High-model abundance may permit the gradual evolution of Batesian mimicry: an experimental test

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In Batesian mimicry, a harmless species (the ‘mimic’) resembles a dangerous species (the ‘model’) and is thus protected from predators. It is often assumed that the mimetic phenotype evolves from a cryptic phenotype, but it is unclear how a population can transition through intermediate phenotypes; such intermediates may receive neither the benefits of crypsis nor mimicry. Here, we ask if selection against intermediates weakens with increasing model abundance. We also ask if mimicry has evolved from cryptic phenotypes in a mimetic clade. We first present an ancestral character-state reconstruction showing that mimicry of a coral snake (Micrurus fulvius) by the scarlet kingsnake (Lampropeltis elapsoides) evolved from a cryptic phenotype. We then evaluate predation rates on intermediate phenotypes relative to cryptic and mimetic phenotypes under conditions of both high- and low-model abundances. Our results indicate that where coral snakes are rare, intermediate phenotypes are attacked more often than cryptic and mimetic phenotypes, indicating the presence of an adaptive valley. However, where coral snakes are abundant, intermediate phenotypes are not attacked more frequently, resulting in an adaptive landscape without a valley. Thus, high-model abundance may facilitate the evolution of Batesian mimicry.

Keywords: adaptive landscape; Batesian mimicry; coral snake mimicry; predation; colour pattern

1. INTRODUCTION

Batesian mimicry occurs when a harmless species (the ‘mimic’) resembles a dangerous one (the ‘model’) and thereby co-opts the protection from predation that is often afforded to conspicuous, toxic species (Bates 1862; reviewed in Ruxton et al. 2004). Although Batesian mimicry has been called ‘the greatest post-Darwinian application of natural selection’ (Fisher 1958), the route evolution takes in producing mimicry is unknown in many systems.

Much of the debate surrounding the evolution of Batesian mimicry centres on explaining whether it can evolve through a gradual process of incremental evolution. In particular, if the starting point for the evolution of mimicry is a cryptic phenotype (as is often assumed; e.g. Nicholson 1927; Charlesworth & Charlesworth 1975; Charlesworth 1994), then it is unclear how a population can transition from an ancestral cryptic phenotype to a derived mimetic one if the population must pass through a phase in which it expresses a phenotype that is intermediate between these two extremes. Such intermediate phenotypes should generally be disfavoured because they should fail to receive the fitness benefits of either crypsis or mimicry (Nicholson 1927; Charlesworth & Charlesworth 1975; Mappes & Alatalo 1997; but see Fisher 1958; Schmidt 1958, 1960). The low fitness of intermediate forms would seem to preclude the gradual evolution from an ancestral cryptic phenotype to a derived mimetic form.

In order to bypass the problem of evolution through intermediate forms of presumably low fitness, some have suggested a two-step evolutionary model in lieu of the gradual process of incremental evolution (Nicholson 1927; Charlesworth & Charlesworth 1975). According to this model, Batesian mimicry evolves when a major mutation of large effect takes the evolving population over the adaptive valley associated with intermediate phenotypes. This initial mutational leap is followed by smaller mutations that perfect resemblance to the model. Indeed, this two-step mechanism is the reigning paradigm for explaining the evolution of Batesian mimicry (Ruxton et al. 2004; Turner 2005). Nevertheless, documentation of a system in which there is no adaptive valley between crypsis and mimicry would imply that Batesian mimicry might also be able to evolve gradually. However, few have specifically questioned if ecological circumstances exist in which intermediate phenotypes would not reside in an adaptive valley.

Generally, the factors that can increase the fitness of intermediate phenotypes can also favour the evolution of imperfect mimicry. Two such factors are likely to be particularly beneficial to intermediate phenotypes: (i) high-model toxicity (Duncan & Sheppard 1965; Plecki & O’Donell 1971; Goodale & Sneddon 1977; Lindström et al. 1997) and (ii) high-model abundance (Brower 1960; Lindström et al. 1997; Harper & Pfennig 2007). Selection against imperfect mimics decreases under these two conditions for the same reason: the probable payoff to a predator for attacking prey with a given resemblance to the model decreases (Oaten et al. 1975; Sherratt 2002). A way of visualizing this is that the ‘cone of protection’ around the model’s phenotype comes to envelop more and more of the phenotypic space around it as
models become more numerous and noxious. If models are highly abundant and noxious, the cone of protection may grow so wide that selection acts on intermediate phenotypes to form a smooth slope instead of an adaptive valley.

We studied these issues in a well-documented Batesian mimicry complex (Greene & McDiarmid 1981; Pfennig et al. 2001; Brodie & Brodie 2004; Harper & Pfennig 2007, 2008). We begin by presenting a phylogenetic analysis of colour-pattern evolution, which strongly suggests that mimics evolved from cryptic forms in our study system. We then present an empirical test of the hypothesis that predation on intermediate phenotypes relative to cryptic and mimetic phenotypes changes with the abundance of a deadly model. We specifically predicted that in areas of high-model abundance, there would be relaxed selection pressure by predators against intermediate phenotypes and that therefore we would not observe a difference between attack rates on intermediates relative to cryptic and mimetic phenotypes; i.e. in such areas, there would be no adaptive valley associated with intermediate phenotypes. We also predicted that where models are rare, we would find an adaptive valley caused by increased predation on intermediate phenotypes relative to those protected by crypsis and good mimicry. These predictions focus on the presence or absence of an adaptive valley between crypsis and mimicry, not the efficacy of crypsis versus mimicry as alternative strategies for avoiding attack. We are more concerned with the potential existence of an adaptive valley because this dictates the mode of adaptation by which species can transition between these two predator avoidance strategies. To test our predictions, we placed replicas of cryptic, intermediate and mimetic phenotypes in natural areas with high- and low-model abundances to evaluate the selective pressures exerted by predators in each area.

2. MATERIAL AND METHODS

(a) Study system

In the southeastern USA, the highly venomous eastern coral snake (Elapidae: Micrurus fulvius) is the model for a non-venomous mimic, the scarlet kingsnake (Colubridae: Lampropeltis elapsoides). The coral snake’s venom is lethal to most predators (Roze 1996). Not surprisingly, many potential predators show an innate aversion to coral snake colour patterns (Gehlbach 1972; Smith 1975, 1977). Such a potent model is an excellent candidate for generating a wide cone of protection.

The geographical distribution of L. elapsoides overlaps entirely with that of M. fulvius, which ranges from Florida to southern North Carolina. The former’s geographical range also extends north into southern Virginia and west to the Mississippi River (see range map in Harper & Pfennig 2007). Lampropeltis elapsoides is avoided by predators in sympatry with coral snakes, but not in allopatry (Pfennig et al. 2001), confirming that it is indeed a Batesian mimic of M. fulvius. Also, M. fulvius is relatively more abundant than L. elapsoides in Florida than it is in southern North Carolina (Harper & Pfennig 2007). Museum collection data indicate that ratio of the abundance of M. fulvius to L. elapsoides is approximately six times higher in Florida than in North Carolina (Harper & Pfennig 2007). In Florida, L. elapsoides is more variable in colour patterns than in southern North Carolina, where they are more precise mimics, indicating that the cone of protection in North Carolina may be narrower. Previous field experiments have shown that slightly imperfect mimics are selected against in southern North Carolina (Harper & Pfennig 2007), suggesting that variation in the cone of protection can be measured in this system. However, it is unknown whether predation pressure against intermediate phenotypes is relaxed in areas of high-model abundance (e.g. Florida), such that no adaptive valley exists in these areas.

(b) Ancestral character-state reconstruction of colour pattern

To test whether the mimic, L. elapsoides, likely evolved from a cryptic ancestor (as assumed; see §1), we performed an ancestral character-state reconstruction of colour pattern in the snake tribe Lampropeltini. To do so, we used a recently published phylogeny (Pyron & Burbrink 2009) that was built with maximum-likelihood methods and based on three nuclear and six mitochondrial loci. This phylogeny includes all 31 traditionally described species in the Lampropeltini.

We quantified the colour pattern of each species in the phylogeny. We used an ordinal scale developed by Savage & Slowinski (1992) to rank how closely each species’ colour pattern matched that of the model, M. fulvius. We scored pattern and colour separately. For pattern, a score of 3 was given to species with rings that completely encircle the body (i.e. the same pattern as M. fulvius); a score of 2 was given to species with bands that do not completely encircle the body; a score of 1 was given to species with dorsal saddles that encircle the body less than bands (with or without interspersed lateral blotches); and a score of 0 was given to species with any other pattern not matching one of the above three categories. For colour, a score of 1 was given to species with all three of the colours typical of M. fulvius (red, yellow/white and black) and a score of 0 was given to species that lacked at least one of these colours.

Because both colour and pattern are important in predator avoidance of coral snakes and their mimics (Smith 1975; Hinman et al. 1997), our separate pattern and colour scores were combined. We did so by weighting and summing each so that they contributed equally to a continuous composite colour-pattern variable that ranged from 0 (most cryptic) to 2 (most mimetic). Although our classification scheme assumes that coral snake colour patterns are conspicuous and other colour patterns found in the Lampropeltini are cryptic, these assumptions appear to be valid. Experiments have shown that predator avoidance of coral snake colour patterns is probably due entirely to their aposematic function (Brodie 1993), and that drab, blotched colour patterns of other snakes are probably cryptic (Brodie 1992; King 1992).

The evolutionary history of colour pattern in the Lampropeltini was determined using a parsimony reconstruction for continuous characters in Mesquite 2.6 (Maddison & Maddison 2009). We used a squared-change parsimony model that assigns a cost of \((x−y)^2\) to a transition from character state \(x\) to character state \(y\). For taxa whose colours or patterns were difficult to classify, we performed separate analyses with possible alternative values to evaluate the stability of our character-state reconstruction.

(c) Experimental evaluation of predation on intermediate phenotypes in high- and low-model abundance areas

To evaluate attack rates on intermediate phenotypes relative to cryptic and mimetic ones, we measured predation rates...
on different snake colour-pattern phenotypes in the wild. Specifically, we placed artificial snake replicas in natural areas where they would be subjected to potential predation by naturally occurring, free-ranging predators. Moreover, as the replicas were made of a soft substance (clay) that takes impressions, predation events were recorded even though the replicas were left unobserved for the long periods of time necessary for predation to occur. This method has been employed successfully to document both avian and mammalian predators on at least three continents (e.g. Madsen 1987; Brodie 1993; Brodie & Janzen 1995; Hinman et al. 1997; Pfennig et al. 2001, 2007; Wüster et al. 2004; Niskanen & Mappes 2005; Buasso et al. 2006; Harper & Pfennig 2007), indicating that it is robust to different predator guilds and environments. We constructed our replicas with pre-coloured, non-toxic polymer clay (Polyform Products, Elgin, IL) that were coated with a thin film of clear, low-odour spray latex enamel (Krylon Products Group, Cleveland, OH) to minimize any smell emitted by the clay.

To determine the appropriate colour patterns for our cryptic, intermediate and mimetic phenotypes (figure 1), we used morphometric analyses (D. Kikuchi 2009, unpublished data) of museum specimens of eastern milksnakes (Lampropeltis triangulum triangulum; n = 16) and published data on L. elapsoides (Harper & Pfennig 2007). We included 12 dimensions to describe colour pattern. Phylogenetic analyses show that L. t. triangulum is an appropriate representative of a cryptic ancestral phenotype (see §3). To create a 50 percent intermediate phenotype, we used the average value of the cryptic and mimetic phenotype for each dimension of colour pattern measured. Our intermediate phenotype resembled Lampropeltis triangulum sspila and Lampropeltis triangulum temporalis, indicating that it was within the natural range of variation in this genus (compare figure 1c with d).

Before placing replicas in the field, we first arranged them into triads (consisting of one replica of each different phenotype) by tying them to 1 m lengths of clear monofilament fishing line and then attaching one of each phenotype to the same large nail. This ensured that within triads, replicas of each phenotype would share similar micro-habitats. Members of each triad were placed in realistic micro-habitats such as the edges of logs and grass clumps (figure 1). Triads were placed in 750 m transects of 10 triads each so that they were separated from one another by about 75 m. Placing replicas in triads and transects allowed us to use a statistical model in analysing predation that accounted for the possibility that predation events might have non-random spatial distributions. Our sites were protected natural areas such as state parks and national forests. Most of the areas contained longleaf pine forest. Lampropeltis elapsoides is closely associated with longleaf pine forests (Palmer & Braswell 1995), which range from Florida to North Carolina and provide relatively constant habitat throughout the range of the mimicry complex, thus controlling for the visual environment in which predators perceive the snakes. In other words, a colour pattern that is cryptic where models are abundant
(Florida) is likely to be equally cryptic where models are rare (southern North Carolina), as the habitat in both regions is similar. We chose 13 sites in Florida (high-model abundance) and 13 sites in southern North Carolina (low-model abundance) to conduct our experiment (appendix SA in the electronic supplementary material). We left the replicas in the field for 30–36 days. At the end of this time, we collected each replica and, based on the presence/absence of tooth and beak marks, scored each as having been attacked or not (for details, see Pfennig et al. 2007).

Given our prediction that there would be an adaptive valley in areas of low-model abundance but not in areas of high-model abundance, we analysed our data using an a priori contrast to compare the fitness of the intermediate phenotype with the combined fitness of the cryptic and mimic phenotypes. We used the lmer function in the lme4 package (Bates 2005) for R 2.8.1 (R Development Core Team 2008) to build generalized linear mixed models with binomial error distributions for predation data from high- and low-model abundance areas. We used replica fate (attacked or not attacked) as the binary response variable, replica phenotype as the predictor variable and trial nested within transect as random effects.

3. RESULTS
(a) Ancestral character-state reconstruction of colour pattern
Mapping colour pattern onto a phylogeny of the Lampropeltini showed that all strong mimetic resemblances (colour-pattern value > 1) are within a single clade that contains the mimic, *L. elapoides* (figure 2). Outside this clade, some snakes have colour-pattern values above 0 (where 0 denotes a cryptic pattern), but these snakes have three drab colours arranged as dark saddled blotches with black edges on a light background, much as they are in *L. t. triangulum* (see figure 1b). Such colour patterns are still highly cryptic and do not resemble the model, *M. fulvius*.

The last common ancestor of the clade containing mimetic snakes and the clade formed by *Bogertophis* and *Pseudelaphe* was probably very similar to *L. t. triangulum* in phenotype, most likely having a saddled pattern of three drab colours. This result was robust to altering values of colour pattern for *P. flavivirga* and *Pantherophis guttatus*, both of which were difficult to classify owing to variability in appearance (results not shown). Furthermore, reconstructed values of colour pattern at deeper nodes are all close to zero, indicating that the basal character state for the Lampropeltini was cryptic. We consider this strong evidence that conspicuous mimics evolved from cryptic ancestral phenotypes, and also that *L. t. triangulum* is an appropriate representation of that ancestral phenotype.

(b) Experimental evaluation of predation on intermediate phenotypes in high- and low-model abundance areas
Of 780 replicas that we placed in the field, we discarded 32 (4.1%) owing to loss, fire or human interference. Of the 748 that remained for analysis, 104 (13.9%) were attacked. Attacks were evenly distributed, with 55 replicas attacked in Florida and 49 attacked in North Carolina (*p* > 0.5). Most attacks were by large mammals such as black bear, *Ursus americanus*, and small mammals such as opossum, *Didelphis virginiana*. Attacks by birds were rare. Markings consistent with rodent or insect activity were ignored, as these would not constitute threats to real snakes.

In Florida, where coral snakes are relatively common, the intermediate phenotype was not attacked more than the cryptic and mimetic phenotypes (figure 3a; *n* = 389, *Z* = −0.01, *p* > 0.9). By contrast, in southern North Carolina, where coral snakes are relatively rare, the intermediate phenotype was attacked more frequently than the other two (figure 3b; *n* = 359, *Z* = 1.95, *p* = 0.05).

4. DISCUSSION
Our results demonstrate that Batesian mimicry can arise from cryptic ancestral phenotypes, and that predation on intermediate phenotypes relative to cryptic and mimetic phenotypes changes with the abundance of a deadly model. In particular, an ancestral character-state reconstruction revealed that mimetic coloration evolved in a single clade of Lampropeltini from cryptic ancestors. Moreover, our field experiment confirmed our prediction (see §1) that when a strongly aversive model is common, there is no increased predation associated with phenotypes that are intermediate between cryptic and mimetic forms. By contrast (and also consistent with our prediction), when such models are rare, there is an adaptive valley associated with intermediate phenotypes, verifying that model abundance probably influenced the changes in the adaptive landscape. This study therefore indicates that an adaptive valley may not be present in all circumstances under which Batesian mimicry might evolve, widening the number of scenarios that can explain its evolution.

As noted in §1, evolutionary biologists have long debated how Batesian mimicry evolves. Because it is generally assumed that there will be an adaptive valley associated with phenotypes that are intermediate between cryptic and mimetic forms, recent opinion has considered a two-step mechanism as the likely explanation for the evolution of Batesian mimicry (Ruxton et al. 2004; Turner 2005). According to this model, the first step occurs when a major mutation of large effect takes an evolving population over the adaptive valley associated with intermediate phenotypes. Later, the second step occurs when mutations of small effect perfect the resemblance of the mimic to the model. It is important to note that the raison d’être for a two-step hypothesis is the supposed constant presence of a valley in the adaptive landscape. Yet prior to the present study, research had not evaluated empirically whether conditions might exist under which no such adaptive valley is present.

Our study fills this gap. By demonstrating that conditions do indeed exist under which there is no adaptive valley associated with phenotypes that are intermediate between cryptic and mimetic forms, our data imply that the two-step process need not be considered essential for the evolution of Batesian mimicry. Thus, our study suggests that there are situations under which Batesian mimicry can evolve gradually through a process of incremental evolution. We hasten to add, however, that our results in no way demonstrate that a two-step process could not have unfolded in our (or any other) system.
Our results merely suggest that such a two-step process need not have occurred because of the constant presence of a valley in the adaptive landscape, as long assumed.

Debate over whether mimicry evolves gradually or begins with a mutation of large effect is essentially about what mechanism is responsible for enabling a population to access the adaptive peak of mimicry. Hypothesizing that mimicry evolves without the first, major mutation implicitly invokes another mechanism to create the requisite smooth adaptive landscape.

Changes in selective pressures, such as those caused by varying model abundance, appear to lie behind many populations’ transitions to new adaptive peaks (Fear & Price 1998). It appears that mimicry, long regarded as an exception among adaptations, has the potential to evolve in the same manner as many other traits.

This study provides further evidence that the coral snake mimicry complex in the southeastern USA conforms to the theoretical expectations of a Batesian mimicry system. Previous research established the
Do the conclusions from this study apply to other mimicry systems with less noxious models? After all, coral snakes are highly toxic, and they should generate a much wider cone of protection than less toxic models. Our results should apply to other mimicry systems for the simple reason that even models less deadly than coral snakes can still be strongly aversive. For example, Lepidopteran larvae (which often serve as models and are often considered to be less toxic) have been known to cause death in potential predators (Poulton 1890), and therefore may still incur high costs on their attackers. Moreover, other Batesian mimicry systems feature models dangerous enough to warrant the evolution of innate aversion in potential predators (Schuler & Hesse 1985; Nelson & Jackson 2006). In any event, it is not the noxiousness of the model per se that determines the cone of protection around the model; both model noxiousness and abundance impinge on the shape of the probability density function that describes the predator’s likelihood of sampling prey as prey approach the model in resemblance. It is the shape of that distribution itself, in conjunction with that associated with cryptic coloration, which ultimately decides the shape of the adaptive landscape. Even weakly aversive models may fill in a valley in the adaptive landscape if they are highly abundant. Therefore, both types of landscapes observed in this study should occur in other Batesian mimicry systems.

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