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Developmental plasticity compensates for selected low levels of behavioural avoidance in a freshwater snail

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The way in which an organism responds behaviourally to environmental stimuli may either be innate (i.e. expressed without the need for previous experience) or affected by prior experience; but the relative importance of these two mechanisms in controlling antipredator behaviour is, as yet, unclear. We investigated how the avoidance behaviour of juvenile great pond snails, *Lymnaea stagnalis* (L.), artificially selected for either high or low innate avoidance behaviour, where the snail crawls above the waterline in response to predation cues, differed in terms of their response to prolonged exposure to predator kairomones during their development. Specifically, we tested: (1) whether snails from lines selected for low response showed enhanced crawl-out behaviour following exposure to predator kairomones during development; and (2) whether snails from high-response lines were able to increase their response further when raised in predator kairomone or whether these snails were constrained in their response. Following developmental exposure to kairomones from a predatory fish (i.e. tench, *Tinca tinca* L.), low-response snails showed an increased avoidance response when exposed to predation (i.e. predator kairomone plus alarm cues), equivalent to that seen in lines selected for high innate responses. In contrast, snails from high-response selection lines showed no change in crawl-out behaviour. These findings suggest that for antipredator behaviour, developmental exposure can compensate for the selected reduction in innate response in *L. stagnalis*, and that there may be constraints limiting the maximum level of crawl-out behaviour.

Predation imposes high fitness costs and, as such, prey taxa are under strong selection to evolve effective antipredator traits (Lima & Dill 1990; Lass & Spaak 2003; Benard 2004). These traits may be innate and therefore expressed without the need for prior experience; alternatively they may be induced defences (Tollrian & Harvell 1999) whereby the phenotypic expression of the genotype varies depending on the experience of individuals during development (Bradshaw 1965). Selection on a trait that alters the mean value of that trait may also affect the degree of plasticity seen in that trait in response to the developmental environment (Scheiner 2002; Garland & Kelly 2006). At present, however, few studies have explicitly assessed how such selection on avoidance behaviour influences the ability of subsequent generations to respond to altered environmental conditions.

Phenotypic plasticity is thought to be associated with taxa that experience variable environmental conditions (Kawecki & Ebert 2004; Zhang 2006), and selection for greater trait plasticity can occur rapidly. For example in the great tit, *Parus major*, plasticity in laying time has increased significantly in response to variability in the timing of food availability (Nussey et al. 2005). In this case the increased plasticity appears to be correlated with an innate earlier laying date. Plasticity may also enable animals to survive in a novel environment by responding to environmental variation more rapidly than constitutive traits (Parsons & Robinson 2006). *Rana aurora* tadpoles, for example, evolved induced defences in response to cues from the invasive predatory bullfrog, *R. catesbeiana*, in less than 70 years (Kiesecker & Blaustein 1997), and *Gammarus pulex* have evolved higher levels of induced defences towards predatory fish in populations that coexist with fish (Åbjörnsson et al. 2004). Theoretically, directional selection will favour plastic traits that alter phenotype in the same direction as selection on the mean (Garland & Kelly 2006), and traits under strong selection on the mean response may also show the greatest degree of plasticity (Stearns 1983; Van Buskirk & Relyea 1998).

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The great pond snail, *Lymnaea stagnalis*, is able to respond rapidly to artificial selection for innate antipredator behaviour, crawling above the water line in response to predation cues (Dalesman et al., in press), producing, in just two generations, levels of divergence in innate behaviour found naturally between populations in high- and low-risk environments (Dalesman et al. 2007). *Lymnaea stagnalis* juveniles also increase antipredator behaviour following acute (48 h) exposure to a simulated predation event, where both predator kairomone and alarm cues are presented together (Dalesman et al. 2006). In the latter study acute exposure of juvenile snails to predator kairomones alone did not alter the behavioural response.

We used F3 generation snails from lines selected for high or low levels of innate antipredator behaviour (Dalesman et al., in press), to test whether this selection led to different responses following exposure of snails to predator kairomones during development from oviposition. We specifically investigated whether: (1) snails selected for low levels of innate antipredator behaviour increased their levels of avoidance behaviour following developmental exposure to predator kairomones; and (2) whether snails selected for a high level of response could increase crawl-out behaviour following developmental exposure to predator kairomones, or were constrained in their ability to increase this avoidance behaviour further.

METHODS

Study Species

Great pond snails from two source populations on the Somerset Levels, U.K., South Drain (51.18°N, 2.88°W) and Chilton Moor (51.19°N, 2.88°W) were selected for either high or low behavioural responses to fish predation cues (fish kairomones plus conspecific alarm cues) over two generations in the laboratory (see selection regime below), and a nonselected (random) line was also bred from each population to control for effects of laboratory rearing (Dalesman et al., in press). In the laboratory, adults were kept in aquaria with 4 litres of aerated artificial pond water (ASTM 1980) with 90 mg/litre [Ca^{2+}] (Rundle et al. 2004) at 20 ± 1 °C under a 12:12 h light:dark cycle, and fed on iceberg lettuce and spinach. Wild-caught adult *L. stagnalis* were retained as laboratory stock at the end of this study.

Tench, *Tinca tinca*, were taken from a laboratory stock originally obtained from Emperor Tropicals & Water Garden Centre, Plymouth, U.K. They were maintained in aerated and filtered water at 15 ± 1 °C under ambient light levels in 25-litre aquaria at a stocking density of 12 fish per aquarium (0.006 kg/litre). Gravel substrate and shelters positioned in the aquarium were used to mimic natural conditions. The tench were fed Nutri-flake (Hozelock Cyprio, Birmingham, U.K.), which contains no mollusc extracts, and hence avoided potential dietary cues affecting the trials. No experimental manipulations were carried out on these fish and the welfare of the fish was carefully considered throughout. After the trials the tench were retained as laboratory stock.

Selection Regime on F1 and F2 Generations

We carried out the selection regime as outlined in Dalesman et al. (in press). Briefly, two replicate selection lines, one from each original population, were used for each selection level to produce high- and low-selected individuals. Behavioural trials to select high and low responders using individual juvenile snails (6 ± 0.5 mm) were identical to those outlined below; however, in the case of selection trials all individuals were exposed to alarm plus tench cue combined, and it is the response to these combined cues that was selected for. Selection trials were carried out on 24 individuals at a time from each selection line. Selection criteria for high and low responses were such that high-responding individuals were those that crawled above the waterline within the first 30 min of the behavioural trial and did not re-enter the water for the 2 h duration; low-response individuals were those that never crawled out of the water during the 2 h trial period.

In the F1 generation both high- and low-responding individuals were selected during the same behavioural trials; however, in the F2 generation high-response individuals were selected only from the high-response selection lines, and low-response individuals were selected only from the low-response selection lines. Selection trials for each population were run concurrently to ensure that snails in all selection lines would be approximately equal in age, such that reproduction of the subsequent generation in each selection line would occur at the same time. Unselected individuals to produce the subsequent generation were selected at random from the F1 generation and from the unselected lines only in the F2 generation. In both cases 24 randomly chosen individuals were used for each unselected line.

Juvenile Development of the F3 Generation

F3 egg masses were collected from F2 adult *L. stagnalis* aquaria on the day they were laid and transferred into individual 1.5-litre aquaria with 1 litre of artificial pond water maintained at 20 ± 1 °C under a 12:12 h light:dark cycle. We used three aquaria per population/selection line combination for the predator exposure treatment (fish kairomones) and three aquaria for each of the controls (artificial pond water) giving a total of 36 (18 per developmental exposure treatment). We produced fish kairomone by placing three tench, (10 ± 1 cm) in 4 litres of water for 1 h. We then added 100 ml of either kairomone water, or control water, to give a 10% concentration, to each 1-litre aquarium every 4 days. This concentration did not cause juvenile snails to crawl above the waterline in rearing aquaria (S. Dalesman, personal observation) and did not significantly affect crawl-out behaviour in a previous study (Dalesman et al. 2006). On hatching, 21 ± 0.5 days after oviposition, the number in each aquarium was reduced to 20 individuals to control density. Kairomone or control addition continued every 4 days until snails reached 6 ± 0.5 mm spire height, 25 ± 0.5 days after hatching, at which point the juvenile snails were used for behavioural trials. The duration between oviposition and behavioural trials (46 ± 1 days) meant that each individual had been exposed to predator kairomone 12–13 times during the total developmental period.

Behavioural Trials

Sixty individuals per population/selection line combination (five from each aquarium per behaviour trial treatment) were exposed to one of four treatments using alarm cue from crushed conspecifics (A) and tench kairomone (T) in an orthogonal design: (1) control A−T− (artificial pond water alone, no tench kairomone or alarm cue added), (2) alarm cue only A+T− (no tench kairomone), (3) tench kairomone alone A−T+ (no alarm cue added) or (4) tench kairomone plus alarm cue A+T+. We produced tench cue in exactly the same way as in the exposure protocol above, by placing three tench into 4 litres of artificial pond water for 1 h, alarm cue by mixing three crushed conspecifics (6 ± 0.5 mm spire height) into 4 litres of pond water and tench plus alarm cue by mixing three crushed conspecifics into 4 litres of tench cue water; the control was the addition of artificial pond water alone.

Individual snails were selected at random from the exposure aquaria to experience each of the four treatments during the behavioural trial; the treatment protocol ensured that the position of each behavioural chamber was randomized. Each snail was used