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Fixed behavioural plasticity in response to predation risk in the three-spined stickleback

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I experimentally tested the repeatability and plasticity of two antipredator behaviours, shoaling and risk taking, in a sample of 443 juvenile three-spined sticklebacks, *Gasterosteus aculeatus*. I quantified between-individual variation in these behaviours as well as behavioural changes over time in two groups of sticklebacks that were either exposed or not exposed to simulated predation pressure. Shoaling and risk taking were repeatable within individuals in both experimental and control fish. Individual willingness to shoal increased over time in both experimental and control groups, but there was no evidence that shoaling changed in response to predation risk. Risk taking also showed temporal changes: sticklebacks exposed to simulated predation risk became increasingly fearful, unlike the control fish, suggesting that this behaviour is plastic. There was, however, no evidence of between-individual variation in the behavioural changes over time in either the control or experimental condition, suggesting that behavioural plasticity is a fixed response in the individuals of this population.

Phenotypic plasticity is the ability of a single genotype to produce more than one phenotype in response to environmental conditions (Pigliucci, 2001; Scheiner, 1993). Behavioural plasticity, in particular, may be linked to fitness, for example by adjusting foraging behaviour to the level of predation risk or competition or by changing mating behaviour according to sociosexual environments (Bell & Sih, 2007; Han & Brooks, 2013; Krebs & Davies, 1997; Laskowski & Bell, 2013). Recent studies have shown that individuals from the same population may differ in the level of behavioural plasticity (Dingemanse & Wolf, 2013). Within-population variation in behavioural plasticity can have important consequences for animal populations by increasing or decreasing individual differences in behavioural strategies or by affecting the consistency of the behaviours in different environmental contexts (Nussey, Wilson, & Brommer, 2007).

Predators play an important role in the evolutionary process of shaping behavioural patterns. Predator-mediated directional selection on behaviour (Bell & Sih, 2007; Huntingford, Wright, & Tierney, 1994; Lima, 1998; Wolf, van Doorn, Leimar, & Weissing, 2007) can reduce behavioural variation in a population under a constant level of predation pressure. On the other hand, short-term effects of predation pressure on the phenotypic expression of behaviours (Dingemanse, Barber, Wright, & Brommer, 2012; Relyea, 2005) may be important for the viability of individuals in a heterogeneous environment in which predation risk varies over time (Pigliucci, 2001; Roff, 1997). Adaptive plasticity in antipredator behaviour could reduce mortality when predators are present, but maximize fitness gains via increased feeding rate in other circumstances (Luttbeg & Sih, 2010; Stamps, 2007).

To improve our understanding of how predation pressure influences prey behaviour, it is necessary to test whether individuals vary in the pattern of behavioural change in response to predation risk (Dingemanse et al., 2012). The behaviour of individuals can vary in multiple ways as a function of personality and plasticity. For instance, the average level of antipredator behaviour, which represents personality, may vary between individuals (I: individual variation). Individuals may also vary in the level of their environment-behaviour gradient representing behavioural plasticity (I∗E: individual∗environment interaction). If a simple behavioural rule is favoured according to predation risk (Houston & McNamara, 1999), selection should erode within-population variation in behavioural plasticity (Dingemanse, Kazem, Réale, & Wright, 2010). Individual and genetic variation in behavioural plasticity can be maintained if selection depends on the frequency of different types of behaviour within a population (Wolf, van Doorn, & Weissing, 2011) or if selection fluctuates (Sasaki & Ellner, 1997). The variation may also be maintained when

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the behavioural plasticity reflects alternative strategies with comparable average performance over evolutionary time (Oliveira, Taborsky, & Brockmann, 2008).

I experimentally tested behavioural plasticity in response to predation risk in the three-spined stickleback, *Gasterosteus aculeatus*. Juvenile sticklebacks used in this study were born in captivity, but originated from a natural population preyed on by piscivores. Predation pressure could thus have shaped the behavioural plasticity in this population during their evolutionary history. I studied the expression of behavioural phenotypes in full-sib and half-sib families in different environments. Juvenile fish from different genetic families were exposed to two environments that differed in predation risk. Experimental fish were exposed to simulated predation risk and control fish were kept in the predator-absent condition. During the experiment, changes over time in social responses to conspecifics (i.e. shoaling) and willingness to take risks for foraging (i.e. risk taking) were observed. By comparing behavioural changes over time in the control and experimental fish, I tested whether these behaviours are plastic in relation to predation risk. I also tested whether the behavioural plasticity varies between individuals by testing behavioural reaction norms based on temporal changes in behaviour within each treatment.

METHODS

Study Population and Breeding Design

Sexually immature three-spined sticklebacks were captured with hand nets from the Rio Ulla, Galicia (Spain), in February 2013 (for a map see the Supplementary material). Once mature, 16 males and 16 females (of the 70 fish originally collected) were used for breeding. The breeding design and fish husbandry of adults and juveniles are fully described in a previous paper (Kim & Velando, 2015). Each fish bred twice with two different mates, producing 32 full-sib families of the F1 generation, during April–May 2013. Thus, each full-sib family had a maternal and a paternal half-sib family. At age 40 days, fry from each full-sib family were divided among two ($N = 7$ families) or four ($N = 25$ families) 'growth tanks' ($N = 114$ tanks; 24 × 16.5 cm and 17.5 cm high), depending on the brood size. Each tank housed 11 or 12 juvenile fish. The tanks were connected to closed water systems equipped with the combined continuous function of a mechanical filter, a circulation pump and a flow-through water-cooling device. Juvenile fish were fed to satiation twice daily until 5 months old then once a day. They were fed on a progressive diet of newly hatched *Artemia* from hatching to 3 months old and a commercial pelleted diet (Gemma Micro, Skretting, Norway) from 2 months old onwards. The natural photoperiod was simulated by programmed illumination.

Experimental Protocol

The experiment was carried out during September–November with 448 juvenile sticklebacks around 5 months old (143–160 days) from 31 full-sib families (and 112 different growth tanks) in seven weekly experimental sessions. One family from the stock was excluded from this experiment to match the number of fish across all different experimental tanks and weeks. Age effects on behaviours were not significant in preliminary analyses; therefore age was not included in further analyses. Prior to each weekly experimental session, I created four experimental and four control tanks (33 × 18 cm and 19 cm high). Each tank contained eight sticklebacks from four or five different full-sib families (see also Kim & Velando, 2015). Four individuals were randomly selected from each growth tank; two individuals were then allocated to two experimental tanks and the other two to two control tanks. Before allocation to a tank, individuals were weighed and permanently marked with colour elastomer tags (Northwest Marine Technologies, Shaw Island, WA, U.S.A.) under a low dose of benzocaine anaesthetic. Each individual was marked with a coloured tag on either the anterior or posterior dorsal of both lateral sides to allow rapid identification of the eight different individuals in the same tank. Body weight did not differ between the experimental and control groups (mean ± SE; experimental: 0.307 ± 0.005 g, $N = 224$; control: 0.308 ± 0.005 g, $N = 224$; Student's t test: $t_{446} = 0.086$, $P = 0.931$).

Each experimental tank contained a sponge filter, an artificial plant and a transparent food cup to which bloodworms were added as food once a day. The front wall of the tanks was transparent to enable observation. The other walls were opaque. Large opaque dividers were inserted between the tanks to prevent interference from different experimental treatments. During the acclimatization period of 6 days in the experimental tank, the fish were accustomed to feeding on bloodworms from the food cup.

After acclimatization, two different behaviours were recorded in all fish (day 0; shoaling was recorded between 0900 and 1200 hours and risk taking at 1500–1700 hours); the experimental treatment began immediately after the behavioural tests (the first predator attack simulation was performed at 1800 hours on day 0). The chemical and visual simulation treatments consisted of adding 20 ml of water from an aquarium holding brown trout, *Salmo trutta*, before introducing a model trout (13 cm long) into the tank and chasing the sticklebacks for 10 min with this model. I ensured that all the sticklebacks in the tank were chased during each treatment. The control tanks were treated by adding the same amount of clean water and omitting the visual stimulus. The treatments were executed repeatedly at randomly chosen times of day between 0900 and 1800 hours. Each experimental tank was subjected to 12 treatments (120 min) throughout days 0–4. One treatment on day 0, four treatments each on day 1 and day 2, two treatments on day 3 and one treatment on day 4 were scheduled.

Behavioural Observations

Behavioural observations were made repeatedly for all individual sticklebacks before the treatment began (day 0), during the treatment (day 3) and after the treatment (day 4). Sticklebacks that died during the experiment were excluded from the analyses (experimental: $N = 3$ individuals; control: $N = 2$ individuals). A total of 2658 observations made on 443 individuals (two behaviours × three repeated measures) were used for statistical analyses.

The tests for shoaling and risk taking are fully described in a previous paper (Kim & Velando, 2015). In summary, shoaling was tested for each individual in an observation tank, which contained three unfamiliar conspecifics of similar size. A focal fish was allowed to swim between the acclimatization and conspecific zones (16 cm distance); the time taken to reach the conspecific zone was measured up to 180 s. This test assesses the individual's willingness to join the conspecific group. Individuals were returned to their experimental tanks after this test. At least 3 h after the shoaling test, I assessed individual willingness to forage under predation risk simulated by a model avian predator (the grey heron, *Ardea cinerea*) in the experimental tanks (see also Bell, 2005). I attached a dummy head of a grey heron over the experimental tank and then added bloodworms to the food cup. When at least one fish took a bite of food, an attack was simulated by quickly releasing the predator's head. I observed individual behaviours for 300 s while the predator model was still present above the tank and recorded the time taken since the attack for each individual to take the first bite of food. Risk taking was measured simultaneously in all