A RARE MODEL LIMITS THE DISTRIBUTION OF ITS MORE COMMON MIMIC: A TWIST ON FREQUENCY-DEPENDENT BATESIAN MIMICRY

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Batesian mimics are predicted to lose their fitness advantage not only in the absence of an unpalatable model, but also when the mimic becomes relatively abundant. The phenotypic hybrid zone between mimetic and nonmimetic admiral butterflies, comprising the polytypic *Limenitis arthemis* species complex, offers an ideal opportunity to test these predictions because the position of the hybrid zone is hypothesized to be controlled by the geographic range of *Battus philenor*, the chemically defended model. We used 29 years of observational field data from a continental-scale butterfly monitoring program, the 4th of July Butterfly Counts, to show that (1) the advantage of mimicry does not extend beyond the range of the model, (2) in contrast to expectations, the mimicry complex is maintained even where the model is rare and (3) the sharp phenotypic transition between mimetic and nonmimetic admiral populations occurs over a very narrow spatial scale corresponding to the limit of the model's range. These results suggest that, even at very low densities, there is selection for Batesian mimicry and it maintains the geographic position of this hybrid zone. Our findings highlight the value of large-scale, long-term citizen science monitoring programs for answering basic ecological and evolutionary questions.

KEY WORDS:　Frequency-dependent selection, gene flow, hybridization, mimicry, wing pattern evolution.

Batesian mimicry is a classic example of adaptation that provides compelling evidence for evolution by natural selection (Bates 1862; Fisher 1930; Mallet and Joron 1999). Palatable mimics that resemble unpalatable models gain a fitness advantage because predators often rely upon visual cues to recognize, and avoid, the model's phenotype. However, protection from predation is hypothesized to be frequency dependent (Fisher 1927, 1930), and selection favoring mimicry is predicted to break down not only in the absence of the model (Wallace 1870; Waldbauer and Sternburg 1987; Waldbauer 1988a; Pfennig et al. 2001, 2007) but also when the mimic becomes common relative to the model (Fisher 1930; Brower and Brower 1962; Huheey 1964; Oaten et al. 1975; Getty 1985; Mallet and Joron 1999; Harper and Pfennig 2007).

Although numerous theoretical (reviewed in Huheey 1988; Ruxton et al. 2004) and laboratory (Brower 1960; Nonacs 1985; Lindström et al. 1997; Lindström et al. 2004; Rowland et al. 2007) studies support these hypotheses, demonstrations that frequency-dependent Batesian mimicry operates in natural populations are rare. This is largely due to the difficulties associated with accurately estimating, and/or manipulating, the relative frequency of a model and its mimics in nature. Observational studies that have directly measured both models and mimics in the field sampled at only one or a few sites and show variation in the relative abundances of each (Brower and Brower 1962; Darst and Cummings 2006). Experimental field studies that have attempted to address this issue (Jeffords et al. 1979; Pfennig et al. 2001; Harper and Pfennig 2007; Pfennig et al. 2007) have relied upon preexisting geographic variation in the relative abundance of a noxious model

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(e.g., present vs. absent, or common vs. rare) and use experimentally introduced mimics (so the abundance of actual mimics is unknown) to test predictions about frequency dependence. Therefore, conclusions about how the frequency or density of a model influences the degree of protection provided for palatable mimics in natural populations have been limited to qualitative rather than quantitative inferences.

Mimetic and nonmimetic populations of North American admiral butterflies present an ideal opportunity to examine how the distribution and abundance of a chemically defended model influences the strength of selection for a Batesian mimic. White-banded admiral butterflies (*Limenitis arthemis arthemis*) occur in the northeastern United States and throughout Canada as far west as Alaska (Scott 1986, Fig. 1) These butterflies are characterized by broad, white bands that traverse both the dorsal and ventral surfaces of the wing. In contrast, Red-spotted Purples (*L. a. astyanax*), distributed throughout the southeastern United States (Fig. 1), possess vibrant blue to blue-green iridescent scales along the outer portion of the hindwing and phenotypically resemble the chemically defended Pipevine swallowtail (*Battus philenor*). Pipevine swallowtails are highly toxic (Fordyce 2000; Sime et al. 2000; Fordyce et al. 2005), and early ecological studies (Brower and Brower 1962) and behavioral experiments with caged avian predators (Brower 1958; Platt et al. 1971) indicate that *L. a. astyanax* is a Batesian mimic of *B. philenor*.

Recent genetic work (Mullen et al. 2008) has shown that the southeastern, mimetic subspecies originated in allopatry, and that the ranges of these two color morphs have subsequently come together in a secondary zone of contact along a narrow geographic band (Fig. 1) where the two subspecies are known to hybridize. Historically, subspecies boundaries in this complex have been heavily debated (Remington 1958; Platt and Brower 1968; Remington 1968) and, although other factors may play a role, it has long been hypothesized that selection related to mimicry limits gene flow between these two wing pattern races and, therefore, is responsible for maintaining the position of the phenotypic hybrid zone (Platt and Brower 1968; Remington 1968; Mullen et al. 2008). If so, then we expect to see a relationship between the distribution of *Battus* and where the mimetic subspecies begins to transition to the nonmimetic subspecies.

Here, we tested two central predictions of Batesian mimicry theory. First, we determined if the distribution of mimics is dependent of the presence of the model. Second, we determined if this relationship was frequency dependent, with mimicry only advantageous where the model is common relative to the mimic. In doing so, we also examined the hypothesis that selection for mimicry maintains the position of the hybrid zone between the two subspecies (Fig. 1). These tests were carried out by estimating the relative abundance of the mimic, the Red-spotted Purple (*L. a. astyanax*), its chemically defended model, the Pipevine Swallowtail (*B. philenor*), and the nonmimetic subspecies, the White admiral (*L. a. arthemis*) across the entire range of each species using direct field observations, made as part of the annual 4th of July Butterfly Surveys over a 29-year time span.

Materials and Methods

We used the 4th of July Butterfly Surveys to obtain estimates of abundances of the model, the mimetic and the nonmimetic
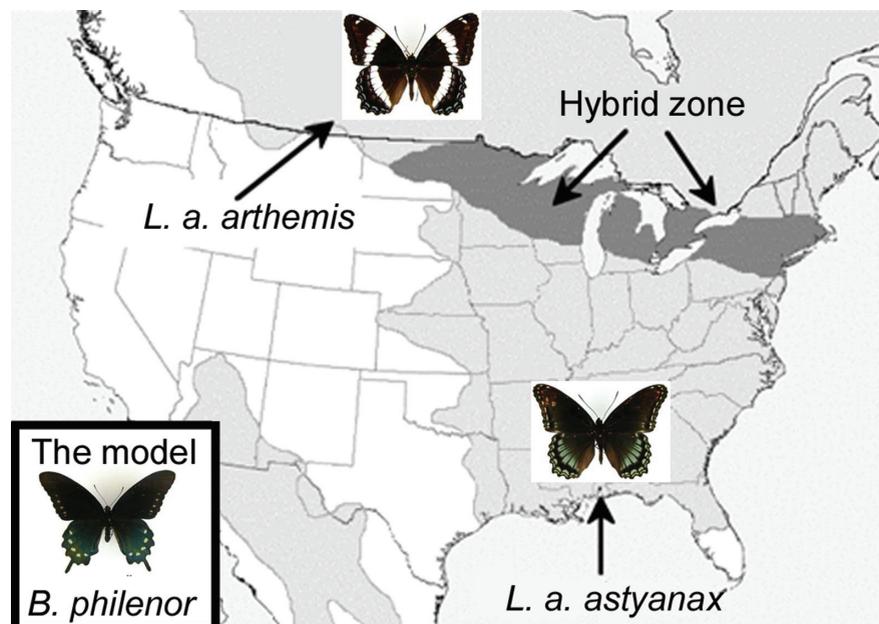

Figure 1. The phenotypic hybrid zone between *Limenitis arthemis arthemis* and *L. a. astyanax*. The unpalatable model, *Battus philenor*, is shown in the inset.

subspecies throughout their ranges. The 4th of July Butterfly Surveys were patterned after the Christmas Bird Counts (Swengel 1990). Volunteers establish a count site and once per year (usually in June or July) spend a full day counting as many individuals of each species that they can find within a 25-km radius of the count center. Latitude and longitude coordinates are provided by each count coordinator. There are no standardized survey protocols, but effort (in terms of number of groups, individual participants, and time) is quantified. The program began in 1975 with 28 count sites. Currently, approximately 450 count sites are monitored each year throughout the United States and Canada. We obtained the count records from the North American Butterfly Association in text files and used PERL (ver. 5.8) to parse data into an Access database. Latitude and longitude coordinates were checked using Terraserver (www.terraserver-usa.com) and all butterfly names were rectified using the North American Butterfly Association's checklist.

From the 4th of July butterfly data, we located 3627 surveys where either *B. philenor* or either subspecies of the *L. a. arthemis-astyanax* complex were recorded. Most surveys identified 100% of individual *L. arthemis* to subspecies; however, surveys where fewer than 90% were identified to subspecies were dropped from the analysis. This left 3332 surveys for analysis. To calculate proportions of the model (*B. philenor*) relative to the mimetic morph, we selected survey sites in which at least 10 individuals of either species had been sighted over the course of that count. We determined the proportion of *B. philenor* relative to the total of both species at each site for each year, then took a mean of the proportion over years. The same method was used to select survey sites and calculate proportions of the mimetic morph (*L. a. asyanax*) relative to the nonmimetic morph (*L. a. arthemis*). Proportions based on each survey site were mapped and graphed relative to latitude. We calculated quantile regressions (Cade and Noon 2003) to determine latitudes where critical proportions transitioned. We used a 95% quantile to determine the latitude where *B. philenor* transitioned to effectively becoming zero proportion of the survey and a 5% quantile to determine at what latitude *L. a. arthemis* began to enter the system. Analyses were done in R (ver. 2.4.0).

Results

Our data clearly show the mimetic relationship between *B. philenor* and *L. a. astyanax* is not favored in the absence of the model (Fig. 2). Inside the latitudinal range where the model appears in the surveys (up to ~41°N), the mimetic morph comprises 100% of the *L. arthemis* population. Within a degree (~110 km) of this point, mimics seem to lose their protective advantage and the nonmimetic morph becomes an increasingly dominant portion of the *L. arthemis* population (Fig. 2B,C). This transition point sets the southern limit of the hybrid zone between the mimetic and
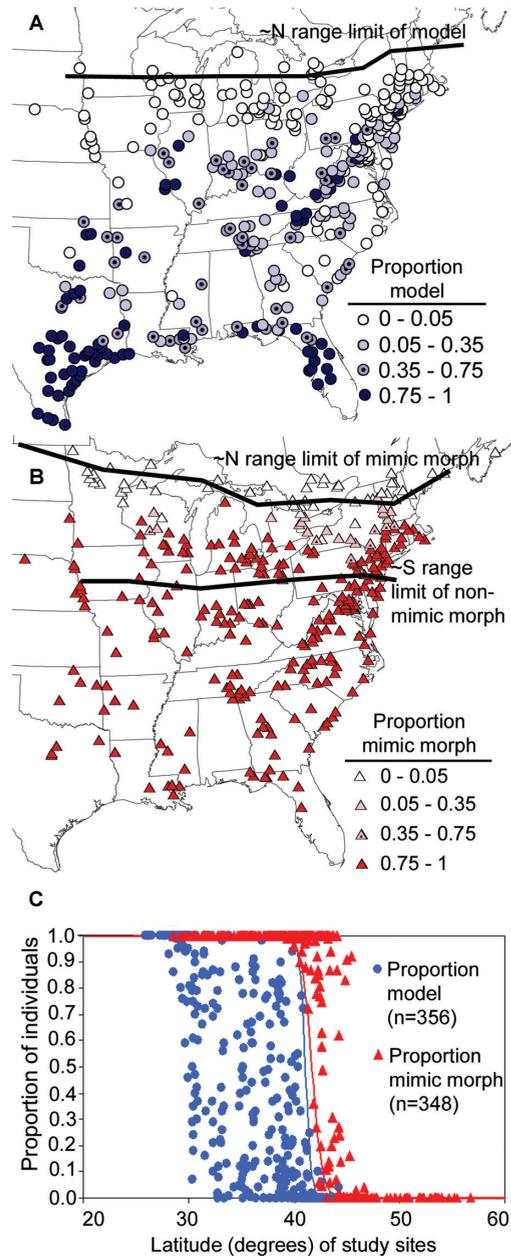

Figure 2. (A) Proportions of the distasteful model, *Battus philenor*, relative to totals of the model and its mimic, *Limenitis arthemis astyanax*, based on survey data from the 4th of July butterfly counts. The approximate range limit of *B. philenor* is also shown. (B) Proportions of the mimic morph, *L. a. astyanax*, relative to totals of the mimetic and nonmimetic morph *L. a. arthemis*, based on survey data from the 4th of July butterfly counts. The northern range limit of the mimic morph and southern range limit of the nonmimetic morph are also shown. (C) Proportions of individuals based on the latitude of the 4th of July Survey site. The proportions of pipevines (the model) to totals with Red-spotted Purples (the mimic) are shown in blue circles. The blue line represents the 95% quantile regression. The proportion of red-spotted purples (the mimetic morph) to totals of the mimetic and nonmimetic morph (white admirals) are shown in red triangles. The red line represents the 5% quantile regression.

nonmimetic morphs of *L. arthemis* (because individuals can only hybridize where they co-occur) and indicates strong congruence between the presence of the model and the position of the hybrid zone. This result is consistent between years (analysis not shown), so is not influenced by year-to-year variation in abundances. Note that we determined model "presence" by a recorded occurrence in the 4th of July data; published range maps include a larger area (see range limit in Fig. 2A) reflecting any record of occurrence no matter how rare. This is further demonstrated by the fact that the known range of the nonmimetic morph overlaps extensively with that of *B. philenor* (see range limits in Fig. 2A,B), but they only co-occur in three of 3332 surveys compiled from 29 years of July 4 counts (the mimetic and nonmimetic morphs co-occur frequently where *B. philenor* is absent). This highlights the danger of using published range maps to determine how the range of a model may influence the range of a mimic; for this purpose, abundance data are critical.

The second prediction, that mimicry should not be maintained where the model is rare, is refuted by our data. Although the term "rare" is certainly subjective, our data show that even where the model (*B. philenor*) is extremely rare compared to the mimic (*L. a. astyanax*), the mimicry complex is maintained. Only when the proportions of the model drop nearly to zero across its range does the nonmimetic subspecies (*L. a. arthemis*) begin to show up in the surveys (Fig. 2A,B). This is clearly shown in Figure 2C and highlighted by the juxtaposition of the 95% quantile (below which 95% of observations occur) for the proportion of *B. philenor* and the 5% quantile for the proportion of *L. a. astyanax*. Additionally, proportions of *B. philenor* relative to its mimic *L. a. astyanax* are highly variable throughout its range (Fig. 2A,C), so even within the range where *B. philenor* regularly occurs, there are pockets in which it is very rare or absent. Yet throughout the range where *B. philenor* is observed in our surveys (up to ∼41°N), the mimicry complex is completely maintained.

Discussion

Our geographic analysis contradicts the generally held view that unpalatable models must be common relative to palatable mimics for Batesian mimicry complexes to be maintained. Merely the presence of the model seems to be sufficient to provide a selective advantage for the mimetic morph. The striking congruence between the range of *Battus* and the sharp phenotypic transition between mimetic and nonmimetic wing pattern races of *Limenitis* provides strong evidence (albeit observational) that, even at very low proportions, selection related to wing pattern mimicry maintains the geographic position of this hybrid zone. We are aware of no other dataset that is able to show this pattern over the entire range of a mimicry complex.

There are several possible explanations for why mimicry is maintained throughout the range of the model, even in areas in which the model is extremely rare. For example, predators may sample from different populations that vary in the relative abundance of the model over a geographically widespread area. If predator learning is strongly influenced by their total experience, then protection may extend to populations where *Limenitis* is common and *Battus* is rare or nearly absent. This is particularly plausible for avian predators that can be both highly vagile and live for several years. Furthermore, evidence suggests that predators can learn to avoid mimics when models and mimics are temporally segregated (Waldbauer 1988b), and this may hold true for spatial segregation as well.

Another possibility is that the mimetic phenotype may be locally maintained throughout the range of *Battus* via migration among mimics, especially in isolated pockets in which the model is relatively rare or absent (see Fig. 2A). In fact, migration likely also explains the presence of individuals with the mimetic phenotype in populations adjacent to the limits of the model's range. Recent genetic work (Mullen et al. 2008) has shown that migration between wing pattern races of *Limenitis* is significant and highly asymmetrical, with genes from *L. a. astayanax* (the mimetic form) able to infiltrate populations of the nonmimetic form, but not vice versa. Given the substantial hybridization in this zone, selection acting against the introgression of nonmimetic alleles may be very strong, even in areas in which the model is very rare or absent. Some authors have suggested that the sharp transition between mimetic and nonmimetic morphs is due to an adaptive advantage to the nonmimic outside the range of the model (e.g., disruptive coloration, Platt and Brower 1968), although there is no direct evidence for this. A more plausible hypothesis is that selection acts against the mimetic phenotype outside of the model's range as a consequence of increased conspicuousness or other physiological costs associated with mimicry (e.g., differences in thermoregulation).

Finally, it may be that mimics simply do not have to be rare as generally assumed for mimicry complexes to be maintained (Darst 2006). Hoverfly mimics, for example, often outnumber their wasp models (Azmeh et al. 1998) although this has been attributed to human-induced climate change. Several studies have also shown that models can be rare near their range limits due to environmental gradients (Hespenheide 1986; Harper and Pfennig 2007; Pfennig et al. 2007) and that selection for the best mimics can actually be strongest near these range limits because protection requires a stronger resemblance to the rare model (Harper and Pfennig 2007). Alternatively, protection for Batesian mimics may be strengthened where there is an abundant availability of alternative prey (Lindström et al. 2004) and/or the risk of predation may be diluted (Rowland et al. 2007). In these butterfly communities, *B. philenor* and its most common mimics comprise only 6%, on average, of the butterflies sampled (analyses not presented). Thus, it is likely that the cost of avoiding fully or partially

palatable mimics in this system is low. A final factor to consider is that the model in this particular case, *B. philenor,* has been shown to be highly unpalatable (Sime et al. 2000), and because palatability directly impacts the efficacy of predator learning (Mallet 1999; Pough et al. 1973) even low densities may be sufficient for protection, given the abundance of alternative prey.

Conclusions

We have shown a striking concordance between the distribution of the chemically defended Pipevine Swallowtail (*B. philenor*) and its associated Batesian mimic, the Red-spotted Purple (*L. a. astyanax*). This suggests selection for mimicry is responsible for maintaining the position of the phenotypic hybrid zone between subspecies of the polytypic *L. arthemis* species complex. However, we did not find evidence that models need to be common relative to their mimics, with our results indicating that selection for mimicry is sufficiently strong to maintain the fixation of the mimetic phenotype even in areas in which the model is locally very rare. Our results suggest that theoretical models of Batesian mimicry could be improved by placing them in a spatial context to allow for movement of both predators (to address learning) and mimics (to assess the impacts of gene flow and colonization). Finally, we suggest that the expansion of large-scale, long-term monitoring programs, in combination with field studies addressing the role of gene flow, will increase our ability to explore the dynamics of mimicry at more appropriate spatial scales.

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