Assessing the potential for an evolutionary response to rapid environmental change: invasive toads and an Australian snake

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ABSTRACT

Extinctions are ultimately caused by a change in an organism's environment. Species that can adapt are more likely to persist indefinitely in the face of such changes. We argue that an understanding of the factors encouraging and/or limiting the potential for adaptation is an important consideration in assessing the long-term outcomes of environmental change. Such an approach suggests a cohesive way of assessing the potential for an impact and the long-term consequences of a particular environmental change. We illustrate this approach with a case study of a native Australian snake (the keelback, *Tropidonophis mairii*) faced with the invasion of an extremely toxic prey item (the cane toad, *Bufo marinus*). We examine the likely strength of selection, the heritability of toxin resistance and the likelihood of trade-offs or pre-adaptation. We assess an internal trade-off (between toxin resistance and locomotor performance) and an external trade-off (between resistance to the toxin of toads and a native prey species, *Litoria dahlii*). Our analysis reveals weak selection, high heritability and no trade-offs in resistance to toad toxin, suggesting that keelbacks are capable of mounting a rapid adaptive response to invasion by the cane toad.

Keywords: conservation, contemporary evolution, invasive species, predator–prey.

INTRODUCTION

Whether a species will persist or go extinct in the face of rapid environmental change will largely depend upon the species' ability to adapt to the change. While the question of whether or not a species will go extinct in the short term is important, whether or not it will adapt should be the ultimate concern. Adapting to the new environment is clearly the best 'strategy' for long-term persistence of a species.

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   This perspective suggests an approach to assessing the potential long-term impact of an environmental change. Whether a species is likely to adapt to a given change will depend on several factors, including:

1. The strength of selection imposed by the change: excessively high selection may lead to extinction, whereas lower levels of selection will encourage adaptive evolution.
2. The ability of the population to respond to the selective force: that is, does the population have sufficient heritable variation at the characters under selection?
3. Whether the population has other constraints limiting adaptive potential: for example, trade-offs, long generation-time relative to the pace of change.

   Invasive species offer an excellent opportunity to study the evolutionary implications of rapid environmental change for several reasons. First, the timing and spatial pattern of the invasion is often well documented. Second, invaders often have a large impact on native species and the mechanism of impact is often well understood. Finally, invasions of species can happen naturally in ecosystems and thus it is expected that some capacity to adapt to them should exist.

   In this paper, we describe a case study in which we assess the likelihood of a rapid adaptive response by a native species to an invader. To do so we assess the heritability of an adaptive trait and the likelihood of trade-offs (both intrinsic and extrinsic) acting against adaptive change. This approach is logistically simpler than documenting short-term impacts, and can clarify probable long-term effects of the invader on the native species.

A CASE STUDY: CANE TOADS AND AUSTRALIAN SNAKES

The history of the cane toad in Australia represents an excellent example of the invasion of a dangerous prey item. Cane toads (*Bufo marinus*) were introduced into Australia in 1935. Since then, they have spread throughout large areas of Queensland and have entered the Northern Territory and New South Wales, currently occupying a range of approximately 1 million square kilometres (Lever, 2001). The exact impact of toads on the native fauna has been poorly elucidated, mainly due to logistical difficulties and a lack of baseline data for comparison (van Dam *et al.*, 2002). Nevertheless, there is a very clear inference that the invasion of the toad has had a massive impact on species of Australian snakes. A recent study suggests that 49 species of snake are potentially impacted by the toad and that the majority of these species are poorly equipped to deal with a likely dose of toad toxin (Phillips *et al.*, 2003). The main active principal of toad toxin is a class of steroid-derived compounds known as bufogenins (or bufodienolides; Chen and Kovarikova, 1967), unique to toads and biochemically very different from the active peptides that constitute the main defensive secretions of Australian frogs (Daly and Witkop, 1971; Erspamer *et al.*, 1966, 1984). Bufogenins are extremely toxic, exerting strong cardiac effects. Thus with the arrival of the cane toad, Australian snakes were faced with a novel and extremely powerful toxin in potential prey items.

   Australian frog-eating snakes are thus under selective pressure to adapt to the presence of the toad. Four possible adaptive solutions are identifiable:

1. Populations can increase resistance to toad toxin.
2. Populations can evolve to avoid/exclude toads as a prey item.

3. Populations can evolve modified habitat preferences (spatial and/or temporal), such that exposure to toads is reduced.
4. Populations can evolve shifts in morphology (particularly relative gape width) that reduce ingestible prey size and hence exposure to lethal doses of toxin.

None of these solutions is mutually exclusive and the presence of toads is likely to drive populations towards all four. Whether each solution is achievable will depend upon the magnitude of response required (the strength of selection), the heritable variance for each relevant trait and the presence or absence of trade-offs or pre-adaptations. However, any one of these solutions, on its own, would allow a population to persist with toads.

This paper explores the possibility of adaptation by increased resistance to toad toxin in one species of snake (the keelback, *Tropidonophis mairii*). In doing so we examine the strength of selection, the heritable variation and the possibility of trade-offs or pre-adaptation as a result of co-evolution with dangerous native prey (Dahl's aquatic frog, *Litoria dahlii*).

A relevant pre-adaptation to toad toxin could exist through previous long-term exposure to other toxic amphibians. Although the effects of native frog toxins on Australian snakes are poorly understood, most Australian frogs contain skin toxins, yet many are still eaten by frog-eating snakes (Greer, 1997). One exception to this generality is *Litoria dahlii*. This frog is extremely toxic to most sympatric snake species and is abundant in the floodplains of tropical Australia (Madsen and Shine, 1994). The only snake species found capable of consistently surviving the ingestion of *Litoria dahlii* was the keelback, *Tropidonophis mairii* (Madsen and Shine, 1994). This species is also extremely resistant to the toxin of the cane toad (Phillips *et al.*, 2003).

Examining this system (*T. mairii, L. dahlii* and *B. marinus*) allows us to ask the specific question: Are keelbacks likely to adapt to the presence of toads? To assess the potential for adaptive response in toxin resistance, we:

- calculate the heritability of toxin resistance (heritability of a trait is the proportion of the variation in the trait that is directly heritable and thus gives an indication of the 'ability' of the trait to respond to selection);
- examine possible trade-offs (strong trade-offs may constrain adaptive options); and
- examine the possibility of pre-adaptation through co-evolution with native prey (pre-adaptation potentially reduces the strength of selection).

In making these assessments and using *T. mairii* as a model, we are also able to address several questions of general relevance to the adaptive solutions available to Australian snakes. First, *Tropidonophis* is highly resistant to both *L. dahlii* and *Bufo* toxins. This raises the possibility that resistance to one toxin confers resistance to the other (i.e. pre-adaptation to *Bufo* through co-evolution with *L. dahlii*). If this is the case, we might expect a lowered impact on snakes in areas where *L. dahlii* is abundant due to similar selective forces imposed by both toads and *L. dahlii*.

Secondly, the relationship between resistance to the two toxins may be the reverse of that suggested above: adaptation to one toxin may reduce resistance to the other. The presence of such a trade-off would restrict adaptive options for Australian snakes faced with the selective force of the presence of toads – adapting to one dangerous prey would reduce their ability to tolerate the other dangerous prey taxon.

Thirdly, adaptation to either prey may entail decreased performance in other traits related to fitness. One such trait might be decreased locomotor performance associated with higher levels of resistance to the toxin. Brodie and Brodie (1999b) argue that there is a strong trade-off between resistance to tetrodotoxin and locomotor performance in garter snakes (i.e. faster snakes are disproportionately affected by toxin).

METHODS

Toxin extraction

We extracted toxin from freshly killed *L. dahlii* and *B. marinus* by removing the dorsal skin (front of parotoids to knees in *B. marinus* and back of tympanum to knees in *L. dahlii*) and drying it at room temperature before weighing. Dried skins were cut into pieces and placed in a blender with 10 times v/w of 40% ethanol. Skins were rehydrated for 2–3 h before blending. The resulting liquid was strained and then reduced to 50% of initial volume by evaporation at room temperature. These preparations were stored at 4°C between use. A control solution was created using 40% ethanol evaporated to 50% of its original volume at room temperature.

Collection of snakes

Gravid female keelbacks were collected by hand near Humpty Doo, in the Northern Territory. Females were measured and weighed and kept in captivity until they oviposited (usually within 1 week of capture). At the time of collection, toads were not yet present in this area; the invasion front was approximately 300 km south and west of Humpty Doo.

Newly laid eggs were measured and weighed and placed on a mixture of vermiculite and water in a plastic bag to incubate. Thirteen clutches were split into four incubation treatments – two hydric regimes (wet = 1 : 1 ratio of vermiculite to water by mass; dry = 2 : 1 ratio) were run orthogonally with two temperature regimes (high variation, mean 23.4°C, variance 9.4; and low variation, mean 23.5°C, variance 5.1). The remaining 13 clutches were incubated under conditions identical to the wet substrate/low temperature variance treatment.

Toxin resistance assay

Resistance to toxin in newly hatched keelback snakes was assayed using post-dose reduction in locomotor performance, a methodology modified from that of Brodie and Brodie (1990). Individuals were swum along a 2-m swimming trough and were timed with an electronic stopwatch over three consecutive 50-cm segments of the trough with a stopwatch. Animals were encouraged to swim by tapping them on the tail. Water temperature was maintained at 23 ± 1°C.

All individuals were weighed before testing to the nearest 0.1 g on a digital scale. A swimming trial consisted of two consecutive laps of the trough. This yielded six measurements of swim speed over 50 cm, of which only the fastest was retained. All animals (1–2 days post-hatching) were initially subjected to three swim trials 1 h apart. This yielded three maximum sprint speed times, which were averaged to generate the pre-dose estimate of maximum swim speed (b).

On the following day, snakes were given a specific dose of toxin or control solution. Dosing was achieved by use of a micropipette attached to a thin rubber feeding tube. The tube was inserted into the stomach to a depth of 5 cm from the snout before toxin was expelled. Animals were observed for 1 min following this procedure to ensure the toxin was not regurgitated. Two swim trials were then undertaken for each individual, 30 and 90 min post-dosing, respectively. A third trial was not run due to the increased possibility of recovery by this time. Once again only the fastest speed for each trial was taken. This yielded two time measurements which were averaged to give post-dose swim speed (a). The percentage reduction in swim speed (%redn) was calculated from these times using the formula, %redn $= 100 \times (1 - (b/a))$.

Locomotor effects

Several experiments were performed. The first two were used to determine whether the administration of toxin caused a reduction in swim speed. For toad toxin we were also able to assess the effect of increasing dosages on the decrement in swim speed. Due to limited toxin extract, we did not assess this factor for *L. dahlii* toxin.

For *L. dahlii* toxin, two clutches of keelbacks (previously untested, single incubation treatment, 19 individuals) were used. Each clutch was split into two groups: group 1 was given 50 μl of *L. dahlii* toxin and group 2 was given the same volume of control solution in the manner described above. The post-dose decrement in swim speed for each individual was recorded. A one-factor analysis of variance (ANOVA) was used to determine the effects of toxin versus control on the decrement in swim speed.

For toad toxin, five clutches of keelbacks (previously untested, single incubation treatment, 24 individuals) were split by clutch into four groups. Each of the four groups received either 100 μl of control solution, 25, 50 or 100 μl of toad toxin. Decrement in swim speed was assessed as previously described. A one-factor ANOVA was used to assess the effect of dose on the decrement in swim speed.

Toxin resistance trade-offs: *Litoria dahlii* versus *Bufo marinus*

To assess the correlation between responses to toad toxin and responses to *L. dahlii* toxin within individual snakes, we used 15 clutches (from both the mixed and single incubation treatments) and tested all 115 individuals for resistance to both toad and *L. dahlii* toxins. On the day after the pre-dose swimming trials, neonates were tested with 50 μl of toad toxin. On the fourth day after dosing with toad toxin, the animals were given 50 μl of *L. dahlii* toxin and tested in the same manner as for toad toxin.

The order of dosing was not staggered because our first priority was to develop a large data set on toad resistance for the heritability analysis (see below). However, we did conduct a small experiment to determine whether being previously tested for toad toxin affected *L. dahlii* resistance measures. In this experiment, we split four clutches (18 individuals) into two groups. One group was tested first for resistance to toad toxin and then for resistance to *L. dahlii* toxin. The second group had the test order reversed. In both groups, 4 days elapsed between each test. The data for the individuals tested for toad toxin resistance first were also used in the heritability analysis (see below).

Data analysis

Heritability

We measured locomotor decrements of 167 individuals representing 24 clutches (including individuals assessed for resistance to *L. dahlii* toxin trade-offs and toad toxin locomotor effects) to estimate heritability using a full-sib design. Full-sib designs are unable to control for the effect of a common maternal environment, potentially inflating estimates of heritability. Of the 24 clutches used for heritability analysis, 13 had been incubated under varying conditions (see above). This process effectively minimized the common incubation environment of clutches and allowed us to explicitly test the effect of incubation treatment on toxin resistance. Variance components under the full-sib design were estimated by restricted maximum likelihood in SPSS. Restricted maximum likelihood is the best technique for generating unbiased estimates of variance parameters with an unbalanced design (Shaw, 1987). We used a jackknife approach (iteratively removing one family) to estimate heritability and its standard error. Both neonate mass and maternal mass may influence resistance to toad toxin. Before calculation of heritability, these factors were removed from the toxin resistance data by taking the residuals of a multiple regression of both neonate mass and maternal mass on the percentage reduction in swim speed.

Locomotor trade-offs

To assess the possibility of a trade-off between toxin resistance and locomotor performance, we regressed pre-dose speeds against post-dose speeds for 15 clutches (115 individuals) tested with both toxins. The gradient of the line in each case was assessed against null expectations under the following model:

$$A = mB + c$$

where A is the post-dose speed, B is the pre-dose speed, m is the effect of the toxin (null: equal to the average proportion of original speed for each toxin) and c is a constant. This method for inferring the presence of a trade-off differs from that used previously for similar data (Brodie and Brodie, 1999b), with the crucial difference being in construction of the null slope. Our approach is more conservative, because the null slope under our model will always be less than the null of 1 assumed by Brodie and Brodie (1999b). Furthermore, the test is made more conservative because individual mass could not be removed from the analysis without compromising the logic of calculating the null. The inclusion of mass will bias the slope upwards, resulting in conservative inference under this analysis.

A randomization test was performed to assess the significance of any deviation of m from that expected under the null hypothesis (Manly, 1991). In this case, a trade-off is evidenced by a slope lower than the null. The effect of individual mass on response to the standard dose of toxin could not be removed without sacrificing the ability to calculate a null slope. The effect of individual mass is likely to bias the observed slope in a positive direction, making this a conservative test for the presence of a trade-off in this system. To determine the power of the data to detect a deviation from the null slope, the randomized distribution was advanced by successive decreases of 0.05. For each of these increments, the overlap of the new distribution with non-significant values of the test distribution was calculated (β). The power was calculated as $1 - \beta$ for each increment (Sokal and Rohlf, 1995).

Toxin resistance trade-offs: Litoria dahlii versus Bufo marinus

Because both toxins were tested on each individual in this experiment, there is no need to adjust post-dose times by pre-dose times. In fact, doing so would cause the measures for the two toxins to become correlated through the effect of the common pre-dose speed. Thus post-dose times (*a*) for each toxin for each individual were compared. Because post-dose speed is likely to be correlated with both pre-dose speed and snake mass, we removed their effect by taking the residuals of a multiple regression of pre-dose speed and snake mass on post-dose speed for each toxin. The Pearson product–moment correlation coefficient was calculated to determine the strength of the correlation between *L. dahlii* and toad toxin post-dose times after correcting for pre-dose speed and snake mass. A randomization test (Manly, 1991) was used to test the significance of the observed correlation and to assess the power of the data to detect a correlation of various strengths. The value of the observed correlation was compared with that of 1999 randomized sets of the data. For non-significant results, power was assessed as above.

RESULTS

The effect of toxins

The toad skin extract amounted to 144.7 mg of skin per millilitre of final extract. The extract of *L. dahlii* skin equated to a final concentration of 119.0 mg per millilitre of final extract. Three of 208 snakes tested died after we gave them a dose of toxin. Whether this was due to the toxin or simply handling stress is not discernible. Most animals that we tested recovered over the course of 8–24 h.

The mean masses of snakes did not differ significantly between treatment groups in the *L. dahlii* toxin experiment ($F_{1,17} = 0.90$, $P = 0.35$). The dose of toxin (equating to 5.95 mg of dried *L. dahlii* skin) gave an average reduction in speed of 27.9% in the toxin group as opposed to a 9.4% increase in speed in the control group ($F_{1,17} = 5.88$, $P = 0.026$).

A similar pattern was observed for toad toxin. The percentage reduction increased with increasing dose of toxin (Fig. 1). The difference between doses was significant overall ($F_{3,20} = 16.003$, $P < 0.0001$). Fisher's PLSD revealed significant differences between all doses and the control ($P = 0.037$ in all cases) and significant differences between doses ($P = 0.023$ in all cases) except for the 50/100 μl comparison ($P = 0.091$). Fifty microlitres of toxin gave an average reduction of 66.1%, compared to a reduction of 19.5% in the control group.

Heritability

Multiple regression revealed significant effects of both individual (neonatal) mass and maternal mass on toxin resistance (mass, $P = 0.0025$; mother's mass, $P = 0.0187$; $R^2 = 0.058$). The residuals from this analysis were used in subsequent analyses.

A total of 83 eggs hatched in the temperature/moisture experiment. Residual toxin resistance of the hatchlings was not significantly affected by the interaction between temperature and moisture treatments ($F_{1,79} = 1.378$, $P = 0.24$). After removal of this factor, we detected no significant effect on residual toxin resistance of either temperature or moisture treatments (temperature, $F_{1,80} = 0.94$, $P = 0.33$; moisture, $F_{1,80} = 0.009$, $P = 0.93$).
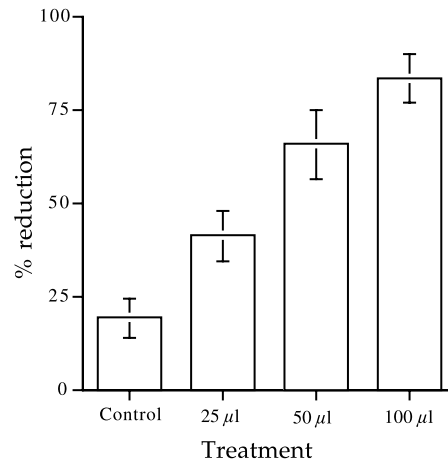
Fig. 1. Percentage reduction in swimming speed in neonate keelbacks across a range of doses of toad toxin. Different individuals were tested at each dose. Error bars represent standard errors.

Restricted maximum likelihood provided an estimate of between-clutch variance of 43.85, compared with a within-clutch variance of 148.36. This yielded an estimation of 0.456 for full-sib heritability. Jackknifing provided a standard error of 0.0488 for this heritability estimate. This high heritability does indicate partial pseudoreplication in the design of our locomotor experiments (above), which treated siblings as independent in the statistical analyses. As such, these results (particularly for the experiment on the effects of *L. dahlii* toxin, which only used two families but for which the heritability is unknown) should be interpreted with caution.

Locomotor trade-offs

The average percentage reduction in swim speed across all snakes tested with 50 μl of toad toxin was 62.5%. The null slope of after-speeds on before-speeds was thus 0.375. The observed slope was 0.325 and was not significantly less than the null ($P = 0.101$). Power analysis suggested that our data had >95% power to detect a negative deviation from the null of 0.16 and had >50% power to detect a deviation of 0.08. In comparison, the average percentage reduction across all animals tested with *L. dahlii* toxin was 30.8%. In this case, the observed slope of 0.4 was significantly less than the null of 0.69 ($P < 0.0005$; Fig. 2).

Toxin trade-offs

Testing snakes for resistance to *Bufo* toxin before testing them for resistance to *L. dahlii* toxin had no significant effect on the estimate of resistance to *L. dahlii* toxin ($F_{1,16} = 0.084$, $P = 0.78$). Including data from this experiment, a total of 115 neonate keelbacks were tested for their resistance to both *L. dahlii* and toad toxin. Of these, three snakes were unable to swim after dosing with toad toxin. Because we were unable to record a time for these animals, they were deleted from the analysis rather than being assigned a large but arbitrary time. Multiple regression revealed significant effects of pre-dose speed and snake mass on post-dose speed for *L. dahlii* toxin (pre-dose speed, $P < 0.0001$; mass, $P = 0.0002$). For toad
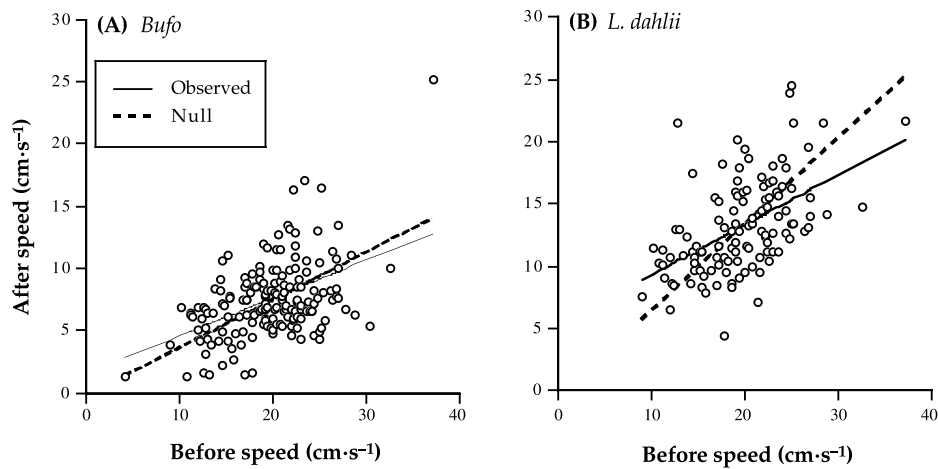
Fig. 2. Scatterplot of pre-dose versus post-dose swim speeds for snakes given (A) *B. marinus* toxin and (B) *L. dahlii* toxin. Null and observed slopes are plotted. The observed slope is significantly smaller only for snakes given *L. dahlii* toxin ($P < 0.0005$).

toxin, however, only pre-dose speed was significant (pre-dose speed, $P = 0.0045$; mass, $P = 0.5689$). Using the residuals from these regressions, the product–moment correlation coefficient was calculated and compared with the null hypothesis of no correlation (H_0: $r = 0$; H_a: $r \neq 0$). The observed correlation for the data was $r = 0.044$ (Fig. 3). The significance of this r-value was compared with the distribution of r-values obtained from 1999 randomizations of the data set. We found that 31.35% of random r-values were greater than that observed, equating to a two-tailed probability of $P = 0.628$. Because this is a non-significant result, it is important to assess the power of the data set to detect various levels of r. The power analysis suggested that the data had $>95\%$ power to detect an r-value ≥ 0.35. The data had $>53\%$ power to detect r-values ≥ 0.2.

DISCUSSION

The effect of toxins

Our results show that the methodology provides a sensitive and non-lethal assay of the effect of toxin on snakes. Both toxins elicited a reduction in speed in hatchling keelbacks relative to a control dose. For toad toxin at least, this reduction strongly depended on dosage. Small differences in dosage rate caused detectable changes in post-dose speed even with relatively small sample sizes, suggesting that this methodology will be suitable for assaying variation in response to a standard dose of toxin.

A very similar methodology has been used to assay tetrodotoxin resistance in several genera of North American snakes, yielding similar results. That is, increased doses elicited a greater reduction in locomotor speed (Motychak *et al.*, 1999). This same pattern has also been shown for nine other species of Australian snake exposed to toad toxin (Phillips *et al.*, 2003). While the mechanism contributing to the decrease in speed is likely to be different between toad toxins (action primarily cardiac), tetrodotoxin (a neurotoxin) and *L. dahlii* toxins (action under investigation), the basic effect appears to be simply that a sick animal
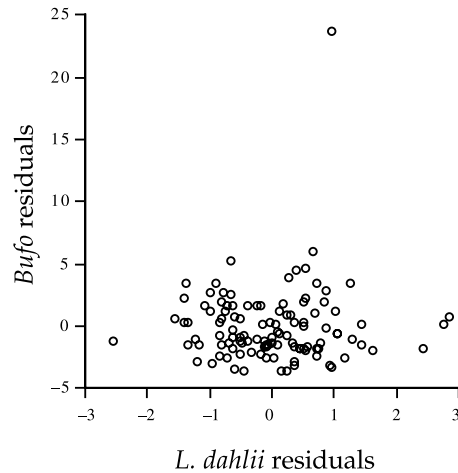
Fig. 3. Scatterplot of post-dose speeds for *L. dahlii* toxin and *B. marinus* toxin after correcting for pre-dose speed and body mass.

swims more slowly than a healthy animal. On this basis, it is likely that the assay will also be useful in detecting variation in resistance to most toxins.

Keelbacks are more resistant to toad toxin than are any of the other Australian snakes studied to date (nine species across three families). It is unlikely that an individual would be able to ingest a large enough volume of cane toad to acquire a lethal dose (Phillips *et al.*, 2003), although some instances of keelbacks apparently dying following the ingestion of a toad have been observed (Ingram and Covacevich, 1990; B.L. Phillips, personal observation). Irrespective, individuals ingesting toads are likely to incur short-term locomotor deficits. The long-term effects of a diet of toads are unknown but there may well be serious fitness costs; keelbacks maintained exclusively on toads became sick and died (Shine, 1991). Thus we expect the imminent presence of toads to exert at least mild selection on keelbacks.

Heritability

Full-sib heritability estimates also include portions of dominance and environmental variance that are thus likely to overestimate heritability (Falconer and Mackay, 1996). We have no information on the contribution of dominance variance and are forced to make the common assumption that it is small. We were able to remove variance attributable to maternal mass however, and incubation treatments appeared to have little influence on toxin resistance. This result suggests that covariance due to a common environment will be minimal in our estimate.

Additionally, it is likely that many of our full-sib groups are in fact half-sib groups. Multiple paternity is common in snakes; multiple matings are often observed (including in *T. mairii*) and molecular data often reveal multiple paternity (e.g. Barry *et al.*, 1992; McCracken *et al.*, 1999). The effect of these cryptic half-sib groups is to give an underestimate of heritability under a full-sib analysis (Brodie and Garland, 1993), making our estimate conservative.

Despite these qualifications, our estimate of heritability for resistance to toad toxin (0.456 ± 0.1) suggests relatively high levels of heritable variation for this trait in this population. Heritability estimates for physiological traits are generally around 0.3 (Roff, 1997). The lack of a heritable basis to variation is thus unlikely to be a major impediment to adaptive change in this trait.

Locomotor trade-offs

Locomotor trade-offs were not detected for response to toad toxin, but we found a strong trade-off between locomotor performance and resistance to *L. dahlii* toxin. This strong trade-off in locomotor performance for *L. dahlii* toxin contrasts with the minimal or non-existent trade-off detected for toad toxin. The Humpty Doo keelback population was naïve to toads at the time of testing but has been exposed to *L. dahlii* for many generations. It is tempting to speculate that selection for increased resistance to *L. dahlii* toxin has resulted in resistance levels being driven upwards by selection to the point where they are balanced by trade-offs. The lack of a trade-off for toad resistance may be due simply to the fact that resistance to toads is not yet under directional selection in this population.

Toxin trade-offs

The power analysis suggests that our data have excellent power to detect reasonable levels of correlation between responses to the two toxins. Our results thus clearly indicate a very poor correlation (if any) between an individual snake's response to toad and *L. dahlii* toxins. Given that the action of the two toxins is likely to be different, this result is not altogether surprising. However, it suggests that selection for resistance to *L. dahlii* toxin will not pre-adapt a population for resistance to toad toxin. The lack of correlation also suggests that there is likely to be no trade-off between resistance to these two toxins. That is, selection for increased resistance to one toxin does not equate to reduced ability to deal with the other toxin, at least in keelbacks. In this system at least, snakes appear free to evolve resistance to toad toxin without sacrificing resistance to the toxins of native prey.

While pre-adaptation in terms of resistance to toad toxin is unlikely, sympatry with an extremely toxic prey item may cause selection for other traits that will pre-adapt a population to the invasion of the toad. Most important of these is a change in prey preference or foraging tactics – the presence of *L. dahlii* may have led to the evolution of 'fussiness' in attack and feeding responses. Such evolved predator tactics may reduce the impact of the toad or allow a more rapid recovery following the toad invasion. Further research is required to assess these possibilities.

The potential for adaptation

Humpty Doo keelbacks exhibit significant and relatively high heritability for resistance to toad toxin. Thus, in the absence of trade-offs or constraints, toxin resistance is likely to respond to selection. We found no evidence for either a trade-off or pre-adaptation due to the presence of the native frog, *Litoria dahlii*. Thus, snakes sympatric with *L. dahlii* are unlikely to have either an advantage or disadvantage in terms of an adaptive increase in toxin resistance. Additionally, in keelbacks there appears to be no intrinsic trade-off

between locomotor performance and resistance to toad toxin, suggesting no impediment to adaptive change through this factor either.

Thus we find evidence for heritable variation, probable mild selection and no obvious trade-offs for resistance to toad toxin in keelbacks. The clear inference is that this species is capable of adaptively responding to toad invasion by increasing toxin resistance. The relatively short generation time of keelbacks (< 18 months; Brown and Shine, 2002) suggests that rapid adaptive response will be possible in this species. In keeping with this prediction, keelbacks (but not most other frog-eating snake species) remain abundant in areas that toads have occupied for more than 50 years.

While rapid adaptive response is possible for toxin resistance, it remains possible that one or more of the other three traits listed (see Introduction) may be more labile and respond more rapidly to the presence of toads. Our current results simply mean that an adaptive response is possible in keelbacks. To persist through environmental change, one adaptive solution is all that is necessary.

Our approach, using experimental data, is a level of abstraction away from direct measurement of population-level impact but clarifies the likelihood of an adaptive response. Also, by examining factors that may be influential (e.g. the presence of a native, toxic frog), we can make a broader level of inference, beyond the particular study species. For example, the lack of relationship between resistance to the two toxins in the keelback suggests that a similar lack of relationship is likely in other species (i.e. the toxins are very different). Thus the presence of the toxic native is likely to have little bearing on resistance to toad toxin for other snake species in the community.

Examining the problem of environmental change in this manner places individual studies within the broader unifying framework of evolutionary theory. Ultimately, this should allow meaningful comparisons to be made across species and systems. Such a unified approach is necessary to further our understanding of the impacts of invasive species and the consequences of environmental change generally (Caughley, 1994; D'Antonio and Kark, 2002).

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