Ecology, Evolution and Organismal Biology
Publications

Ecology, Evolution and Organismal Biology

4-2014

Predator Perception of Batesian Mimicry and Conspicuousness in a Salamander

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Mimicry from the Predator's Perspective

Aposematism, *Notophthalmus*, *Plethodon*, polymorphism, visual model

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Word count: 4349

Table count: 2

Figure count: 2

Destination of archived data: Dryad

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Abstract – In Batesian mimicry a palatable mimic deceives predators by resembling an unpalatable model. The evolution of Batesian mimicry relies on the visual capabilities of the potential predators, as prey detection provides the selective force driving evolutionary change. We compared the visual capabilities of several potential predators to test predictions stemming from the hypothesis of Batesian mimicry between two salamanders: the model species *Notophthalmus viridescens*, and polymorphic mimic, *Plethodon cinereus*. First, we found mimicry to be restricted to coloration, but not brightness. Second, only bird predators appeared able to discriminate between the colors of models and non-mimic *P. cinereus*. Third, estimates of salamander conspicuousness were background-dependent, corresponding to predictions only for backgrounds against which salamanders are most active. These results support the hypothesis that birds influence the evolution of Batesian mimicry in *P. cinereus*, as they are the only group examined capable of differentiating *N. viridescens* and non-mimetic *P. cinereus*. Additionally, patterns of conspicuousness suggest that selection from predators may drive the evolution of conspicuousness in this system. This study confirms the expectation that the visual abilities of predators may influence the evolution of Batesian mimicry, but the role of conspicuousness may be more complex than previously thought.

Batesian mimicry, in which a palatable species resembles an unpalatable model species in its phenotype or behavior, has long been recognized as an example of adaptation and convergent evolution (Ruxton et al. 2004). This form of mimicry first requires that the unpalatable model provide a warning signal detectable by potential predators that indicates significant costs to consuming it as prey. Frequently, this warning signal is visual in nature (e.g., conspicuous coloration: Rowe and Guilford 2000). Second, the mimic must copy the model's signal to deceive potential predators. Importantly, both the warning signal and subsequent mimicry depend upon the visual capabilities of potential predators, as predators are the selective agents in this system.

Observations of Batesian mimicry have stimulated considerable interest among ecologists and evolutionary biologists (e.g., Bates 1862; Edmunds 1969; Greene and McDiarmid 1981), and theoretical and empirical work on both predators and their prey have led to many predictions of interspecific interactions in Batesian mimicry systems (e.g., reviewed in Mallet and Joron 1999; Speed 1999; Ruxton et al. 2004; Pfennig and Mullen 2010; Stoddard 2012). In particular, three predictions of warning signal design are expected in most systems of Batesian mimicry. First, mimics are predicted to generally match model species in phenotype (Ruxton et al. 2004). In particular, because predators are driving the evolution of mimicry, Batesian mimics are predicted to resemble their models in ways that potential predators can perceive (e.g. Kikuchi and Pfennig 2010). Second, unpalatable models are often predicted to be conspicuous and therefore aposematic (Roper and Redston 1987; Ruxton et al. 2004; Mappes et al. 2005; but see Wüster et al. 2004). When this is the case, mimics are predicted to evolve

conspicuousness as well (reviewed in Ruxton et al. 2004 and discussion in Franks et al. 2009). Finally, since conspicuousness is perceived as the degree of contrast between a target and its background (Ruxton et al. 2004), different backgrounds may influence prey detectability (e.g. Siddiqi et al 2004, Macedonia et al. 2009, Willink et al. 2013). Therefore, the patterns of conspicuousness predicted between models and mimics may be background-specific. The evolution of Batesian mimicry has been extensively studied in numerous taxa (e.g. Tilley et al. 1982; Pfennig et al 2001; Kunte 2009), and some of these studies have examined the visual capabilities of potential predators to test predictions of mimicry (e.g. Cheney and Marshall 2009; Papadopulos et al. 2013). However, to date the role of conspicuousness in the evolution of Batesian mimicry remains under-examined, particularly from the perspective of potential predators.

The salamanders *Notophthalmus viridescens* and *Plethodon cinereus* present an appealing group with which to test predictions of Batesian mimicry in the wild. *Notophthalmus viridescens,* a widespread salamander in Eastern North America, has a triphasic life cycle, where aquatic larvae often transform into a terrestrial juvenile (eft) stage, which then matures into an aquatic newt (Petranka 1998). Efts of *N. viridescens* are strikingly red-orange in coloration (Fig. 1C), and contain tetrototoxin (TTX) in their skin, which is noxious to most potential predators (Brodie 1968; adult newts can also contain TTX: Yotsu-Yamashita and Mebs 2003). Terrestrial *N. viridescens* efts are frequently avoided in feeding experiments using vertebrate predators (e.g. Brodie 1968; Hurlbert 1970; Tilley et al. 1982), which is presumably due to the unpalatability conferred by tetrodotoxin and their distinctive coloration as a signal of unpalatability (Hurlbert 1970).

Thus, red-orange coloration in *N. viridescens* is likely a conspicuous warning signal to predators, thereby making *N. viridescens* an aposematic species with bright warning coloration and noxious defensive chemicals.

By contrast, *Plethodon cinereus,* whose geographic distribution overlaps broadly with that of *N. viridescens*, is not aposematic. This species displays three color-morphs; the two most common are the striped morph (Fig. 1A) and the unstriped morph, which are typically not considered conspicuous (Petranka 1998). However, in some localities a third red-orange morph (erythristic: Fig. 1B) is found that appears similar in coloration to *N. viridescens.* Unlike *N. viridescens*, *P. cinereus* is not noxious and does not contain tetrodotoxin (Brodie and Brodie 1980; Tilley et al. 1982). However, because the two species can be found sympatrically, and because of their similar coloration, the erythristic morph of *P. cinereus* is thought to be a Batesian mimic of *N. viridescens* (Lotter and Scott 1977). Lending support to this hypothesis, two empirical field studies have found that wild and captive bird predators avoid *N. viridescens* and erythristic *P. cinereus* similarly, while they readily consume striped *P. cinereus* (Brodie and Brodie 1980, Tilley et al. 1982). Thus, behavioral studies (e.g. Brodie and Brodie 1980, Tilley et al. 1982) and toxicity assays (e.g. Brodie 1968) provide evidence consistent with the hypothesis of Batesian mimicry in this system, and suggest that bird predators may select salamander prey based on their coloration and toxicity.

In this study we tested three predictions of the hypothesis of Batesian mimicry between *N. viridescens* and *P. cinereus.* First, we evaluated whether erythristic *P. cinereus* and the

eft stage of *N. viridescens* are similar in color and brightness, particularly from the perspective of potential predators. Second, we tested whether *N. viridescens,* and subsequently *P. cinereus*, are conspicuous to potential predators. Third, we determined whether different natural backgrounds could affect estimates of conspicuousness for each predator. In particular, we identified if such variation could facilitate identification of the predators driving selection for color similarity and under which conditions they do so. Although birds are hypothesized to generate selection that may drive the evolution of Batesian mimicry in this system (Brodie and Brodie 1980; Tilley et al. 1982), several vertebrate predators are also implicated as natural salamander predators (including UV-sensitive birds: Lotter and Scott 1977, Fenster and Fenster 1996; snakes: Arnold 1982; and mammals: Brodie et al. 1979). We therefore used visual models generated for multiple potential predator classes and examined conspicuousness and similarity between *N. viridescens* and *P. cinereus* with respect to the visual capabilities of three predator groups relative to three potential backgrounds. This analytical framework allows us to determine not only if erythristic *P. cinereus* appear as mimics of *N. viridescens* to potential vertebrate predators on the basis of color or brightness, but also whether salamander conspicuousness is dependent on the background against which it is viewed.

Materials and Methods

Salamander Collection and Coloration Quantification – In summer 2011 we obtained salamanders from 14 sites in western Massachusetts where both species are widely distributed. In this region the erythristic morph of *P. cinereus* is uncommon but is sympatric with *N. viridescens* more often than expected by chance (*P* = 0.0342;

supplementary material). We collected a total of 521 adult *P. cinereus* and eft-stage *N. viridescens* for field measurements of coloration (N = 123 erythristic *P. cinereus*, 281 striped *P. cinereus*, and 117 *N. viridescens;* unstriped *P. cinereus* were not present at these sites). All individuals were first anesthetized using tricaine methanesulfonate (MS-222: *P. cinereus*) or by applying benzocaine to the ventral surface of the head (*N. viridescens*). There is a possibility that differences in anesthetization could result in uneven changes in coloration between salamander groups. However, such physiologically-based color changes have only been observed in an unrelated salamander lineage (Garcia et al. 2003), and we did not observe any physiological color-change in our salamanders. We then measured spectral reflectance from a single point in the mid-dorsal region of each salamander using a portable JAZ-PX spectrometer (OceanOptics, Dunedin, FL) with a 100 μm entrance slit, a pulsed xenon lamp, and a QR400-7-UV-BX reflectance probe. This probe was fitted with a tip that restricted the measured patch to a 2 mm diameter circle, maintained a constant distance (20 mm) between probe tip and measured patch, and excluded ambient light. We held the probe perpendicular to the surface of the salamanders and used a Spectralon white reflectance standard between each animal to correct for drift in lamp intensity (see e.g., Kraemer et al. 2012). Measuring reflectance at a perpendicular angle can introduce specular glare, particularly when coloration is influenced by structural pigmentation in the target animal (Endler 1990). However, the dorsal coloration of these salamanders is pigment-based, with limited structural elements (Bagnara and Taylor 1970), which in this case, minimized the potential for specular glare. We measured each spectrum at 1nm intervals from 300 to 700 nm. We acknowledge that color variation among sites may be present. However,

insufficient sample sizes within sites precluded a formal analysis of the multivariate color variation among geographic sites and within morphs (supplementary material). Therefore, only comparisons among morphs were performed. From one site where salamanders were collected (Lilly Pond Wildlife Management Area, Goshen, Massachusetts) we obtained 10 reflectance spectra each of naturally wet and dry leaf litter on 9 June 2012. We also collected 20 spectra of bare soil from a surrogate locality (Pammel Woods, Ames, Iowa; 11 July 2013) to represent backgrounds that predators might view salamanders against (Fig. 1B). We acknowledge that sampling of background spectra is somewhat limited. However, all sites consisted of a similar compliment of hemlock-northern hardwood forest, and thus we assumed that the backgrounds against which salamanders were viewed did not vary among sites.

Multivariate brightness and color quantification – To obtain a set of color and brightness variables for statistical comparison (*sensu* Endler and Thery 1996; Grill and Rush 2000) we performed principal components analysis on the raw reflectance spectra. In this representation, PC1 is interpreted as brightness, or the total amount of light reflected from the measured patch, while the remaining PC-axes describe aspects of chroma and hue (henceforth coloration: see Endler 1990; Endler and Thery 1996; Grill and Rush 2000). In our case, PC1 was highly correlated with an independent measure of overall brightness (Q_t; *sensu* Endler 1990; $r > 0.99$). The remaining principal component axes (PC_2 - PC_{401}) describe the shape of the spectral curve and thus the color that each spectrum represented (Grill and Rush 2000). For our analyses we used Q_t, or the total

reflectance under each spectrum, to evaluate brightness and PC_2 - PC_{401} to evaluate coloration.

Visual model – We estimated the ability of potential predators to discriminate between various prey items and typical background surfaces with a visual model developed by Vorobyev et al (2001). This analytical model is derived from the signal to noise ratio of predator photoreceptors, and assumes that the ability of predators to discriminate between objects depends on this ratio (Vorobyev et al. 1998). The model yields estimates of visual contrast between a target and a background (as in Maan and Cummings 2012) or as a contrast between two targets (as in Siddiqi et al. 2004). These contrasts are calculated for each of two visual channels, chromatic (ΔS) and achromatic (ΔL), in which the chromatic visual channel summarizes the aspects of visual stimuli pertaining to coloration (i.e. chroma and hue), while the achromatic visual channel summarizes the aspects of visual stimuli pertaining to brightness. In each case, large contrast values correspond to clear and discriminable differences between targets and backgrounds (or between targets) from the perspective of the predator, while smaller values represent similar coloration between targets and backgrounds (or between targets) and are thus potentially indistinguishable. Contrast values of 1 or smaller are thought to describe indistinguishable pairs of spectra because photoreceptor processing of visual stimuli will result in more noise than original signal, while values greater than 1 correspond to more signal than noise (Vorobyev et al. 1998).

As input data, the visual model requires reflectance measures of the target and background, background habitat irradiance, and photoreceptor sensitivities of the predator. We used spectral data obtained for each salamander as described above. Average wet leaf litter, dry leaf litter, and bare soil spectra served as backgrounds. We used two irradiance measures: the standard D65 daylight irradiance (Judd et al. 1964) and a forest shade irradiance measure reported elsewhere (Maan and Cummings 2009). We report only the contrasts calculated with the forest irradiance because the relationships among contrasts did not differ as a result of irradiance measurement.

Three classes of vertebrates have been implicated as potential predators of North American terrestrial salamanders, and differ substantially with respect to their visual capabilities. While we were unable to estimate the spectral sensitivities of specific salamander predator species for this system, we were able to use known spectral sensitivities of related vertebrates to compare the visual capabilities for each predator class: birds (salamander predators: tetrachromatic blue jays and brown thrashers, Howard and Brodie 1973, Chen and Goldsmith 1986 – visual model: the tetrachromatic blue tit, Hart et al. 2000), snakes (salamander predators: garter snakes, Arnold 1982 – visual model: eastern garter snake, Sillman 1997), and mammals (salamander predators: shrews and other small, opportunistic mammals, Brodie et al. 1979, Petranka 1998 – visual model: thirteen-lined ground squirrel, Jacobs et al. 1985). We used a rhodopsin template (based on vitamin A1; Govardovskii et al. 2000) to estimate the spectral sensitivities for the garter snake (*Thamnophis sirtalis*), with photoreceptor peak sensitivities of: long-wave sensitive (LWS) λ_{max} = 554, short-wave sensitive (SWS) λ_{max} = 482, and UV-wave

sensitive (UWS) $\lambda_{max} = 360$ (Sillman et al. 1997). We used the same rhodopsin template to estimate spectral sensitivities for the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*), which is an opportunistic, diurnal forager with LWS $\lambda_{max} = 517$ and SWS $\lambda_{max} = 437$ (Jacobs et al. 1985). A full description of the visual model calculations and corresponding code are found in the supplementary material. All analyses were conducted in R 2.15 (R Development Core Team 2012).

Statistical Analyses

Prediction 1a: Model-mimic color and brightness similarity – To evaluate the similarity of warning signals predicted for Batesian mimicry, we compared predator-independent estimates of color (PC-color data) and brightness (Q_t values as estimated by integrating across each spectrum from 300-700 nm) across salamander groups (eft *N. viridescens,* striped *P. cinereus,* erythristic *P. cinereus*) and background groups (wet leaf litter, dry leaf litter, soil) using Permutational-MANOVA (for color data; Anderson 2001) or analysis of variance (ANOVA; for brightness data). Permutational-MANOVA was used because of the high-dimensionality of the color space. We then evaluated pairwise differences between groups using randomization. Briefly, the observed pairwise differences between groups (measured as Euclidean distances) were first obtained from the least-squares (LS) means for each group. Specimens were then permuted with respect to group identity and the model was recalculated (permutations = 999), and pairwise distances between LS means were obtained. The proportion of randomly generated distances greater than the observed was then treated as the significance of each pairwise comparison (for statistical details see Anderson and Ter Braak 2003; Adams and Collyer

2009; Adams 2010; Adams and Nistri 2010). A sequential Bonferroni correction was used to account for multiple testing during pairwise comparisons.

Prediction 1b: Salamander discriminability –For each predator visual model, we used a one-sample t-test to evaluate color and brightness contrasts obtained for *P. cinereus* relative to *N. viridescens*. Contrast scores significantly greater than 1.0 suggest that the predator can differentiate between these two salamanders. This analysis was repeated for brightness and color components of each *P. cinereus* morph and for each predator model. A sequential Bonferroni correction was used to account for multiple testing during t-tests.

Predictions 2 and 3: Salamander conspicuousness – We also compared salamander color and brightness to various backgrounds using a one-sample t-test. Contrast scores significantly greater than 1.0 suggest that salamanders are conspicuous and therefore visible to the predator against that background. This analysis was repeated for each salamander group against each background and for each predator model. Additionally, we compared the conspicuousness of *P. cinereus* color-morphs using two-sample t-tests. A sequential Bonferroni correction was used to account for multiple testing during t-tests. Statistical analyses evaluating all predictions were performed in R 2.15 (R Development Core Team, 2012).

Results

Prediction 1a: Model-mimic color similarity

We found significant differences in brightness among salamander and background groups ($F = 90.3$, $P < 0.001$). Pairwise comparisons revealed that erythristic *P. cinereus* were brighter than all other groups, while *N. viridescens* was similar in brightness to all backgrounds examined (Table 1A: Fig. 1E). Additionally, striped *P. cinereus* were not different in brightness from dry leaf litter. Using Permutational-MANOVA we also found large and significant differences in overall coloration among salamander and background groups ($F = 2.127$, $P < 0.001$). Pairwise comparisons revealed that the coloration of both *N. viridescens* and erythristic *P. cinereus* differed significantly from striped *P. cinereus* and soil. Additionally, dry leaf litter and soil coloration were significantly different (Table 1B: Fig. 1F). All other pairwise comparisons between groups were non-significant.

Prediction 1b: Salamander discriminability

Based on the visual models, both *P. cinereus* morphs were significantly different from *N. viridescens* in brightness from the perspective of all three predators (results not shown). However, the visual models indicated that neither mammals nor snakes could differentiate between the coloration of *N. viridescens* and either *P. cinereus* morph. Importantly, while birds could differentiate between *N. viridescens* and striped *P. cinereus* coloration, they were unable to differentiate between *N. viridescens* and erythristic *P. cinereus* (Fig. 2A).

Predictions 2 and 3: Salamander conspicuousness

Using the visual models for potential predators we found that all salamander groups were conspicuous against all backgrounds with respect to brightness (results not shown). By contrast, when viewed against dry leaf litter, all salamander groups were conspicuous in their coloration from the perspective of birds, while only striped *P. cinereus* were conspicuous in coloration for snakes. No salamander groups were conspicuous in coloration from the perspective of mammals (Fig. 2B). Against both soil and wet leaf litter, *N. viridescens* coloration was conspicuous to all three predators (Fig. 2C,D). Against soil both striped and erythristic *P. cinereus* coloration were conspicuous to birds (Fig. 2C). Erythristic *P. cinereus* brightness was significantly more conspicuous than striped *P. cinereus* against wet leaf litter and soil for all predators (Table 2A,B). Against dry leaf litter, erythristic *P. cinereus* brightness was significantly more conspicuous than striped *P. cinereus* for birds (Table 2C). Finally, for mammals against dry leaf litter and soil and for snakes against dry leaf litter, striped *P. cinereus* coloration was more conspicuous than erythristic *P. cinereus* coloration (Table 2B,C).

Discussion

How predators perceive and classify Batesian mimics and their models is of central importance to understanding mimicry. In this study we used a visual model to test three predictions of Batesian mimicry from the perspective of predators. First, we found models (*N. viridescens*) and mimics (erythristic *P. cinereus*) to be similar in coloration while dissimilar in brightness. Second, models and mimics were indistinguishable in coloration from the perspective of all three vertebrate predators, and only birds appeared

able to discriminate between models and non-mimic (striped) *P. cinereus* on the basis of coloration. Third, *N. viridescens* coloration was more conspicuous than either *P. cinereus* morph when viewed against both wet leaf litter and soil for all predators, but not when viewed against dry leaf litter. Finally, erythristic *P. cinereus* were more conspicuous than striped *P. cinereus* under most conditions for brightness, but not for coloration.

Model – Mimic Color Similarity

The finding that the coloration of *N. viridescens* and erythristic *P. cinereus* did not differ statistically is not surprising, as similarity of color between the two is what initially prompted biologists to suggest erythristic *P. cinereus* were Batesian mimics of *N. viridescens* (Lotter and Scott 1977). Conversely, significant differences in brightness between *N. viridescens* and erythristic *P. cinereus* were unforeseen and suggest that mimicry in this system is restricted to coloration and not brightness, as these species are most similar in coloration but not brightness. Interestingly, *P. cinereus* are not the only mimics of *N. viridescens*. *N. viridescens* may also serve as the model for three additional salamander species (*Pseudotriton ruber*: Howard and Brodie 1971; *Gyrinophilus porphyriticus*: Howard and Brodie 1973*; Pseudotriton montanus*: Huheey and Brandon 1974) and a leech (*Macrobdella diploteria*: McCallum et al. 2008). Further research that disentangles the elements of warning signal design (i.e. color, brightness, and pattern) in these species may shed light on how mimicry evolves in this system and why some aspects of warning signals are mimicked while others are not.

Salamander Discriminability

Our results show that when viewed from the perspective of predators, models and mimics were quite similar in coloration, though dissimilar in brightness, indicating that vertebrate predators likely classify erythristic *P. cinereus* as mimics of *N. viridescens* on the basis of coloration and not brightness. Additionally, we found that snakes and mammals appear incapable of differentiating between the coloration of *N. viridescens* and non-mimic *P. cinereus*. This suggests that while these predators prey on salamanders (Brodie et al. 1979; Arnold 1982), they do not view erythristic *P. cinereus* as mimics of *N. viridescens*. Conversely, bird predators are able to differentiate between the coloration of *N. viridescens* and striped, non-mimic *P. cinereus*, but are not capable of distinguishing between *N. viridescens* and erythristic *P. cinereus.* These observations imply that birds view erythristic *P. cinereus* as being similar in coloration to *N. viridescens*, and thus, the model to mimic relationship is preserved when considering bird predators. We note however that our results rely on the manner in which the light environment (i.e. irradiance) is estimated, and as such are somewhat preliminary in scope because we used a single irradiance measure in our analyses. Indeed, variation in the light environment by site, time of day, and season can alter salamander discriminability by predators (for a review of the effect of variation in light environment on animal vision, see Endler 1993), but the influence of such variation is currently unknown for mimicry systems. Nevertheless, our preliminary findings lend support to the hypothesis that birds are the dominant selective force driving the evolution of mimicry in *P. cinereus* (Brodie and Brodie 1980; Tilley et al. 1982). Visually hunting and diurnal litter-searching birds, such as thrushes and robins, are known to search for and consume salamanders (e.g. Coker

1931; Wilson and Simon 1985). By searching through litter during the day, birds are likely to expose individuals of both salamander species on a regular basis. The ability of birds to differentiate between *N. viridescens* and striped *P. cinereus,* but not between *N. viridescens* and erythristic *P. cinereus,* may thus provide the erythristic morph with a selective advantage over striped individuals, thereby helping to maintain erythristic individuals in *P. cinereus* populations.

Salamander Conspicuousness

Since *N. viridescens* is unpalatable to predators (Brodie 1968), its red-orange coloration may serve as a conspicuous warning signal to predators. Conspicuousness is commonly assumed for defended model species of Batesian mimics (Ruxton et al. 2004; but see Wüster et al. 2004). Consistent with this prediction, we found that *N. viridescens* were conspicuous for all three vertebrate predator types examined. However, they were most prominent when viewed against wet leaf litter and soil, while against dry leaf litter, *N. viridescens* coloration was no more conspicuous than that of *P. cinereus*. This result may appear puzzling, until one considers the activity periods of terrestrial salamanders. Salamanders like *N. viridescens* and *P. cinereus* are most active, and thus likely to encounter predators, when the environment is cool and wet (Petranka 1998). Consequently, selection should drive *N. viridescens* to appear most conspicuous against wet, and not dry, leaf litter. Our data are consistent with this prediction, as *N. viridescens* were most conspicuous against wet leaf litter and soil. We note that this hypothesis could be tested by identifying the conditions in which *N. viridescens* and *P. cinereus* experience the strongest selection pressure from predators, for example, through studies that expose

clay salamander replicas to predators under various environmental conditions (e.g. *sensu* Brodie 1993; Pfennig et al. 2001; Kuchta 2005).

In contrast to *N. viridescens*, *P. cinereus* coloration was generally not conspicuous to predators. This result is surprising, as it implies that erythristic *P. cinereus* coloration is less conspicuous than *N. viridescens* coloration. This result is also potentially important, as it is commonly assumed that the evolution of Batesian mimicry necessitates mimics to overcome the 'cost of conspicuousness,' in which mimicking a conspicuous species requires mimics to adopt a conspicuous phenotype, which should increase their exposure to predators (Speed and Ruxton 2010). Our results suggest that *N. viridescens* may be more visible to predators than erythristic *P. cinereus*. The ability to mimic defended species while simultaneously appearing less conspicuous to predators may make Batesian mimicry more likely to evolve than conditions in which the mimic is as conspicuous as the defended model (Sherratt and Franks 2005). The ability to mimic *N. viridescens* while remaining relatively inconspicuous may have contributed to the evolution of Batesian mimicry in this species. Interestingly, erythristic *P. cinereus* were brighter than striped *P. cinereus* (Table 1; Fig. 1). Color and brightness are tightly linked (Duellman and Trueb 1986), perhaps constraining the evolution of brightness in this species. Consequently, there may be conspicuous costs for erythristic *P. cinereus* apart from coloration.

Conclusions

Batesian mimicry requires mimics to resemble unpalatable models, particularly from the perspective of natural predators, and this resemblance may require mimics to appear conspicuous to predators. Here we provide an explicit test of three predictions of mimicry in a system consisting of two salamander species. We found mimics and models to be similar in overall coloration and from the perspective of potential predators. Additionally, unpalatable models were found to be conspicuous to predators, while mimics may appear less conspicuous, particularly under the conditions that they are more likely to encounter predators, which supports the hypothesis that predators can drive the evolution of conspicuousness in prey species. These findings also indicate that while conspicuousness may be an important factor for the evolution of mimicry, other factors likely influence how predators perceive conspicuousness. By examining the impact of conspicuousness in natural systems we will better understand why mimicry evolves in some species but not others.

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Figure 1

Panels A-C: representative salamanders and their spectra, with striped *P. cinereus* (A; *),

erythristic *P. cinereus* (B; §), and *N. viridescens* (C; †). Shaded regions in each plot

correspond to the standard deviation of spectra for each group. Panel D: background

spectra used in the analyses. Panel E: mean spectra for each salamander and background

group, with mean brightness for each group to the right of each spectrum. Panel F:

Multivariate coloration of each salamander and background group. Least squares means

and standard error bars for each group are plotted.
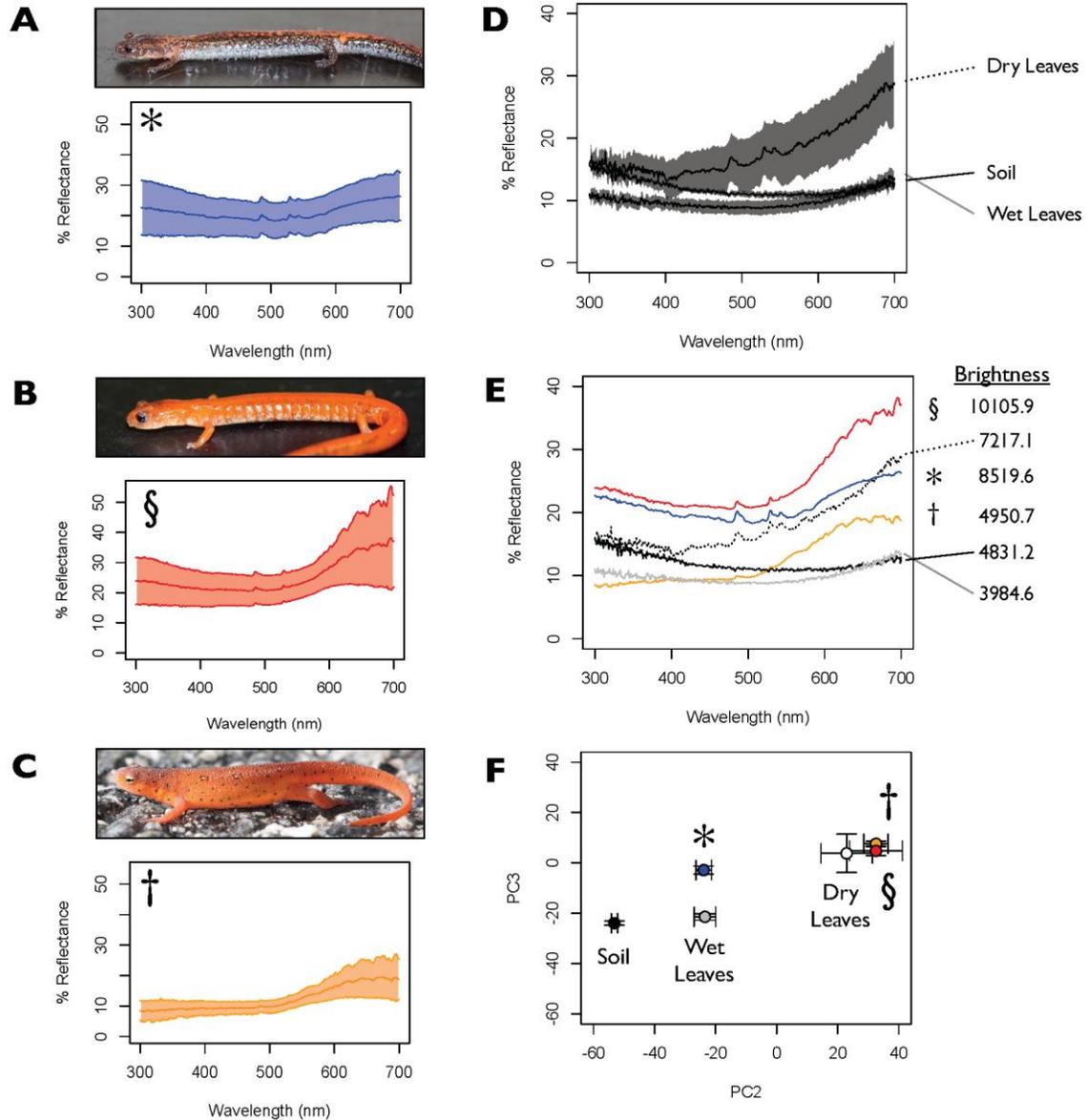
Figure 2

Salamander color contrasts as predicted for each group from predator visual models. Contrasts were estimated against A) *N. viridescens*, B) dry leaf litter, C) soil, and D) wet leaf litter. Contrasts predicted to be distinguishable lie above the horizontal dotted line and are denoted with an asterisk. White bars correspond to *N. viridescens*, light gray bars to erythristic *P. cinereus*, and dark gray bars to striped *P. cinereus*.
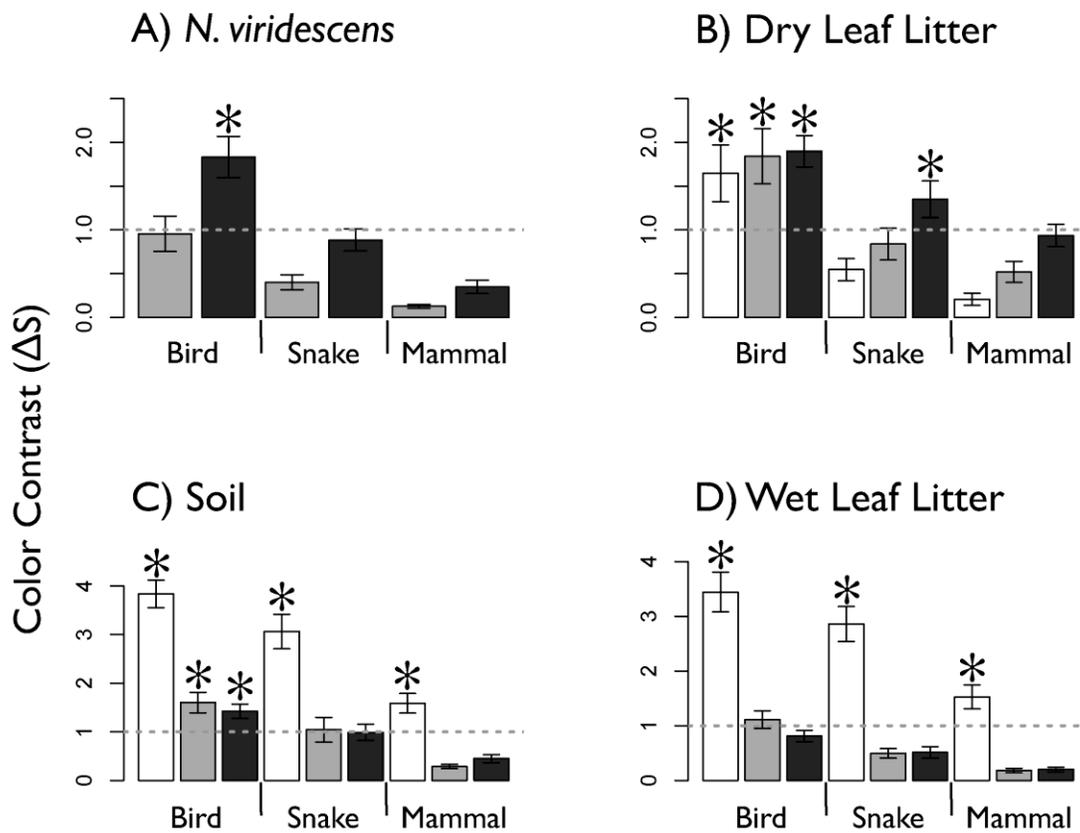
Table 1 – Differences between salamander and background groups in (A) brightness and (B) color. Values below the diagonal are the Euclidean distances between least squares means, while values above the diagonal are the pairwise P-values from randomization. 'Erythristic' and 'Striped' denote respective color morphs of *P. cinereus*, and '*N.v.*' denotes *N. viridescens.* Bold values indicate significance.

A	*N.v.*	Erythristic	Striped	Wet Leaves	Dry Leaves	Soil
N.v	-	**0.001**	**0.001**	0.294	0.021	0.860
Erythristic	**5155.24**	-	**0.001**	**0.001**	**0.003**	**0.001**
Striped	**3568.83**	**1586.41**	-	**0.001**	0.038	**0.001**
Wet Leaves	966.11	**6121.35**	**4534.94**	-	0.013	0.459
Dry Leaves	2266.34	**2888.90**	1302.49	3232.45	-	0.038
Soil	119.59	**5274.83**	**3688.42**	846.52	2385.93	-

B	*N.v.*	Erythristic	Striped	Wet Leaves	Dry Leaves	Soil
N.v.	-	0.477	**0.001**	0.011	0.218	**0.001**
Erythristic	6.92	-	**0.001**	0.012	0.276	**0.001**
Striped	**57.90**	**56.99**	-	0.300	0.021	0.018
Wet Leaves	**64.67**	**62.66**	21.29	-	0.061	0.231
Dry Leaves	26.13	23.84	**51.44**	56.52	-	**0.003**
Soil	**92.03**	**90.83**	**37.59**	30.84	**84.11**	-

Table 2 – Color and brightness differences in conspicuousness between erythristic and striped *P. cinereus* against (A) wet leaf litter, (B) dry leaf litter, and (C) soil. Bold values indicate significant differences.

A		*t*	df	*P*-value
Bird viewer	Color	3.05	225.4	0.003
	Brightness	**10.45**	**329.4**	**<0.001**
Snake viewer	Color	-0.27	382.4	0.786
	Brightness	**6.74**	**328.5**	**<0.001**
Mammal viewer	Color	-0.69	334.2	0.493
	Brightness	**5.40**	**329.1**	**<0.001**

B		*t*	df	*P*-value
Bird viewer	Color	-0.31	204.2	0.755
	Brightness	**5.55**	**205.4**	**<0.001**
Snake viewer	**Color**	**-3.61**	**375.2**	**<0.001**
	Brightness	2.42	259.5	0.016
Mammal viewer	**Color**	**-4.69**	**352.6**	**<0.001**
	Brightness	1.10	228.5	0.271

C		*t*	df	*P*-value
Bird viewer	Color	1.37	246.4	0.172
	Brightness	**10.47**	**347.1**	**<0.001**
Snake viewer	Color	0.34	230.7	0.731
	Brightness	**7.38**	**341.4**	**<0.001**

Mammal viewer	Color	-3.45	387.1	<0.001
	Brightness	5.12	328.9	<0.001