

Individual boldness affects interspecific interactions in sticklebacks

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Received: 26 June 2008 / Revised: 13 November 2008 / Accepted: 14 November 2008
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Abstract Within populations of many species, individuals that are otherwise similar to one another in age, size or sex can differ markedly in behaviours such as resource use, risk taking and competitive ability. There has been much research into the implications of such variation for intraspecific interactions, yet little investigation into its role in influencing interspecific interactions outside of a predator–prey context. In this study, we investigated the role of individual-level behavioural variation in determining the outcomes of interactions between two ecologically similar fishes, the threespine and ninespine sticklebacks (*Gasterosteus aculeatus* and *Pungitius pungitius*). Experiment 1 asked whether individuals of both species were consistent in their expression of two behaviours: activity in novel surroundings and latency to attack prey. For each behaviour, focal individuals were assayed twice, 10 days apart. Performances were positively correlated between exposures, suggesting behavioural consistency within individuals, at least over this timescale. Experiment 2 revealed not only differences in habitat use described both by

species-level variation, with ninespines spending more time in vegetated areas, but also by individual differences, with more active individuals of both species spending more time in open water than in vegetation. Experiment 3 revealed that when heterospecific pairs competed for prey, bolder individuals consumed a greater share, irrespective of species. These findings suggest that individual-level variation can facilitate overlap in habitat use between heterospecifics and also determine the outcomes of resource contests when they meet. We discuss how this might vary between populations as a function of prevailing selection pressures and suggest approaches for testing our predictions.

Keywords Bold–shy axis · Personality · Temperament · Behavioural syndrome

Introduction

Within a given species, individuals that are otherwise similar to one another in terms of age, size or sex can differ consistently and substantially from one another in their behaviour (Wilson et al. 1994; Wilson 1998; Sih et al. 2004; Realé et al. 2007). This heterogeneity, termed variously boldness, or the bold/shy continuum (Wilson et al. 1993), temperament (Box 1999), coping style (Koolhaas et al. 1999) or personality (Lowe and Bradshaw 2001), can encompass numerous behavioural traits, including habitat use, sociality, response to predators, intraspecific aggression and neophobia. These traits can be arranged into behavioural syndromes, such that behaviour in one context predicts behaviour in another (reviewed by Sih et al. 2004), or they can vary independently of one another (e.g. Coleman and Wilson 1998; Webster et al. 2007). There seems to exist a general fitness tradeoff, in that individuals

Communicated by T. Bakker

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whose behaviours tend to be more risk prone have greater reproductive success whilst also incurring a greater risk of early mortality compared to those exhibiting more risk-averse behaviours (meta-analysis by Smith and Blumstein 2008). The emergence and persistence of individual variation in risk taking may result from adaptive differences in reproductive tradeoffs, with risk-prone or risk-averse behaviour being associated with strategies to maximise short- or long-term fitness (Wolf et al. 2007).

Lately, there has been an upsurge of interest in the fitness consequences of individual differences in behaviour, as recent findings have begun to highlight the relevance and significance of this variation in evolutionary ecology (Realé et al. 2007). Research in this area has focused upon the role of individual behavioural variation in determining, for example, dispersal (Dingemanse et al. 2003), reproductive success (Boon et al. 2007), response to environmental perturbation (Dingemanse et al. 2004) and divergence in habitat use and resource polymorphism (McLaughlin 2001; Wilson and McLaughlin 2007). Whilst numerous studies have considered interactions between conspecifics, little attention has been paid to the role of individual behavioural variation in interspecific interactions outside of a predator–prey context. Understanding the processes underlying resource competition between ecologically similar species is important, since the outcome of such competition has implications for character displacement and community structuring. In this study, we investigated the influence of individual behavioural variation upon interactions between two ecologically similar fishes, the threespine and the ninespine stickleback (*Gasterosteus aculeatus* and *Pungitius pungitius*).

Three- and ninespine sticklebacks occur in sympatry through much of their ranges. Whilst a degree of habitat segregation is thought to exist where they co-occur, with the more poorly armoured, and predator-vulnerable ninespine using vegetated areas to a greater extent than the generally longer spined and heavily plated threespine (Benzie 1965; Hart 2003), these preferences are not definitive, and in nature, the two species often shoal together. Both are generalist predators of a range of limnetic and benthic invertebrates (Delbeek and Williams 1987). This overlap in resource use means that interactions between them are likely to be common where they are found together.

Our study had two aims. Our first aim was to determine the extent to which the previously demonstrated interspecific differences in preference for vegetated or open habitats were affected by individual differences in activity levels and boldness. In this study, boldness was defined as the expression of a behaviour under conditions of potential risk (Realé et al. 2007). Our second aim was to determine the relationship between individual levels of activity and

boldness and competitive ability in prey contests between heterospecifics. We performed three experiments. Experiment 1 considered the consistency of behavioural variation. Individual fish of both species were tested on two occasions, 10 days apart, and their activity or boldness score recorded. We predicted that these behavioural measures would be correlated between exposures. In Experiment 2, we tested the previously reported difference in habitat use. In this study, we not only predicted that ninespines would spend more time in the vegetation but also that individual variation in activity and boldness would determine the extent to which the open and vegetated habitats were used. In Experiment 3, we predicted that bolder or more active individuals would consume the larger share of prey in interspecific prey contests and that there would be no species difference in competitive ability.

Materials and methods

Study system

Young-of-the-year three- and ninespine sticklebacks were collected from Melton Brook, Leicestershire, UK in late summer 2005 using dip-nets and were transported to the laboratory.

Sticklebacks in this channel co-occur with a number of predatory fish (Eurasian perch, *Perca fluviatilis*, and Northern pike, *Esox lucius*), which are present at low densities.

In the laboratory, they were divided into conspecific groups of 20, and each group was assigned to its own chemically and visually isolated holding tank (40×25×25 cm, water depth 20 cm, with artificial vegetation for cover). The water temperature and light/dark regimes were held at 10°C and 12:12 h, respectively, over the duration of the study. Maintaining this temperature and photoperiod was sufficient to inhibit reproductive behaviour and prevented the fish from developing nuptial colouring. Fish were fed live *Daphnia* spp. and frozen Chironomid larvae once per day, unless otherwise stated below. They were held under these conditions for 3 months before the experiments began.

Behavioural assays

We performed four behavioural assays. The first considered general activity (assay A), while the second investigated boldness (assay B). In designing the boldness assay, we were careful to choose a target behaviour that was not directly dependent upon activity levels, such as exploration rate. Our chosen boldness assay, outlined below, focussed instead upon the latency to emerge from cover, cross an

open, exposed area and attack a group of prey. A pilot study revealed that fish generally spent <6 s over the open area during the trial ($n=15$ of each species; mean±standard error=4.4±0.7 s and 3.8±0.8 s for threespines and nine-spines, respectively), and all attacked the prey within 5 s after crossing (3.0±0.5 s and 2.3±0.5 s, respectively). This suggests that the latency to break cover and attack prey is a discreet behavioural action and is not simply an artefact of the activity levels of the fish simply determining when it strays into proximity with the prey patch. A third assay, assay C, considered the use of open versus vegetated habitat and investigated the differences in habitat preferences previously reported in our chosen study species (Hart 2003). Our final assay focused upon interspecific prey competition (assay D). All behavioural assays, with the exception of prey competition, were performed in test tanks measuring 60×30×30 cm, with a water depth of 27 cm and a 2-cm deep substrate of 5-mm gravel. Test tanks were covered with black screening on the sides and rear, and observations were made via a slit in a black screen in order to prevent disturbance. The configuration of the tanks varied between assays, as described below. Following each trial, the water in the tank was replaced. Fish were deprived of food for 24 h prior to being tested in assays A–C. Details of the feeding regime used in assay D are given below.

Activity (assay A)

Fish were tested in an open environment; aside from the gravel substrate, the tank was unfurnished. The fish was introduced to the centre of the tank within a 7×7 cm, 27-cm tall holding unit. This was constructed of clear, perforated plastic, allowing the fish to assimilate visual and chemical cues from the test tank. The fish was held in the unit and allowed to settle for 5 min, before this was removed, releasing the fish and beginning the trial. The trial ran for 5 min, and we used point sampling (Lehner 1996) at 15 s intervals ($n=20$ measurements) to record whether the fish was swimming or freezing.

Boldness: latency to attack prey (assay B)

Shyness and boldness describe the relative degree to which individuals incur risk when performing behaviours. In this assay, we recorded the latency of fish to leave a shaded refuge and cross an open, illuminated area, to reach a prey patch. In the open area, they were conspicuous and had no shelter from predators. Individuals faced a tradeoff between leaving shelter earlier, risking predation but gaining access to food sooner or leaving later, a strategy that might allow them to better gauge predation risk but at the cost of delayed feeding. The test tank was divided into three sections along its longest axis. Those at either end were 15-cm

wide. One contained a prey patch in the form of a centrally placed 5-cm diameter 27-cm tall clear plastic cylinder containing approximately 200 live *Daphnia* spp. The other end section formed a refuge and contained artificial vegetation constructed from 5-mm-thick strands of green twine, arranged in five clumps of ten strands each, running to the water surface and weighted at the bottom. Three were placed 5 cm apart and 5 cm from the end of the tank, and the remaining two were placed 5 cm in front of these and 10 cm apart from each other. The refuge end section was shaded by a 15-cm wide strip of black plastic placed across the top of the tank. The central section contained a 30×30 cm white plastic sheet laid flat upon the substrate. Both the central section and the prey patch were illuminated from above by a single 750-mm long, 60 W, white light striplight positioned centrally, 120 cm above the water surface. In order to reach the prey patch the fish had to leave the refuge and enter the illuminated area of the tank, crossing this open area. A 7×7 cm, 27-cm tall holding unit, described above, was placed in the refuge section, between the two forward-most clumps of vegetation. The fish was held in the unit for 5 min, before this was removed, releasing the fish and beginning the trial. We recorded the time taken for the fish to leave the refuge, cross the central section and attempt to strike the *Daphnia* within the prey patch cylinder.

Habitat preferences: open versus vegetated water (assay C)

This assay was designed to compare the influence of species-specific differences (Hart 2003) and individual behavioural variation in determining habitat use. One half of the tank contained artificial vegetation constructed from 5-mm-thick strands of green twine. These were arranged in 16 clumps of ten strands each, running to the water surface and weighted at the bottom. They were spaced 5 cm apart in a grid within one 30×30 cm half of the tank. The other half of the tank was open, containing no structure apart from the gravel substrate. The fish was introduced to the centre of the tank within a 7×7 cm, 27-cm-tall holding unit, as previously. The fish was held in the unit for 5 min, before this was removed, releasing the fish and beginning the trial. The trial ran for 5 min, and we used point sampling (Lehner 1996) at 15-s intervals ($n=20$ measurements) to record whether the fish was in the vegetated or the open half of the tank.

Prey competition (assay D)

We tested pairs of heterospecifics, as described in Experiment 3, below. Fish were tested within their own holding tank so as to minimise stress. Fish were provided with single, sequentially delivered *Daphnia* spp., a common prey type of both species. Thirty minutes before the fish

were tested, the artificial vegetation was removed from their tanks. This ensured that both fish had a clear line of sight to the prey. Prey suspended in 2 ml of tank water were added via a 5-ml syringe through one of five holes, spaced 2 cm apart at the water line of each aquarium. The order in which each hole was used was randomised to prevent fish from monopolising or predicting optimum feeding positions (Webster and Hart 2006; 2007). We introduced a total of six prey items, with a 30-s period between the ingestion of one item and the introduction of the next. We recorded the species in each pair that consumed the prey item in each instance. This test was performed three times according to the Experiment 3 schedule and was used to calculate the mean prey intake per partner per pair. Immediately after testing, we returned the artificial vegetation and provided excess *Daphnia* to the tank, so that both fish could feed until satiated. Uneaten *Daphnia* were removed after 30 min. This served to standardise hunger levels between trials.

The experiments

These behavioural assays were employed in the following three experiments.

Experiment 1: Are individual behavioural differences consistent over time?

The ecological study of individual behavioural variation assumes that the behavioural responses expressed by individuals exposed repeatedly to the same stimuli should be consistent within individuals and correlated across them between exposures. If individual responses vary unpredictably between exposures, then attempting to use individual variation in the studied behaviours to predict the outcomes of subsequent behaviours or interactions might be misleading. In this experiment, we measured the behaviours of individuals of each species on two occasions, 10 days apart, in the activity and boldness assays described above. Four groups of 15 size-matched fish (37–40 mm standard length) were established, two of each species. Body length did not differ significantly between the two species [one-way analysis of variance (ANOVA): $F_{(1, 58)}=1.03$, $P=0.18$]. Fish were housed at a density of three conspecifics per tank (30×30×30 cm; water depth, 20 cm, artificial vegetation for cover). Within each tank, individuals were identified by their standard length and by their body markings. One group of each species was then tested twice in one of the two behaviour assays, for activity (A) and boldness (B), according to the following schedule:

- Day 1. Groups established
- Day 2. First test
- Day 12. Second test

Statistical analysis

We used Spearman rank correlations to look for correlations between the response variables recorded for each individual of each species in each of the two assays.

Experiment 2: Does individual behavioural variation predict habitat use?

Three- and ninespine sticklebacks have been shown to differ in their preferences for open versus vegetated habitats (Hart 2003). Here, we asked whether this pattern was exclusively a species-specific difference or whether habitat use also varied within species as a function of individual differences in behaviour. Habitat use is an indirect measure of the potential for competition between species, since it influences how often they are likely to meet in space, and the potential for both to exploit the same habitat-specific resources. We tested 34 threespine and 35 ninespine sticklebacks, measuring between 31 and 50 mm standard length. Body length did not differ significantly between the two species (one-way ANOVA: $F_{(1, 67)}=2.06$, $P=0.21$). For each fish, we recorded the blotted mass to the nearest 0.001 g and then placed it in its own separate chemically and visually isolated holding tank measuring 30×20×20 cm, water depth 17 cm with a 1-cm deep fine sand substrate. Artificial vegetation was provided for cover to minimise stress. For each individual, we performed a number of behavioural assays according to the following experimental schedule:

- Day 1: Fish assigned to its own holding tank
- Day 3: Behaviour assay: A. Activity
- Day 5: Behaviour assay: C. Habitat preferences: open versus vegetated water
- Day 7: Behaviour assay: B. Boldness: latency to attack prey

Statistical analysis

We used a general linear model to look for predictors of habitat use by individuals. Proportional habitat use scores obtained from assay C provided the dependent variable. Species was assigned as a fixed factor, and the proportion of time spent moving in assay A and the latency to attack prey obtained from assay B, along with individual body mass measured on day 1 were included as covariates. We also used one-way ANOVAs to look for species differences in activity, habitat preferences and boldness. Proportional habitat use scores and the proportion of time spent moving were first normalised with arcsine transformations.

Experiment 3: Does individual behavioural variation predict the outcome of interspecific prey contests?

In this experiment, we considered prey contest interactions between individuals of the two species. This is a direct measure of resource use competition. Given the overlapping prey use patterns of the two species and the fact that they shoal together in nature, it is likely that prey contests are frequent in the wild. We asked whether the two species differed in their ability to compete for a common prey type and whether individual behavioural variation also played a role in determining contest outcomes. We formed 24 pairs of one threespine and one ninespine stickleback, size matched to <5 mm standard length. Body length did not differ significantly between the two species (one-way ANOVA: $F_{(1, 47)}=2.52$, $P=0.16$). Each pair was housed in a chemically and visually isolated tank measuring (30×20×20 cm, water depth 17 cm, with artificial vegetation added for cover). Pairs of fish were always housed together, except during assays A and B where they were tested alone. For each pair, we ran three prey competition trials and performed other behavioural assays according to the following experimental schedule:

Day 1: Fish were measured to the nearest 0.1-mm standard length, weighed to the nearest 0.001 g and assigned in pairs to their own holding tank

Day 3: Behaviour assay: D. Prey competition trial 1

Day 5: Behaviour assay: D. Prey competition trial 2

Day 7: Behaviour assay: D. Prey competition trial 3

Day 9: Behaviour assay: A. Activity

Day 12: Behaviour assay: B. Boldness: latency to attack prey

Statistical analysis

Within each pair, we subtracted the mean proportional prey share of the ninespine stickleback from that of the threespine stickleback. This gave a value where a positive score corresponded to a greater prey share for the threespine stickleback, and a negative score indicated a greater prey share for the ninespine stickleback. We also did this for body mass, as measured on day 1, and for the proportion of time spent moving in assay A and the latency to attack prey obtained from assay B. These were used as covariates in a general linear model with the difference in prey share designated the dependent variable, with differences in activity, boldness and body mass included as covariates. Data for the proportion of time spent moving were first normalised by arcsine transformation. We used a Wilcoxon-signed rank test to compare the prey share difference values against a null expected value of zero in order to determine whether prey share differed between the two species.

Results

Experiment 1: Are individual behavioural differences consistent over time?

Activity levels were consistent within individuals and were therefore correlated between exposures performed 10 days apart (Spearman rank correlation: threespines, $N=15$, $r=0.91$, $P<0.001$; ninespines, $N=15$, $r=0.81$, $P<0.001$, Fig. 1). Latency to emerge from cover, cross open water and attack prey was also correlated between exposures (threespines, $N=15$, $r=0.75$, $P=0.002$; ninespines, $N=15$, $r=0.66$, $P=0.016$, Fig. 1).

Experiment 2: Does individual behavioural variation predict habitat use?

A one-way ANOVA revealed that the ninespines spent significantly more time in the vegetation half of the tank than did the threespines [$F_{(1, 67)}=9.77$, $P=0.003$, Fig. 2a]. We observed no interspecific differences in activity levels [one-way ANOVA: $F_{(1, 67)}=1.31$, $P=0.25$, Fig. 2b] or latency to approach the prey patch sooner in the boldness test [one-way ANOVA: $F_{(1, 67)}=3.26$, $P=0.07$, Fig. 2c].

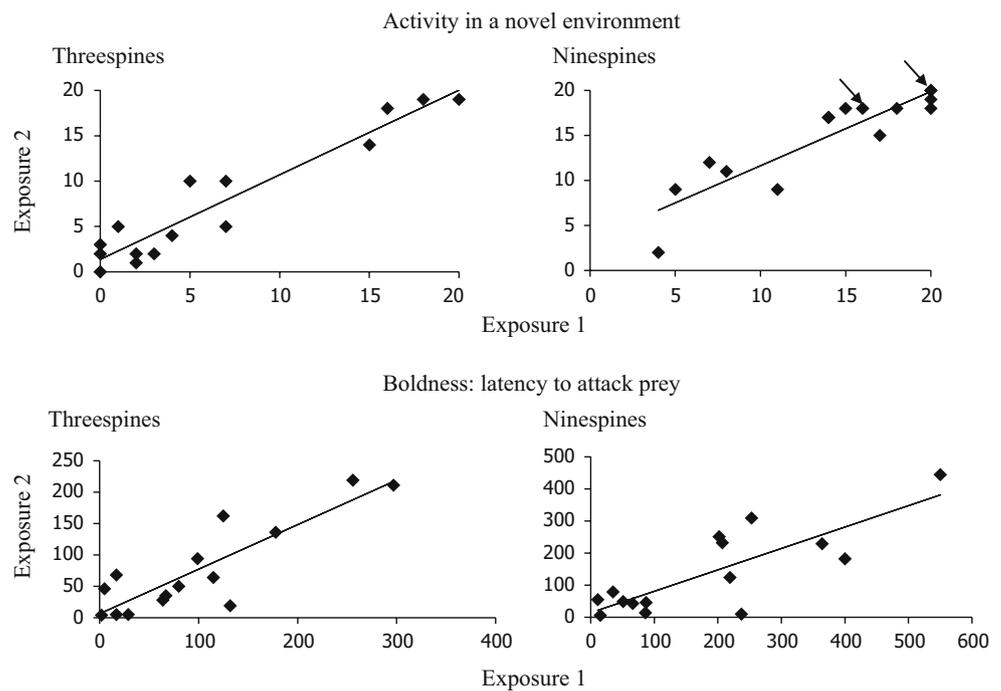
A general linear model (presented in Table 1.) supported the observed species difference in habitat use and also revealed that individual variation in activity levels were correlated with habitat use. Specifically, individuals that were more active in a novel habitat were also more likely to spend more time in open water in the habitat use test (Fig. 3). Together, species and individual activity levels explained almost half of the variation in habitat use ($R^2=0.49$). Neither individual variation in latency to approach a prey patch nor body mass were correlated with habitat use (see Table 1).

Experiment 3: Does individual behavioural variation predict the outcome of interspecific prey contests?

We saw no evidence for a species-specific difference in competitive ability when heterospecific pairs were competing for sequentially delivered *Daphnia* prey (Wilcoxon-signed rank test: $N=24$, $Z=-1.01$, $P=0.30$, Fig. 4). Competitive interactions took the form of scramble competition (Ward et al. 2006), with both individuals pursuing a prey item, until one of them captured it and consumed it.

Differences in prey share recorded during the first prey competition test were correlated with those obtained for the third prey competition test (Spearman rank correlation: $n=24$, $r=0.20$, $P=0.012$, Fig. 5). This suggests that the relative differences in competitive ability between individuals within pairings were stable over the study period.

Fig. 1 Measures of individual activity in a novel environment (number of sampling points recorded as active out of a total of 20) and latency to emerge from cover, cross open ground and attack prey were positively correlated between the two exposures, indicating that these behaviours were expressed consistently within individuals. Each data point represents one individual fish, except in the ninespine activity levels panel, where two pairs of individuals had identical scores. These points are indicated by the arrows, and each represents two individuals



A general linear model (presented in Table 2.) revealed that differences in boldness between the competing individuals were correlated with differences in mean prey share. Individuals that emerged from cover and attacked prey sooner than their partners in the boldness assay (where fish were tested individually) also consumed a greater mean share of prey than their partners during paired competition trials (Fig. 6). Variation in boldness explained one third of the observed prey share disparity ($R^2=0.33$), while we saw no effect of differences between individuals in activity levels or body mass.

Discussion

The findings of our study highlight the ways in which variation in individual behaviour might influence the outcome of interactions that occur between ecologically similar species. Our first experiment demonstrated that differences in the assayed behaviours of both three- and ninespine sticklebacks were relatively stable within individuals over time, at least on a scale of days to weeks. This finding is consistent with previous work on boldness in threespine sticklebacks (Ward et al. 2004).

In accordance with the findings of earlier research (Hart 2003), our second experiment identified a species difference in habitat use, with the ninespines seen to spend more time in the vegetated habitat to a greater extent than the threespines. In addition to this, however, we also saw that individual variation in activity levels was correlated with

habitat use, with individuals that were more active in movement rate assay also spending more time in open water in the habitat use test. One explanation for this correlation is that the putatively anti-predatory behaviours seen in both contexts (i.e. remaining inactive in the open surroundings of the novel habitat test or restricting movement to the vegetated half of the tank in the habitat use test) are different components of a broader risk-averse exploration or foraging strategy. Spending more time immobile might be the most effective means of avoiding detection by predators when cover is not available. Species-specific differences in habitat preferences imply niche separation, with perhaps limited scope for encounters and direct interaction between heterospecifics under natural conditions. In fact, the findings of our second experiment might lead us to predict that whilst the mean habitat use preferences of two sympatric species can differ, individual differences in behavioural tendencies might nevertheless facilitate a degree of overlap and therefore interaction between certain individuals. The degree of overlap and subsequent potential for interaction are likely to be determined by a range of environmental pressures, and this is an idea we explore further below.

Our third experiment revealed that individual variation in boldness was correlated with competitive ability. Individuals that emerged from cover to attack prey sooner in the boldness test were more likely to consume a greater share of prey items in the paired heterospecific prey contests. Again, this correlation can be viewed as reflecting related components of a risk-mediated foraging strategy.

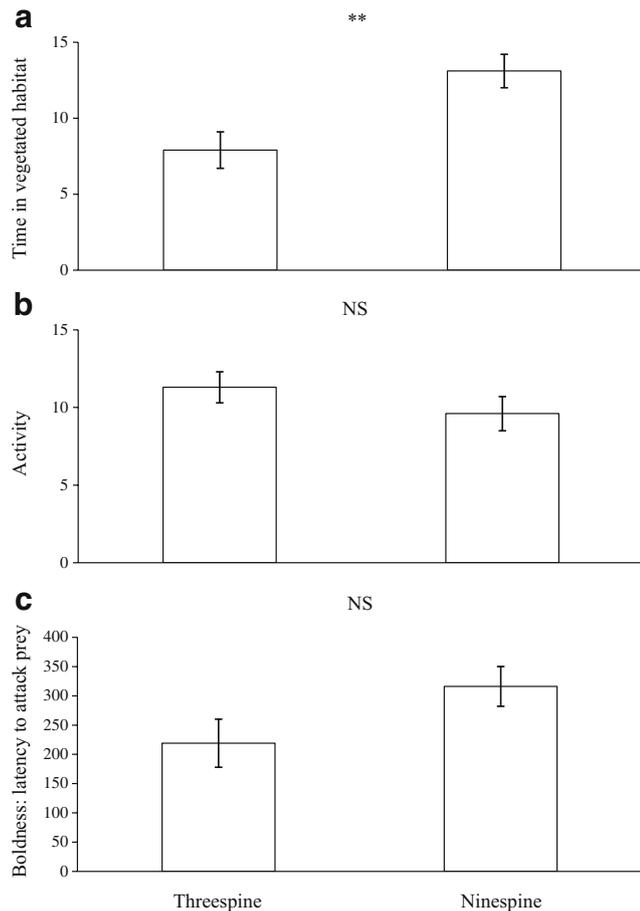


Fig. 2 **a** Ninespines spent more time in the vegetated half of the tank than did threespines (mean number of sampling points in vegetation out of a total of 20, \pm standard error). **b** There were no differences in activity rates seen between threespines or ninespines (number of sampling points recorded as active out of a total of 20, \pm standard error). **c** There was no difference in the latency to emerge from cover, cross open ground and attack prey (s, \pm standard error) seen between either species. ** $P < 0.01$, NS non-significant

Table 1 Results of a general linear model investigating factors influencing individual variation in the time spent in the vegetated half of a test tank in threespine and ninespine sticklebacks

Source	Degrees of freedom	<i>F</i>	<i>P</i> value
Corrected model	4	15.53	<0.001
Intercept	1	24.57	<0.001
Species	1	7.71	0.007
Activity	1	35.08	<0.001
Boldness	1	0.50	0.479
Body size	1	2.06	0.105
Error	64		
Total	69		

$R^2 = 0.49$. We saw a significant effect of species, with ninespines spending more time in the vegetated half of the tank than threespines, and also an effect of individual activity, with more active individuals spending more time in the open half of the tank than less active ones

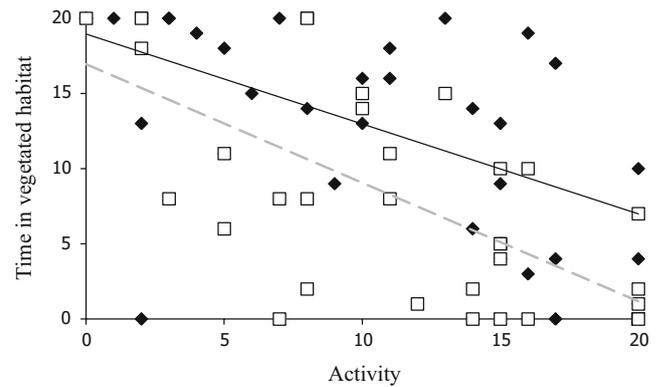


Fig. 3 Individuals that were more active in the novel environment (number of sampling points recorded as active out of a total of 20) spent less time in the vegetation in the habitat use test (mean number of sampling points in vegetation out of a total of 20). Threespine sticklebacks (white squares and grey, broken line) and ninespine sticklebacks (black diamonds and black, solid line)

Emerging from cover into an unfamiliar, open environment to attack prey is potentially costly in terms of predation risk. Individuals might delay emergence in order to gather valuable information about their surroundings but do so at the cost of delayed and potentially lost foraging opportunities. Coolen et al. (2003) found that ninespine sticklebacks preferentially observe feeding conspecifics from cover before selecting prey patches, a behavioural strategy that is in keeping with this idea.

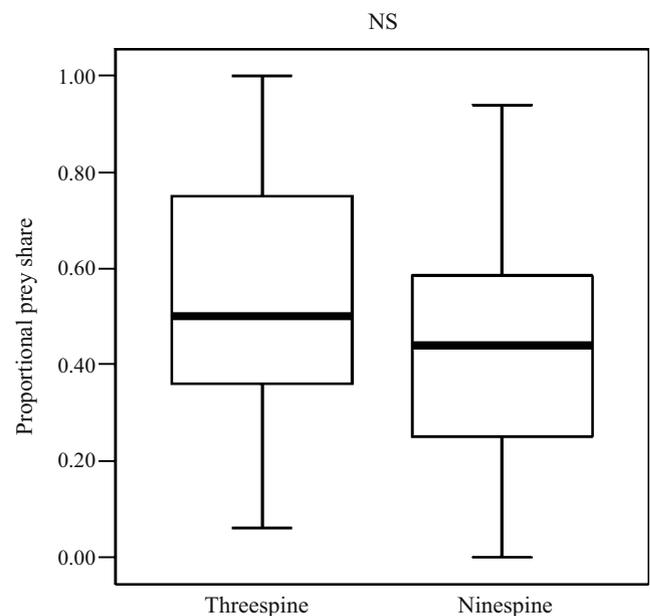


Fig. 4 The proportional prey shares of threespine and ninespine sticklebacks competing for prey in heterospecific pairs. The bold line indicates the median value, the box shows the interquartile range and the lower and upper error bars indicate the fifth and 95th percentiles, respectively. NS non-significant

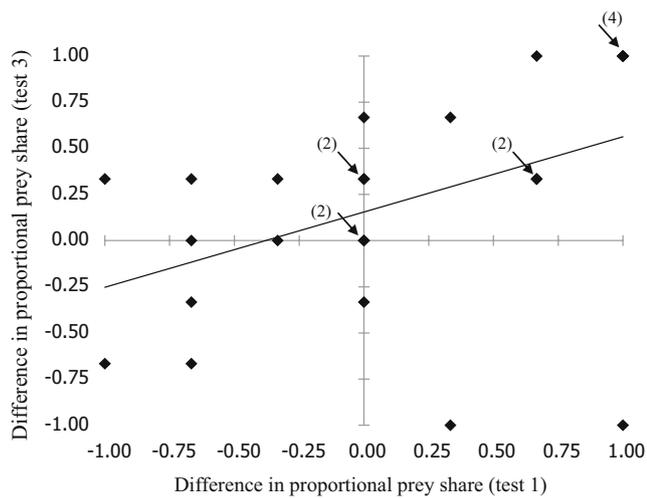


Fig. 5 Disparity in relative prey share within pairs of competing threespine and ninespine sticklebacks was positively correlated between the first and third tests, indicating that relative competitive ability was consistent between exposures. Relative prey share was calculated by subtracting the prey share of the ninespine from that of the threespine, such that a positive value indicates a greater prey share obtained by the threespine and a negative value indicates a greater prey share for the ninespine. Note that some pairs have identical scores. Their points therefore overlap, and these are indicated by arrows. The numbers in parentheses indicate the number of pairs represented by that point

In the paired feeding trials, competitive interactions took the form of scramble competition, rather than agonistic contest competition or kleptoparasitism. Costs associated with competing were not therefore linked to potential injury from competitors. Instead, cost might be incurred through energy expenditure, since scramble competition involves bursts of rapid locomotion in order to beat competitors to prey items or predation risk, since such movements could make individuals more conspicuous. As such, the same

Table 2 Results of a general linear model investigating factors influencing prey share in competition between paired threespine and ninespine sticklebacks

Source	Degrees of freedom	<i>F</i>	<i>P</i> value
Corrected model	3	3.42	0.037
Intercept	1	1.97	0.175
Activity	1	1.71	0.205
Boldness	1	7.04	0.015
Body size	1	0.32	0.576
Error	20		
Total	24		

$R^2 = 0.33$

Difference in boldness (latency to emerge from cover and attack prey, measured in a separate assay) was the only variable seen to be related to prey competition. Within each heterospecific pair, we saw that individuals that were bolder were also more likely to gain a greater share of the contested prey resource

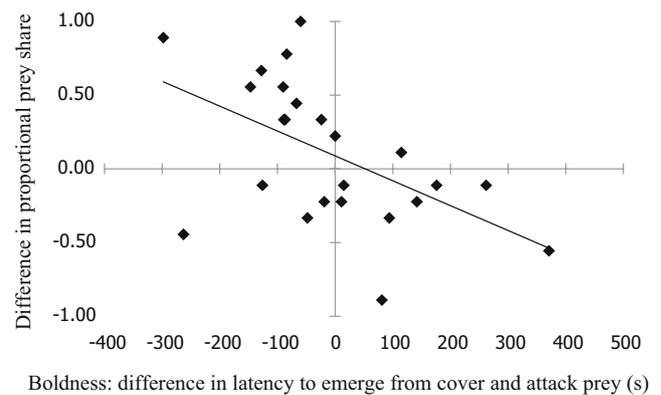


Fig. 6 Proportional prey share (mean of three trials) within pairs of competing threespine and ninespine sticklebacks was negatively correlated with the difference in latency to emerge from cover and attack prey between the two competing individuals. That is, individuals within each pair that attacked prey sooner when tested alone in the boldness test also tended to gain a greater share of prey when competing with their partner in the prey competition test. Relative prey share was calculated by subtracting the mean prey share of the ninespine from that of the threespine, such that a positive value indicates a greater prey share obtained by the threespine, and a negative value indicates a greater prey share for the ninespine. The difference in latency to emerge from cover and attack prey was calculated using the same method. Each data point represents one heterospecific pair

tradeoff exists between remaining relatively inconspicuous (and saving energy), at the cost of lost feeding opportunity, versus increasing prey intake, but at the risk of greater exposure to predators.

An alternative possibility to this ‘risk-mediated behavioural strategy’ idea is that the correlations between habitat use and activity seen in Experiment 2 and boldness and competitive ability seen in Experiment 3 are linked components of a behavioural syndrome. These behaviours may be constrained by, and vary in concert with, other behaviours under the control of common endocrinal or genetic mechanisms. In this study, selection acting upon one behaviour can exert influence upon other linked behavioural traits expressed in other contexts. Research by Huntingford (1976) and Bakker (1986) revealing positive correlations between territoriality and boldness, and general aggression and boldness, respectively, supports this idea. Previous work by our group (Webster et al. 2007) focussing upon threespine sticklebacks from a different population to the one used in the current study found that individuals could to some extent behave strategically, adjusting their behaviours with group size and by inference per capita risk, despite observed correlations between boldness when tested alone. This finding suggests that behavioural syndromes are not present in all populations and/or that their influence upon behaviour is not inflexible. It is not clear whether the correlations observed in this study were due to tradeoffs associated with evolved risk-mediated behavioural strate-

gies or the results of the constraining effects of a behavioural syndrome, and further work will be needed to elucidate this.

Could individual variation in behaviour play a significant role in interspecific interactions in natural systems? This will almost certainly depend upon the ecological similarities between the species in question. The competitive exclusion principle (Gause 1934) dictates that no two species can occupy exactly the same niche. Resource use overlap and the potential for competitive interactions are likely by definition to be minimal between ecologically specialised species, but where ecological generalist species co-occur, overlap in the use of resources such as food, territory or refuge may be sufficient for competitive interactions to occur regularly, allowing for the potential for individual level differences to exert measurable influence upon contest outcomes. Another factor critical in determining whether individual level differences are likely to play any meaningful role in such interactions is the degree to which species differ in terms of competitive ability and motivation. Where competitive interactions occur between species that differ substantially in size or aggression, then the outcome of contests is likely to be consistently predictable, with little scope for any significant effects of individual behavioural variation. In other cases, where species differences are less extreme and contest outcomes less obvious, traits particular to the competing individuals may become more important in deciding contest outcomes. Competing individuals can also differ in their motivation, the extent to which they are willing to trade off the benefits they might accrue by winning a contested resource against the potential costs of losing. For example, territory holders, having more to lose, might fight harder than intruders, while hungry individuals might compete more vigorously for food than their recently fed opponents (Parker 1974). This is as true for interactions between heterospecifics as it is for competing conspecifics.

The three- and ninespine sticklebacks used in this study are examples of animals where we might expect potential for individual level differences to impact upon interspecific interactions in nature. Both forage for the same range of prey types, and though differences in habitat preferences exist and differences in levels of aggression have been reported (Huntingford 1977), both species are known to shoal together under natural conditions. Our experiments used fishes of similar size. In nature, heterospecific shoal mates are likely to be size-matched in order to minimise oddity effects, an anti-predation response, and because body size determines swimming speed, with associated costs for larger or smaller, and therefore faster or slower swimmers (Krause et al. 2005). This could serve to reduce the potential for gross differences in body size or strength alone to determine the outcomes of competitive interac-

tions. Indeed, mixed species groups might be useful study systems for researchers looking to study the effects of individual variation in behaviour upon competitive interactions in natural systems. In the wild, heterospecific aggregations are regularly seen amongst shoaling fishes (Hoare et al. 2000; Krause et al. 2005), passerine birds (Dolby and Grubb 1998) and ungulate, cetacean and primate mammals (Stensland et al. 2003). The composition of such groups affords the opportunity for frequent interspecific interactions, while resource use overlap is likely, especially in groups which form primarily because of shared food preferences amongst the constituent species.

Within sets of interacting species, niche overlap and the potential for interaction between heterospecifics can vary between sympatric populations (Delbeek and Williams 1987) as a function of differences in environmental factors such as heterogeneity in habitat structure, prey assemblage, predation pressure and community structure. When interspecific competition is intense, character displacement is likely, leading to the restriction of niche breadths and reducing the likelihood of interactions between species (Schluter and McPhail 1992; Schluter 2000). When predation pressure is severe, species-specific differences in vulnerability can also lead to character displacement and habitat shifts, resulting in species segregation and having similar consequences for resource use overlap (Persson and Eklöv 1995). In the case of three- and ninespine sticklebacks, differences in spine and lateral plate morphology mean that they differ in their vulnerability to predation by gape-limited predators (Hoogland et al. 1957). This difference most likely accounts for the observed differences in habitat preferences (Hart 2003; our experiment 2), and greater shoaling responses elicited by predators (Godin and Clark 1997), and greater reliance upon social information (Coolen et al. 2003) seen in ninespines relative to threespines. The fish used in our study came from a site in which predators are present at low densities. It is probable that populations that are subject to greater predation pressure might exhibit greater differences in habitat use and behaviour and that the opportunity for, and resolution of, interspecific interactions will be less dependent upon individual level variation and more likely to be decided by species-level differences. Similarly, where predation pressure is lower or absent, we might expect to find a reduction in the importance of species-specific differences and greater scope for individual level variation to affect the frequency and outcome of interactions. The tractability of the stickleback study system means that this hypothesis can easily be tested, and we predict that there will be a negative correlation between the intensity of predation pressure acting upon populations and the relative importance of individual level variation in determining the outcomes of interspecific resource contests. Such an

approach could give us insight into the role of behavioural variation in determining interspecific interactions in natural systems.

Acknowledgements Mike Webster was supported by a NERC studentship. All procedures carried out in this study conformed to the current laws of the UK. We thank Theo Bakker and two anonymous referees for their helpful comments.

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