ORIGINAL ARTICLE

THE DIVERSITY AND EVOLUTION OF BATESIAN MIMICRY IN *PAPILIO* SWALLOWTAIL BUTTERFLIES

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Papilio swallowtail butterflies exhibit a remarkable diversity of Batesian mimicry, manifested in several sex-limited and polymorphic types. There is little understanding of how this diversity is distributed within *Papilio*, and how different mimicry types have evolved in relation to each other. To answer these questions, I present a graphical model that connects various mimicry types by hypothetical character state changes within a phylogenetic framework. A maximum likelihood analysis of evolution of mimicry types on the *Papilio* phylogeny showed that sexually monomorphic mimicry and female-limited mimicry have evolved repeatedly but predominantly independently in different clades. However, transitions between these mimicry types are rarely observed. The frequency distribution of character state changes was skewed in favor of the evolution of mimicry, whereas many theoretically plausible character state changes, especially evolutionary loss of mimicry, were not evident. I discuss these findings in relation to studying the tempo of evolutionary change, loss of traits, and directionality and connectivity among character states. The pathway approach and phylogenetic patterns of mimicry demonstrated in *Papilio* are useful to test novel hypotheses regarding the diversity and evolutionary directionality of Batesian mimicry in other systems.

KEY WORDS: Character state path network, microevolution, polymorphism, speciational evolution.

Natural selection seems to favor certain evolutionary trajectories and outcomes. For example, the evolution of larger body size (Cope's rule) or brain size within lineages is ubiquitous in several animal groups (Alroy 1998; Striedter 2005; Clauset and Erwin 2008). Competitive advantage, red-queen dynamics, or enemy-free space in adaptive landscapes accompanying such changes are believed to favor such evolutionary directionality in many complex adaptations. In an intriguing proposal, Vane-Wright suggested that one such adaptation with evolutionary directionality is Batesian mimicry (Vane-Wright 1971, 1979). Batesian mimicry, in which palatable species gain protection from predators due to their resemblance to aposematic species, is common among insects (Wickler 1968; Rettenmeyer 1970). Perhaps, the peak of Batesian mimetic perfection, diversity, and complexity is seen in butterflies, in which mimicry manifests either in both sexes

or it is sex-limited, and it may be monomorphic or polymorphic (Eltringham 1910). There is little understanding about the organization of mimetic diversity and the evolutionary paths that different mimicry types follow. While studying sexually dimorphic mimicry in *Mycalesis drusillodes*, Vane-Wright proposed two evolutionary trajectories that could produce five Batesian mimicry types (Vane-Wright 1971). The first evolutionary trajectory was: sexually monomorphic nonmimetic ancestors → sexually monomorphic mimics with a single mimetic form → sexually monomorphic mimics with multiple mimetic forms → sexually dimorphic mimics in which the sexes mimic different models ("dual mimics," such as *M. drusillodes*). The second evolutionary trajectory was: sexually monomorphic nonmimetic ancestors → female-limited mimics → mimetic female polymorphism. Vane-Wright also suggested that once mimicry evolves, sexually

monomorphic mimicry and female-limited mimicry could inter-convert in response to changing selective pressures.

Why would one expect such diversity of and directionality in mimicry types? Although Vane-Wright did not specifically address this question, Batesian mimicry theory offers several reasons (Bates 1862; Wallace 1889; Fisher 1958; Edmunds 1974; Sheppard 1975; Turner 1978; Ruxton et al. 2004). Mimicry is an adaptive peak that offers relative enemy-free space; hence the evolution of Batesian mimicry should enhance the life expectancy of mimetic individuals and increase their fitness relative to non-mimetic individuals. However, the fitness advantage to Batesian mimics is frequency-dependent, so that the frequency of mimics cannot exceed a certain threshold beyond which mimicry is no longer advantageous. This is because at a very high mimic frequency, predators are more likely to encounter and consume a mimic before they encounter a model. At this stage, mimicry may become polymorphic or female-limited, both of which would maintain greater relative fitness of mimetic phenotype. Thus, the frequency-dependent advantage of mimicry may direct the evolution of monomorphic mimicry toward polymorphism and female-limitation, giving rise, in the process, to different mimicry types.

Vane-Wright formalized his hypotheses in a pathway model in which evolutionary change in wing color patterns was postulated to follow certain character state paths, similar to the two mentioned above (Vane-Wright 1979). Although the predicted state path changes could potentially occur over microevolutionary timescales (i.e., between two speciation events), many of Vane-Wright's predictions were testable only at macroevolutionary scales, involving changes in wing color patterns across at least one speciation event. Vane-Wright, in fact, believed that species-level phylogenies, which capture speciational patterns in wing color evolution (cf. Jiggins et al. 2006), could test the directionality and pathway models of Batesian mimicry. These, however, remained untested because such phylogenies with a large representation of mimetic species were unavailable until recently. Evolutionary relationships of many mimetic and nonmimetic *Papilio* swallowtail butterflies, a group with extensive Batesian mimicry, are now known from a molecular phylogeny (Zakharov et al. 2004), offering an opportunity to test Vane-Wright's pathway model.

Approximately, 25% of ∼200 *Papilio* swallowtail butterfly species are mimetic, with independent origins of mimicry in several species groups (Zakharov et al. 2004). Together they exhibit all the forms of Batesian mimicry known in butterflies, forming an ideal group to study mimicry. Studies on *Papilio* have addressed many challenging questions in mimicry theory and evolutionary biology in general, and *Papilio* continue to instruct biologists in the mechanisms of evolution (Wallace 1865; Fisher 1958; Sheppard 1975; Scriber et al. 1995; Mallet 2004; Kunte 2008). Here, I present a version of character state paths model that is modified to study the evolution of *Papilio* mimicry types using phylogenetic methods. Then, I map the diversity of mimicry types to test their evolutionary directionality, including gains, losses, and transitions between mimicry types. I show that during the repeated independent evolution of mimicry types among *Papilio*, certain character state paths have been followed particularly frequently, transitions between different mimicry types have been rare, and evidence for the loss of mimicry, as recently proposed in *Limenitis* butterflies (Prudic and Oliver 2008), is lacking. Finally, I will discuss the implications of the observed state path networks for phylogenetic inference using maximum likelihood methods, especially in relation to studying the evolution and diversity of Batesian mimicry.

Materials and Methods
MOLECULAR PHYLOGENY OF *PAPILIO* BUTTERFLIES

I used a previously published molecular phylogeny of *Papilio* butterflies that included 51 *Papilio* species representing all major species groups, and two outgroups: *Pachliopta neptunus* and *Eurytides marcellus* (Zakharov et al. 2004). The phylogeny was based on data from ∼2.3 kilobases of mitochondrial genes (cytochrome oxidase subunits I and II and tRNA-leucine) and ∼1.0 kilobases of the nuclear gene elongation factor 1 alpha. Phylogenetic analyses with maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods produced congruent trees from combined datasets. MP and ML analyses of combined data each resulted in a single tree. A consensus tree constructed from the Bayesian analysis had topology and branch lengths matching the MP bootstrap consensus and ML trees. Most of the internal nodes were well-supported, although deeper relationships among some clades were weakly supported. See Zakharov et al. (2004) for further details.
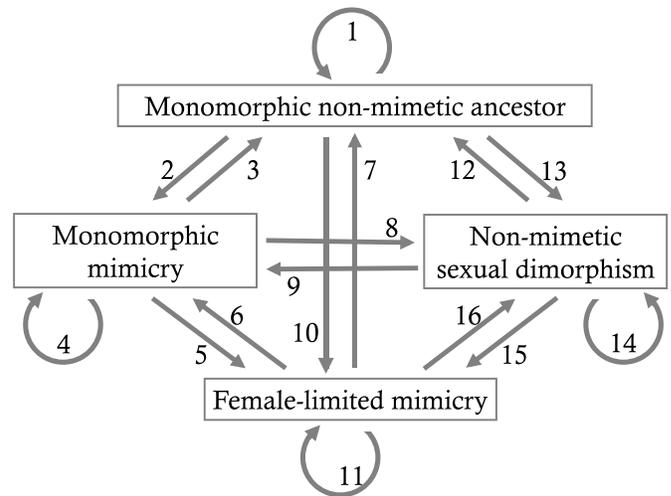
MIMICRY TYPES

Species were assigned to four mimicry and two nonmimicry types based on their wing color patterns. The mimicry types were: (1) sexually monomorphic mimicry: sexually monomorphic species in which both sexes are mimetic with a single mimetic form, e.g., *P. scamander* and *P. epycides*, henceforth referred to as "monomorphic mimicry," (2) female-limited mimicry: this is found in sexually dimorphic species in which males are non-mimetic and at least one female form is mimetic, e.g., *P. polytes* and *P. aegeus*, (3) polymorphic mimicry: sexually monomorphic species with multiple mimetic forms that are shared between the sexes, e.g., *P. clytia*, and (4) sexually dimorphic mimicry: species are sexually dimorphic but both sexes are mimetic, mimicking the same or different models, e.g., *P. erostratus* females mimic *Parides photinus* and males mimic *Battus polydamas*. Henceforth, I will refer to this mimicry type as "dimorphic mimicry;"

Vane-Wright's dual mimicry being its subset. The two non-mimicry types were: (1) sexually monomorphic nonmimetic and (2) sexually dimorphic nonmimetic. Henceforth, I will refer to the mimicry and the nonmimicry types together as "mimicry types" for convenience, the nonmimicry types being the null states. The six mimicry character states were unordered and unweighted. I chose to map these six mimicry types because they are represented in the current phylogeny by at least one example. In the present analysis, I did not account for female-limited mimetic polymorphism because a previous analysis has specifically dealt with it (Kunte 2008). I assigned character states to species from several monographs (Eltringham 1910; Wynter-Blyth 1957; Tyler et al. 1994), and specimens deposited in the McGuire Center for Lepidoptera and Biodiversity in the University of Florida at Gainesville. Specific character state assignments included sub-specific and geographical variation.

Note that not all mimetic *Papilio* have been experimentally demonstrated to be Batesian mimics. The cases of two nymphalids, *Hypolimnas bolina* (Marsh et al. 1977) and *Limenitis archippus* (Ritland and Brower 1991), caution that the nature of mimicry may be more complex than usually assumed. These two species were traditionally classified as Batesian mimics of aposematic *Danaus* and *Euploea*, but were experimentally shown to be themselves chemically defended, at least under certain conditions. Thus, these species may be considered Müllerian rather than Batesian mimics (in Müllerian mimicry unpalatable species mimic each other). Nonetheless, I have included the above mimicry types in *Papilio* under Batesian mimicry. This is because experimental evidence invariably shows the *Papilio* species tested so far to be palatable to predators and therefore Batesian mimics (Brower 1957, 1958; Codella and Lederhouse 1989; Chai 1990; Uesugi 1995). This includes *P. polyxenes*, in which caterpillars are aposematic but adults are palatable and female-limited Batesian mimics of *Battus philenor* (Brower 1957; Codella and Lederhouse 1989). Most *Papilio* with aposematic caterpillars (Prudic et al. 2007) are nonmimetic (see Kunte 2008 and Results below). On the other hand, caterpillars of most mimetic *Papilio*, including the largest mimetic radiations in subgenera *Menelaides* and *Heraclides*, feed on rutaceaous and related plants with little noxious chemical properties (Anonymous 2009), and species tested so far from these groups have been shown to be palatable to predators (Brower 1957, 1958; Chai 1990; Uesugi 1995). Given the current evidence, I have assumed *Papilio* to be Batesian mimics.

CHARACTER STATE PATHS, NETWORKS AND DIRECTIONALITY IN MIMICRY EVOLUTION

The evolution of mimicry may proceed in several "steps," that is, following several character state changes, potentially leading to the evolution of sexual dimorphism or monomorphism as a by-product of selection for specific mimicry types. To study the


Figure 1. A theoretical character state path network showing 16 paths leading to the evolution of common Batesian mimicry types in *Papilio* swallowtail butterflies.

character state changes associated with mimicry types in *Papilio*, I constructed a character state path network that linked the various mimicry types (Fig. 1). I excluded polymorphic mimicry and dimorphic mimicry from this network for simplicity because these two mimicry types are very rare in the present *Papilio* phylogeny, and their inclusion would have unnecessarily increased the number of possible character state paths. The remaining four mimicry types produced 16 paths, numbered in Figure 1 for reference, which may be followed during speciational change (sensu Jiggins et al. 2006) or stasis in wing color patterns. The empirical frequencies of these paths were then calculated by ML method (see next section).

PHYLOGENETIC ANALYSES OF CHARACTER EVOLUTION AND STATE PATHS

I studied the evolution of mimicry types in *Papilio* and traced character state changes by reconstructing ancestral states using ML criterion with one-parameter Markov k-state model, implemented as "Mk1 (est.)" in Mesquite (Maddison and Maddison 2006). The internal nodes in Figure 2 represent proportional maximum likelihoods of various ancestral mimicry types. I used this ancestral reconstruction to calculate the empirical frequency of character state changes between various mimicry types. The resultant frequency histograms of character state changes across the *Papilio* phylogeny, depicted in Figure 3, were generated with the following rules. The log likelihoods for ancestral states can be used in Mesquite to set a decision threshold, T, such that if the log likelihoods of two character states differ by T or more, the character state with a higher likelihood (i.e., lower negative log likelihood) is chosen as the ancestral state at the particular internal node. I used the default, recommended value of $T = 2$ in Mesquite to get the most likely ancestral states at internal nodes. The default value
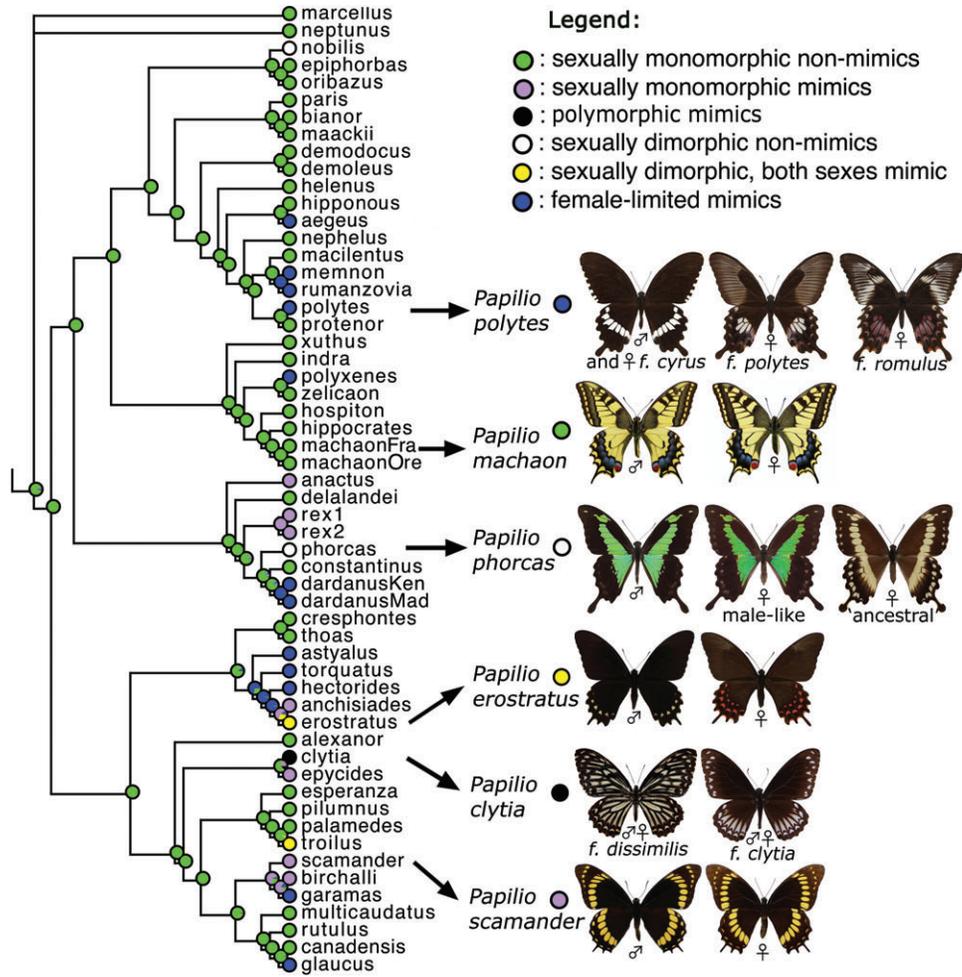
Figure 2. Distribution of mimicry types among *Papilio* butterflies. Internal nodes show proportional likelihoods for the character states as estimated by the maximum likelihood method. Representative species for each mimicry type is also illustrated.

of $T = 2$ resolved most internal nodes; that is, it assigned each node a single character state as most likely using the decision threshold ($T = 2$), which produced the proportional maximum likelihood score ≥ 0.85 for that character state at the specific node. The character states of the extant species were always unambiguous (proportional maximum likelihood score $= 1$). After this, starting from the base of the tree toward the extant species, I calculated the frequency of each character state change or stasis by summing over the character state paths that had been followed between every speciation event in the *Papilio* phylogeny, that is, between each of the ancestor–descendent pair of nodes. For example, the most recent common ancestor of the species pair *P. hipponous* and *P. aegeus* was most likely monomorphic and nonmimetic (proportional maximum likelihood score $= 0.975$). A change from this ancestral state to female-limited mimicry in *P. aegeus* was counted as one character state change following state path 10, and the stasis leading to the monomorphic nonmimetic *P. hipponous* was counted as one character state change following state path 1.

Three species, *P. dardanus*, *P. rex*, and *P. machaon* were each represented by two specimens in the original phylogeny. Because mimicry types of both specimens of each of the three species were the same, state changes were considered only up to the first internal node leading to the two specimens, that is, up to the "species node."

Five of the 50 internal nodes in Figure 2 had ambiguous ancestral character states, that is, they had more than one character state with log likelihoods at least twice as large as the log likelihoods for all other character states. The five ambiguous nodes were the most recent common ancestors of: (1) (*birchalli*, *garamas*)), (2) (*scamander*, (*birchalli*, *garamas*)), (3) (*clytia*, *epycides*), (4) (*anchisiades*, *erostratus*), and (5) (*astyalus*, (*torquatus*, (*hectorides*, (*anchisiades*, *erostratus*))))). In these cases, all the state paths that contributed to the proportional maximum likelihood values ≥ 0.85 from the ancestral to the descendent species were included in the dataset following the default setting in Mesquite. For example, the most recent common ancestor of *P. birchalli* and *P. garamas* was more likely monomorphic and
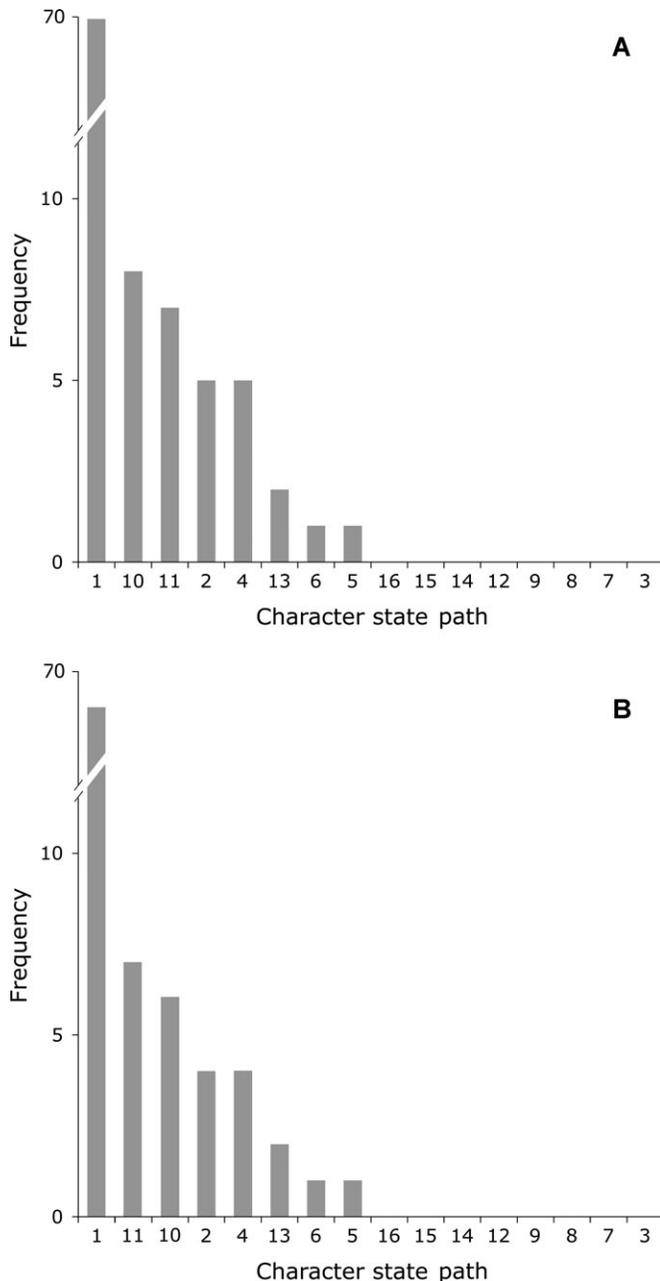
Figure 3. Character state changes or stasis during the evolution of wing color patterns associated with various mimicry types in *Papilio*. The numbers on the x-axis correspond to the numbered theoretical character state paths in Figure 1. Frequencies of character state paths on the y-axis are derived empirically from Figure 2 using a maximum likelihood criterion with a decision threshold value of either $T = 2$ (A), or $T = 1$ (B).

(state paths 5, 11, and 10). I tried two alternative approaches to resolve the ancestral character state ambiguities: (1) to exclude clades with ambiguous internal nodes and (2) to lower the value of the decision threshold to a level that would resolve the ambiguous nodes (for the *Papilio* phylogeny, $T = 1$). Given the importance of the above five ambiguous internal nodes and the specific ambiguities themselves in the present study (see Results), I have presented the results below using all clades and a default value of $T = 2$ (Fig. 3A). The second approach of using $T = 1$ (Fig. 3B) resolved ambiguities in favor of the character state that had a higher negative log likelihood than the competing character states, and was better in the sense that it avoided inclusion of the same datapoint in frequencies of multiple character state paths. However, it was unsatisfactory in four out of the five cases in which the differences between the negative log likelihoods of the competing character states was very small. The use of $T = 1$ slightly affected frequencies of a few character state paths but it did not affect the overall patterns of mimicry evolution in *Papilio* (compare Fig. 3A with Fig. 3B).

Results

Figure 2 shows the maximum likelihood reconstruction of ancestral mimicry types on the *Papilio* phylogeny (–log likelihood = 74.62). Figure 3 presents the empirical frequency of character state changes in mimicry types, drawn from the theoretical character state paths as numbered in Figure 1. Two important patterns are evident from these analyses. First, although wing color patterns vary across species and species groups, most speciational evolution in *Papilio* retains sexual monomorphism in wing coloration (character state path 1, $n = 70$ with $T = 2$ in Fig. 3A, $n = 69$ with $T = 1$ in Fig. 3B). This is perhaps indicative of selective pressures, such as mutual sexual selection, that favor sexual monomorphism across most of the *Papilio* phylogeny. Second, the distribution of mimicry types is highly unequal: monomorphic mimicry (state path 2, $n = 5$) and female-limited mimicry (state path 10, $n = 8$), have evolved repeatedly in *Papilio* whereas polymorphic mimicry ($n = 1$) and dimorphic mimicry ($n = 2$) have evolved rarely. Transitions between mimicry types are also rare: there is only one instance of monomorphic mimicry changing to female-limited mimicry (state path 5), in *P. garamas* and one instance of female-limited mimicry changing to monomorphic mimicry (state path 6), in *P. anchisiades*.

Several character state paths, on the other hand, are absent. For example, the evolution of monomorphic mimicry from nonmimetic sexual dimorphism (state path 9), or the evolution of nonmimetic sexual monomorphism from sexual dimorphism (state path 12), is not evident. More prominently, state paths that represent loss of monomorphic mimicry (paths 3 and 8) or female-limited mimicry (paths 7 and 16) are missing from the *Papilio*

mimetic (proportional maximum likelihood score = 0.722) but could also have been female-limited mimetic (proportional maximum likelihood score = 0.149) or monomorphic and nonmimetic (proportional maximum likelihood score = 0.102). The change from this ambiguous ancestral state to the female-limited mimicry in *P. garamas* was counted in each of the three possible state paths

phylogeny. The absence of the loss of mimicry is significant: there were 14 instances on the *Papilio* phylogeny in which mimetic ancestors gave rise to descendent lineages. In these 14 instances, if the loss of mimicry was as probable as any other state paths, the probability of not observing the loss of mimicry was very low ($P < 0.0001$).

The evolution of dimorphic mimicry has followed different evolutionary trajectories than the one suggested by Vane-Wright. Vane-Wright speculated that, because dimorphic mimicry is much less common than monomorphic mimicry, the former is dependent on the occurrence of the latter, and expected it to follow a three-step evolutionary path: sexually monomorphic nonmimetic ancestors → monomorphic mimicry → polymorphic mimicry → dimorphic mimicry. Contrary to his expectation, *P. erostratus* has followed a different two-step evolutionary path, as follows. *Papilio erostratus* is nested within the large South American *Heraclides* subgenus of *Papilio*, with ~30 species (Häuser et al. 2005). *Heraclides* has a few monomorphic nonmimetic species and a large number of female-limited mimics. From the current taxon sampling in *Heraclides*, it appears that female-limited mimicry first evolved in *P. astyalus*, *P. torquatus,* and *P. hectorides* from the *P. cresphontes*- and *P. thoas*-like monomorphic nonmimetic ancestors (Fig. 2). The internal nodes connecting the ancestors of these female-limited mimics to *P. achisiades* and *P. erostratus* are unresolved. However, current reconstruction of ancestral states suggests that the most recent common ancestor of *P. achisiades* and *P. erostratus* was either a monomorphic mimic similar to *P. achisiades* (proportional likelihood = 0.56) or a dimorphic mimic similar to *P. erostratus* (proportional likelihood = 0.31). A denser phylogeny of *Heraclides* may resolve this uncertainty.

Papilo troilus shows yet another character state path to dimorphic mimicry: that of a one-step change from sexually monomorphic nonmimetic wing coloration directly to dimorphic mimetic coloration. *Papilio troilus* is sexually dimorphic, although both sexes mimic the same model, *B. philenor*, the males being poor mimics. There are two scenarios to explain this dimorphic mimicry: (1) the mimetic wing coloration of *P. troilus* evolved initially as monomorphic mimicry but the mimetic resemblance of males is gradually degrading in response to changing selective pressures and (2) it initially evolved as female-limited mimicry and is now evolving toward monomorphic mimicry by breaking up the genetic female-limitation of mimicry (cf. Vane-Wright 1971). *Papilio troilus* is the only mimetic species in its species group; hence, unlike in *Heraclides*, denser taxon sampling will not resolve the uncertainty about the evolutionary direction of mimicry in *P. troilus*.

Polymorphic mimicry is represented in the present *Papilio* phylogeny solely by *P. clytia*, which has two mimetic forms that are shared between the sexes (Fig. 2). Form *dissimilis* mainly mimics *Tirumala limniace* and *T. septentrionis*, whereas form

clytia mimics *Euploea* species. This species belongs to *Chilasa*, a small Oriental subgenus with 11 species, eight of which are Batesian mimics. Six of these are monomorphic mimics and two are polymorphic mimics: *P. clytia* and *P. paradoxa*; the latter also mimics *Euploea* species. The representation of *Chilasa* in the current *Papilio* phylogeny by *P. epycides* and *P. clytia* has not resolved the ancestral state (proportional likelihood for the ancestral state being sexually monomorphic nonmimicry = 0.66, for monomorphic mimicry = 0.15, and for polymorphic mimicry = 0.13). However, given the prevalence of monomorphic mimicry in *Chilasa*, it is most plausible that the evolution of polymorphic mimicry in *P. clytia* has followed the evolutionary path hypothesized by Vane-Wright: sexually monomorphic nonmimetic ancestors → monomorphic mimicry → polymorphic mimicry. This needs to be confirmed with a species-level phylogeny of *Chilasa*.

Discussion

The complexity of Batesian mimicry in butterflies has lead to much speculation about evolutionary pathways leading to the various mimicry types (Vane-Wright 1971, 1979). Here, I have tried to provide phylogenetic framework and data to test the theories about evolution and diversity of Batesian mimicry types. Using a molecular phylogeny of *Papilio*, I was able to test for the first time predictions regarding the organization of mimicry types within clades, supporting Vane-Wright's original proposals in showing that: (1) female-limited mimicry and monomorphic mimicry mostly follow independent evolutionary routes; (2) transitions between various mimicry types do occur, albeit infrequently; and (3) mimicry seems to favor the evolution of sexual dimorphism and polymorphism among monomorphic clades, following the expectation from the frequency-dependent advantage of mimicry. The evolution of dimorphic mimicry, however, appears to have followed unexpected character state paths. Thus, the present analysis joins a growing number of studies that have used phylogenetic or population genetic methods to test specific predictions about the evolution and diversity of mimicry (Vane-Wright et al. 1999; Flanagan et al. 2004; Jiggins et al. 2006; Ceccarelli and Crozier 2007; Kunte 2008; Prudic and Oliver 2008).

Phylogenetic reconstruction is a statistical process that produces several tree topologies with varying level of nodal support. The empirical relative frequencies of state paths in Figure 3 are sensitive to tree topologies; hence, their distributions will change slightly based on specific tree topologies used, just as they change slightly when different decision threshold values (T) are used to reconstruct ancestral states (Figs. 3A and 3B). Nonetheless, the patterns presented above seem robust and will most likely persist when a denser *Papilio* phylogeny based on more gene sequences is available. However, a denser phylogeny is likely to resolve

several critical ambiguous internal nodes, particularly those leading to *P. erostratus* and *P. clytia*. It may also reveal novel character state paths leading to diverse mimicry types. Based on the above framework and analysis, phylogenetic studies of mimicry in two groups of swallowtail butterflies: subgenera *Menelaides* and *Heraclides*, seem especially promising. These subgenera have a large number of mimetic species and contain taxa that have fueled research on mimicry and natural selection for over a century. Further phylogenetic work in these groups will present a more complete understanding of the evolution and diversity of mimicry in swallowtail butterflies.

One of the main findings of this article—that although the evolution of Batesian mimicry is prevalent in *Papilio*, its loss is not evident—is remarkable. This is because the evolutionary loss of mimicry must undoubtedly frequently occur due to a number of causes, including changes in selective pressures resulting from extinction of the model or the model's phenotypic movement away from the aposematic pattern that is being mimicked by the Batesian mimics, and a mismatch between the distributional ranges of models and mimics (Pfennig et al. 2001; Pfennig et al. 2007; Ries and Mullen 2008). Recently, such an evolutionary loss of mimicry was proposed to have taken place in the North American *Limenitis arthemis*, which presumably reverted to an ancestral nonmimetic phenotype (Prudic and Oliver 2008). *Limenitis arthemis* has two mimetic subspecies (*L. a. astyanax* and *L. a. arizonensis*) at lower latitudes where their ranges overlap with that of their model, *B. philenor*, and a nonmimetic subspecies (*L. a. arthemis*) at higher latitudes where the model is absent. Prudic and Oliver generated molecular data for different *Limenitis* populations from one mitochondrial gene (COII) and two nuclear genes (EF-1alpha and *wingless*), although the nuclear genes were phylogenetically uninformative and the nodal support for COII tree topology was weak. From this ambiguous phylogenetic tree, Prudic and Oliver concluded that nonmimetic *arthemis* was derived from mimetic *astyanax* and *arizonensis*, *arthemis* having reverted to the non-mimetic cryptic coloration in the absence of the model. This interesting conclusion, unfortunately, is not based on evidence: the branches separating the mimetic *astyanax* and *arizonensis* from the nonmimetic *arthemis* were unresolved for mimicry states in Prudic and Oliver's analysis. Hence, it was erroneous to conclude that mimicry was ancestral in this species and that *arthemis* had lost it. Furthermore, a comprehensive study based on three mitochondrial and seven nuclear regions sequenced from more specimens, and published almost concurrently, has shown that the nonmimetic *arthemis* is, in fact, basal (Mullen et al. 2008). It also revealed that haplotypes of the mimetic *astyanax* and *arizonensis* populations are embedded among the *arthemis* populations. Another recent study has further shown with a robust AFLP-based phylogeny that the nonmimetic *arthemis* has retained, not regained, the shared ancestral white-banded pattern of the genus,

and there is a single origin of mimicry in *astyanax* and *arizonensis* (Savage and Mullen 2009). Both these studies disfavor Prudic and Oliver's notion of *arthemis* having lost mimicry. Outside of *Limenitis*, the much larger phylogeny of *Papilio* was likely to have revealed some examples of the loss of mimicry, but the above analysis offers no examples. How could we explain this lack of evidence for the loss of mimicry, when mimicry is expected to be lost frequently? Mimicry in butterflies is a complex adaptation, possibly involving supergenes and dramatic coding and regulatory changes in a number of genes, each with multiple alleles (Fisher 1958; Ford 1965; Sheppard 1975; Koch et al. 2000; Joron et al. 2006; Clark et al. 2008). Thus, it is possible that having once evolved, the genetic architecture of mimicry is difficult to break down rapidly. It is also possible that in the absence of protected models, mimics that have acquired conspicuous wing color patterns suffer heavy predation aided by their slow flight. This may drive the mimics extinct before genetic changes that would enable reversion to nonmimetic wing patterns could take place. It is, therefore, plausible that the selective pressures for the loss of mimicry almost inevitably result not as a phylogenetic signature in the form of nonmimetic species nested among mimetic species, but as invisible signs of extinctions of mimetic species that leave no descendent species.

This brings forth an important caveat regarding the use of phylogenetic methods in inferring evolutionary patterns: that of undocumented extinctions. Extinct taxa are rarely represented on species phylogenies, thereby potentially biasing the conclusions of phylogenetic studies. Ecologically biased extinctions may especially produce a very different pattern of character state evolution compared to scenarios in which extinctions may be completely random. In the context of mimicry, biased extinction would imply that certain mimicry types are more likely to go extinct, whereas if extinctions are just as likely irrespective of mimicry type or even the presence of mimicry, extinctions could be said to be random. Mimicry theory currently does not predict whether extinctions should be random with respect to the presence and type of mimicry. Hence, it is difficult to gauge whether, and how, extinctions – if biased in favor of mimicry – might have affected the phylogenetic patterns presented above.

The above analysis and conclusions based on standard phylogenetic methods raise two other important issues, namely, the tempo of evolutionary change in relation to speciation events, and the validity of using species-level or higher phylogenies to study evolution of traits that may evolve at macro- and/or microevolutionary scales. The first issue is especially critical in the present context in relation to the evolution of sexual dimorphism that accompanies female-limited mimicry. Current standard population genetic models posit that "even when the evolution of the average phenotype of the two sexes is geologically rapid, on the order of a few thousand to several thousand generations, the time

scale for the evolution of sexual dimorphism may be on the order of millions of generations" (Lande 1980). This might imply that traits that face intense selection in both sexes have a very high rate of evolutionary change, and that such traits may evolve at microevolutionary scales. The character state changes in these traits would be captured as one-step evolutionary transitions on species-level phylogenies, as state changes $0 \rightarrow 1$ from ancestors A to B. In contrast, the rate of evolution of sexually dimorphic traits may be much slower compared to average time intervals between speciation events. Thus, for female-limited mimicry to evolve from an ancestral monomorphic nonmimetic species, the two-step evolutionary series of state changes $0 \rightarrow 1 \rightarrow 2$ might follow the sequence of the ancestor–descendent species relationship in a lineage: $A \rightarrow B \rightarrow C$, with A being a monomorphic nonmimetic (state 0) ancestor and B being its descendent with monomorphic mimicry (state 1), which may in turn give rise to C, a female-limited mimic (state 2). Such a two-step evolutionary change would be expected in sexually dimorphic traits as long as the assumptions and conclusions of the current population genetic models regarding the tempo of evolution of sexually dimorphic traits are correct. Indeed, many studies have recently claimed that these "genetic correlations" models can explain bright, male-like sexual ornaments in female birds and other taxa, some of which are subsequently lost in more derived species (Amundsen 2000). If these models were to be applied to the evolution of female-limited mimicry to predict the sequence of character state evolution just mentioned, notice that the sequence reflects state paths 2 and 5 in tandem (Fig. 1). The sequence of these paths is in fact rare, while state path 10 is common in *Papilio*. One might argue that this trend is an artifact of missing taxa: if the *Papilio* phylogeny included all species, the monomorphic mimetic links between monomorphic nonmimetic ancestors and female-limited descendents would indeed be discovered. I have surveyed the occurrence and mimicry type in every species group in all of *Papilio* species, and find that this argument would not be true even if all the species were to be included in the *Papilio* phylogeny. For example, in species groups in which all the relevant species have been included in the current phylogeny, such as the *P. glaucus* and *P. polyxenes* species groups, female-limited mimicry still shows the one-step pattern of evolution (state path 10), with no monomorphic mimetic intermediates (Fig. 2). This might suggest that the rate of evolutionary change toward sexually dimorphic traits may potentially be more rapid than current models predict. Recent observational and experimental evidence showing that sexually dimorphic traits may evolve or be enhanced rapidly within a few generations (Badyaev 2005; Chenoweth et al. 2008), lends credibility to this suggestion. Until this matter is settled, caution may be warranted when making firm assumptions about sexually monomorphic or dimorphic traits and their evolutionary rates.

This article underlines two critical questions: how is mimetic diversity organized within clades, and what patterns of directionality in mimicry types are seen in Batesian mimicry in *Papilio*, or butterflies in general? The above analysis shows that character state path networks, combined with phylogenetic analysis, are a powerful set of tools to understand the organization of Batesian mimetic diversity. From this analysis I conclude that directionality is indeed evident in various Batesian mimicry types in *Papilio*. The two common mimicry types – sexually monomorphic and female-limited mimicry – follow single-step state paths from the ancestral monomorphic nonmimetic wing color patterns. Female-limitation of mimicry apparently evolves rapidly at microevolutionary scales. More complex mimicry types such as polymorphic and sexually dimorphic mimicry, however, evolve by multi-step state paths, which might explain their rarity. The broad patterns in *Papilio* show that the frequency of various character state paths is highly unequal, some state paths being very rare. There is no evidence of mimicry having been lost in *Papilio* without the mimetic species going extinct. Based on these observations I suggest that selection for mimicry and its complex genetic architecture may act in a ratcheting manner toward generating greater diversity in Batesian mimicry. The initial evolution of one mimicry type may facilitate evolution of other mimicry types when selection for mimicry changes, sometimes leading to more complex mimicry types. Selection against mimicry, on the other hand, may result in extinction of the mimetic species rather than loss of mimicry. Such type of selection—diversification–extinction dynamics may have driven speciation and wing color pattern diversity in Batesian mimetic butterflies.

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