

Once a Batesian mimic, not always a Batesian mimic: mimic reverts back to ancestral phenotype when the model is absent

Kathleen L. Prudic^{1,2,*} and Jeffrey C. Oliver³

¹Department of Ecology and Evolutionary Biology, ²BIO5: Institute for Collaborative Bioresearch, and ³Interdisciplinary Program in Insect Science, University of Arizona, Tucson, AZ 85721, USA

Batesian mimics gain protection from predation through the evolution of physical similarities to a model species that possesses anti-predator defences. This protection should not be effective in the absence of the model since the predator does not identify the mimic as potentially dangerous and both the model and the mimic are highly conspicuous. Thus, Batesian mimics should probably encounter strong predation pressure outside the geographical range of the model species. There are several documented examples of Batesian mimics occurring in locations without their models, but the evolutionary responses remain largely unidentified. A mimetic species has four alternative evolutionary responses to the loss of model presence. If predation is weak, it could maintain its mimetic signal. If predation is intense, it is widely presumed the mimic will go extinct. However, the mimic could also evolve a new colour pattern to mimic another model species or it could revert back to its ancestral, less conspicuous phenotype. We used molecular phylogenetic approaches to reconstruct and test the evolution of mimicry in the North American admiral butterflies (*Limenitis*: Nymphalidae). We confirmed that the more cryptic white-banded form is the ancestral phenotype of North American admiral butterflies. However, one species, *Limenitis arthemis*, evolved the black pipevine swallowtail mimetic form but later reverted to the white-banded more cryptic ancestral form. This character reversion is strongly correlated with the geographical absence of the model species and its host plant, but not the host plant distribution of *L. arthemis*. Our results support the prediction that a Batesian mimic does not persist in locations without its model, but it does not go extinct either. The mimic can revert back to its ancestral, less conspicuous form and persist.

Keywords: character evolution; Lepidoptera; parametric bootstrap; wing pattern evolution

1. INTRODUCTION

Many organisms have evolved defensive colour patterns to mitigate the persistent risk of predation. Aposematic, or warning, coloration is one such defensive strategy used by noxious organisms to visually communicate their toxicity or distastefulness to potential predators (Cott 1940; Guilford 1990). An aposematic pattern confers survival benefits to the prey because it is both easier for the predator to learn and less likely to be forgotten (Gittleman & Harvey 1980; Roper & Redstone 1987; Roper 1990; Alatalo & Mappes 1996; Prudic *et al.* 2007). These benefits are believed to have facilitated the evolution of aposematic coloration from ancestrally cryptic patterns (Fisher 1930; Sherratt & Beatty 2003). Closely related to aposematic coloration is visual defensive mimicry that is often a deceptive signal, defined as a close physical resemblance among unrelated species (Bates 1862). Batesian mimicry is a specialized signal with complex interactions between the model, the mimic and the predators (Ruxton *et al.* 2004 and references therein). In this mimicry system, an unprotected species (mimic) evolves a physical resemblance

to an aposematic species (model), and the mimic gains protection from predation by deceiving the predators. This relationship is often considered parasitic because the unprotected species undermines the effectiveness of the aposematic signal.

Much theoretical and empirical research has focused on the conditions necessary for Batesian mimicry to arise and how it functions once it exists, but much less is known about how these relationships change through time, especially when the model is no longer present (Ruxton *et al.* 2004; Mappes *et al.* 2005). It is presumed that the protection afforded to the mimic should break down in locations where the model is absent because the predators do not recognize either the model or mimic as unprofitable prey and the mimic has a highly conspicuous phenotype (Pfennig *et al.* 2001, 2007; Ruxton *et al.* 2004). The geographical range of the mimic is predicted to be limited by the geographical range of the model even though suitable habitat for the mimic may exist outside that range. Yet, there are examples where a mimic has been documented occurring in locations without its model (Ritland 1995; Pfennig *et al.* 2001; Prudic *et al.* 2002). The mimetic lineage has several potential outcomes in response to this change in model presence. This species could remain a Batesian mimic in locations without its model, especially when predation is weak (Pfennig *et al.* 2007). A mimic could also go extinct in these locations

* Author and current address for correspondence: Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06511, USA (kathleen.prudic@yale.edu).

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due to intense predation (Pfennig *et al.* 2001). Less explored are the expectations that the mimic could evolve a new colour pattern to mimic another model species or revert back to its ancestral, non-mimetic phenotype (Ruxton *et al.* 2004). The latter two scenarios are traditionally considered unlikely and have the least empirical support because it is assumed that considerable genetic change is responsible for the specialized phenotype of a Batesian mimic (Fisher 1930; Ruxton *et al.* 2004; Pfennig *et al.* 2007).

The North American admiral butterflies (Nymphalidae: *Limenitis*) provide a unique opportunity to investigate the evolution and maintenance of Batesian mimicry relationships. The ancestral wing pattern of a black ground colour and dorsal–lateral white band is commonly found in Eurasian congeners (Platt 1983; Mullen 2006). This wing pattern is regarded as an example of disruptive coloration because the butterfly is difficult to detect and attack especially when it is flying through its native riparian habitat (Platt & Brower 1968; Platt 1983). This type of cryptic pattern reduces prey detectability by visually inhibiting the predator from seeing the prey's actual body outline (Cuthill *et al.* 2005; Stevens & Cuthill 2006). However, in North America, there has been a divergence away from this cryptic defensive strategy (Platt 1983; Mullen 2006). Three of the four *Limenitis* species are conspicuously coloured mimics, each of a different model species, and all with very specialized wing pattern morphologies (Prudic *et al.* 2002 and references therein). Unlike other well-studied butterfly mimicry systems, such as *Heliconius*, these three mimetic species also have different larval host plant requirements than their respective models (Opler *et al.* 2004). Since the geographical distribution of the host species plays a large role in determining the geographical distribution of a phytophagous insect species (Bernays & Chapman 1994; Loxdale & Lushai 1999), disjunctions in the geographical distributions of model and mimic phytophagous insects may arise due to differences in larval host plant requirements. Mimetic herbivorous insects may exist in locations without their respective model, if hosts are available for the mimetic but not the model species. Of particular interest are the different phenotypes possessed by admiral subspecies of *Limenitis arthemis*. The red-spotted purple (*Limenitis arthemis astyanax* and *Limenitis arthemis arizonensis*) is a conspicuously black and purple-coloured Batesian mimic of the pipevine swallowtail (Papilionidae: *Battus philenor*; Platt *et al.* 1971). The white admiral (*Limenitis arthemis arthemis* and *Limenitis arthemis rubrofasciata*) exhibits the ancestral white-banded cryptic form of the Eurasian congeners and is a close non-mimetic relative of the red-spotted purple. Based on wing colour information, it has been hypothesized that the white admiral or its nearest relative spread east out of Eurasia across Beringia and south to its current range. Subsequently, the red-spotted purple arose in sympatry with the model, the pipevine swallowtail (Remington 1958; Platt & Brower 1968; Platt 1983). However, recent phylogenetic evidence indicates that this assumption of evolutionary history may not be the case. The white admiral may have arisen from the red-spotted purple lineage, but this hypothesis warrants further investigation (Mullen 2006).

Here we reconstructed the evolutionary history of North American *Limenitis* using molecular phylogenetic

methods. We documented the direction of the evolutionary transitions from cryptic to mimetic forms and vice versa. Using parametric bootstrapping, we also evaluated the likelihood that a mimetic form changed back to the ancestral cryptic form. We then investigated if Batesian mimicry transitions from mimetic to cryptic were correlated with the absence of the model species, based on the geographical distribution of the model species.

2. MATERIAL AND METHODS

(a) *Taxon sampling*

We sampled all the North American members of the genus *Limenitis* and three species of Palaearctic *Limenitis* (electronic supplementary material, table). We sampled all subspecies of *L. arthemis* and most subspecies of *Limenitis archippus* and *Limenitis weidemeyerii* (Hodges *et al.* 1983). Putative hybrids of all North American *Limenitis* have been observed in the field, but the extent to which they are viable is currently unknown (Platt 1983). We did not include any specimens from overlap or hybrid zones, as classification of hybrid individuals is difficult and may hinder phylogenetic inference (Grant & Grant 1998). Additionally, we included samples from two closely related genera, *Neptis* and *Adelpha*, as outgroups (Willmott 2003).

(b) *DNA extraction and sequencing*

We inferred the evolutionary relationships among North American *Limenitis* using one mitochondrial gene, cytochrome oxidase subunit II (*COII*), and two nuclear genes, elongation factor 1 alpha (*EF1a*) and *wingless*. We extracted total genomic DNA from leg or thoracic tissue using DNeasy tissue extraction kit (Qiagen, Inc., CA, USA) according to the manufacturer's protocol. PCRs (50 µl) were performed on a Mastercycler (Eppendorf, NY, USA), in 1X Eppendorf Hotmaster Taq Buffer containing 0.2 µM of each primer, 0.1 mM dNTPs, 2.5 mM MgCl₂ and 1 unit of Eppendorf Hotmaster Taq DNA polymerase. We amplified *COII* using the amplification primers Pierre and Eva (Caterino & Sperling 1999). The thermal cycle profile was an initial 1.5 min denaturation at 94°C; 32 cycles of 40 s at 94°C, 40 s at 45°C and 45 s at 72°C; a 7 min final extension at 72°C. To amplify *EF1a*, we used the amplification primers e44f and efcM4r (Monteiro & Pierce 2001) with the profile: initial 2 min denaturation at 94°C; 35 cycles of 20 s at 94°C, 15 s at 50°C and 60 s at 65°C; a 5 min final extension at 65°C. We amplified *wingless* using the amplification primers LepWG1 and LepWG2 (Brower & DeSalle 1998) with the profile: initial 2 min denaturation at 94°C; 15 cycles of 30 s at 94°C, 30 s at 65°C – 1°C per cycle, 1 min at 65°C; 25 cycles of 30 s at 94°C, 30 s at 50°C, 1 min at 65°C; and a 10 min final extension at 65°C. PCR products were purified with a Millipore size-exclusion filtration (Millipore, MA, USA) prior to direct sequencing. Both strands were sequenced using an Applied Biosystems BigDye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, CA, USA) and run on an Applied Biosystems 3730XL DNA Analyzer (Applied Biosystems). We generated consensus sequences from the two strands and aligned these sequences by eye with the aid of the program BioEDIT (Hall 1999). Representative sequences for all alleles of each locus have been deposited in GenBank (accession numbers: DQ117851–DQ117897, EF643261–EF643361 and EU433934–EU433946).

(c) Phylogenetic analyses

We performed maximum parsimony and Bayesian MCMC on each gene separately. For each gene, we began by removing redundant sequences from the alignment. Maximum parsimony analyses were performed in PAUP* v. 4.0b10 (Swofford 2001). We initially performed a heuristic tree search with 1000 random-addition replicates, saving all the most parsimonious trees per replicate. To assess node support, we performed 1000 non-parametric bootstrap pseudoreplicates, each with 10 stepwise addition, nearest-neighbour interchange heuristic search replicates, saving the first 1000 most parsimonious trees per heuristic search replicate.

We used a hierarchical likelihood ratio test to estimate the model of evolution for the Bayesian analyses (Huelsenbeck & Rannala 1997). *COII*, *EF1a* and *wingless* all fit a GTR+ Γ model, with empirical base frequencies. Using this model in MRBAYES v. 3.01 (Huelsenbeck & Ronquist 2001), we analysed each gene separately; in each analysis, we ran four chains (three hot and one cold) for 2 000 000 generations, sampled trees every 100 generations, and discarded the trees sampled in the first 1 000 000 generations (burnin). To estimate the posterior probability of clades, we used the post-burnin sampled trees to generate a 50% majority rule consensus tree using the 'sumt' command in MRBAYES.

We used the three gene trees to generate a species tree in MESQUITE (Maddison & Maddison 2006) to infer the relationships among North American *Limenitis*. Briefly, because the three genes are evolving independently, and may not always accurately reflect the history of the species' relationships, we used the gene trees to search for the 'best-fit' species tree that minimizes the number of deep coalescences of the gene trees (Maddison 1997). Gene trees of recently diverged taxa may not be monophyletic, due to incomplete lineage sorting (Hudson & Coyne 2002; Funk & Omland 2003), although a significant phylogenetic signal may still be present (Maddison & Knowles 2006). By accounting for the possibility of incomplete lineage sorting, this approach infers of species' relationships without requiring the gene trees to be monophyletic. We used the heuristic search function in MESQUITE to find the optimal species tree that minimizes the number of deep coalescences of the gene trees, using a nearest-neighbour interchange branch swapping algorithm, treating gene trees as unrooted, and allowing polytomies to be automatically resolved before the coalescence cost is assessed (Maddison & Maddison 2006). This approach has proved useful for inferring true species relationships when gene trees do not recover the monophyly of species (Maddison & Knowles 2006).

(d) Character evolution

We coded wing morphology into three states: 0 = white-banded (*L. a. arthemis*, *L. a. rubrofasciata*, *L. weidemeyerii*, *Limenitis lorquini*, *Limenitis camilla*, *Limenitis populi*, *Limenitis reducta*); 1 = black, *Battus mimic* (*L. a. astyanax*, *L. a. arizonensis*); and 2 = *Danaus mimic* (*L. archippus*). Ancestral character states were reconstructed on the species tree in MESQUITE (Maddison & Maddison 2006), using a parsimony model of unordered states.

(e) Parametric bootstrapping

Phylogenetic reconstruction of *COII* demonstrated strong support against a clade of the black mimetic phenotype (see §3). To determine whether our reconstructions were significantly better than those reflecting monophyly of the mimetic

phenotype, we performed phylogenetic parametric bootstrapping (Huelsenbeck *et al.* 1996) on the *COII* gene tree. Briefly, we compared parsimony tree lengths of an unconstrained search to tree lengths of a search constrained to retain only those trees in which the black mimetic taxa formed an exclusive clade. This difference in tree lengths represented our observed test statistic (δ_{obs}). From the most parsimonious constrained trees, we used the tree with the highest likelihood to estimate the model of evolution using hierarchical likelihood ratio tests (Huelsenbeck & Rannala 1997). This model (GTR+ Γ +I) was then implemented in MESQUITE v. 1.12 (Maddison & Maddison 2006) to simulate 1000 character matrices on the most parsimonious reconstruction with an exclusive black mimetic clade. For each matrix, we performed two parsimony tree searches, one unconstrained and one constrained as above, and the difference in tree lengths were used to generate a distribution of the test statistic, δ . We determined the significance of our results by comparing δ_{obs} with the simulated distribution of δ . A significant value of δ_{obs} leads to the rejection of the hypothesis that the group tested is monophyletic.

(f) Distribution and geographical range of***L. arthemis* forms, the model and the host plants**

We used published geographical information of North American butterfly and host plant county records to determine whether the transition from mimetic to cryptic *L. arthemis* form was correlated with the absence of its Batesian model, *B. philenor*, or some other ecological factor such as host plant availability. We evaluated this question at two spatial scales: at the level of our taxonomic sampling used to reconstruct the gene trees and at the level of the entire geographical range of *L. arthemis*. For the butterfly information, we used two sources of county records (Layberry *et al.* 1998; Opler *et al.* 2004) and for the plant information, we used one source of county records (USDA 2007). By combining information from these databases, we were able to document the presence or absence of (i) *L. arthemis*' larval host plant, (ii) the model *B. philenor*, and (iii) *B. philenor*'s larval host plant (*Aristolochia* spp.) for two sets of locations: (a) the exact locations where we sampled our specimens used to reconstruct the phylogeny and (b) across the entire range of *L. arthemis* in North America. For the smaller geographical analysis, 17 different counties were examined, six with the white-banded form and 11 with the black mimetic form. For the larger geographical analysis, 1509 out of 3135 counties in North America (excluding Hawaii) had records of *L. arthemis*: 144 counties with the white-banded form only; 1166 counties with the black mimetic form only; and 199 counties with both forms together. We used linear regression in JMP-In (JMP 2002) to evaluate factors predicting the geographical distributions of the mimetic and cryptic forms of *L. arthemis*.

3. RESULTS**(a) Phylogenetics and character evolution**

We reconstructed the gene trees for all North American *Limenitis* (figure 1). Parsimony and Bayesian reconstructions for each gene were topologically congruent, although Bayesian reconstructions showed support for some clades that were not supported in the parsimony analyses. For *COII*, there were no shared haplotypes among the species of *Limenitis*. Within *L. arthemis*, the white-banded cryptic form and the black mimetic form also shared no haplotypes. There were no shared haplotypes

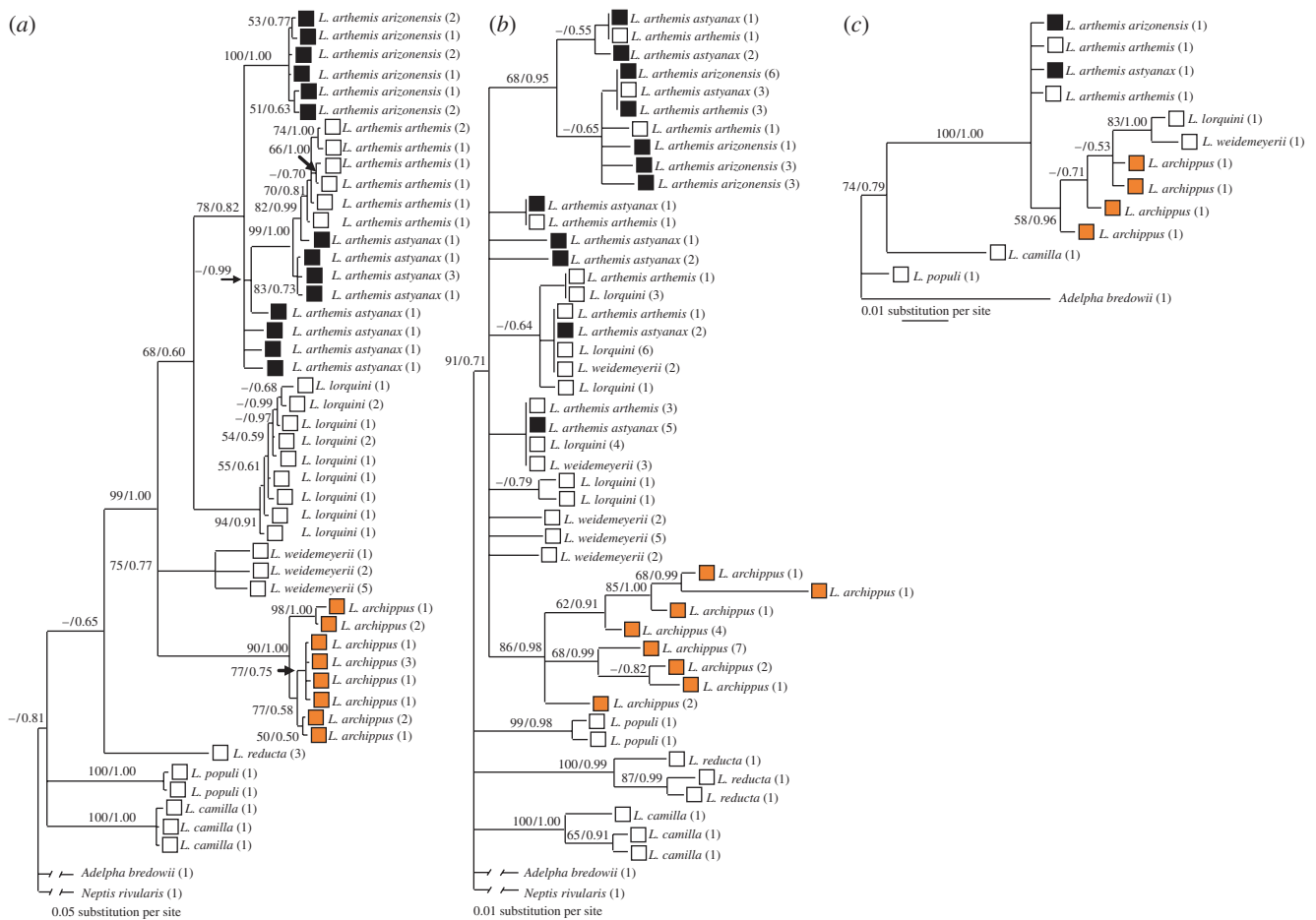


Figure 1. Consensus trees from Bayesian inference of (a) *COII*, (b) *EF1a* and (c) *wingless*. Branch labels show maximum parsimony bootstrap support/Bayesian posterior probability. Wing morphology character states for terminal taxa are shown. White, white-banded; black, *Battus* mimic; orange, *Danaus* mimic.

between the two mimetic subspecies (*L. a. astyanax* and *L. a. arizonensis*). In contrast, many *EF1a* haplotypes were found in more than one species, and only *L. archippus* was recovered as monophyletic. The *wingless* data showed the least phylogenetic resolution, although there was significant support for the monophyly of North American *Limenitis*. The four specimens of *L. arthemis* (two *L. a. arthemis*, one *L. a. arizonensis* and one *L. a. astyanax*) all possessed identical *wingless* haplotypes. In the species tree search, minimizing the number of deep coalescences of the *COII*, *EF1a* and *wingless* Bayesian gene trees, we recovered two best-fit trees, which differed only in the arrangement of out-group taxa (*Adelpha bredowii* and *Neptis rivularis*). The inferred relationships among North American *Limenitis* species are shown in figure 2, along with ancestral reconstructions of wing morphology. Our results confirm that the white-banded cryptic form is the ancestral phenotype of North American *Limenitis* (Mullen 2006). The two equally parsimonious ancestral reconstructions of the wing pattern in *L. arthemis* on this species tree are: (i) the black mimetic form (*L. a. astyanax* and *L. a. arizonensis*) evolved once from a white-banded cryptic ancestor, and reverted back to the white-banded cryptic form in the lineage leading to *L. a. arthemis* or (ii) the black mimetic form evolved twice from a white-banded cryptic ancestor (figure 2).

The most parsimonious trees in unconstrained searches of the *COII* data were 276 steps and the most parsimonious trees in constrained searches were 284 steps

($\delta_{\text{obs}} = 284 - 276 = 8$). This falls well outside of our simulated distribution of δ (mean $\delta = 0.242$, 95% CI = 0–3), so we reject an exclusive black mimetic clade at $p < 0.001$.

(b) Distribution and geographical range of *L. arthemis* forms, the model and the host plants

At the smaller geographical scale using only the locations of the specimens used for phylogenetic analyses, the black mimetic form was positively correlated with the presence of the model ($N = 11$, $r^2 = 0.75$, $p < 0.001$), whereas the presence of the white-banded cryptic form of *L. arthemis* was negatively correlated with the presence of the model, *B. philenor* ($N = 6$, $r^2 = 0.45$, $p = 0.004$; table 1a). This was also explained by the distribution of the model's host plant. *Aristolochia* spp. were positively correlated with the presence of the black mimetic form of *L. arthemis* ($N = 11$, $r^2 = 0.33$, $p = 0.024$), but were negatively correlated with the presence of the white-banded cryptic form ($N = 6$, $r^2 = 0.31$, $p = 0.025$; table 1a). However, the willow, the major *L. arthemis* larval host plant, was present in every county in which *L. arthemis* was found. The presence of the host plant did not explain the different geographical distributions of the two *L. arthemis* forms ($N = 17$).

Focusing on the entire geographical range of *L. arthemis*, the presence of the white-banded cryptic form was again negatively correlated with the presence of the model, *B. philenor* ($N = 346$, $r^2 = 0.15$, $p < 0.001$),

Table 1. Presence and absence county records of the model *B. philenor* and its larval host plant, *Aristolochia* spp., in relation to county records of the mimetic and non-mimetic forms of *L. arthemis*. (a) The data from the specimens used in the molecular phylogeny; (b) the data across the entire geographical range of *L. arthemis*.

| | | county presence of <i>L. a. astyanax</i> and <i>L. a. arizonensis</i> (mimetic black form) | county presence of <i>L. a. arthemis</i> and <i>L. a. rubrofasciata</i> (non-mimetic white-banded form) |
|--|---------|---|---|
| (a) | | | |
| <i>Battus philenor</i> Batesian model | present | 10 | 0 |
| | absent | 1 | 6 |
| <i>Aristolochia</i> spp. (<i>Battus</i> host) | present | 8 | 0 |
| | absent | 3 | 6 |
| (b) | | | |
| <i>Battus philenor</i> Batesian model | present | 897 | 90 |
| | absent | 465 | 256 |
| <i>Aristolochia</i> spp. (<i>Battus</i> host) | present | 788 | 35 |
| | absent | 574 | 311 |

and the black mimetic form was positively correlated with the model ($N=1362$, $r^2=0.75$, $p<0.001$; table 1b). These patterns were also explained by the distribution of the model's larval host plant (white-banded form: $N=346$, $r^2=0.22$, $p<0.001$, slope negative; black mimetic form: $N=1362$, $r^2=0.27$, $p<0.001$, slope positive; table 1b). However, willow always occurred in the same county as any *L. arthemis* and did not explain the different geographical distributions of the *L. arthemis* forms ($N=1509$), and thus there are suitable host plants for *L. arthemis* in locations both with and without the Batesian model, *B. philenor*.

4. DISCUSSION

Batesian mimicry is a highly specialized interaction between the model, the mimic and the predator. The mimic gains protection from predation by resembling a dangerous or unpalatable species. Since it is dependent on the honest signal of the model, the Batesian mimic should not occur in locations without its model. The predators will not recognize the mimic as potentially dangerous in areas without the model and will predate on the mimics more intensely because this prey is highly conspicuous and palatable (Ruxton *et al.* 2004; Pfennig *et al.* 2007). Yet, there are documented examples of a Batesian mimic occurring in locations without its model in ecological time (Ritland 1995; Pfennig *et al.* 2001; Prudic *et al.* 2002). Here we examined whether a Batesian mimic could persist outside the range of its model from a phylogenetic perspective considering the four potential evolutionary outcomes outlined in the literature. A Batesian mimetic species could go extinct if predation was intense or it could persist as a Batesian mimic if predation was weak (Pfennig *et al.* 2007). Although thought to be more unlikely owing to genetic constraints, a mimic could also evolve a new colour pattern to mimic another model species; or it could revert back to its ancestral non-mimetic phenotype (Fisher 1930; Ruxton *et al.* 2004). The data presented here support the hypothesis that a Batesian mimic does not persist in locations without its model, but it does not go extinct either (figure 1; table 1). Instead, in locations where the model does not occur, the mimic may have reverted back to its ancestral form that is less conspicuous to predators.

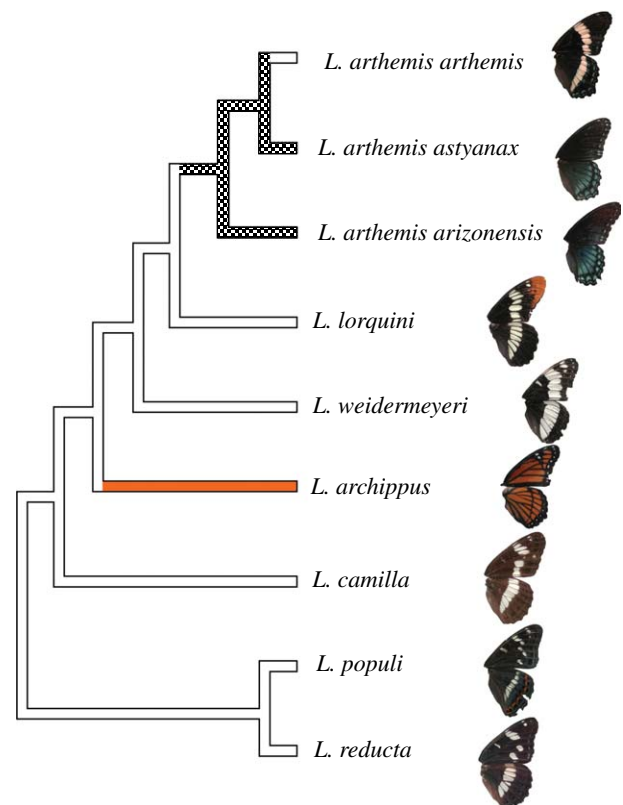


Figure 2. Species tree of North American *Limenitis* inferred from minimizing deep coalescences of gene trees. Branch colours show maximum parsimony character reconstructions of ancestral states (white, non-mimetic disruptive coloration; black, *Battus* mimic; orange, *Danaus* mimic; checkered, equivocal between white and black).

Previous studies have hypothesized that the black mimetic form of *L. arthemis* evolved from the white-banded cryptic form of *L. arthemis* (Platt & Brower 1968; Platt 1983). However, our results from the phylogenetic analyses are consistent with the hypothesis that the white-banded cryptic form evolved from a black mimetic form. The inferred species tree and parametric bootstrapping results demonstrate that cryptic *L. a. arthemis* did not give rise to the mimetic subspecies *L. a. astyanax* and *L. a. arizonensis*; rather, the lineages leading to the two

mimetic subspecies diverged before the evolution of *L. a. arthemis* (figure 2). Although the nuclear gene trees revealed little resolution among the North American species of *Limenitis*, there was no uncertainty about these relationships in the species tree inference, demonstrating the phylogenetic signal present in gene trees (Maddison & Knowles 2006). Parsimony character reconstructions of wing phenotypes are equivocal, but it is unlikely that the black mimetic phenotype evolved twice given current distributions of North American *Limenitis* species. When the model, *B. philenor*, co-occurs with *Limenitis* species, those *Limenitis* species are always mimics, of *B. philenor* or other distasteful model species over a broad geographical scale (Platt & Brower 1968). Thus, the hypothesis of two independent origins of the black mimetic phenotype would have required long-term coexistence of *B. philenor* and a non-mimetic ancestor of *L. a. arthemis* and *L. a. astyanax*. This pattern of coexistence of *B. philenor* and white-banded cryptic *Limenitis* is not observed across the contemporary permanent range of *B. philenor*. Given the inferred history of North American *Limenitis* (Mullen 2006, this study), the lack of any cryptic *Limenitis* co-occurring with *B. philenor*, and the rejection of a black mimetic clade by parametric bootstrapping, we propose that a black mimetic lineage gave rise to the white-banded cryptic lineage observed in *L. arthemis*.

Based on the adaptive function for this evolutionary pattern, we predicted that the ecological data of both model and mimic would also link strongly with our phylogenetic results. The reversion to a white-banded cryptic form is consistent with the geographical distributions of the model (*B. philenor*) whose distribution is limited by its larval host plant (*Aristolochia* spp.). The geographical distribution of the white-banded cryptic *L. arthemis* is negatively correlated and the black mimetic form is positively correlated with the geographical range of the model (table 1 and electronic supplementary material, figure). A similar geographical pattern is found in another Batesian mimic of *B. philenor*, the eastern tiger swallowtail (Papilionidae: *Papilio glaucus*), which is a female-limited Batesian mimic of *B. philenor* (Brower 1958). Both mimetic and non-mimetic female forms of *P. glaucus* are present in locations where *B. philenor* occurs. However, in the same geographical region where *L. arthemis* changes from the black mimetic form to the white-banded cryptic form and *B. philenor* becomes increasingly rarer, *P. glaucus* females also shift from their mimetic to non-mimetic form (Brower & Brower 1962).

Reversion to a cryptic wing phenotype in *L. arthemis* is not unexpected given our knowledge of the genetic architecture underlying wing pattern variation. Even though butterfly wing patterns are a complex trait, the genetic mechanisms may be easy to change (Nijhout 1991). In butterfly Batesian mimicry, the wing pattern of the mimic is thought to be produced by a single gene of major effect with a suite of modifier genes that refine the wing pattern (Turner 1977). Although this mechanism has not been demonstrated definitively in *Limenitis* (but see Platt 1983), it has been shown in other Batesian mimics such as *Papilio dardanus* (Nijhout 2003). Also, in *Heliconius* butterflies, a homologous gene or complex of genes regulates wing pattern diversity in three different species with different phenotypes (Joron *et al.* 2006). Rather than a constraining role, this locus seems to provide wing phenotype flexibility across species and

environments. It presumably functions by responding to a wide range of selection pressures, including predation, to produce radically divergent, locally adapted wing patterns from a very similar genetic mechanism. The genes regulating butterfly wing patterns do not appear to become constrained when these lineages become either Batesian or Müllerian mimics.

Our results also relate to a previously unreported phenomenon regarding butterfly distribution and range. Butterfly species, like many phytophagous insects, are limited by the distribution of their larval host plants (Bernays & Chapman 1994; Loxdale & Lushai 1999). Host acceptance and selection are determined by a wide variety of ecological and physiological factors (e.g. Futuyma & Moreno 1988; Bernays 1989; Thompson 2005). However, all of these factors relate to the interaction between insect species and host plant species. Our results suggest that the distribution of the black mimetic form of *L. arthemis* is determined not by the distribution of its own larval host plant (*Salix* spp.), but by the distribution of its model's larval host plant (*Aristolochia* spp.; table 1). Thus, the broad geographical distributions of phytophagous insect species and subspecies also depend on other indirect interactions in their communities, in addition to the relationship with their own larval host plant(s). This distributional pattern has been shown many times at a smaller community scale (e.g. Bernays 1989), but not across the larger geographical range of a species or subspecies.

To conclude, our results demonstrate that a Batesian mimic does not persist in locations without its model. The distribution of the mimic is correlated with the presence of the model and, in this system, the model's host plants. Batesian mimicry is a specialized signal with complex interactions between the model, the mimic and the predators across a broad geographical range. Thus, the distribution of the mimic may be limited by the distribution of the model species in many cases. However, our findings also demonstrate that a mimic does not necessarily go extinct without its model. When the model is absent, the mimic may re-evolve an ancestral defensive strategy and become less conspicuous to predators thereby persisting in locations without its Batesian model.

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