

# A single origin of Batesian mimicry among hybridizing populations of admiral butterflies (*Limenitis arthemis*) rejects an evolutionary reversion to the ancestral phenotype

Wesley K. Savage and Sean P. Mullen

*Proc. R. Soc. B* published online 15 April 2009  
doi: 10.1098/rspb.2009.0256

## References

**This article cites 66 articles, 15 of which can be accessed free**  
<http://rsjb.royalsocietypublishing.org/content/early/2009/04/08/rspb.2009.0256.full.html#ref-list-1>

## P<P

Published online 15 April 2009 in advance of the print journal.

## Subject collections

Articles on similar topics can be found in the following collections

[taxonomy and systematics](#) (72 articles)  
[ecology](#) (502 articles)  
[evolution](#) (584 articles)

## Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

To subscribe to *Proc. R. Soc. B* go to: <http://rsjb.royalsocietypublishing.org/subscriptions>

# A single origin of Batesian mimicry among hybridizing populations of admiral butterflies (*Limenitis arthemis*) rejects an evolutionary reversion to the ancestral phenotype

Wesley K. Savage\* and Sean P. Mullen

Department of Biological Sciences, Lehigh University, B217 Iacocca Hall, 111 Research Drive, Bethlehem, PA 18015-4732, USA

Batesian mimicry is a fundamental example of adaptive phenotypic evolution driven by strong natural selection. Given the potentially dramatic impacts of selection on individual fitness, it is important to understand the conditions under which mimicry is maintained versus lost. Although much empirical and theoretical work has been devoted to the maintenance of Batesian mimicry, there are no conclusive examples of its loss in natural populations. Recently, it has been proposed that non-mimetic populations of the polytypic *Limenitis arthemis* species complex represent an evolutionary loss of Batesian mimicry, and a reversion to the ancestral phenotype. Here, we evaluate this conclusion using segregating amplified fragment length polymorphism markers to investigate the history and fate of mimicry among forms of the *L. arthemis* complex and closely related Nearctic *Limenitis* species. In contrast to the previous finding, our results support a single origin of mimicry within the *L. arthemis* complex and the retention of the ancestral white-banded form in non-mimetic populations. Our finding is based on a genome-wide sampling approach to phylogeny reconstruction that highlights the challenges associated with inferring the evolutionary relationships among recently diverged species or populations (i.e. incomplete lineage sorting, introgressive hybridization and/or selection).

**Keywords:** wing pattern evolution; mimicry; amplified fragment length polymorphism; *Limenitis*; phylogeny; gene flow

## 1. INTRODUCTION

Batesian mimicry is a classic example of adaptation because the mimic gains a direct fitness advantage (e.g. reduced predation) due to its phenotypic resemblance to the protected model. Although the relationship between a Batesian mimic and its model can vary both temporally (Waldbauer 1988) and spatially (Ritland & Brower 2000; Ries & Mullen 2008), in the simplest iteration, the fitness of a palatable Batesian mimic is dependent on the frequency of a chemically defended, and typically aposematic, model (Bates 1862; Fisher 1930; reviewed by Mallet & Joron 1999).

Under frequency-dependent selection (Fisher 1930; Ayala & Campbell 1974), protection from predation for Batesian mimics is expected to break down (i) in the absence of the model (Wallace 1870; Waldbauer & Sternburg 1987; Waldbauer 1988; Pfennig *et al.* 2001, 2007) or (ii) when the mimic becomes abundant relative to the model (Fisher 1930; Brower & Brower 1962; Huheey 1964; Oaten *et al.* 1975; Getty 1985; Mallet & Joron 1999; Harper & Pfennig 2007). Although numerous laboratory (Brower 1960; Nonacs 1985; Lindström *et al.* 2004; Rowland *et al.* 2007) and theoretical (Huheey 1976, 1988; Ruxton *et al.* 2004; Mappes *et al.* 2005) studies support these predictions, empirical examples of mimetic breakdown in natural populations are relatively rare (but see Harper & Pfennig 2007, 2008; Joron 2008).

One putative example of mimetic breakdown is represented by the phenotypic transition zone between mimetic and non-mimetic admiral butterfly populations, which comprise the polytypic *Limenitis arthemis* complex. White-banded populations of these butterflies (*Limenitis arthemis arthemis* and *Limenitis arthemis rubrofasciata*) range widely from northeastern to northwestern North America, as far west as Alaska (Scott 1986), and closely resemble the ancestral wing pattern phenotype characteristic of the entire genus (Mullen 2006). By contrast, red-spotted purples (*Limenitis arthemis arizonensis* and *Limenitis arthemis astyanax*) are allopatrically distributed in southwestern North America and the southeastern United States, and are well-known Batesian mimics of the chemically defended Pipevine swallowtail (*Battus philenor*; Brower & Brower 1962; Platt *et al.* 1971; Platt 1975; Mullen 2006). A recent study on the evolutionary relationships among the North American representatives of this genus has tested the hypothesis that an adaptive Batesian mimicry phenotype was lost in white-banded populations of the *L. arthemis* species complex (Prudic & Oliver 2008). The results of this study posit that selection for mimicry breaks down outside the range of the model (*Battus*), and consequently that white-banded populations of *L. arthemis* are the result of an evolutionary reversal by loss of the mimetic phenotype (figure 1).

If true, this finding represents the only documented example (in butterflies) of a reversion to the ancestral phenotype because of selection *against* mimicry. Although several recent studies have found evidence for a

\* Author for correspondence (wksavage@lehigh.edu).

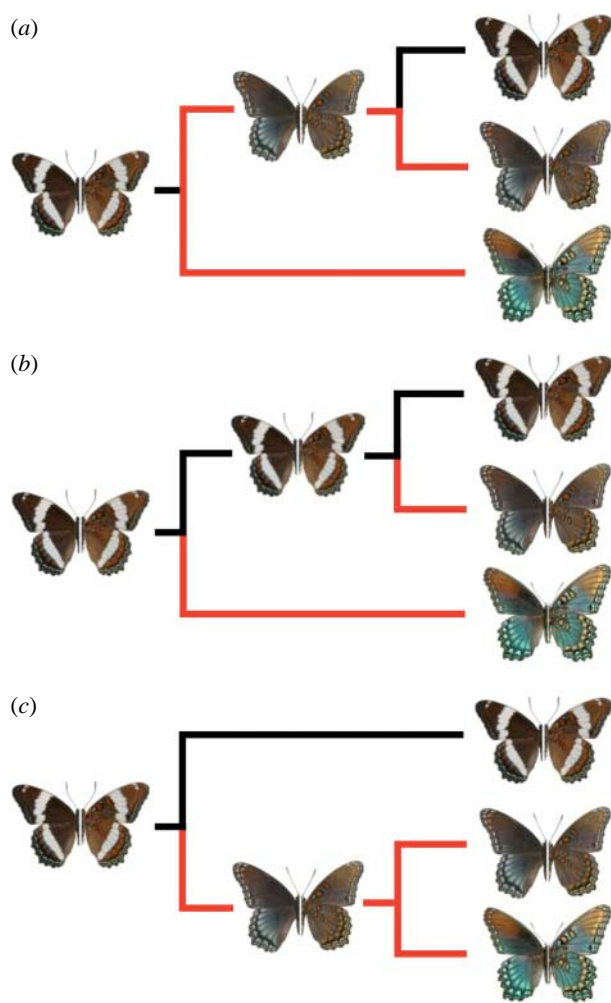


Figure 1. Addressing the gain/loss of mimicry based on parsimony inference of a single locus leaves several alternative explanations that are equally likely. (a) The first scenario demonstrates the reversion to the ancestral white-banded wing pattern proposed by Prudic & Oliver (2008), which does not address the two mimetic forms in the complex demonstrated in (b) the second scenario. In this case, two independent origins of mimicry with no reversal in the white-banded form is an equally parsimonious scenario to (a). (c) The third scenario is the only topology that resolves both the origin of mimicry and the maintenance of the ancestral wing pattern. Resolving which of these scenarios are supported by genetic data will provide important conclusions to inferring the evolution of mimicry in the *L. arthemis* complex. For each of the above scenarios, the terminal taxa are *L. a. arthemis*, *L. a. astyanax* and *L. a. arizonensis*. Red branches indicate adaptive mimicry evolution.

breakdown of selection favouring Batesian mimicry phenotypes in allopatry (with respect to the model; Harper & Pfennig 2007, 2008; Pfennig *et al.* 2007), none of these studies explicitly addressed the origin of the allopatric mimetic populations. Allopatric Batesian mimics could arise via several mechanisms, including range expansion of the mimic, range contraction of the model or gene flow into non-mimetic allopatric populations. Thus, although there is clear evidence that selection for mimicry breaks down outside of the range of the *Battus* model in this system (Prudic & Oliver 2008; Ries & Mullen 2008), there are several reasons to question the conclusion that the absence of mimicry in white-banded populations represents an evolutionary loss of mimicry.

First, if the absence of mimicry in white-banded populations of *L. a. arthemis* was the result of population expansion to regions outside the range of the model, then there is no *a priori* reason to expect evidence of genetic differentiation between mimetic and non-mimetic populations except for those regions of the genome controlling wing pattern. This is not the case because significant population structure corresponding to wing pattern phenotypes exists in this complex (Mullen 2006; Mullen *et al.* 2008; Prudic & Oliver 2008), consistent with the hypothesis that the hybrid zone between mimetic (*L. a. astyanax*) and non-mimetic (*L. a. arthemis*) subspecies is the result of secondary contact between two evolutionarily distinct lineages that diverged in allopatry (Mullen *et al.* 2008). Second, significant levels of both contemporary and historical gene flow have occurred between these two wing pattern forms, indicating that, although partially isolated, for many loci individual gene tree topologies will not accurately represent the true 'species' history (Pamilo & Nei 1988; Maddison 1997; Nichols 2001). Third, despite its wide use in phylogeny reconstruction, mitochondrial DNA (mtDNA) is notoriously unreliable when used to infer the history of a phenotypic trait among recently diverged hybridizing taxa (Ballard & Whitlock 2004). Finally, the conclusion that mimicry was lost in this complex was largely based on (i) a species tree estimated from mtDNA, and (ii) the assumption that no mtDNA introgression occurs between mimetic and non-mimetic phenotypes (figure 1a; Prudic & Oliver 2008). However, an equally parsimonious interpretation is that mimicry evolved independently to give rise to both mimetic subspecies in this complex and was not lost in *L. a. arthemis* (figure 1b). Alternatively, it is possible that mimicry evolved once and the mimetic lineages diverged in allopatry (figure 1c).

Given that inferences about wing pattern evolution in this group so directly hinge upon a robust reconstruction of the relationships among the three subspecies, we used amplified fragment length polymorphism (AFLP) markers to assay genetic variation at a genome-wide scale. AFLP fingerprinting often provides resolution in molecular phylogenies where nucleotide sequence data fail to recover relationships in recently evolved taxa (Sullivan *et al.* 2004; Bonin *et al.* 2005; Mendelson & Shaw 2005; Mendelson *et al.* 2005; Althoff *et al.* 2007; Meudt & Clarke 2007; Holland *et al.* 2008). Here, we present a phylogeny of the North American species of *Limenitis*, which indicates that mimicry evolved once in the *L. arthemis* complex, and that the hybrid zone between mimetic and non-mimetic populations is the result of secondary contact between two distinct lineages, rather than the loss of mimicry due to range expansion and primary differentiation.

## 2. MATERIAL AND METHODS

### (a) Taxon sampling

Because the species-level phylogeny of this genus has previously been published (Mullen 2006), this study focused specifically on the unresolved relationships among mimetic and white-banded phenotypes within the polytypic *L. arthemis* species complex. Previous work also indicates ongoing gene flow among subspecies within this group (Mullen *et al.* 2008), so we restricted our phylogenetic sampling of the two eastern subspecies (*L. a. arthemis* and

Table 1. Specimen information for eight wing pattern races of *Limenitis* examined in this study. (Listed by taxon is the sample size (*N*), collection locality, collection date, phenotype and the pattern of mimicry (with the model species).)

species	<i>N</i>	locality	date	wing pattern	mimicry pattern
<i>Limenitis arthemis</i> group					
<i>L. a. arizonensis</i>	3	Arizona, USA	2001	black	Batesian ( <i>Battus philenor</i> )
<i>L. a. arthemis</i>	4	New Brunswick, CA	2004	white-banded	non-mimetic
<i>L. a. astyanax</i>	4	Mississippi, USA	2001	black	Batesian ( <i>Battus philenor</i> )
<i>Limenitis lorquini</i>	4	British Columbia, CA	2002	white-banded, orange tip	Batesian ( <i>Adelphia</i> )
<i>Limenitis weidemeyerii</i>	3	South Dakota, USA	2003	white-banded	non-mimetic
<i>Limenitis archippus lahontani</i>	4	Nebraska, USA	2003	orange	Müllerian ( <i>Danaus</i> )
<i>Limenitis populi</i>	2	Sweden	2002	white-banded	non-mimetic
<i>Limenitis reducta</i>	2	France	2002	white-banded	non-mimetic

Table 2. Summary statistics derived from eight AFLP primer pair combinations for eight *Limenitis* species. (The number of fragments and the genetic differentiation estimates ( $F_{ST}$ ) are listed along with the percentage of polymorphic bands for each primer pair combination. The percentage of polymorphic bands is also listed for each taxon at each primer pair combination.)

	5-FAM-5'				NED-5'				total
	ACT-CTC	ACT-CTG	ACA-CTC	ACA-CTG	AAC-CAA	AAC-CAT	AAC-CTA	AAC-CTT	
<i>EcoRI-MseI</i> primers									
number of fragments	342	277	332	314	214	231	236	316	2262
$F_{ST}$	0.12	0.15	0.37	0.19	0.22	0.22	0.30	0.23	0.18
percent polymorphic	86.3	79.1	86.4	90.1	81.3	77.1	80.1	79.1	82.9
<i>Limenitis arthemis</i> group									
<i>L. a. arizonensis</i>	28.4	26.4	21.1	30.6	30.4	22.5	34.3	25.0	27.1
<i>L. a. arthemis</i>	25.7	23.8	21.5	28.3	22.4	23.4	30.1	28.8	25.6
<i>L. a. astyanax</i>	25.7	21.3	25.1	28.7	28.5	29.0	29.7	23.7	26.2
<i>Limenitis lorquini</i>	35.7	26.7	34.4	33.8	15.9	28.1	29.7	30.7	30.2
<i>Limenitis weidemeyerii</i>	20.8	17.3	15.4	26.1	16.8	18.6	24.2	21.2	20.1
<i>Limenitis archippus lahontani</i>	21.6	21.3	39.3	32.2	22.9	16.9	21.6	21.5	25.3
<i>Limenitis populi</i>	17.0	12.3	15.1	18.5	18.2	24.2	25.8	16.5	18.0
<i>Limenitis reducta</i>	13.7	15.2	—	18.2	9.8	23.8	11.0	14.9	15.7

*L. a. astyanax*) to populations well outside the region where the two subspecies are known to hybridize (Mullen *et al.* 2008; Ries & Mullen 2008); the third subspecies, *L. a. arizonensis*, is reproductively isolated de facto due to its range allopatry.

In total, we included eight *Limenitis* taxa, three of which are contained in the *L. arthemis* complex. Each taxon is represented by at least two specimens from a single locality. The total dataset consists of 24 *Limenitis* specimens, out of which 20 represent six North American wing pattern races (i.e. species and subspecies) and four Palaearctic representatives of admiral butterflies (*Limenitis*). The four Palaearctic specimens were used for Nearctic–Palaearctic out-group comparison/character polarization. Our geographical sampling and total sample sizes are restricted because three recent works have thoroughly established the weak geographical population structure of each wing pattern race, and that sampling large numbers of individuals from throughout the range does not improve resolution (Mullen 2006; Mullen *et al.* 2008; Prudic & Oliver 2008). In this study, we are conducting a genome-wide analysis of mimicry evolution, not of phylogeographic relationships. Specimens, locality information and wing pattern phenotypes are listed in table 1.

#### (b) AFLP methods

Genomic DNA was isolated from flight muscle tissue using a commercially available extraction kit (DNeasy Tissue

Kit, Qiagen). DNA concentrations were standardized by spectrophotometry for subsequent molecular applications. We used the AFLP Plant Mapping Kit (Applied Biosystems) and a standard protocol (Vos *et al.* 1995; Bonin *et al.* 2005) to genotype the 24 specimens with eight *EcoRI-MseI* selective primer combinations (table 2). The basic steps were performed as follows: (i) restriction enzyme digestion of genomic DNA and ligation with *EcoRI* and *MseI* adaptors, (ii) dilution of restriction–ligation products and preselective amplification with primers containing single nucleotide additions, and (iii) selective amplification with three nucleotide additions to the fluorescently labelled 5' oligonucleotides. The AFLP fragments were sized with ROX-500 (–250) standard and capillary electrophoresis on an ABI Prism 3730 DNA analyser (Applied Biosystems), and scored by the absence/presence of peaks using GENEMAPPER software v. 3.7 (Applied Biosystems). Fragments were scored according to GENEMAPPER settings suggested by Holland *et al.* (2008), using bin widths of 0.5 but raising the minimum relative fluorescent units value to 100 to exclude incorrectly assigned peaks that result from background noise and stutter. Fragments were scored in the 100–400 bp range; raising the minimum size threshold was recently suggested as a step that minimizes homoplasmy that is more prevalent in smaller fragments (Althoff *et al.* 2007).

**(c) Data analysis**

The presence-absence of peaks for each AFLP primer pair was converted into a binary data matrix (0 or 1) for all individuals. We used AFLP-SURV v. 1.0 (Vekemans 2002) to calculate genetic diversity and basic summary statistics for each of the eight taxa. Genetic diversity was estimated with Zhivotovsky's (1999) Bayesian method of computation with a non-uniform prior distribution.

We analysed the binary matrix with four different phylogenetic methods: tree reconstructions using distance; neighbor-joining (NJ); and parsimony were calculated in PAUP\* v. 4.0b10 (Swofford 2000); and Bayesian inference (BI) was performed using MRBAYES v. 3.1.2 (Ronquist & Huelsenbeck 2003). The restriction-site distance measure of Nei & Li (1979) was used for computing distance and NJ trees with the minimum evolution objective function. Clade support was evaluated by 5000 bootstrap pseudoreplicates for both distance (full heuristic search) and NJ methods, both under the Nei & Li (1979) distance measure. Parsimony estimation was performed under default assumptions and settings for the binary character state matrix, and clade support was also estimated with 5000 bootstrap pseudoreplicates.

BI was conducted assuming the F81-like restriction site model implemented in MRBAYES with the noabsencesites coding bias correction (Ronquist *et al.* 2005; Koopman *et al.* 2008). The two-state model implemented in MRBAYES understates the complex genetic processes of AFLP evolution, and thus is less likely than distance and NJ methods to provide accurate inference of phylogeny (Althoff *et al.* 2007; Luo *et al.* 2007; Holland *et al.* 2008). A new Bayesian model using AFLP marker lengths was recently described by Luo *et al.* (2007), but it has yet to overcome the computational challenges associated with large datasets (it takes weeks to analyse a small dataset; Luo *et al.* 2007; Koopman *et al.* 2008). The Dirichlet prior for the state frequencies set to (7.3, 1.0) to match the empirical (0-1) frequencies in the dataset. The Bayesian analysis was performed with two runs of  $2 \times 10^7$  generations each, with 10 independent chains per run, sampling frequency of 2000 generations and a burn in of 5000 samples. Burn in was determined by post-run evaluation of the potential scale reduction factor convergence diagnostic (Gelman & Rubin 1992) and stationarity of log-likelihoods. Clade credibility values are discussed where they conflict with bootstrap results.

**3. RESULTS****(a) AFLP variability**

The eight AFLP primer pairs yielded 1875 segregating fragments out of 2262 total amplified bands; for each per primer pair, the number of amplified fragments ranged from 214 to 342. The total number of polymorphic fragments per taxon ranged from a low of 356 in *Limenitis reducta* to a high of 682 in *Limenitis lorquini* (table 2). Total gene diversity for the complete taxon set was 0.133, and overall  $F_{ST}$  was 0.184. Nei's genetic distances ranged from 0.008 to 0.012 within the *L. arthemis* complex, with the maximum genetic distance between *Limenitis weidemeyerii* and *L. reducta* (0.063; data not shown). Primer pair and per taxon results of AFLP are summarized in table 2. The AFLP distances are, as expected, much greater between *Limenitis* taxa, but are similar in relative differences to the uncorrected  $p$ -distances for mitochondrial and nuclear sequence data between taxa (table 3; Mullen 2006).

**(b) AFLP phylogeny**

All four phylogenetic methods (distance, NJ, parsimony and BI) based on the AFLP data recovered the same general topology illustrated in figure 2. The major result of these methods indicates that (i) named *Limenitis* species and subspecies all form monophyletic lineages, and (ii) the two mimetic lineages of *L. arthemis* (*L. a. astyanax* and *L. a. arizonensis*) form a clade that is sister to the white-banded form (*L. a. arthemis*). For the first of these results, nodes are strongly supported under all four methods of phylogeny reconstruction with high bootstrap (distance, NJ and parsimony) and clade credibility values (BI). The second result is moderately supported under both distance-based methods, which are widely used methods with dominant data-like AFLPs (e.g. Mendelson *et al.* 2005), but poor support under the parsimony and BI analyses. However, both of these methods did recover the same topological relationship of the *L. arthemis* complex: (*L. a. arthemis* (*L. a. astyanax* and *L. a. arizonensis*)), and the weaker support is not unexpected given the poor fit of these two models for AFLP data (Althoff *et al.* 2007; Luo *et al.* 2007; Koopman *et al.* 2008) and the evidence for hybridization between the two eastern wing pattern races (Mullen *et al.* 2008).

Figure 2 illustrates the general AFLP-based topology of the *Limenitis* taxa sampled here, as well as the lack of resolution at deeper phylogenetic depths among *Limenitis* lineages. While distance and NJ methods resolved the same topologies and similar bootstrap support values among lineages within the Nearctic *Limenitis* group, none of the four phylogenetic methods resolved the Nearctic *Limenitis* as a monophyletic clade (yellow node, figure 2); however, this relationship is well supported by previous phylogenetic analyses that used mitochondrial and nuclear DNA sequence data (see Mullen 2006; Prudic & Oliver 2008). Similarly, our AFLP phylogeny did not resolve the relationship between the Palaearctic taxa (*Limenitis populi* and *L. reducta*) and the Nearctic *Limenitis* taxa, but previous work by Mullen (2006) and Prudic & Oliver (2008) suggests that *L. populi* shared an ancestor with the Nearctic species more recently than *L. reducta*. Among the Nearctic taxa, each species is recovered as a strongly supported monophyletic lineage, but the relationships among *L. lorquini*, *L. weidemeyerii*, *L. archippus* and *L. arthemis* were unresolved. This is consistent with previous efforts to reconstruct the relationships among the Nearctic taxa (Mullen 2006; Prudic & Oliver 2008), which each failed to strongly resolve the pattern of speciation in this group.

In general, nodes supported by distance and NJ bootstrap pseudoreplicates based on the AFLP data had lower support in parsimony and Bayesian methods. Specifically, these latter two methods both recovered the focal mimetic clade (*L. a. astyanax* and *L. a. arizonensis*), but with lower clade credibility and bootstrap values (0.64 and 56%, respectively) relative to the higher bootstrap support values obtained by distance and NJ analyses (86 and 77%, respectively). Thus, two phylogenetic methods recover moderate support for a mimetic clade of *L. arthemis*, and two provide weaker inference but still recover the same topology.

Table 3. Pairwise divergence values (uncorrected  $p$ -distances) for combined mitochondrial *cob1-cob2* sequences (below diagonal) and translation elongation factor 1 alpha (data from Mullen 2006). (Members of the *L. arthemis* complex are the first three taxa listed.)

taxon		1	2	3	4	5	6	7	8
1	<i>L. a. arizonensis</i>	—	0.2	0.2	0.4	0.7	0.4	1.4	2.3
2	<i>L. a. arthemis</i>	2.6	—	0.2	0.8	1.0	0.8	1.8	2.7
3	<i>L. a. astyanax</i>	1.8	2.6	—	0.8	1.0	0.8	1.8	2.7
4	<i>L. lorquini</i>	2.6	3.1	2.4	—	0.4	0.8	1.2	2.1
5	<i>L. weidemeyerii</i>	2.5	3.3	2.6	2.0	—	1.0	1.4	2.3
6	<i>L. archippus lahontani</i>	3.6	3.9	3.4	3.4	3.1	—	1.7	2.5
7	<i>L. populi</i>	8.3	8.5	8.3	8.1	7.7	8.2	—	1.9
8	<i>L. reducta</i>	8.6	8.9	8.8	8.2	8.2	8.6	10.9	—

#### 4. DISCUSSION

Recently, it has been proposed that the absence of mimicry among white-banded populations of *L. arthemis* represents the evolutionary loss of mimicry, which would be the first documented example of a reversion to an ancestral wing pattern phenotype (Prudic & Oliver 2008). If true, this conclusion has important implications for the maintenance of Batesian mimicry and the flexibility of the genome to respond to differential environmental pressures (i.e. selection). However, as Barton & Hewitt (1985) first pointed out, it is important to distinguish between the history of a phenotypic trait (e.g. mimicry) and the history of populations exhibiting that trait. Because the former will often be inaccessible due to the confounding effects of reticulate population histories and stochastic evolutionary and ecological processes, the resolution of how many times mimicry arose and/or was lost in the *L. arthemis* complex remains elusive.

The results of this study, based on a large dataset consisting of a genome-wide sampling of nuclear markers, indicate that the two mimetic (*L. a. arizonensis* and *L. a. astyanax*) subspecies in the *L. arthemis* complex are a monophyletic lineage, which is a sister group to the white-banded, non-mimetic subspecies (*L. a. arthemis*; figure 2). Thus, we find a singular origin of mimicry in the *L. arthemis* clade (*L. a. arizonensis* and *L. a. astyanax*) and retention of the ancestral white-banded phenotype (*L. a. arthemis*), where the model species (*B. philenor*) does not occur. However, consistent with previous attempts to reconstruct the phylogenetic relationships in this genus (Mullen 2006; Mullen *et al.* 2008; Prudic & Oliver 2008), we were unable to resolve the basal relationships among the Nearctic species (figure 2).

Although concerns about AFLPs have been raised, recent work indicates that homoplasy can be significantly reduced by implementing strict scoring parameters (Althoff *et al.* 2007; Holland *et al.* 2008). In the case of this study, the genome size of *Limenitis* is approximately 380 Mb (S. P. Mullen 2009, unpublished data), placing it well within the range where the average fragment homology is quite high across species (89%; Althoff *et al.* 2007). In addition, the advantage of employing a large number of genome-wide markers for phylogeny reconstruction is that it avoids many of the problems associated with single-locus inference among recently diverged taxa, as demonstrated by an increasing number of recent studies (Kardolus *et al.* 1998; Albertson *et al.* 1999; Baayen *et al.* 2000; Bakkeren *et al.* 2000; Ganter & Lopes 2000; Hodkinson *et al.* 2000;

van Raamsdonk *et al.* 2000; Giannasi *et al.* 2001; Parsons & Shaw 2001; Buntjer *et al.* 2002; Allender *et al.* 2003; Després *et al.* 2003; Brouat *et al.* 2004; Sullivan *et al.* 2004; Bonin *et al.* 2005; Mendelson & Shaw 2005; Mendelson *et al.* 2005). In fact, given the expectation that hybridization among mimetic and non-mimetic populations should lead to substantial introgression of neutral markers, the nodal support for a single origin of mimicry is surprisingly high, a result that is inconsistent with the conclusion that mimicry was lost in white-banded populations. Therefore, it is likely that incomplete lineage sorting and/or introgression (Funk & Omland 2003; Anderson *et al.* 2009) have confounded previous efforts to recover the history of wing pattern evolution in this group.

Although examples of mitochondrial introgression are common (e.g. Krosby & Rohwer 2009; Shaw 2002), an alternative interpretation of the mitochondrial data presented in Mullen (2006), Mullen *et al.* (2008) and Prudic & Oliver (2008) is that southeastern populations of mimetic *L. a. astyanax* historically came into contact with a lineage of white-banded *L. a. arthemis* in sympatry with *Battus*, causing a selective sweep of alleles underlying the mimetic phenotype. Results from this study and previous work (Mullen 2006; Mullen *et al.* 2008; Prudic & Oliver 2008) support the following history for wing pattern evolution in the *L. arthemis* complex. First, the ancestral white-banded form gave rise to a mimetic lineage because of strong selection in the range of the codistributed model, *B. philenor*. Pleistocene vicariance of the mimetic lineage gave rise to allopatric populations of mimetic *L. a. arizonensis* in southwestern North America and mimetic *L. a. astyanax* in the southeast. Subsequently, hybridization between white-banded and mimetic populations would have resulted in sympatric mimetic forms fixed for different mtDNA haplotypes (e.g. IB and II) because of selection for the mimicry phenotype in the range of the model (figure 3). Non-mimetic populations of *L. a. arthemis* could have persisted in northwestern refugia, corresponding to mitochondrial haplotype IA, and expanded their range to give rise to the current zone of secondary hybridization between the two wing pattern phenotypes. Indeed, a history of episodic contact between mimetic and non-mimetic lineages is highly consistent with both the findings presented by Mullen *et al.* (2008) and Prudic & Oliver (2008), and also explains how mimetic phenotypes at the range limit of the model often possess non-mimetic mitochondrial haplotypes, as well as the lack of genealogical exclusivity for each phenotype

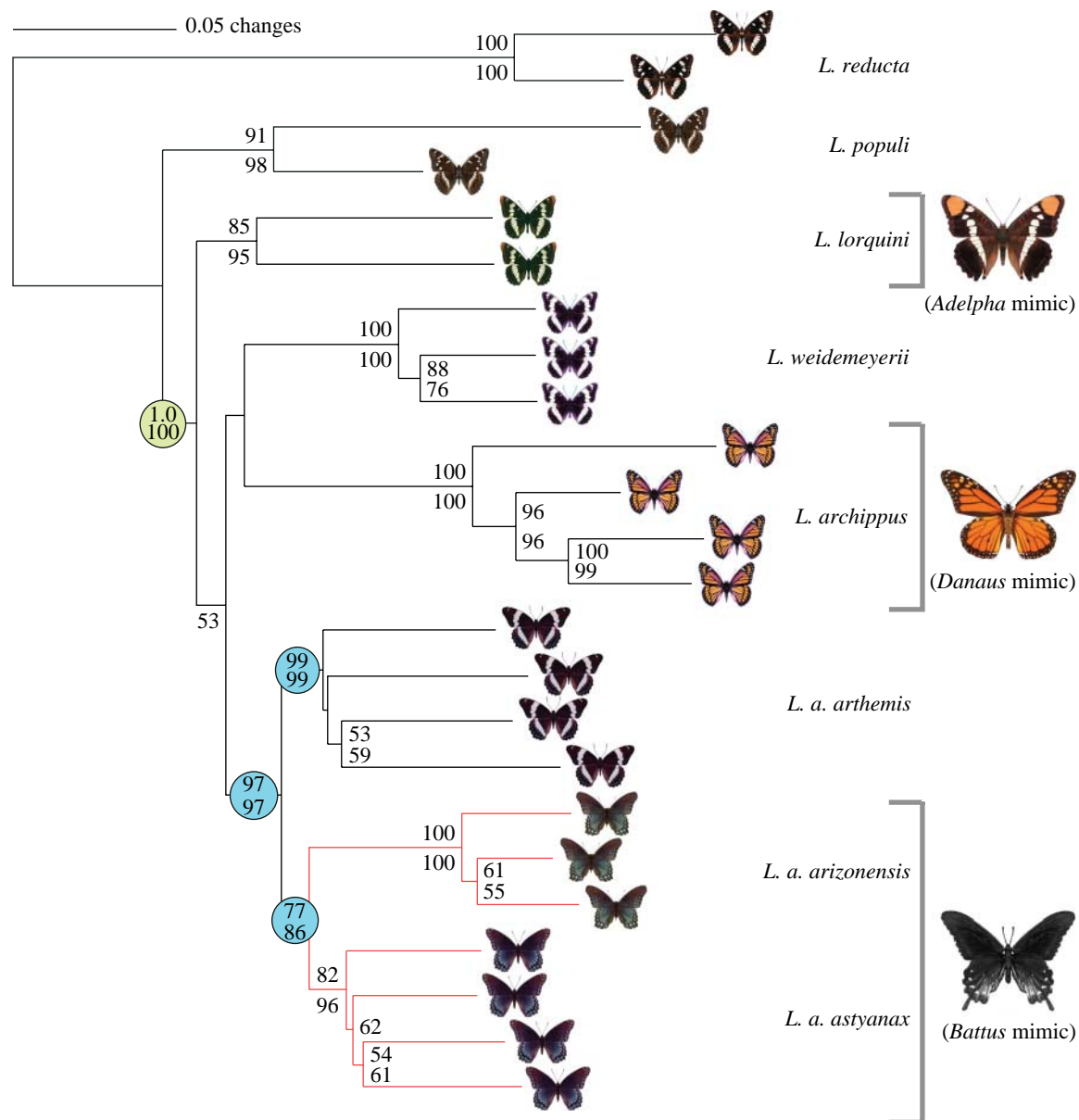


Figure 2. Inferred relationships for eight species of admiral butterflies (*Limenitis*) based on 2262 AFLP fragments. The white-banded wing pattern is the ancestral state (vertical black bar on right of tree), with mimetic wing patterns and the model (on right). Branch support values were derived from Nei & Li (1979) restriction-site distances for both NJ (above) and distance bootstrap indices (below). The monophyly of the Nearctic *Limenitis* is supported by analyses of nuclear and mitochondrial genes (yellow circle; Mullen 2006; Prudic & Oliver 2008); reported at this node are the Bayesian posterior probability and parsimony bootstrap values for this clade from both studies. *Limenitis arthemis* is a well-supported group (blue circles), with the white-banded race sister to a monophyletic clade that contains both of the mimetic forms of *L. arthemis* (red branches). The loss of mimicry is not supported, but in fact there is a monophyletic group of mimics (*L. a. arizonensis* and *L. a. astyanax*) that suggest mimicry evolved once in the mimetic *L. arthemis*.

and the highly asymmetrical gene flow between these two forms (*L. a. astyanax* → *L. a. arthemis*; Mullen *et al.* 2008). Ultimately, conclusive resolution of the history of wing pattern evolution in this group will almost certainly require identification of the specific locus/loci underlying the trait for mimicry.

#### (a) *The causes and consequences of allopatric Batesian mimicry*

Given the dramatic potential consequences of frequency-dependent selection on Batesian mimicry phenotypes with respect to both individual fitness and the potential origin of reproductive barriers among populations, it is essential

to understand the conditions under which mimicry is expected to evolve and be maintained. Here, we focused on elucidating the evolutionary origins and fate of a Batesian mimetic phenotype among hybridizing populations of the *L. arthemis* butterfly species complex, and provided compelling evidence against an evolutionary loss of mimicry by reversion to the ancestral phenotype in this system. Batesian mimicry is a classic example of adaptive phenotypic evolution but, as yet, we have only an incomplete understanding of the conditions that maintain or break down the mimetic relationship between palatable Batesian mimics and unpalatable models. This is particularly true with respect to allopatric Batesian mimics that

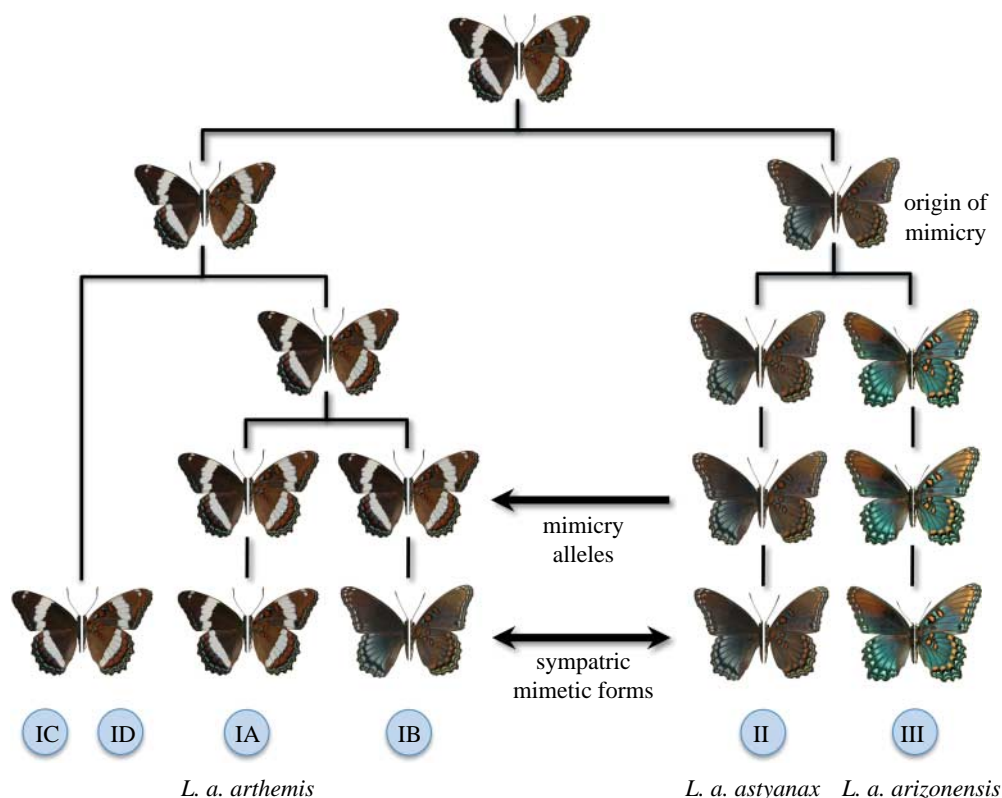


Figure 3. Scenario depicting the evolution of mimicry in the *L. arthemis* complex. A white-banded phenotype (ancestral state) gave rise to forms that evolved mimetic phenotypes when sympatric with a chemically defended species (*B. philenor*). Codistributed white-banded and mimetic phenotypes hybridized, resulting in the sweep of mimetic alleles into the white-banded form, and subsequently the capture of white-banded mitochondria (haplotype II) in mimetic forms that are sympatric with *L. a. astyanax* (haplotype II). Mimetic phenotypes are not recovered in other white-banded populations of *L. a. arthemis* (haplotypes IA, IC and ID). *Limenitis a. arizonensis* is allopatric with respect to *L. a. astyanax* and *L. a. arthemis*, and possesses an exclusive mtDNA haplotype (III). (Inferred from results in Mullen *et al.* 2008.)

exist outside the geographical range of their respective models (Fisher 1930; Harper & Pfennig 2007, 2008; Pfennig *et al.* 2007). Specifically, it is unclear to what extent allopatric mimicry exists across diverse taxonomic groups, and how often allopatric mimics arise as a result of range expansion, range contraction of the model and/or through gene flow into non-mimetic populations.

The results of this study mean that we still have no unequivocal example of the loss of mimicry in natural populations (but see Harper & Pfennig 2008; reviewed in Joron 2008), and although it may well occur, results from a comprehensive analysis of mimicry evolution in *Papilio* butterflies strongly indicate otherwise (Kunte *in review*). Our results also caution against inferring character evolution using gene trees for recently diverged taxa, which are unlikely to resolve the true species phylogeny. Although recent advances in Bayesian and coalescent methodologies have improved our ability to recover phylogenetic relationships among closely related taxa with incongruent gene trees (Edwards & Beerli 2000; Maddison & Knowles 2006; Edwards *et al.* 2007; Liu & Pearl 2007), it is still true that gene trees and species trees are often not the same (Pamilo & Nei 1988; Maddison 1997; Edwards & Beerli 2000; Nichols 2001).

We are grateful to B. Evans, S.-P. Quek and M. Kronforst for their technical and laboratory assistance. D. W. Pfennig, M. Kronforst, M. Hassel and three anonymous reviewers offered detailed comments and suggestions that greatly improved the manuscript. Part of this work was carried out by using the resources of the Computational Biology Service

Unit from Cornell University, which is partially funded by Microsoft Corporation.

## REFERENCES

- Albertson, R. C., Markert, J. A., Danley, P. D. & Kocher, T. D. 1999 Phylogeny of a rapidly evolving clade: the cichlid fishes of Lake Malawi, East Africa. *Proc. Natl Acad. Sci. USA* **96**, 5107–5110. (doi:10.1073/pnas.96.9.5107)
- Allender, C. J., Seehausen, O., Knight, M. E., Turner, G. F. & Maclean, N. 2003 Divergent selection during speciation of Lake Malawi cichlid fishes inferred from parallel radiations in nuptial coloration. *Proc. Natl Acad. Sci. USA* **100**, 14074–14079. (doi:10.1073/pnas.2332665100)
- Althoff, D. M., Gitzendanner, M. A. & Segraves, K. A. 2007 The utility of amplified fragment length polymorphisms in phylogenetics: a comparison of homology within and between genomes. *Syst. Biol.* **56**, 477–484. (doi:10.1080/10635150701427077)
- Anderson, T. M. *et al.* 2009 Molecular and evolutionary history of melanism in North American gray wolves. *Science* **323**, 1339–1343. (doi:10.1126/science.1165448)
- Ayala, F. J. & Campbell, C. A. 1974 Frequency-dependent selection. *Ann. Rev. Ecol. Syst.* **5**, 115–138. (doi:10.1146/annurev.es.05.110174.000555)
- Baayen, R. P., O'Donnell, K., Bonants, P. J. M., Cigelnik, E., Kroon, L., Roebroek, E. J. A. & Waalwijk, C. 2000 Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic formae speciales causing wilt and rot disease. *Phytopathology* **90**, 891–900. (doi:10.1094/PHYTO.2000.90.8.891)

- Bakkeren, G., Kronstad, J. W. & Levesque, C. A. 2000 Comparison of AFLP fingerprints and ITS sequences as phylogenetic markers in Ustilaginomycetes. *Mycologia* **92**, 510–521. (doi:10.2307/3761510)
- Ballard, J. W. O. & Whitlock, M. C. 2004 The incomplete natural history of mitochondria. *Mol. Ecol.* **13**, 729–743. (doi:10.1046/j.1365-294X.2003.02063.x)
- Barton, N. H. & Hewitt, G. M. 1985 Analysis of hybrid zones. *Ann. Rev. Ecol. Syst.* **16**, 113–148. (doi:10.1146/annurev.es.16.110185.000553)
- Bates, H. W. 1862 Contributions to an insect fauna of the Amazon valley (Lepidoptera: heliconidae). *Trans. Linn. Soc. Lond.* **23**, 495.
- Bonin, A., Pompanon, F. & Taberlet, P. 2005 Use of amplified fragment length polymorphism (AFLP) markers in surveys of vertebrate diversity. In *Methods in enzymology*, vol. 395 (eds E. A. Zimmer & E. H. Roalson), pp. 145–161. New York, NY: Academic Press. (doi:10.1016/S0076-6879(05)95010-6)
- Brouat, C., Mckey, D. & Douzery, E. J. P. 2004 Differentiation in a geographical mosaic of plants coevolving with ants: phylogeny of the *Leonardoxa africana* complex (Fabaceae: Caesalpinioideae) using amplified fragment length polymorphism markers. *Mol. Ecol.* **13**, 1157–1171. (doi:10.1111/j.1365-294X.2004.02113.x)
- Brower, J. V. Z. 1960 Experimental studies of mimicry. IV. The reactions of starlings to different proportions of models and mimics. *Am. Nat.* **94**, 271. (doi:10.1086/282128)
- Brower, L. P. & Brower, J. V. Z. 1962 The relative abundance of model and mimic butterflies in natural populations of the *Battus philenor* mimicry complex. *Ecology* **43**, 154–158.
- Buntjer, J. B., Otsen, M., Nijman, I. J., Kuiper, M. T. R. & Lenstra, J. A. 2002 Phylogeny of bovine species based on AFLP fingerprinting. *Heredity* **88**, 46–51. (doi:10.1038/sj.hdy.6800007)
- Després, L., Gielly, L., Redoutet, B. & Taberlet, P. 2003 Using AFLP to resolve phylogenetic relationships in a morphologically diversified plant species complex when nuclear and chloroplast sequences fail to reveal variability. *Mol. Phylogenet. Evol.* **27**, 185–196. (doi:10.1016/S1055-7903(02)00445-1)
- Edwards, S. V. & Beerli, P. 2000 Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* **54**, 1839–1854. (doi:10.1111/j.0014-3820.2000.tb01231.x)
- Edwards, S. V., Liu, L. & Pearl, D. K. 2007 High-resolution species trees without concatenation. *Proc. Natl Acad. Sci. USA* **104**, 5936–5941. (doi:10.1073/pnas.0607004104)
- Fisher, R. A. 1930 *The genetical theory of natural selection*. Oxford, UK: Clarendon Press.
- Funk, D. J. & Omland, K. E. 2003 Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annu. Rev. Ecol. Syst.* **34**, 397–423. (doi:10.1146/annurev.ecolsys.34.011802.132421)
- Ganter, P. F. & Lopes, M. D. 2000 The use of anonymous DNA markers in assessing worldwide relatedness in the yeast species *Pichia kluyveri* Bedford and Kudrjavzev. *Can. J. Microbiol.* **46**, 967–980. (doi:10.1139/cjm-46-11-967)
- Gelman, A. & Rubin, D. B. 1992 Inference from iterative simulation using multiple sequences. *Stat. Sci.* **7**, 457–472. (doi:10.1214/ss/1177011136)
- Getty, T. 1985 Discriminability and the sigmoid functional response: how optimal foragers could stabilize model–mimic complexes. *Am. Nat.* **125**, 239–256. (doi:10.1086/284339)
- Giannasi, N., Thorpe, R. S. & Malhotra, A. 2001 The use of amplified fragment length polymorphism in determining species trees at fine taxonomic levels: analysis of a medically important snake, *Trimeresurus albolabris*. *Mol. Ecol.* **10**, 419–426. (doi:10.1046/j.1365-294x.2001.01220.x)
- Harper, G. R. & Pfennig, D. W. 2007 Mimicry on the edge: why do mimics vary in resemblance to their model in different parts of their geographical range? *Proc. R. Soc. B* **274**, 1955–1961. (doi:10.1098/rspb.2007.0558)
- Harper, G. R. & Pfennig, D. W. 2008 Selection overrides gene flow to break down maladaptive mimicry. *Nature* **451**, 1103–1106. (doi:10.1038/nature06532)
- Hodkinson, T. R., Renvoize, S. A., Ni Chonghaile, G., Stapleton, C. M. A. & Chase, M. W. 2000 A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). *J. Plant Res.* **113**, 259–269. (doi:10.1007/PL00013936)
- Holland, B., Clarke, A. & Meudt, H. 2008 Optimizing automated AFLP scoring parameters to improve phylogenetic resolution. *Syst. Biol.* **57**, 347–366. (doi:10.1080/10635150802044037)
- Huheey, J. E. 1964 Studies of warning coloration and mimicry. IV. A mathematical model of model–mimic frequencies. *Ecology* **45**, 185–188. (doi:10.2307/1937125)
- Huheey, J. E. 1976 Studies in warning coloration and mimicry. VII. Evolutionary consequences of a Batesian–Müllerian spectrum: a model for Müllerian mimicry. *Evolution* **30**, 86–93. (doi:10.2307/2407675)
- Huheey, J. E. 1988 Mathematical models of mimicry. *Am. Nat.* **131**, S22. (doi:10.1086/284765)
- Joron, M. 2008 Batesian mimicry: can a leopard change its spots—and get them back? *Curr. Biol.* **18**, R476–R479. (doi:10.1016/j.cub.2008.04.009)
- Kardolus, J. P., Van Eck, H. J. & Van den Berg, R. G. 1998 The potential of AFLPs in biosystematics: a first application in *Solanum* taxonomy (Solanaceae). *Plant Syst. Evol.* **210**, 87–103. (doi:10.1007/BF00984729)
- Koopman, W. J. M. *et al.* 2008 AFLP markers as a tool to reconstruct complex relationships: a case study in *Rosa* (Rosaceae). *Am. J. Bot.* **95**, 353–366. (doi:10.3732/ajb.95.3.353)
- Krosby, M. & Rohwer, S. 2009 A 2000 km genetic wake yields evidence for northern glacial refugia and hybrid zone movement in a pair of songbirds. *Proc. R. Soc. B* **276**, 615–621. (doi:10.1098/rspb.2008.1310)
- Kunte, K. In review. The diversity and evolution of Batesian mimicry in *Papilio swallowtail* butterflies.
- Lindström, L., Alatalo, R. V., Lyytinen, A. & Mappes, J. 2004 The effect of alternative prey on the dynamics of imperfect Batesian and Müllerian mimics. *Evolution* **58**, 1294–1302. (doi:10.1554/03-271)
- Liu, L. & Pearl, D. K. 2007 Species trees from gene trees: reconstructing Bayesian posterior distributions of a species phylogeny using estimated gene tree distributions. *Syst. Biol.* **56**, 504–514. (doi:10.1080/10635150701429982)
- Luo, R., Hipp, A. L. & Larget, B. 2007 A Bayesian model of AFLP marker evolution and phylogenetic inference. *Stat. Appl. Genet. Mol. Biol.* **6**, 1–32. (doi:10.2202/1544-6115.1152)
- Maddison, W. P. 1997 Gene trees in species trees. *Syst. Biol.* **46**, 523–536. (doi:10.2307/2413694)
- Maddison, W. & Knowles, L. 2006 Inferring phylogeny despite incomplete lineage sorting. *Syst. Biol.* **55**, 21–30. (doi:10.1080/10635150500354928)
- Mallet, J. & Joron, M. 1999 Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. *Annu. Rev. Ecol. Syst.* **30**, 201–233. (doi:10.1146/annurev.ecolsys.30.1.201)

- Mappes, J., Marples, N. & Endler, J. A. 2005 The complex business of survival by aposematism. *Trends Ecol. Evol.* **20**, 598–603. (doi:10.1016/j.tree.2005.07.011)
- Mendelson, T. C. & Shaw, K. L. 2005 Rapid speciation in an arthropod: the likely force behind an explosion of new Hawaiian cricket species revealed. *Nature* **433**, 375–376. (doi:10.1038/433375a)
- Mendelson, T. C., Shaw, K. L., Elizabeth, A. Z. & Eric, H. R. 2005 Use of AFLP markers in surveys of arthropod diversity. In *Methods in Enzymology*, vol. 395 (eds E. A. Zimmer & E. Roalson), pp. 161–177. San Diego, CA: Academic Press. (doi:10.1016/S0076-6879(05)95011-8)
- Meudt, H. M. & Clarke, A. C. 2007 Almost forgotten or latest practice? AFLP applications, analyses and advances. *Trends Plant Sci.* **12**, 106–117. (doi:10.1016/j.tplants.2007.02.001)
- Mullen, S. P. 2006 Wing pattern evolution and the origins of mimicry among North American Admiral butterflies (Nymphalidae: *Limnitis*). *Mol. Phylogenet. Evol.* **39**, 747–758. (doi:10.1016/j.ympev.2006.01.021)
- Mullen, S. P., Dopman, E. B. & Harrison, R. G. 2008 Hybrid zone origins, species boundaries, and the evolution of wing-pattern diversity in a polytypic species complex of North American admiral butterflies (Nymphalidae: *Limnitis*). *Evolution* **62**, 1400–1417. (doi:10.1111/j.1558-5646.2008.00366.x)
- Nei, M. & Li, W. H. 1979 Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl Acad. Sci. USA* **76**, 5269–5273. (doi:10.1073/pnas.76.10.5269)
- Nichols, R. 2001 Gene trees and species trees are not the same. *Trends Ecol. Evol.* **16**, 358–364. (doi:10.1016/S0169-5347(01)02203-0)
- Nonacs, P. 1985 Foraging in a dynamic mimicry complex. *Am. Nat.* **126**, 165–180. (doi:10.1086/284407)
- Oaten, A., Pearce, C. E. M. & Smyth, M. E. B. 1975 Batesian mimicry and signal detection theory. *Bull. Math. Biol.* **37**, 367–387. (doi:10.1007/BF02459520)
- Pamilo, P. & Nei, M. 1988 Relationships between gene trees and species trees. *Mol. Biol. Evol.* **5**, 568–583.
- Parsons, Y. M. & Shaw, K. L. 2001 Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. *Mol. Ecol.* **10**, 1765–1772. (doi:10.1046/j.1365-294X.2001.01318.x)
- Pfennig, D. W., Harcombe, W. R. & Pfennig, K. S. 2001 Frequency-dependent Batesian mimicry. *Nature* **410**, 323–323. (doi:10.1038/35066628)
- Pfennig, D., Harper, G., Brumo, A., Harcombe, W. & Pfennig, K. 2007 Population differences in predation on Batesian mimics in allopatry with their model: selection against mimics is strongest when they are common. *Behav. Ecol. Sociol.* **61**, 505–511. (doi:10.1007/s00265-006-0278-x)
- Platt, A. P. 1975 Monomorphic mimicry in Nearctic *Limnitis* butterflies: experimental hybridization of the *L. arthemis-astyanax* complex with *L. archippus*. *Evolution* **29**, 120–141. (doi:10.2307/2407146)
- Platt, A. P., Coppinger, R. P. & Brower, L. P. 1971 Demonstration of the selective advantage of mimetic *Limnitis* butterflies presented to caged avian predators. *Evolution* **25**, 692–701. (doi:10.2307/2406950)
- Prudic, K. L. & Oliver, J. C. 2008 Once a Batesian mimic, not always a Batesian mimic: mimic reverts back to ancestral phenotype when the model is absent. *Proc. R. Soc. B* **275**, 1125–1132. (doi:10.1098/rspb.2007.1766)
- Ries, L. & Mullen, S. P. 2008 A rare model limits the distribution of its more common mimic: a twist on frequency-dependent Batesian mimicry. *Evolution* **62**, 1798–1803. (doi:10.1111/j.1558-5646.2008.00401.x)
- Ritland, D. B. & Brower, L. P. 2000 Mimicry-related variation in wing color of viceroy butterflies (*Limnitis archippus*): a test of the model-switching hypothesis (Lepidoptera: Nymphalidae). *Hol. Lepid.* **7**, 5–11.
- Ronquist, F. & Huelsenbeck, J. P. 2003 MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. (doi:10.1093/bioinformatics/btg180)
- Ronquist, F., Huelsenbeck, J. P. & Van der Mark, P. 2005 *MRBAYES 3.1 manual*. Tallahassee, FL: School of Computational Science, Florida State University.
- Rowland, H. M., Ihalainen, E., Lindstrom, L., Mappes, J. & Speed, M. P. 2007 Co-mimics have a mutualistic relationship despite unequal defences. *Nature* **448**, 64–67. (doi:10.1038/nature05899)
- Ruxton, G. D., Sherratt, T. N. & Speed, M. P. 2004 *Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry*. Oxford, UK: Oxford University Press.
- Scott, J. A. 1986 *The butterflies of North America: a natural history and field guide*. Stanford, CA: Stanford University Press.
- Shaw, K. L. 2002 Conflict between nuclear and mitochondrial DNA phylogenies of a recent radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. *Proc. Natl Acad. Sci. USA* **99**, 16 122–16 127. (doi:10.1073/pnas.242585899)
- Sullivan, J. P., Lavoué, S., Arnegard, M. E. & Hopkins, C. D. 2004 AFLPs resolve phylogeny and reveal mitochondrial introgression within a species flock of African electric fish (Mormyroidea: Teleostei). *Evolution* **58**, 825–841. (doi:10.1554/03-313)
- Swofford, D. 2000 *Phylogenetic analysis using parsimony (PAUP 4.0\* b10)*. Sunderland, MA: Sinauer Associates.
- Van Raamsdonk, L. W. D., Van Ginkel, M. V. & Kik, C. 2000 Phylogeny reconstruction and hybrid analysis in *Allium* subgenus *Rhizirideum*. *Theor. Appl. Genet.* **100**, 1000–1009. (doi:10.1007/s001220051381)
- Vekemans, X. 2002 AFLP-SURV version 1.0. Distributed by the author, Laboratoire Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.
- Vos, P. *et al.* 1995 AFLP: a new technique for DNA fingerprinting. *Nucleic. Acids Res.* **23**, 4407–4414. (doi:10.1093/nar/23.21.4407)
- Waldbauer, G. P. 1988 Asynchrony between Batesian mimics and their models. *Am. Nat.* **131**, S103. (doi:10.1086/284768)
- Waldbauer, G. P. & Sternburg, J. G. 1987 Experimental field demonstration that two aposematic butterfly color patterns do not confer protection against birds in Northern Michigan. *Am. Mid. Nat.* **118**, 145–152. (doi:10.2307/2425637)
- Wallace, A. R. 1870 *Contributions to the theory of natural selection*. London, UK: MacMillan Press.
- Zhivotovsky, L. A. 1999 Estimating population structure in diploids with multilocus dominant DNA markers. *Mol. Ecol.* **8**, 907–913. (doi:10.1046/j.1365-294x.1999.00620.x)