



Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour

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We examined relationships between individual differences in antipredator behaviour and prey morphological characters (size, shape) that influence prey vulnerability. Behavioural responses of *Physa gyrina* to chemical cues associated with predation by crayfish *Orconectes rusticus*, were assayed in the laboratory for 6 days over a 13-day period. Snails displayed consistent, individually repeatable responses to the predation cues, including hiding (refuge use) and substratum avoidance (crawling to the water surface or out of the water). We assessed shell morphology using morphometric techniques that isolate geometrically independent components of size and shape variation corresponding to aperture width and apertural obstruction. Previous studies indicate that large size, narrow apertures and obstructed apertures reduce morphological vulnerability to the shell-entry predation tactics used by crayfish. In the present study, small, and thus more vulnerable, prey tended to show stronger antipredator behaviour than large prey (i.e. behavioural compensation for morphological vulnerability). In contrast, behavioural and shape-based defences were positively correlated; snails with narrow apertures showed strong antipredator responses. We refer to this 'double defence' against predators as cospecialization. With either compensation or cospecialization, suites of correlated behavioural or morphological traits must be studied in tandem to understand the adaptive value of prey responses to predators.

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Studies of antipredator behaviour typically find that prey show adaptive behavioural responses such as reducing their activity or increasing their use of refuge in the presence of predators (reviewed by Sih 1987, 1995; Lima & Dill 1990; Werner & Anholt 1993). The appropriate response, or the degree of response shown, often differs from one potential prey organism to the next. Differences in prey behaviour frequently reflect underlying connections with other traits related to vulnerability or energetic reserves. For example, prey in good condition often use costly defences that prey in poor condition do not perform (e.g. Stenzler & Atema 1977; Damsgård & Dill 1998). Vulnerable classes of prey (e.g. based on size, sex, reproductive status) may also be more likely to perform antipredator behaviour than well-defended prey (Stein 1977;

Reist 1983; Sih 1982, 1986; Werner et al. 1983; Alexander & Covich 1991a). Such studies of connections between antipredator behaviour and other traits have tended to focus on average responses shown by classes of prey, or focused on connections between behaviour and highly labile traits (i.e. dynamic state variables; Mangel & Clark 1988). Relatively little is known about individual variation in antipredator behaviour of prey within given classes and states. How consistent (repeatable) are individual differences in antipredator behaviour? If behaviours show repeatability, are individual differences in behaviour related to relatively subtle differences in other inherently repeatable traits, such as morphology?

Individual variation in behaviour is of particular interest for analyses of selection and the evolution of behaviour. To quantify consistent, individual variation in behaviour (i.e. repeatability of behaviour), evolutionary ecologists partition variation in behaviour into within-versus between-individual components (Arnold & Bennett 1984; Lessels & Boag 1987; Boake 1994). Repeatability is the ratio of the between-individual component of variance to total phenotypic variance. Several studies have quantified the repeatability of predator escape behaviours (e.g. Arnold & Bennett 1984; Garland 1988)

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but none that we know of has addressed repeatability of predator avoidance.

If prey show consistent, individual differences in behaviour, then an important issue is to understand factors that explain the differences. For example, are differences in behaviour related to individual differences in prey morphology, physiology or chemistry? Correlations between antipredator behaviour and other antipredator traits are particularly important for understanding natural selection on behaviour (e.g. Brodie 1992). Prey that show weak, apparently suboptimal, behavioural responses to predators might not suffer heavy predation if they have effective morphological or chemical defences. Although many studies have documented relationships between prey behaviour and relatively obvious differences among prey, few studies have examined correlations between prey behaviour and subtle variations in morphology. Given that prey often display intraspecific variation in both behaviour and morphology (and other predation-related traits), it is critical to study correlations and functional connections among traits to understand integrated prey responses to predators (Brodie 1992; McPeck 1995; Cheverud 1996).

We distinguish four functional relationships between traits that mediate a given selective agent: codependence, complementation, cospecialization and compensation. For behaviour and morphology, codependence occurs when traits are mechanically linked. For example, predator escape behaviour in fish (swimming bursts) is dependent upon effective morphology (e.g. large tailfin area and deep caudal peduncle) to enact the behaviour (reviewed by Webb 1982).

Alternatively, behaviour and morphology can be mechanically independent. Mechanically independent traits often must be used in particular combinations to produce an effective antipredator response. We refer to these as complementary traits. For example, individual garter snakes can be either striped or cryptic in coloration. The appropriate behavioural response to predators depends on colour. Crypsis requires prey to be motionless, whereas active escape is enhanced by stripes (Brodie 1992).

In yet other instances, behaviour or morphology can each be effective on its own. Prey can then employ both types of defence simultaneously (presumably enhancing overall defence), or only one type of defence (presumably saving the cost of a second defence). For example, if some individuals (relative to others) both hide more and have superior morphological defences, we refer to this as cospecialization. In statistical terms, cospecialization occurs if there is a positive correlation between the degree of prey defence shown for two types of antipredator traits. If, on the other hand, prey show a negative correlation between two types of antipredator traits, this represents trait compensation. For example, prey with poor morphological defence might show stronger antipredator behaviours than morphologically well-defended prey.

Here, we examined behavioural responses of the freshwater snail *Physa* (= *Physella*) *gyrina* to a common, natural predator, the crayfish *Orconectes rusticus*. We quantified: (1) antipredator behaviour in the presence versus absence

of refuge structures; (2) repeatability of predator avoidance behaviours; and (3) relationships between antipredator behaviour and two aspects of prey morphology that influence vulnerability to crayfish (snail size and shell shape).

Study System

Physid snails are common and important members of aquatic food webs in ponds, lakes and rivers (Sheldon 1987; Gelwick & Matthews 1992; Vaughn et al. 1993; Lodge et al. 1994). These snails are preyed upon by many organisms, including flatworms, leeches, fly larvae, beetles, crayfish, fish, turtles, birds and mammals (reviewed by Snyder 1967). The present study focuses on crayfish, which commonly coexist with snails yet are capable of strongly impacting snail densities in natural populations (Lodge et al. 1994). Crayfish locate snail prey by chemotactically searching the substrata. They attack snails by using their mandibles and first walking legs to extract snail tissue from the aperture (Snyder 1967; Alexander & Covich 1991a; DeWitt et al., in press) or to crush small, thin-shelled individuals (A. P. Covich, personal communication).

Physid snails respond behaviourally to chemical cues from predation by using avoidance behaviours including hiding in refuges (e.g. crevices, tight spaces under rocks), burial into the substrata, and avoidance of the substrata by either crawling to the waterline or out of the water (Snyder 1967; Alexander & Covich 1991a, b; DeWitt 1996a; Turner 1996, 1997).

Morphological defences against crayfish may involve size and shape. Larger snails (≥ 10 mm shell length) are less vulnerable to predation by crayfish than are smaller snails (Alexander & Covich 1991a). Shell shape is important because crayfish and other decapods typically attack snails through the shell aperture. Snails with narrow or obstructed apertures are more difficult to consume (Ebling et al. 1964; Vermeij 1979; Appleton & Palmer 1988; DeWitt et al., in press). Apertural obstruction can be conferred in some snails by opercula or calcareous projections into the aperture (Vermeij 1979; Appleton & Palmer 1988). However, freshwater pulmonate snails such as *Physa* have no opercula and do not produce calcareous projections. Here we suggest an alternative way to block shell entry. Snails with greater whorl overlap (i.e. less drawn-out spires) may have a greater portion of the aperture blocked by the previous whorl. In the following section, we describe methods for quantifying both snail behaviour and aspects of snail size and shape that should reduce vulnerability to crayfish.

METHODS

To quantify snail antipredator responses in the presence of crayfish, we assayed the behaviour of 100 wild-caught, individual snails that were exposed to cues associated with predation. To quantify the repeatability of individual variation in antipredator behaviour, we repeated our behavioural assays on 6 days spread over a 13-day period.

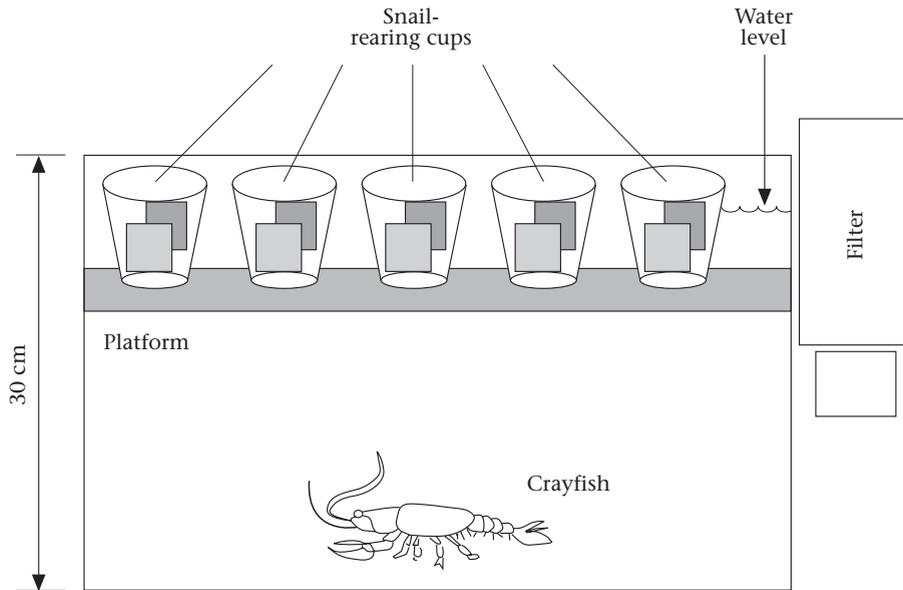


Figure 1. Apparatus used in behavioural assays. This same set-up is used in laboratory rearing of physiol snails (e.g. DeWitt 1996a, 1998).

We then used computer-assisted morphometric methods to measure shell size and shape for each of the snails, and analysed relationships between snail behaviour and shell morphology.

Snails and crayfish were collected from an unnamed, second-order stream in Jessamine County, Kentucky (37.81°N, 84.55°W), approximately 200 m upstream from its entry into the Little Hickman River. Both snails and crayfish were abundant at the time of collection.

One hundred snails (5.2–14.1 mm shell length) were established individually in 250-ml cups. Each cup was covered by a petri dish lid and had two (3 × 4 cm) mesh windows (mesh size=0.15 mm) to allow water exchange with the tanks into which cups were placed. Cups were placed, 10 each, on platforms immersed in 38-litre aquaria with (charcoal-less) power filters to circulate water (Fig. 1). The platforms held cups near the water surface so that approximately 1.5 cm of space was available in the cups for snails to crawl out of the water. A single crayfish (20–30 mm carapace length) was placed into each aquarium, below the platform, so that crayfish could not physically contact snails. Although chemical cues from crayfish can sometimes elicit mild snail responses (Snyder 1967), we assayed snail antipredator behaviour by adding chemical cues from crushed conspecifics to the baseline of cues from crayfish. Previous studies showed that chemical cues from injured conspecific snails are necessary to elicit full antipredator responses (Snyder 1967; Alexander & Covich 1991b; Covich et al. 1994; Turner 1996). Thus, our measure of antipredator response (a response to cues of enhanced predation risk) was the increase in use of safe microhabitats associated with the addition of crushed snail extract to a background containing crayfish cues.

Snail behaviour can depend on individual condition (Snyder 1967; Stenzler & Atema 1977). To minimize differences in condition among snails, we maintained all animals for 10 days with ad libitum food prior to

experimentation. During this time, snails and crayfish were both fed spirulina flakes (Jennings et al. 1970). Half of the cups in each aquarium were provided with sand and a 'refuge structure', which consisted of a ceramic tile with a plastic cross to support it (i.e. the view from above looked like ☒). Snails with this habitat structure could hide or avoid the substrata (crawl to the waterline or out of the water) to reduce the perception of risk. The remaining cups had sand but lacked refuge structures so that the range of avoidance behaviours was restricted. The difference in options (mediated by habitat structure) was included in our experimental design to assess the flexibility of snail antipredator responses. Snails in this study never buried themselves.

Behavioural Assay

Snail behaviour was assayed for 3 consecutive days, and then after a week without observation, for 3 more consecutive days. On each observation day, we conducted direct visual scan samples from 1000 to 1500 hours in which we noted each snail's position every 10 min (=18 000 scan samples). Chemical cues from crushed snails were added at 1130 hours; thus we recorded snail behaviour for 1.5 h before adding crushed snail extract, and for 3.5 h after. Crushed snail extract was made on each observation day by homogenizing five snails (8–12 mm shell length) in 100 ml of water with a mortar and pestle. A sample of 10 ml of extract was administered into each of the 10 tanks by gently releasing it into the plume of the power filter.

Snail positions during scan samples were noted as being either of five locations in the cups: (1) under refuge; (2) on the substrata or on top of the refuge; (3) on the cup wall; (4) at the waterline or surface film; and (5) above the water. Snails that were scored in position 1 had more than 50% of their shell under the tile; those on the side or upper surface of refuge structures, or on the substrata

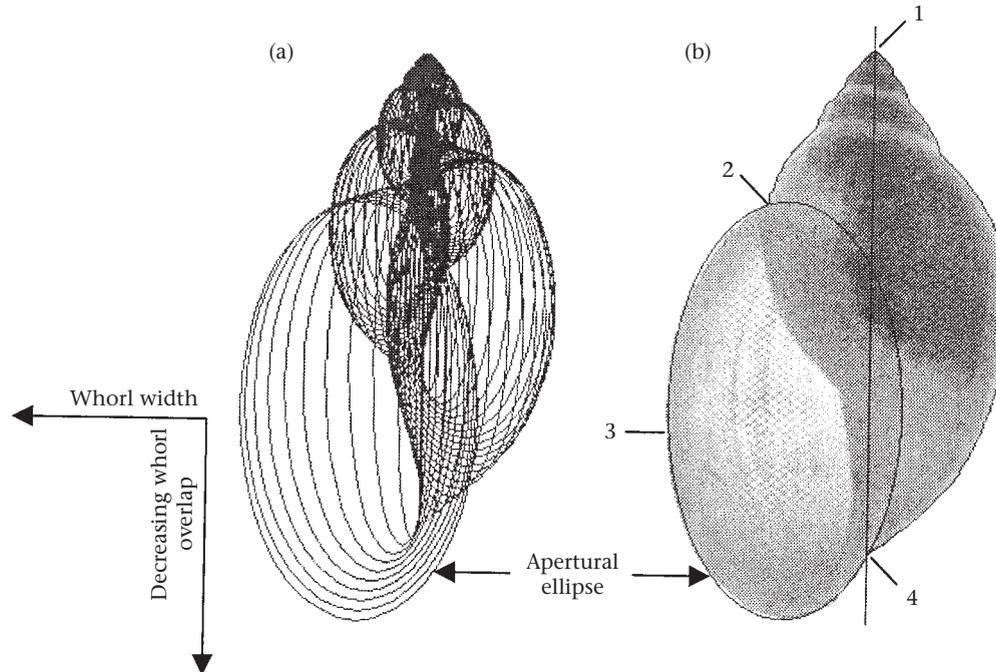


Figure 2. Shell morphology of *Physa*. (a) Computer generated *Physa* shell with vectors indicating directions of ellipse migration about the central (coiling) axis that correspond to increasing whorl width and decreasing whorl overlap (\approx apertural obstruction). (b) Image of an actual *Physa* shell with an ellipse, coiling axis, and important landmarks superimposed. Points 1 and 4 identify the coiling axis, which is the baseline used in our analyses. Joint displacement of landmarks 2 and 3 left of the coiling axis represents greater whorl expansion, hence a wider aperture that would facilitate shell entry by crayfish. Displacement of the landmarks downward represents decreased whorl overlap and a more exposed aperture (i.e. a smaller portion of the previous whorl would block the aperture).

outside the refuge structures were scored in position 2. Snails on the side of the cup were scored in position 3 unless they made contact with the surface film of the water, in which case they were scored in position 4. Finally, snails were scored in position 5 only if more than 50% of their shell was above the waterline.

Four snails died during the 23 days of experimentation, leaving our sample size at 96 individuals.

Morphometric Methods

After the behavioural assay, an image of each snail was captured using a video imaging system composed of an inverted Wild M5 stereoscope, a Sony video camera (model SSC-C370) and a PCVisions Plus, AT OFG image capture board (640×480 pixels). Snail positions were standardized by placing snails on the microscope stage aperture down (facing the camera) and allowing them to rest on the three natural points of contact (DeWitt 1996a). An outline and four landmarks were digitized for each shell using MorphoSys software (Meacham & Duncan 1989; Fig. 2).

Functional considerations and previous experimental studies suggest that with respect to crayfish, the key measures of shell shape are aperture width and apertural obstruction; shells with narrow or blocked apertures should be more difficult for crayfish to consume. To estimate these parameters, we selected two points as a reference to which the remaining points were rotated, translated, and scaled into alignment (Bookstein 1991).

We used the coiling axis as our baseline because it is developmentally meaningful in snails. We defined this axis by points 1 and 4, which were obvious in our captured images (Fig. 2). The two remaining landmarks were on the aperture. Rather than obtaining separate shape information from each landmark, we were interested in their joint motion in the horizontal and vertical directions (see arrows in Fig. 2). Thus we summed the X and Y values of the two 'shape' landmarks on the aperture. The joint motions of these points in the X and Y dimensions relative to the coiling axis therefore correspond conceptually to aperture width and whorl overlap (\approx apertural obstruction), respectively. Because physid shells are left coiled, the sign of the summed X variable was changed to serve as an intuitive metric for aperture width; larger values then correspond to wider apertures.

We estimated each snail's size by the length of its central axis (Bookstein 1991), the distance between points 1 and 4 in Fig. 2. Many past studies used the longest dimension of the shell (shell length) to represent size. Shell length and baseline length for snails in this study were related by the following linear regression equation ($R^2=0.98$, $N=96$, $P<0.0001$):

$$\text{shell length} = -0.575 + (1.183 \times \text{baseline length}) \quad (1)$$

Our measure of shell width was not correlated with size ($r = -0.14$, $N=96$, NS) or whorl overlap ($r = -0.06$, $N=96$, NS), but whorl overlap and size were negatively correlated ($r = -0.69$, $N=96$, $P<0.0001$).

The geometric morphometric methods used herein are more favorable on theoretical and statistical grounds than traditional morphometrics (Bookstein 1991; Rohlf & Marcus 1993). However, we obtained the same qualitative results using a traditional morphometric analysis.

Statistical Analyses

Our experimental design allowed us to address the following three focal questions. (1) How do individual snails respond to cues associated with heightened predation risk in the presence of crayfish? (2) Do individual snails show consistent, repeatable differences in their degree of antipredator response? (3) Are individual differences in antipredator behaviour related to shell morphology?

To quantify snail responses to enhanced predation risk, we compared the behaviour of individual snails before versus after exposure to crushed snail extract. Our experimental design did not include 'control' individuals that were never exposed to crushed snail chemicals. Instead, individual snails served as 'internal controls' for themselves. A potential problem with internal controls is the possibility that differences in snail behaviour before versus after addition of chemicals might represent a time sequence effect, rather than a treatment effect. The likelihood of this potential confound is strongly reduced by the fact that all individuals were examined over six repeated 'before versus after' trials. Our design also did not isolate the effect of crayfish chemicals per se. Crayfish scent without injured snail scent generally does not elicit detectable antipredator responses (Snyder 1967; Crowl & Covich 1990; Alexander & Covich 1991a; DeWitt 1996a) or elicits only weak responses (Snyder 1967; T. J. DeWitt & A. Sih, unpublished data). Our intended focus was on the strong response cued by adding crushed snail scent to an environment with crayfish.

Data on individual snails were condensed into mean proportions of time in each defensive behaviour during the pre-exposure interval and three postexposure intervals of equal (i.e. 70 min) length. Proportions were arcsine square-root transformed for analysis. To test for responses to the enhanced perception of risk, we used repeated measures analyses of variance (RM-ANOVA; SPSS 1997). RM-ANOVA first tests for an overall response, i.e. do any intervals differ from one another? If significant differences are evident, then specific contrasts can be conducted, using an alpha of 0.05, to discern the temporal dynamics of a response, that is, which intervals differed from which others? This procedure is important when alternative behaviours are enacted on different time scales. In particular, we were looking for intervals of peak activity once significant responses were evident from the initial test. To assess the effects of the refuge availability on antipredator behaviour, analyses were done separately for cups with versus without refuge structures.

To generate a single measure of individual snail response to predators, we summed the three types of behavioural response into a single measure of reactivity (see Results) by pooling reaction proportions observed

during the peak periods of activity for each behaviour. We used this pooled index of reactivity to evaluate the repeatability of behaviour across days and to test for associations between behaviour and morphology. Repeatability of behaviour was calculated following Lessels & Boag (1987). We assessed three measures of repeatability: (1) the intraclass correlation across the first 3 sequential days; (2) across all 6 days; and (3) the regular (i.e. Pearson) correlation between 3-day means.

To evaluate relationships between behaviour and morphology, we used a general linear model (SPSS 1997) to partition variance in antipredator behaviour (the mean index of reactivity) into five main effects including refuge presence, a blocking factor for the separate aquaria, and the three morphological variables of interest: shell size, summed X values (\approx aperture width) and summed Y values (\approx whorl overlap). Two interaction terms (size \times width, and size \times whorl overlap) were also included to test for interactive effects of prey size and shape on behaviour. Finally, we included a quadratic term to control for nonlinear effects of size on snail behaviour.

RESULTS

Antipredator Responses

The nature of snail responses to the addition of crushed conspecific scent depended on whether a refuge structure was present (Fig. 3). When refuge was present, snails enacted either of two opposite options for avoiding predators. Some snails crawled out of the water ('crawl-out'). Repeated measures ANOVA indicated significant time-course variation in the proportion of snails above the waterline ($F_{3,114}=10.09$, $P<0.0001$). Specifically, crawl-out behaviour peaked in the first interval after exposure to injured snail scent and then gradually waned over the next 3.5 h. The peak in crawl-out behaviour occurred within 10 min of adding injured snail scent, rising from about 1% before the cue to nearly 10% afterward. Alternatively, some snails responded by hiding under the refuge structure ($F_{3,114}=26.49$, $P<0.0001$). The percentage of snails under refuge immediately increased, from about 50 to 60% upon the addition of crushed snail extract. This response accumulated over the next few hours to about a 70% level of response. Use of the waterline microhabitat did not vary in the presence of refuge structures ($F_{3,114}=0.52$, $P=0.7$).

In the absence of refuge structures, snails only had the option of moving up to the waterline or out of the water. There was a significant increase in crawl-out behaviour from about 2% to nearly 14% immediately after exposure to crushed snail scent (overall 'time' effect, $F_{3,114}=16.34$, $P<0.0001$). This crawl-out response waned over the next 3.5 h in a manner similar to that observed in the presence of refuge. Use of the waterline increased after the addition of crushed snail cues, from 10 to 25% ($F_{3,114}=15.93$, $P<0.0001$), in contrast to behaviour in the presence of refuge, where no change was noted.

Based on the patterns evident from the analysis, we created a summary index of reactivity for individual

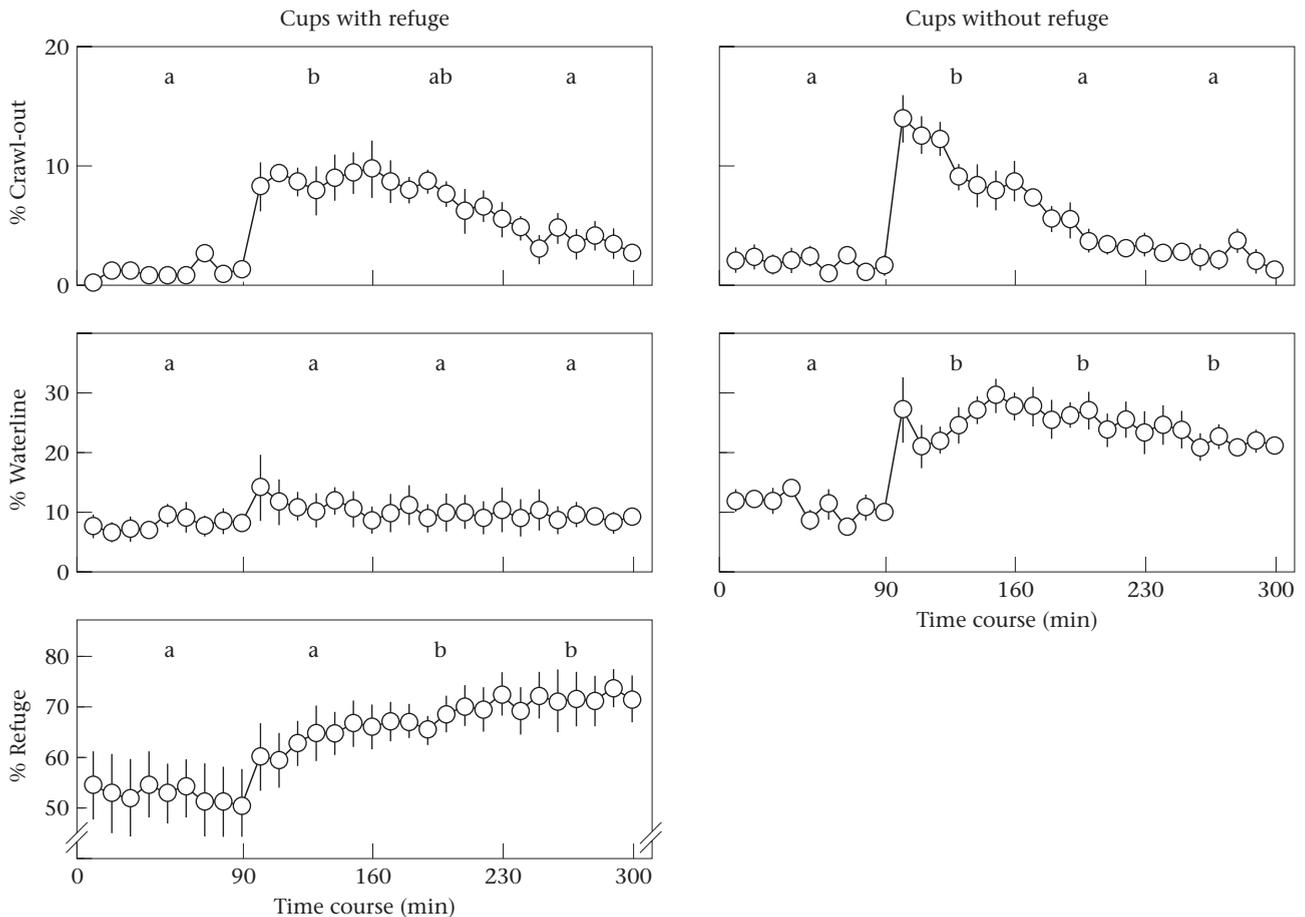


Figure 3. The percentage of snails in each defensive behaviour over a 5-h time course. The scent of injured conspecific snails was added at 90 min. Points represent means for each 10-min census. Error bars on individual points indicate variation (standard error) between the 6 days of sampling. For analysis the data were condensed into mean behaviours during each of four time intervals. Intervals assigned unlike letters differed at $\alpha=0.05$ (see text for analysis details).

snails. As noted earlier, our goal was to quantify the relative response shown by different individuals. Thus we chose an index that ignored time periods when most snails showed little or no response, and focused on time periods when many individual snails showed relatively strong antipredator responses. Specifically, we calculated an index of reactivity for each individual by subtracting the percentage of time spent in defensive positions (under refuge, out of the water, and at the waterline) during the control period from the percentage of time spent in the same positions during critical (peak) times after exposure to crushed snail extract. Critical times were defined to be the first hour postexposure for crawl-out behaviour and in the last hour recorded for refuge and waterline use (see Fig. 3). Hour-long, rather than 70-min, periods were used as our standard to parallel other work on these behaviours that is planned, in progress, or recently completed.

The pooled index of reactivity, which reflects responses to the heightened perception of predation risk, was similar in the presence and absence of refuge structures (Table 1). That is, the number of snails that shifted from exposed to safe positions was similar for cups with and without refuge. However, the nature of response depended on the

presence or absence of refuge structures (if structures were present they were used; if not, snails relied more on substratum avoidance). In subsequent analyses (for repeatability of behaviour and for relationships between behaviour and morphology), we pooled data from the

Table 1. Factors that explain individual variation in antipredator behaviour of physid snails*

Source	<i>df</i>	<i>MSE</i>	<i>F</i>	<i>P</i>	Effect†
Refuge presence	1	0.062	1.84	NS	
Tank	9	0.119	3.51	0.001	
Size	1	0.561	16.53	0.0001	-7.7
Size ²	1	0.237	6.98	0.01	+3.4
Aperture width	1	0.310	9.15	0.003	-1.5
Whorl overlap	1	0.015	0.45	NS	
Size × aperture width	1	0.280	8.25	0.005	+4.9
Size × whorl overlap	1	0.013	0.39	NS	
Error	79	0.033			

*Results of a general linear model (SPSS 1997) evaluating the effects of several variables on variation in mean snail reactivity ($R^2=0.49$, $N=96$, $P<0.0001$).

†Effect estimates (as standardized coefficients) are provided to indicate the direction of significant regression trends.

different refuge treatments rather than divide our analyses for snails with and without refuge structures.

Repeatability of Antipredator Behaviour

Reactivity of snails to alarm cues was repeatable and fairly stable for individuals in both sequential days and sequential weeks. No appreciable gain or loss of repeatability (r_I) was noted at increasing time scales. For the first 3 days, r_I across days was 0.33 ($N=96$, $P<0.0001$) and for the 6-day series it was 0.27 ($N=96$, $P<0.0001$). When calculated as the correlation between 3-day means, r_I was equal to 0.28 ($N=96$, $P<0.006$).

Part of the consistency in individual behaviour could be due to a size effect on behaviour (see the next section). Because size did not change appreciably during the experiment, the effect would be consistent across days and should thus account for at least some of the repeatability of behaviour. To assess the contribution of size relative to other consistent individual differences in generating the repeatability, we regressed behavioural reactivity in a focal week against both size and reactivity in the other week. This test showed that behaviour in one week was related to behaviour in the next, even when statistically controlling for the effect of size (standardized partial regression coefficients, $b'>0.24$, $N=96$, $P<0.02$). Although the stability of relative size across weeks probably contributed somewhat to repeatability in behaviour, other unmeasured individual characteristics also had important effects producing consistency of individual behaviour.

Relationships between Behaviour and Morphology

Roughly half the variation in individual mean reactivity (over all 6 days) was accounted for by effects in the model (Table 1). As noted earlier, the presence of refuge did not significantly influence mean reactivity. Mean behaviour differed significantly among tanks (accounted for statistically by blocking by tanks). We made no attempt to understand factors explaining between-tank variation. Rather, we focused on effects of snail morphology on behaviour.

Individual differences in antipredator behaviour were significantly related to prey size, aperture width, and an interaction between size and aperture width (Table 1). The effect of size was the strongest. Smaller snails (those most vulnerable to crayfish) performed more antipredator behaviour, indicating behavioural compensation for morphological vulnerability to crayfish. The size effect on behaviour was nonlinear, as indicated by the significant quadratic term for size. Specifically, snails above an intermediate size (about 10 mm in baseline length) showed little response to predation cues (Fig. 4a). An exception to this general rule was that large snails with particularly wide apertures were also responsive to predation cues, as indicated by the significant size \times shape interaction (Table 1) and illustrated in Fig. 4b. In Fig. 4c, we show this interaction (the separate shape–behaviour relationships for large and small snails) by taking transects through the topography of Fig. 4b.

With regard to shell shape, on average, snails with narrower apertures showed the strongest behavioural responses. Again, an exception occurred for some large snails, with wider apertures, that were more responsive. Thus most snails seemed to show trait cospecialization, whereas larger snails showed behavioural compensation with regard to aperture width (Fig. 4c).

Whorl overlap, which we suggested may cause apertural obstruction, did not explain variation in individual behaviour once we statistically accounted for effects due to size and aperture width.

DISCUSSION

Our results showed that: (1) snails tended to hide under refuge and crawl out of the water in the presence of refuge, but relied on crawling to the waterline and out of the water when refuge was absent; (2) individual prey showed repeatable differences in their overall response to predation cues over a 13-day period; (3) individual variation in antipredator behaviour was associated with shell size and aperture width, two morphological characters that influence vulnerability to crayfish predation. The relationship between size and behaviour reflects behavioural compensation for low escape success given an encounter with crayfish, while the relationship between aperture width (shape) and behaviour primarily represented cospecialization, in which the two traits should reinforce each other's effects in reducing predation risk. Below, we discuss each of these results in greater detail.

Antipredator Responses

Our observations corroborated patterns seen in earlier studies of physid snail response to predation cues (Snyder 1967; Alexander & Covich 1991a, b; DeWitt 1996a; Turner 1996, 1997). With no refuge structure available, the addition of chemical extract from crushed snails to a background of nonforaging crayfish cues resulted in a significant increase in the proportion of snails at the waterline or out of the water (Fig. 3). Crawl-out behaviour was primarily a short-term response whereas use of the waterline was longer in term. With a refuge structure available, snails climbed out of the water in the short term but sought cover in refuge structures for extended periods of time. All of these responses could reduce predation by crayfish. Although crayfish often go under rocks, our refuge structures were probably too close to the substrata to allow crayfish to attack snails easily in refuge. Similarly, crawling to the waterline per se could put snails out of reach for crayfish (if the water is deep enough) and crawling out of the water should generally make snails unavailable to aquatic crayfish. Crawl-out might be the most effective at reducing encounters with crayfish but may have important costs (e.g. loss of opportunities to feed or mate, risk of desiccation; Covich et al. 1994). Thus, the use of refuge structures and the waterline microhabitat might be favoured during periods of prolonged exposure to cues indicating predation risk.

The presence of refuge also influenced the proportion of snails in defensive positions under default conditions (i.e. crayfish present, but no cues of snail injury). Rela-

tively few snails (about 10%) were at the waterline or out of the water in default conditions, yet when refuge was available, about 50% of the snails used the shelter. That is, about 60% of snails were in defensive positions when refuge was available, compared with about 10% in the absence of refuge. The high proportion of snails using available refuges under default conditions may reflect the presence of crayfish scent, which may indicate some risk, albeit less than when the predator's scent is accompanied by that of injured snails (Snyder 1967). Another contributing factor is that these snails were well fed for several days before assaying their antipredator behaviour. Previous studies show that well-fed snails are more likely to spend time in safe microhabitats (Snyder 1967; Stenzler & Atema 1977).

Although the availability of refuge influenced both the baseline behaviour of snails and the type of response to enhanced risk, interestingly, the amount of increase in response to crushed snail extract did not depend on the presence of refuge. In both refuge treatments, the addition of crushed snail cues produced, on average, a 20–25% absolute increase in the percentage of snails in defensive positions. Individual responses ranged from a 100% increase in mean time spent in defensive positions to as much as a 30% decrease in antipredator behaviour after the addition of crushed snail extract (Fig. 4a). Thus, some individual snails responded dramatically in ways that appeared to be adaptive, yet other individuals seemed (superficially) to show maladaptive behaviour.

Repeatability of Antipredator Behaviour

We found consistent differences among individuals in the magnitude of their response to enhanced predation risk. Although ethologists and behavioural geneticists have a long history of observing individual variation in behaviour and repeatability of behaviour (Arnold & Bennett 1984; Clark & Ehlinger 1987; examples in Boake 1994), few studies have quantified repeatability for the antipredator behaviours, especially predator avoidance responses, most typically studied by behavioural ecologists (e.g. refuge use, microhabitat shifts, reduced foraging activity). Indeed, predator-prey behavioural ecologists usually test each individual only once, to preserve statistical independence of samples assessed in alternative conditions.

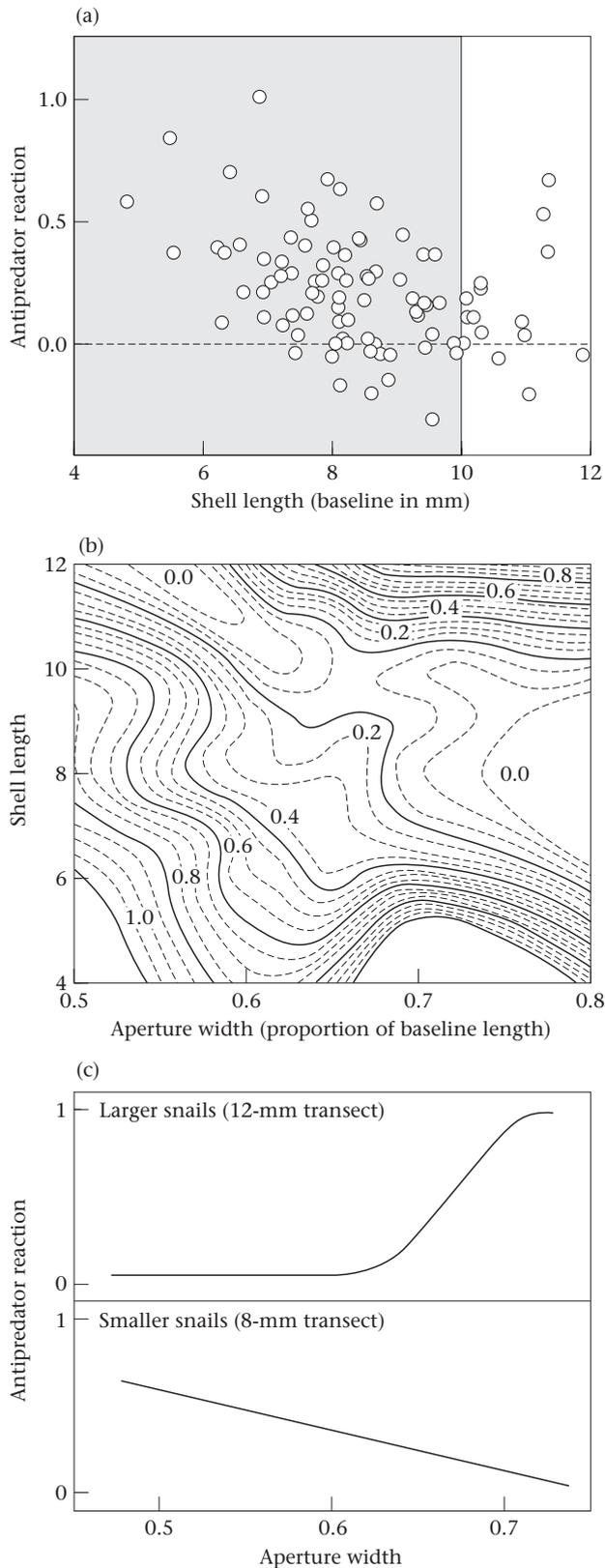


Figure 4. Relationship between morphological traits and antipredator behaviour. (a) Size effect. Values greater than zero (dashed line) indicate snails whose use of safe microhabitats increased after exposure to predation cues. Antipredator behaviour was negatively related to size for snails less than or equal to 10 mm (shaded region; vulnerable sizes). Snails greater than 10 mm (unshaded region; low vulnerability) showed little or no behavioural response to predators. (b) Bivariate surface plot showing the complex relationship between the two morphological variables (size and shape) and antipredator behaviour. Topographic height indicates the level of behavioural response. (c) Transects through the bivariate plot for snails 2 mm above and below the size threshold at 10 mm.

Individual differences in behaviour can stem from many potential causes. One common cause might be size effects. Behaviour is often size dependent (Stein 1977; Reist 1983; Sih 1982, 1987; Garland 1988; examples in Boake 1994; for *Physa* see DeWitt 1996b). If individual differences in relative size are stable over long periods, then size effects on behaviour will generate consistent individual differences in behaviour. In our study, size did influence antipredator behaviour (Fig. 4a, Table 1) and relative size changed little, if at all, over our 13-day experiment. Therefore, stability of relative size could partially explain the observed consistency of behaviour.

Multiple regression showed, however, that the repeatability of antipredator behaviour remained even after size was accounted for statistically. A second factor that could explain repeatability of behaviour could be consistent individual differences in condition that persisted across the 2 weeks of the study (e.g. due to differences in feeding history). We attempted to minimize this by holding all individuals under standardized food conditions for 10 days before assaying their antipredator behaviour. Despite this, some variation may have remained in individual condition.

Other sources of repeatable individual variation in antipredator behaviour could include differences in previous experience with predators and genetic variation in antipredator behaviour. Studies of repeatability are frequently cited as being important because repeatability sets an upper limit on heritability, which in turn is important for understanding the evolution of behaviour (Arnold & Bennett 1984; Lessels & Boag 1987; Boake 1994). Below, we argue that relationships we observed between antipredator behaviour and aperture width could reflect variation in experience with predators, genetics or both.

Regardless of the causes of consistent individual differences in behaviour, the consequence is that natural selection can act systematically (i.e. between individuals) on phenotypic variation. However, if individual differences in behavioural phenotypes are correlated with other traits related to fitness, those correlations must be measured to gain a more complete understanding of prey vulnerabilities.

Relationships between Morphology and Behaviour

We distinguished four types of mechanisms that can generate correlations between morphology and behaviour: codependence, complementation, compensation and cospecialization. When behaviour and morphology are correlated (indeed, when any traits are correlated), the correlation must be quantified to understand selection on each individual trait or joint selection on the suite of correlated traits (Lande & Arnold 1983; Price & Langen 1992). For example, if in a particular system, large individuals are unresponsive to predators while small individuals hide in refuge, then one cannot isolate the effect of size on predation risk without accounting for the associated behaviour and vice versa. If the correlation between traits is strong, then in a sense we have only one trait:

not size or behaviour per se, but a size–behaviour combination.

Here, we observed two of the above four mechanisms (i.e. trait compensation and cospecialization). The traits we observed seem unlikely to exhibit codependence or complementation because they influence different stages of the predation act. The behaviours we observed (refuge use and substratum avoidance) act to reduce encounter rates with crayfish. In contrast, morphological defences (size and aperture width) act to reduce the probability of predation given an encounter. Neither trait requires the other to function (codependence) or to be effective (complementation). Instead, their joint effects arise through compensatory or reinforcing mechanisms (cospecialization).

With regard to behaviour and size, we observed a negative relationship between traits that represent behavioural compensation. Previous studies of predation have a long history of documenting size-based behavioural compensation in other species (e.g. Stein 1977; Reist 1983; Sih 1982, 1986; Werner et al. 1983), including another freshwater snail (*Planorbella trivolvis*, Alexander & Covich 1991a). In our case the effect was nonlinear; small snails compensated for their vulnerability by showing stronger antipredator responses, whereas large individuals generally showed weak responses with no apparent, additional size effect above a threshold of about 10 mm in baseline length (=11 mm total shell length; Fig. 4a).

The weak antipredator response shown by relatively large snails might reflect a reduced risk of predation. Alexander & Covich (1991a) found a sharp drop in ingestion probability at this size. Snails 5–9 mm (total shell length) suffered 100% mortality in their study. In contrast, mortality dropped sharply to nearly zero for snails between 10 and 12 mm long. If prey show effective antipredator responses, then with behavioural compensation, prey size should not have such strong effects on mortality. Small snails might suffer relatively little predation due to their strong antipredator response, while larger snails that do not hide might suffer little predation due to their large size. However, Alexander & Covich's experimental apparatus did not allow avoidance behaviours of prey; no substrata or refuge structures were present and the water was perhaps too shallow (4 cm deep) for snails to exceed easily the reach of crayfish by substratum avoidance. Preventing behavioural responses generally may be a good methodological tool for isolating morphological effects, such as size effects on predation risk.

Given that snail size is constrained on short time scales (size cannot be adjusted to short-term changes in predation risk), behavioural compensation should be adaptive for small snails. Flexible traits such as behaviour are important adaptations to track fine-scale temporal variation in selection pressures (West-Eberhard 1989). Conversely, larger snails that are already defended (at least partially) can save the cost of a second defence by not responding behaviourally. Costs of the antipredator behaviours employed by snails are thought to include desiccation risk and risk of predation by terrestrial predators (Covich et al. 1994). Thus, compensation is a way to

achieve economy of overall defence; only animals that badly need the defence incur the cost of performing the behaviour. Given that desiccation risks of aerial exposure are greater for smaller snails than for larger snails (Tucker et al. 1996), small snails appear to be particularly desperate to avoid crayfish.

Although the antipredator reactions of the largest snails were generally low (Fig. 4a), those that did respond tended to have relatively wide apertures (i.e. >0.6 of baseline length; Fig. 4b, c). Most snails showed the opposite pattern. That is, small to intermediate snails with wide apertures showed weak antipredator responses (Fig. 4b, c). Given that crayfish are known to attack snails through shell entry (Snyder 1967; Alexander & Covich 1991a; DeWitt 1996a), the reaction of larger snails seems to be trait compensation; wider shells are morphologically vulnerable but this effect is compensated by increased antipredator behaviour. The opposite relationship observed for snails of small to intermediate size thus could be considered trait cospecialization. Again, cospecialization is a reinforcing relationship between two defences. Here, snails that were morphologically protected by narrow apertures showed stronger antipredator behaviour. To our knowledge, this is the first study to suggest cospecialization for individual variation in antipredator behaviour and morphology.

Why should some individuals show strong behavioural and morphological defences against crayfish, while others are weakly defended in both ways? We suggest that this could reflect individual variation (among our field-caught snails) in either previous history with crayfish or in their sensory ability to detect crayfish. Previous studies documented that snails show plastic, induced, life history and morphological responses to crayfish (Crowl & Covich 1990; DeWitt 1996a, 1998). If snails vary in their experience with predators, then those that have had more exposure to crayfish should show both narrower apertures and stronger behavioural responses to crayfish. Alternatively, individuals with similar experiences with predators can vary in their ability to detect and gauge predation risk. DeWitt (1998) found that snails raised in a given predation regime had correlated plastic responses, perhaps suggesting a common sensory basis used to cue various forms of plasticity. Either of these two proximate mechanisms could produce trait cospecialization.

Cospecialization can also reflect genetic variation in antipredator behaviour that results from differences in evolutionary history with crayfish predation. Although little is known about population differentiation and evolutionary adaptation to crayfish by these snails, it seems plausible that genotypes that have survived past selection from crayfish might be particularly well defended against crayfish both behaviourally and morphologically. To address these possibilities, we are currently examining the behaviour and morphology of snails collected from populations with or without abundant crayfish, and reared with or without crayfish cues.

Recent evolutionary theory emphasizes the importance of correlated traits and, in particular, the joint evolution of suites of correlated traits (Lande & Arnold 1983; Price & Langen 1992). This view is a major theme in recent

studies of morphological and life history traits (Roff 1992; Cheverud 1996), yet it has not penetrated far into the behavioural ecology literature. Because of its plasticity, behaviour can be particularly important in the evolution of adaptive trait integration. Further study of the causes and consequences of phenotypic and genetic correlations between behaviour and morphology should thus prove to be highly insightful.

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