults, but not in subadults, similarly for boldness and activity. Combining the results of the stability of single behaviours with the stability of behavioural syndromes, it appears that boldness and aggression were consistently associated with each other, even though neither boldness nor aggression was stable across ontogeny.

The Putah Population

As in the fish from the Navarro River, there was little evidence that single behaviours were stable over ontogeny (Table 3). The only significant correlations were between

Table 2. Population differences at each age for each behaviour

	Activity		Aggression		Boldness	
	Navarro	Putah	Navarro	Putah	Navarro	Putah
Juveniles						
N	35	28	35	28	35	28
$\bar{X} \pm SD$	-0.176 ± 1.065	0.451 ± 0.989	-0.109 ± 1.013	0.197 ± 1.030	0.083 ± 1.098	0.024 ± 1.056
Z		-2.608		-1.300		-0.262
P		<0.01*		0.19		0.79
Subadults						
N	32	25	32	25	32	25
$\bar{X} \pm SD$	-0.430 ± 0.736	0.010 ± 0.859	-0.403 ± 0.805	0.286 ± 0.966	-0.410 ± 0.403	-0.044 ± 0.882
Z		-2.529		-3.093		-1.626
P		<0.01*		<0.01*		0.10
Adults						
N	32	20	32	20	32	20
$\bar{X} \pm SD$	0.059 ± 0.889	-0.167 ± 0.204	-0.148 ± 1.020	0.433 ± 1.066	-0.446 ± 0.690	0.518 ± 1.272
Z		-0.631		-2.681		-2.783
P		0.53		<0.01*		<0.01*

Values are means \pm SD of the component scores derived from a principal components analysis.

*Statistically significant after the sequential Bonferroni procedure. Within each population, for each behaviour, the test statistics were replaced by their corresponding P values and then ranked from smallest to largest and the sequential Bonferroni procedure was followed.

juvenile and subadult aggression and subadult and adult boldness.

In addition, there was little evidence for behavioural syndromes, or correlations between behaviours, for Putah fish (Table 4).

Therefore, in the Putah fish, no behavioural syndromes were stable through ontogeny.

DISCUSSION

Our study supports the previous findings that behavioural syndromes can vary even among closely related taxa. For the fish from Putah Creek, there was no indication that either single behaviours or correlations between behaviours were stable across ontogeny. In contrast, for

fish from the Navarro River, there was a consistent positive relation between boldness and aggression at all three stages in the study. Hence, in one of the two populations, a behavioural syndrome involving boldness and aggression was stable across ontogeny.

However, our results also show that a behavioural syndrome can be stable across ontogeny even if the single behaviour patterns that make up that syndrome are not themselves stable across ontogeny. In particular, in the Navarro population, fish that were more aggressive than others at one age were not necessarily more aggressive at other ages. Individuals that were, for example, very aggressive as juveniles did not necessarily grow up to become very aggressive adults, and similarly for boldness. However, there was a positive correlation between aggression and boldness towards predators at all three stages of

Table 3. Spearman rank order correlations between single behaviours at different ages

	Juvenile–Subadult		Subadult–Adult		Juvenile–Adult	
	r_s	P	r_s	P	r_s	P
Activity						
Navarro	0.171	0.37	-0.051	0.79	0.191	0.31
Putah	0.428	0.13	-0.381	0.18	-0.172	0.56
Aggression						
Navarro	0.331	0.07	0.315	0.08	0.389	0.03*
Putah	0.706	<0.01*	-0.021	0.94	0.147	0.62
Boldness						
Navarro	0.075	0.70	0.123	0.52	0.067	0.73
Putah	-0.019	0.95	0.735	<0.01*	0.257	0.38

*Statistically significant correlation between the behaviour measured at different ages after the sequential Bonferroni procedure. Within each population, for each behaviour through time, the test statistics were replaced by their corresponding P values and then ranked from smallest to largest and the sequential Bonferroni procedure was followed.

Table 4. Spearman rank order correlations between different behaviours (behavioural syndromes)

	Juvenile		Subadult		Adult	
	r_s	P	r_s	P	r_s	P
Boldness–Aggression						
Navarro	0.563	<0.01*	0.477	0.01*	0.597	<0.01*
Putah	0.426	0.02	0.124	0.56	0.182	0.44
Activity–Aggression						
Navarro	0.647	<0.01*	0.264	0.14	0.380	0.03*
Putah	0.344	0.07	0.352	0.08	-0.247	0.30
Boldness–Activity						
Navarro	0.476	<0.01*	-0.019	0.92	0.370	0.04*
Putah	0.067	0.73	0.321	0.12	0.390	0.09

*Statistically significant correlation between the different behaviours after the sequential Bonferroni procedure. Within each population, for each pair of behaviours, the test statistics were replaced by their corresponding P values and then ranked from smallest to largest and the sequential Bonferroni procedure was followed.

ontogeny. That is, as juveniles, as subadults and as adults, individual Navarro fish that were more bold were also more aggressive. This means that a juvenile that was bold and aggressive could grow up to become a timid, non-aggressive adult.

One important message from this study is that a stable behavioural syndrome does not necessarily require consistent individual behaviour throughout ontogeny. Even if a behavioural syndrome characterizes a group of animals at all ages, that does not necessarily mean that an individual cannot change its behaviour through time. Instead, it implies that a cluster of behaviours changes in a coordinated fashion. That is, shyness and introversion might always be associated with each other, without necessarily entailing that shy, introverted children must grow up to become shy, introverted adults.

The finding that unstable single behaviours can be coupled with stable syndromes has some parallels with plastic alternative mating strategies (Moore et al. 1998). In many species with such strategies, suites of correlated traits change in concert with each other during the transition between different mating types, often because they are governed by a hormonal signal with effects at several target sites. For example, when a male cichlid, *Haplochromis burtoni*, obtains a territory, he becomes brightly coloured, aggressive, courts females and undergoes dramatic physiological changes (Hofmann et al. 1999). However, territory ownership is reversible, and when a territorial male returns to a nonterritorial state, those same traits change in a coordinated fashion. In other words, the behaviours are plastic through time, but correlations of behaviours within a given context are not.

In some ways, our results support the common assumption (Krebs & Davies 1997) that a particular behaviour is plastic and amenable to conditional changes in response to changes in different environmental conditions. However, individuals could not change some behaviours without also changing others, which means that behaviours seen in different contexts may not be independent of one another. If different types of behaviour are consistently correlated with each other, then at a given time, an individual cannot change levels of one behaviour without also changing levels of a different behaviour. This might mean that an individual cannot adaptively modulate behaviours when in different situations; an aggressive individual might be inappropriately bold because it cannot 'turn off' a generally aggressive tendency.

While the boldness–aggression syndrome appeared to be a general characteristic of one population (the Navarro River), it did not characterize the other population. The boldness–aggression syndrome was present in wild-caught Navarro adults (Bell, in press), it was preserved in the laboratory, it was robust to different types of predation risk and it was stable through ontogeny. In contrast, the boldness–aggression behavioural syndrome was not a defining characteristic of Putah fish. Therefore, the behaviour of fish from Putah Creek appears to be more labile across contexts than that of fish from the Navarro River.

One possible explanation for this difference between the populations concerns differences in predation pressure: behavioural, morphological and quantitative genetic

evidence suggests that predation risk is higher in the Navarro River (Bell, in press). For example, both wild-caught and laboratory-reared Putah fish were significantly bolder under predation risk than Navarro fish, which suggests that there is a strong genetic component to behaviour under predation risk (although maternal effects could also be responsible). Other studies have shown that there is heritable, adaptive variation in antipredator behaviour among different stickleback populations (Huntingford et al. 1994), such that populations exposed to higher predation risk are more timid than low-risk populations. Therefore, perhaps directional selection by predators in the Navarro River might have favoured a tightly integrated phenotype with strong correlations between traits, whereas either relaxed or variable selection in Putah Creek may have produced more flexible phenotypes.

The other key result from this study is that behavioural syndromes were observed at some ages but not others. For example, in the Navarro population, juveniles that were more active were also more aggressive and more bold, and the same was true for adults. However, the correlations between the behaviours weakened at subadulthood. Within the literature on human personality, there are a variety of both proximal and ultimate hypotheses concerning the timing of instability (Caspi & Bem 1990). For example, we might expect behavioural syndromes to break apart across major transitional periods because different suites of traits might be favoured at different ages, especially if different age groups inhabit very different environments. Considering that behaviours change through ontogeny, as well as the fact that individuals acquire different motor and sensory abilities as they mature (Fuiman & Webb 1988), it is not unreasonable to suspect that correlations between behaviours might change through development (Stamps 2003). Another reason to expect syndromes to uncouple around transitional periods is more proximal: if traits are linked together because they are both affected by the same hormones, then periods of rapid hormonal change, as occur at the time of sexual maturation, might produce instability either by uncoupling previous correlations or by generating new ones.

This hypothesis is relevant here because, while high levels of activity in an unfamiliar environment were associated with high levels of boldness and aggression in Navarro juveniles and adults, the syndrome broke apart at about 6 months of age, when the fish were starting to become sexually mature. Therefore, perhaps the onset of sexual maturity, or hormonal reorganization, triggered a restructuring of a suite of behaviours.

Fish from the Putah population also underwent changes in the relation between behaviours through ontogeny. While boldness and aggression were positively correlated in Putah juveniles, the syndrome disappeared at subadulthood and did not reappear at adulthood. This suggests that even when traits are linked together at a young age, factors during development might alter the relation between them. A promising direction for future studies is to examine the influence of experience on single behaviours, and to ask whether experience-induced changes in single behaviours are associated with changes in other behaviours.

The recognition that individual differences in behaviour can extend to different contexts cautions us to question the assumption that an individual's different behaviours are independent of one another. This holistic perspective views the phenotype as an integrated, interdependent set of different traits. However, our results suggest that correlated traits can be readily uncoupled, through both developmental and evolutionary time. The next question, then, is to consider the factors favouring the assembly of a suite of traits at some stages of ontogeny and in some ecological conditions but not others, and to elucidate the proximal mechanisms that determine the ease with which traits can be uncoupled.

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