

Batesian mimics influence the evolution of conspicuousness in an aposematic salamander

A. C. KRAEMER¹, J. M. SERB & D. C. ADAMS

Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA, USA

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Abstract

Conspicuousness, or having high contrast relative to the surrounding background, is a common feature of unpalatable species. Several hypotheses have been proposed to explain the occurrence of conspicuousness, and while most involve the role of conspicuousness as a direct signal of unpalatability to potential predators, one hypothesis suggests that exaggerated conspicuousness may evolve in unpalatable species to reduce predator confusion with palatable species (potential Batesian mimics). This hypothesis of antagonistic coevolution between palatable and unpalatable species hinges on the 'cost of conspicuousness', in which conspicuousness increases the likelihood of predation more in palatable species than in unpalatable species. Under this mimicry scenario, four patterns are expected: (i) mimics will more closely resemble local models than models from other localities, (ii) there will be a positive relationship between mimic and model conspicuousness, (iii) models will be more conspicuous in the presence of mimics, and (iv) when models and mimics differ in conspicuousness, mimics will be less conspicuous than models. We tested these predictions in the salamander mimicry system involving *Notophthalmus viridescens* (model) and one colour morph of *Plethodon cinereus* (mimic). All predictions were supported, indicating that selection for Batesian mimicry not only influences the evolution of mimics, but also the evolution of the models they resemble. These findings indicate that mimicry plays a large role in the evolution of model warning signals, particularly influencing the evolution of conspicuousness.

Introduction

Batesian mimicry is an interspecific relationship in which a palatable 'mimic' species closely resembles an unpalatable 'model' species. In this relationship, models are often conspicuous by exhibiting phenotypes that markedly distinguish them from the surrounding environment such as behaviour, auditory cues or, most commonly, coloration (Ruxton *et al.*, 2004). Because conspicuous organisms stand out against their background, they can incur costs that include increased attention from predators in addition to the cost of resources invested in conspicuousness. Taking these

costs into account, it is unclear why conspicuousness should evolve. One explanation is that despite these costs, a conspicuous trait can serve as an effective signal of unpalatability to potential predators (this relationship is termed 'aposematism'; Poulton, 1890). Conspicuousness is hypothesized to evolve in aposematic species to quickly train potential predators (Gittleman & Harvey, 1980; Roper & Redston, 1987; Lindström *et al.*, 2001) and act as a mnemonic device for communicating unpalatability to predators (Guilford, 1986; Speed, 2000; Prudic *et al.*, 2007).

However, palatable prey species may also influence the evolution of conspicuousness in aposematic species (Fisher, 1930). One hypothesis suggests that mimicry may drive antagonistic coevolution (Dawkins & Krebs, 1979; Thompson, 1989) such that models evolve phenotypes distinct from mimics ('chase-away selection,' e.g. Poulton, 1890; Edmunds, 1974; Sherratt & Franks, 2005; Franks *et al.*, 2009), and that exaggerated

Correspondence: Andrew C. Kraemer, Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50011, USA. Tel.: (612) 720 6507; fax: (208) 885-7905; e-mail: andrew.c.kraemer@gmail.com

¹Present address: Department of Biological Sciences, University of Idaho, Moscow, ID 83844, USA

conspicuousness may evolve because these warning signals are more costly for palatable mimics than unpalatable models as a result of heightened attention from predators (Speed & Ruxton, 2010). Thus, selection for mimicry should drive mimics to track the conspicuousness of models, while selection on models will push them to appear distinct from mimics. Additionally, the predation cost of conspicuousness will inhibit mimics from perfectly matching models with respect to conspicuousness. From the hypothesis of mimicry, we expect two patterns to emerge: (i) predation pressures will drive mimics to converge on local model phenotypes, and (ii) through selection for mimicry, mimic conspicuousness will follow model conspicuousness, leading to a correlation between mimic and model conspicuousness among localities. Further, from the hypothesis of a cost of conspicuousness we expect two additional patterns to emerge: (iii) while in the presence of mimics, selection on models will drive them to appear more conspicuous to remain distinct from mimics. (iv) Due to the predation cost of conspicuousness, for circumstances in which better mimicry results in higher rates of detection from predators and increased predation, mimics will be unable to perfectly match model conspicuousness. This link between model conspicuousness and mimicry has long been predicted, but is understudied in natural systems of mimicry, particularly from the perspective of potential predators (but see Wüster *et al.*, 2004).

In this study, we tested the four predictions above to evaluate the hypothesis that conspicuousness may evolve in aposematic species through selection from predators to appear distinct from Batesian mimics. We evaluated these hypotheses in a mimicry system between two salamander species: *Notophthalmus viridescens* (the aposematic model) and its Batesian mimic, the erythristic colour morph of *Plethodon cinereus*. The accumulated evidence for mimicry in this system indicates that birds select for mimicry, specifically on the basis of coloration (Brodie & Brodie, 1980; Tilley *et al.*, 1982; Kraemer & Adams, 2014). We used a bird visual model (*sensu* Kraemer & Adams, 2014) to estimate colour conspicuousness of mimics and their aposematic models from the perspective of the relevant predator class, a tetrachromatic bird.

Materials and methods

Study system

We examined hypotheses of conspicuousness in two salamander species: *N. viridescens* (the aposematic model) and its Batesian mimic, *P. cinereus*. *Notophthalmus viridescens* has a triphasic life cycle that includes a secondary, juvenile (eft) stage, in which efts are terrestrial, vibrantly red-orange in coloration (Fig. 1), and contain tetrodotoxin, a neurotoxin, which makes them

unpalatable to predators (Brodie, 1968). In contrast, larval and adult *N. viridescens* are aquatic and drab. The mimic species, *P. cinereus*, exhibits several discrete colour morphs (including striped, unstriped and erythristic), only one of which, the erythristic form (which is solid red-orange in coloration) is qualitatively similar to *N. viridescens* efts (Fig. 1; Petranka, 1998). Unlike *N. viridescens*, *P. cinereus* is palatable to predators and lacks tetrodotoxin (Brodie & Brodie, 1980; Tilley *et al.*, 1982). Both species are found in north-eastern North America and overlap at a broad regional scale. In western Massachusetts, *N. viridescens* can be found at a large number of localities whereas erythristic *P. cinereus* are present at a subset of these localities (Kraemer & Adams, 2014). In this region, there are no other potential mimics of *N. viridescens* (Petranka, 1998).

Of the possible vertebrate predators, tetrachromatic birds are the most likely predator class driving the evolution of mimicry in this system (Lotter & Scott, 1977; Brodie & Brodie, 1980; Tilley *et al.*, 1982). Consistent with the hypothesis of mimicry, potential avian predators are able to discriminate between the coloration of nonmimic *P. cinereus* and efts, but not between the coloration of erythristic *P. cinereus* and efts (Kraemer & Adams, 2014). Lending further support to the hypothesis of mimicry, two empirical field studies have found that wild (Brodie & Brodie, 1980) and captive (Tilley *et al.*, 1982) bird predators avoid *N. viridescens* and erythristic *P. cinereus* similarly, while they readily consume striped *P. cinereus*.

Salamander collection and colour quantification

In the summer of 2011, we collected 123 erythristic *P. cinereus* and 318 eft-stage *N. viridescens* salamanders from 32 localities in western Massachusetts, USA. Although *N. viridescens* and the striped (nonmimic) form of *P. cinereus* were present at all 32 localities, erythristic *P. cinereus* were found in a subset of the localities (see Supporting Information). All individuals were first anesthetized using tricaine methanesulfonate (MS-222: *P. cinereus*) or by applying benzocaine to the head (*N. viridescens*). There is the potential for differences in anesthetization to affect estimates of coloration between salamander groups. However, such physiologically based colour changes have only been observed in members of an unrelated salamander lineage (Garcia *et al.*, 2003), and we did not observe such changes in our salamanders.

After anesthetization, we measured the spectral reflectance from a single point in the mid-dorsal region of each salamander using a portable JAZ-PX spectrometer (OceanOptics, Dunedin, FL, USA) fitted with a 100- μ m entrance slit, a pulsed xenon lamp and a QR400-7-UV-BX reflectance probe. This probe was equipped with a tip that standardized the measured patch to a 2-mm diameter circle at a distance of 20 mm between probe

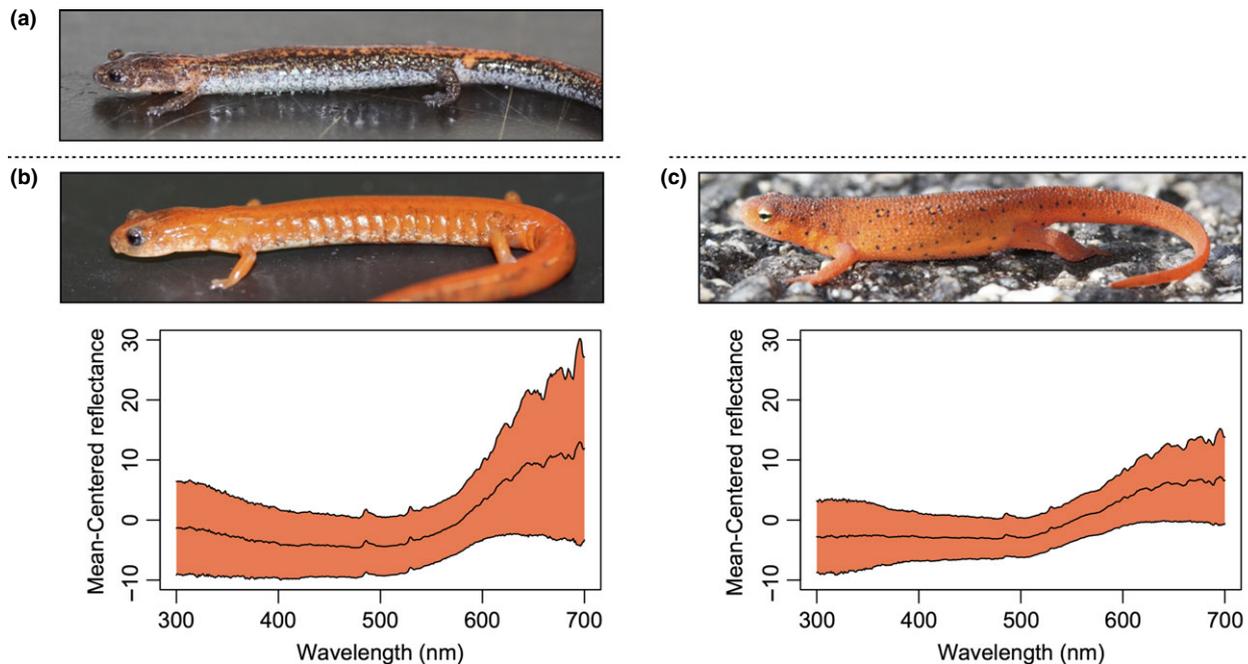


Fig. 1 Representative salamanders from the *Notophthalmus viridescens* – *P. cinereus* mimicry system. (a) Nonmimic *Plethodon cinereus*. (b) Mimic (erythristic) *P. cinereus*. (c) An eft form of the model species *N. viridescens*. Spectrum height can be used to describe the brightness, whereas spectrum curvature is related to colour. This study was focused on salamander coloration; thus, brightness-standardized reflectance spectra and standard deviations are included below salamanders used in the study.

and measured patch while excluding ambient light. We held the probe perpendicular to measured patches and used a Spectralon white reflectance standard between each animal to correct for drift in lamp intensity (see e.g. Kraemer *et al.*, 2012). Collecting reflectance measurements at a perpendicular angle can introduce undesirable spectral glare, in particular under the circumstances in which coloration is influenced by structural elements in the target animal (Endler, 1990). However, the dorsal coloration of these salamanders is composed of pigments with limited structural elements (Bagnara & Taylor, 1970), which reduced the potential for problems associated with spectral glare in this study. We measured each spectrum at 1-nm intervals from 300 to 700 nm (see Fig. 1 for a visualization of spectral reflectance curves used in this study).

Terrestrial salamanders are most commonly found in soils with a thick layer of humus and decaying leaf litter (Grover, 1998; Renaldo *et al.*, 2011). Additionally, they are most active under cool and wet conditions (Grover, 1998; Petranksa, 1998). Therefore, we obtained ten reflectance spectra of the naturally occurring wet leaf litter from one locality where salamanders were collected (Lilly Pond Wildlife Management Area, Goshen, Massachusetts, on 9 June 2012) to represent a typical background against which predators would likely view salamanders. At this locality, both erythristic *P. cinereus* and *N. viridescens* are common. Our sampling

of the background substrate at this locality is somewhat limited, although we note that all localities consisted of a similar complement of hemlock–northern hardwood forest, and thus, it is likely that backgrounds against which salamanders are viewed vary little among localities. However, to further address this issue empirically, we obtained additional background samples from other localities and performed a sensitivity analysis to evaluate the effect of alternative backgrounds on our results (see Effect of Choice of Background below).

Visual model

We used a visual model developed by Vorobyev *et al.* (2001) that was modified to estimate salamander colour conspicuousness (Kraemer & Adams, 2014). This analytical model estimates discriminability of visual signals from the signal to noise ratio of predator photoreceptors (Vorobyev *et al.*, 1998). The model yields estimates of the visual contrast between targets and backgrounds (as in Maan & Cummings, 2012) or between two targets (as in Siddiqi *et al.*, 2004). Erythristic *P. cinereus* mimic *N. viridescens* efts primarily on the basis of coloration and not brightness (Kraemer & Adams, 2014). Selection on model phenotype to appear distinct from mimics will therefore be restricted to coloration. Thus, any patterns of coloration (Prediction 1) or conspicuousness (Predictions 2–4) between models, mimics and backgrounds

are expected to also be restricted to coloration. We therefore used the visual model to calculate contrasts for the chromatic visual channel (ΔS), which summarizes the aspects of visual stimuli pertaining to colour (i.e. chroma and hue). Large contrast values indicate similarly large and easily discriminable differences between targets and backgrounds or between two targets from the predator's perspective, whereas smaller contrasts indicate that they are similar in coloration and are potentially indistinguishable from one another. Contrasts > 1.0 are considered discriminable and apparent to potential predators (Vorobyev *et al.*, 1998).

Estimating the visual model requires several components, including reflectance measures of the target (i.e. salamander) and background (i.e. leaf litter), background habitat irradiance (i.e. light environment) and photoreceptor sensitivities of the predator. We used a forest shade irradiance measure reported elsewhere (*sensu* Kraemer & Adams, 2014) for a constant light environment. Although there are several likely predators of salamanders in this system, prior work indicated that the evolution of mimicry in *P. cinereus* is likely the result of predation from tetrachromatic birds (Brodie & Brodie, 1980; Tilley *et al.*, 1982; Kraemer & Adams, 2014). Although we were unable to estimate the spectral sensitivities of specific bird predator species for this system (e.g. blue jay, hermit thrush or robin), we were able to use known spectral sensitivities for another passeriform species, the blue tit (Hart *et al.*, 2000). Bird predators of these salamanders are tetrachromatic and likely have similar visual capabilities to the blue tit used to generate the visual model (Howard & Brodie, 1973; Chen & Goldsmith, 1986). A full description of visual model calculations and corresponding code is found in Kraemer & Adams (2014). All analyses were conducted in R 3.0.2 (R Development Core Team, 2013).

Statistical analyses

For all analyses, we treated localities as independent, because a prior multivariate analysis of variance found no statistically significant association between the presence of erythristic *P. cinereus* and geographic locality as estimated by latitude and longitude ($F_{1,30} = 2.61$; $P = 0.091$). It is worth noting here that while locality was not a statistically significant factor regarding the presence of erythristic *P. cinereus* individuals, the relatively low P -value suggests a trend towards clustering with regard to mimic phenotype, which would support a previous study finding low levels of gene flow among *P. cinereus* populations (Cabe *et al.*, 2006). With these data, we used the following procedures to test for a relationship between mimicry and model conspicuousness:

Prediction 1: Mimic similarity to local models

We used randomization to test the prediction that mimic coloration was more similar to a local model

than the models at other localities. In this analysis, we first used the visual model to calculate a contrast score between the average erythristic *P. cinereus* and the average *N. viridescens* at each locality where both were found syntopically ($N = 14$). These contrast scores, which represent a difference in coloration between mimics and models from the perspective of bird predators at each locality, were then summed to create an observed difference statistic among all localities. We then randomized the pairing of mimic and model spectra and recalculated the difference statistic 99 999 times to generate a random distribution of difference statistics. We treated the proportion of randomly generated statistics less than the observed difference statistic as the significance of this test.

Prediction 2: Model–mimic colour contrast covariation

We performed a linear regression of erythristic *P. cinereus* and *N. viridescens* colour contrasts across geographic localities to test the prediction that mimic conspicuousness covaried with model conspicuousness. In this analysis, we used the average contrasts between salamander and background at each of the 14 localities where erythristic *P. cinereus* and *N. viridescens* co-occurred.

Prediction 3: Relationship between model colour conspicuousness and mimicry

To test the prediction that models are more conspicuous in the presence of mimics, we performed a nested analysis of variance (ANOVA) using model colour conspicuousness as the dependent variable, mimic presence/absence as the main group factor and localities as the subgroup factor.

Prediction 4: Relative conspicuousness of models and mimics

Finally, to test whether *N. viridescens* were more conspicuous than erythristic *P. cinereus* we performed a nested ANOVA using colour conspicuousness as the dependent variable, species as the main group factor and localities as the subgroup factor. In this analysis, we compared salamanders collected from all 32 localities.

Effect of choice of background

To assess the effect of background differences on salamander conspicuousness, we performed two sensitivity analyses. First, we used each measured background spectrum from Lilly Pond as the background that salamander conspicuousness was estimated against and determined how this affected the resulting patterns of salamander conspicuousness for the statistical analyses described above. Second, we retested predictions using leaf litter and salamander backgrounds collected from two additional sites (Poland Brook and Powell Brook). These additional

comparisons enabled us to determine whether the patterns observed in this study were robust to variation in the backgrounds against which the salamanders were evaluated.

Results

Prediction 1: Mimic similarity to local models

We found that erythristic *P. cinereus* were significantly more similar to local models than to models from other localities ($D = 8.83$; $P = 0.029$). Thus, this analysis provided empirical support for the prediction that mimics more closely matched the phenotype of syntopic models.

Prediction 2: Model-mimic colour contrast covariation

We found a significant and positive relationship between erythristic *P. cinereus* and *N. viridescens* colour conspicuousness among the 14 localities where they were syntopic ($R^2 = 0.357$; $F_{1,12} = 8.22$; $P = 0.014$; Fig. 2, inset). This relationship is stronger when the data are culled to include only localities represented by at least four *N. viridescens* and four erythristic *P. cinereus* individuals ($R^2 = 0.624$; $F_{1,5} = 10.97$; $P = 0.021$).

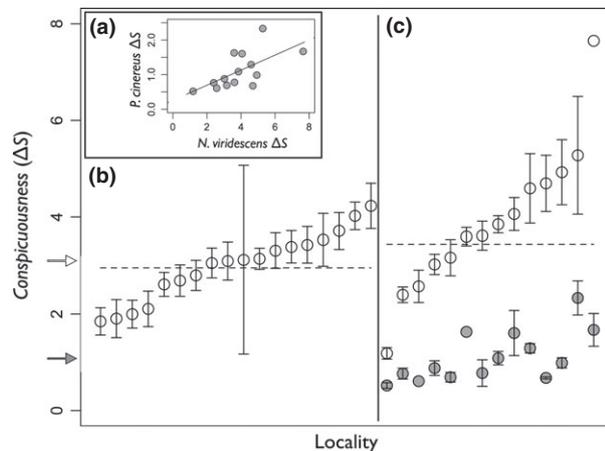


Fig. 2 Colour conspicuousness (ΔS) for *Notophthalmus viridescens* and *Plethodon cinereus* across localities. (a) The inset illustrates the correlation between *N. viridescens* and *P. cinereus* conspicuousness. (b) Estimates of conspicuousness for *N. viridescens* at localities without erythristic *P. cinereus*. (c) Estimates of conspicuousness for *N. viridescens* (white points) and erythristic *P. cinereus* (grey points) at localities where both are found. The horizontal dotted lines illustrate how *N. viridescens* conspicuousness differs between localities with and without erythristic *P. cinereus*. Arrows denote mean conspicuousness scores for *N. viridescens* (white) and *P. cinereus* (grey). Standard error bars are included to show variation in the data.

Prediction 3: Model colour variation and mimic presence

Using nested ANOVA, we found *N. viridescens* was more conspicuous to predators at localities where erythristic *P. cinereus* were present than at localities where erythristic *P. cinereus* were absent (mean *N. viridescens* conspicuousness found with erythristic *P. cinereus* = 3.44; *N. viridescens* conspicuousness without erythristic *P. cinereus* = 2.96; $F_{1,314} = 4.989$; $P = 0.026$; Fig. 2).

Prediction 4: Relative conspicuousness of models and mimics

Using nested ANOVA, we found *N. viridescens* was significantly more conspicuous to predators than erythristic *P. cinereus* (mean *N. viridescens* $\Delta S = 3.13$; mean erythristic *P. cinereus* $\Delta S = 1.11$; $F_{1,437} = 138.383$; $P < 0.001$; Fig. 2).

Effect of choice of background

Finally, the patterns described above remained consistent when evaluated using different background spectra (see Supporting Information). Thus, the results obtained here appeared robust to variation in the backgrounds against which salamanders are viewed.

Discussion

Aposematic species are often conspicuous, even though conspicuousness attracts the attention of potential predators. Higher levels of conspicuousness may evolve in unpalatable species in response to chase-away selection due to the presence of Batesian mimics (e.g. Fisher, 1930; Franks *et al.*, 2009), and we identify empirical evidence supporting this hypothesis in a salamander system. As predicted for mimicry systems, we found that erythristic *P. cinereus* (the Batesian mimic) more closely resembled local *N. viridescens* (the aposematic model species) than *N. viridescens* found at other localities. We also found a significant, positive relationship among localities between the degree of colour conspicuousness of mimics and models from the same localities. Finally, models were more conspicuous in the presence of mimics and were significantly more conspicuous than mimics at all localities. Several implications of these findings are described below.

First, our results demonstrate that erythristic *P. cinereus* were more similar in coloration to syntopic *N. viridescens* than to *N. viridescens* from other localities, providing strong support for the hypothesis that selection for mimicry drives mimics to resemble local models. Although this provides the first evidence of such a relationship in our system, we note that this pattern is not unusual and has been observed in mimicry systems in several taxa including frogs (Darst & Cummings,

2006) and snakes (Greene & McDiarmid, 1981). In these cases, as in our study, the adaptation of mimics to models is highly localized, with localities harbouring different model and mimic phenotypes sometimes separated by only a few kilometres. For example, the tropical coral snake system reviewed by Greene & McDiarmid (1981) contains at least six discrete warning patterns in Central American coral snakes, each locally complemented by a nearly identical nonvenomous snake mimic. These repeated patterns suggest that selection for mimicry can strongly influence the evolution of mimic phenotype.

Second, we found that mimics were not only more similar to local models, but mimic and model conspicuousness covaried among localities, which links the evolution of mimicry to the evolution of conspicuousness in this system. Such a link has long been predicted (Poulton, 1890; Fisher, 1930; Franks *et al.*, 2009; Speed & Ruxton, 2010), though has been little studied in natural mimicry systems. Importantly, selection pressures differ between the mimics and the models, resulting in a coevolutionary arms race in which mimics evolve 'towards' model warning signal design and models evolve 'away' from mimic phenotype (Gavrilets & Hastings, 1998). One prediction of such 'chase-away selection' (Nur, 1970) is that in the presence of mimics, models will evolve phenotypes that distinguish them from local mimics. Predators must learn to avoid models, and models that are easily distinguished from mimics benefit from fewer attacks from predators than models that are more readily confused for palatable prey (Turner & Speed, 1996). One potential outcome of chase-away selection is the evolution of greater conspicuousness in the model species (Franks *et al.*, 2009; Speed & Ruxton, 2010).

Typically, chase-away selection is difficult to measure because of the highly multidimensional nature of warning signal design. In our study, we quantified the outcome of this relationship by examining the univariate measure of colour conspicuousness, which forms an important part of a larger warning signal design that likely includes other dimensions (including colour patterning, organism shape and behaviour). There is the potential for such an evolutionary chase between models and mimics to extend to these phenotypic and behavioural dimensions as well. For example, aposematic species are generally described as bold and slow moving when compared to palatable relatives. Much like other aposematic species, but unlike other salamander species, *N. viridescens* efts can be found slowly traversing the forest floor in broad daylight following precipitation (Petranka, 1998). Conversely, other salamander species are typically secretive, leaving the forest floor leaf litter to forage only during twilight or at night (Petranka, 1998). Such behaviours and other traits may evolve in concert with coloration as part of the 'aposematic syndrome' (*sensu* Santos & Cannatella, 2011) to

ensure aposematic individuals are recognized as unpalatable to potential predators.

Third, our finding that mimics were always less conspicuous than their models is consistent with the hypothesis that there is a 'cost of conspicuousness' for Batesian mimics (Speed & Ruxton, 2010). In most cases, the difference in conspicuousness between models and mimics was greater than one contrast unit (ΔS), which is typically considered to be the limit of discriminability for potential viewers (Vorobyev *et al.*, 1998), indicating that these differences may be apparent to predators. Close resemblance to models may require mimics to become more conspicuous; however, conspicuousness can lead to increased attention from predators (Speed & Ruxton, 2010), resulting in lethal consequences for mimics that do not possess the secondary defences (e.g. toxicity) that models carry. Consequently, selection to resemble models is countered by selection to avoid attention from predators, resulting in mimics that, though resembling their models, are less conspicuous. Although our results are concordant with this hypothesis, research that directly maps the adaptive landscape of model resemblance and conspicuousness in natural populations is needed to empirically test the cost of conspicuousness. Additionally, selection on mimics is influenced by the relative rate of encounters between predators, models and mimics (reviewed in Ruxton *et al.*, 2004). The adaptive landscape of mimicry will, therefore, depend critically on community structure as well as morphological characteristics of the species involved. Alternatively, geographic variation in background or light environment could play a role in influencing the evolution of conspicuousness in this system. However, differences in the selective regimes imposed on models and mimics suggest that background and light environment variation is not sufficient to explain the patterns we observed.

Finally, we found models to be more conspicuous when they were syntopic with mimics than when they were found alone, which suggests that the presence of mimics drives models to appear more conspicuous. Aposematic models are under selection to appear distinct from Batesian mimics when both species are found together, whereas such selection is absent where models are found alone. Because conspicuous warning signals are particularly costly for Batesian mimics, but less costly for the models they resemble, aposematic models may evolve to become more conspicuous when they are mimicked (Franks *et al.*, 2009), which we observed in the mimicry system between *P. cinereus* and *N. viridescens*. Furthermore, we found a positive relationship between mimic : model ratios and the conspicuousness of models among localities (Supporting Information), which suggests that the intensity of selection for model conspicuousness may be driven by the relative rate of encounters between predators and prey. It would be valuable to assess whether these patterns

are general across Batesian mimicry systems and at larger phylogenetic scales (e.g. Are aposematic species that are mimicked generally more conspicuous than aposematic species without Batesian mimics?). If so, Batesian mimicry may influence macroevolutionary patterns in aposematic lineages, perhaps explaining why some aposematic lineages of poison frogs are more conspicuous than other lineages.

Costs of conspicuousness for both models and mimics are further influenced by variation in predator communities; predators with innate avoidance of model phenotype impose little to no cost of conspicuousness for models, whereas predators with learned model avoidance impose some selective cost of conspicuousness for models (Ruxton *et al.*, 2004). Variation in the predator community among sites (the spatial mosaic of coevolution; e.g. Thompson & Cunningham, 2002) may also lead to some of the variation in conspicuousness we observe. Spatial variation in model toxicity also has the potential to influence the maintenance of mimicry (Lindström *et al.*, 1997; Speed & Ruxton, 2010), but is unlikely to play a role at the small scale investigated here (A. C. Kraemer, J. M. Serb & A. C. Adams, submitted). Interestingly, we found a strong negative relationship between model abundance and mimic conspicuousness (Supporting Information). The implications of this observation are not clear, as no scenarios of Batesian mimicry predict this relationship. However, predators may impose limitations on model abundance when mimics are more often encountered and consequently more likely to interfere with the honesty of model warning signals (e.g. when mimics are more abundant or more conspicuous). A more nuanced understanding of predator distribution and behaviour is needed to test the hypothesis that spatial heterogeneity in selective pressures influences the distribution of mimicry we observe in this system. Such links between community ecology, the evolution of mimicry and macroevolutionary patterns of mimicry may lead to insight into the evolutionary dynamics that direct the maintenance of warning signals.

Conspicuousness in aposematic species may evolve for several reasons. Most commonly, conspicuousness is thought to evolve to improve predator recognition and/or memory of unpalatable species (e.g. Guilford, 1986; Roper & Redston, 1987). The relationship between defended species and other potential prey is also considered as influencing the evolution of conspicuousness (e.g. Poulton, 1890; Sherratt & Franks, 2005; Franks *et al.*, 2009). Here, we find evidence supporting the hypothesis that Batesian mimicry can influence the evolution of conspicuousness in an aposematic model species. These findings show that the coevolution of model and mimic warning signal design influences not only the degree to which models and mimics resemble one another, but also how they appear in their natural environment. Thus, changes in

phenotypes that impact how predators recognize models and mimics also influence how visible these phenotypes appear to predators.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Geographic positions of sample localities in western Massachusetts.

Figure S2 (a) Analyses for Prediction 2 using background spectra from additional sites. (b) Influence of individual background spectra on Prediction 2.

Figure S3 (a) Relationship between mimic : model ratio and model conspicuousness. (b) Relationship between the observed number of *N. viridescens* and the conspicuousness of erythristic *P. cinereus* among localities.

Table S1 Localities where salamanders were located and their respective sample sizes.

Table S2 (a) Analyses for Predictions 3 and 4 using background spectra from additional sites. (b) Influence of individual background spectra on Predictions 3 and 4.

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