CONTEMPORARY PATTERNS IN A HISTORICAL CONTEXT: PHYLOGEOGRAPHIC HISTORY OF THE PIPEVINE SWALLOWTAIL, *BATTUS PHILENOR* (PAPILIONIDAE)

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Abstract.—We examined mitochondrial DNA (mtDNA) variation in pipevine swallowtail butterflies (*Battus philenor*) from throughout its extant range to provide a historical, phylogeographical context for ecological studies of the disjunct population in California. We evaluate current hypotheses regarding host plant use, behavior, and mimetic relationships of *B. philenor* populations and generate alternative hypotheses. Compared to populations throughout the rest of the species' range, California populations are ecologically distinct in that they lack mimics, lay significantly larger clutches of eggs, and exclusively use a unique, endemic larval host plant. Analysis of molecular variance, tests of population differentiation, and nested clade analysis of mtDNA variation indicate that, despite low levels of population genetic structure across the species' range, there is evidence of recent range expansion from presumed Pleistocene refuge(s) in southeastern North America. Colonization of California appears to have been a recent event. This phylogeographic investigation also suggests that the evolution of life-history adaptations to a novel larval host has occurred rapidly in California and the lack of mimics in California may be attributable to the recency of colonization.

Key words.—*Battus philenor*, geographic variation, mimicry, phylogeography, pipevine swallowtail, plant-insect interactions, range expansion.

Phylogeographic studies provide ecologists and evolutionary biologists with a historical context in which observed patterns in nature can be placed (Avise 2000). Understanding the temporal component of a species' distribution can be used to explain ecological and phenotypic discontinuities observed across the extant range of a species (Brown et al. 1996; Groman and Pellmyr 2000; Althoff and Thompson 2001; Althoff et al. 2001). Such studies can also reveal extensive periods of allopatry among populations that, until now, were not recognizable (Taylor et al. 1998; Omland et al. 2000). Investigations concerning ongoing ecological processes that lead to differentiation among populations benefit by placing them in such a historical context because different histories can lead to different adaptive strategies, including behavioral, physiological, and life history traits (Althoff and Thompson 1999; Després and Jaeger 1999). Additionally, the magnitude of recent range changes is essential knowledge for investigators employing methodologies that are sensitive to range changes, yet use current distributions to test evolutionary hypotheses (Barraclough and Vogler 2000).

This study attempts to broadly describe the phylogeographic history of the pipevine swallowtail, *Battus philenor* (Papilionidae), throughout its current range. *Battus philenor* is the northernmost member of this Neotropical genus, extending throughout the southeastern portion of North America, as far south as Honduras, and disjunctly in California (Fig. 1) (Scott 1986; Tyler et al. 1994). Although at least five subspecies have been described (Racheli and Pariset 1992; Tyler et al. 1994), based largely on locality, *B. philenor* shows remarkably little phenotypic variation throughout its range (Scott 1986). All members of the genus *Battus* are monophagous, feeding only on plants in the genus *Aristolochia* (Aristolochiaceae) as larvae (Racheli and Pariset 1992). This is in contrast to some other swallowtail genera, such as *Papilio*, which use a number of plant families and where host shifts

have been documented (Shapiro and Masuda 1980; Thompson 1995). Consequently, the range of the pipevine swallowtail is constrained by the range of its host plants, most of which are restricted to tropical or subtropical regions (Pfeifer 1966, 1970). *Aristolochia* species contain toxic alkaloids called aristolochic acids, which *B. philenor* sequester as larvae causing the larvae and adults to be chemically protected from many predators (Rothschild et al. 1970; Fordyce 2000, 2001; Sime et al. 2000; Sime 2002). The unpalatability of *B. philenor* larvae and adults is advertised through aposematic coloration. Larvae are covered with red spines contrasting with a black or dull-red background and adults are mostly black with orange spots on the underside of the hindwing, making them exceedingly conspicuous. As a result, throughout most of its range, *B. philenor* is involved in mimicry complexes with more palatable species of butterflies, including *Papilio polyxenes*, *P. troilus*, *P. glaucus*, *Speyeria diana*, and *Limenitis arthemis* (Poulton 1909; Brower and Brower 1962; Platt et al. 1971; Jeffords et al. 1979; Racheli and Pariset 1992). Indeed, *B. philenor* was a seminal component of Brower's (1958) early investigations into the effectiveness of mimicry. Additionally, *B. philenor* has been used as a model system by numerous investigators studying, for example, female oviposition behavior (Rausher 1981, 1983; Rausher and Papaj 1983; Papaj 1986; Papaj and Rausher 1987; Rausher and Odendaal 1987; Pilson and Rausher 1988; Tatar 1991), larval ecology (Rausher 1980, 1981; Stamp 1986; Fordyce and Agrawal 2001), pupal color polymorphisms (Sims and Shapiro 1983a, b), phenology (Sims and Shapiro 1983c, d), and chemical ecology (Rothschild et al. 1970; Papaj et al. 1992; Sime et al. 2000; Sime 2002; Fordyce 2000, 2001). Although *B. philenor* is well studied, few investigations have examined geographical variation in the phenomena under study (Papaj 1986; Pilson and Rausher 1988;
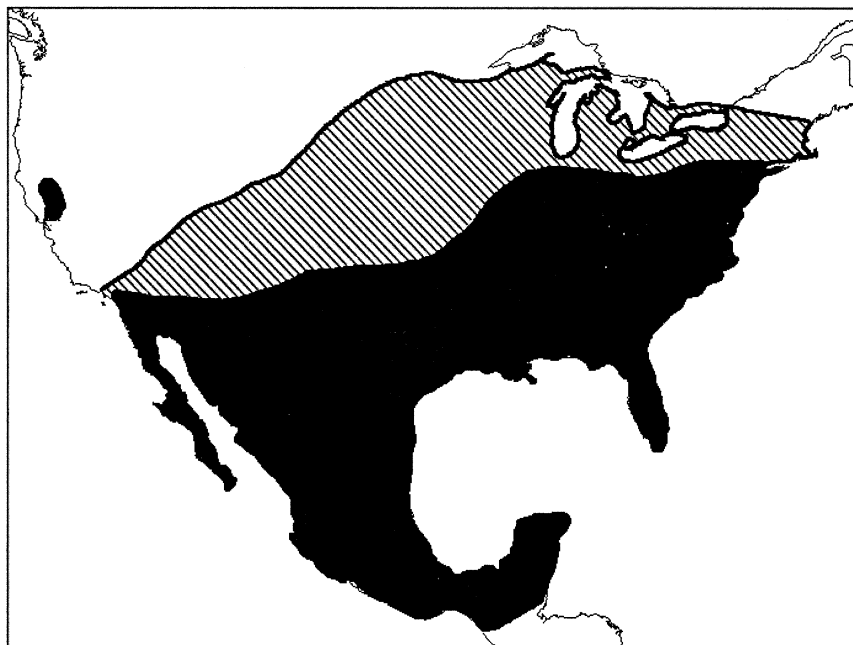
FIG. 1. Range of *Battus philenor*. Solid areas designate permanent range. Diagonal lines designate occasional or nonresident records.

Fordyce 2000) and none have done so with the benefit that a priori knowledge of phylogeographic history provides.

This study was largely motivated by a desire to understand the history of the disjunct population in California. The California population is nominally a distinct subspecies, *B. philenor hirsuta*, hereafter referred to as the California population. A number of factors concerning the ecology of the California population make such an investigation relevant. First, despite its occurring at extremely high densities, *B. philenor* has no mimics in California (Shapiro 1984). It has previously been shown that the California population is at least as toxic as another population involved in mimicry relationships (Fordyce 2000). The absence of mimics may reflect the recent immigration of *B. philenor* to California and thus insufficient time for other butterflies to evolve mimetic

relationships with this model. Alternatively, *B. philenor* may have been associated with the California fauna for a long period of time, implying that the butterflies that occur in sympatry with *B. philenor* in California are genetically or ecologically constrained from evolving such a mimetic relationship. Second, California *B. philenor* females lay significantly larger clutches of eggs compared to females in populations observed elsewhere, and in California larvae benefit by feeding in large aggregations (Fordyce and Agrawal 2001, Fordyce and Shapiro 2003). Third, only one host plant is available to *B. philenor* in California, *Aristolochia californica*, which is endemic to the Central Valley and surrounding foothills of the Coast Range and Sierra Nevada. This plant is believed to be an ancient member of the California flora, and could represent a relict of either the Madrean-Tethyan sclerophyllous vegetation or the Neotropical-Tertiary flora as described by Axelrod (1975). If the large clutch size observed for California *B. philenor* has evolved as an adaptive strategy to exploit this host plant, two hypotheses can describe the evolution of this life-history trait. Either large clutch size reflects a long association with *A. californica*, or *B. philenor* is a relatively recent immigrant to California and modification in clutch size in response to a novel host plant can occur rapidly. This study will place the lack of mimics and large clutch size observed in California in a historical context, and provide a description of post-Pleistocene history of this northernmost member of a largely tropical genus.

MATERIAL AND METHODS

Sampling

A total of 73 *Battus philenor* individuals were obtained from 17 populations to provide a broad geographic sample (Table 1, Fig. 2). All butterflies were frozen and stored at

TABLE 1. Sample localities, sample size, and haplotypes detected.

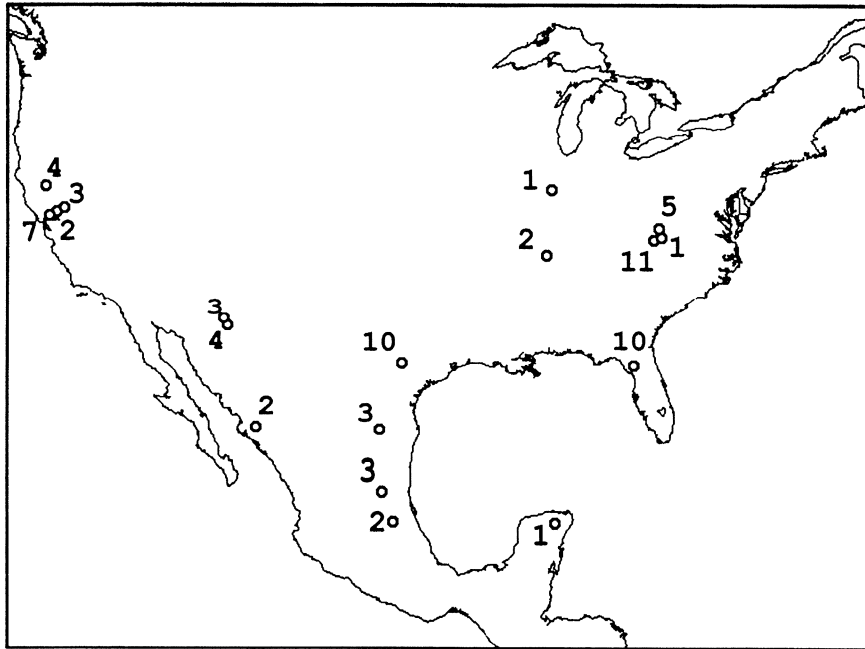| Location	n	Haplotype
Marion Co., Florida	10	A, B, H, K, L, M
Giles Co., Virginia	11	A, G, H, J, N, P, Q
Bedford Co., Virginia	1	Q
Greenbrier Co., West Virginia	5	A, F, I
Marshall Co., Illinois	1	Q
Shelby Co., Tennessee	2	A, J
Hays Co., Texas	10	A, C, J, M, R, S, T
Pima Co., Arizona	4	A, J, S
Maricopa Co., Arizona	3	A, E
Solano Co., California	7	A, B
Shasta Co., California	4	A
Sacramento Co., California	3	A, C
Yolo Co., California	2	A
Sinaloa, Mexico	2	A, D
Nuevo Leon, Mexico	3	A
San Luis Potosi, Mexico	3	A
Hidalgo, Mexico	2	A
Yucatan, Mexico	1	A

FIG. 2. Sampling locations of *Battus philenor*. Numbers indicate number of individuals from each sampling location.

−80°C, except the Mexico samples, all of which were papered museum specimens. One previously published *B. philenor* sequence from a Virginia individual (Caterino et al. 2001) was also included in this study (GenBank accession no. AF170875).

mtDNA Methods

Total genomic DNA was extracted from the thorax of each individual using either chloroform-phenol (Hillis et al. 1996) or DNAzol Genomic DNA Isolation Reagent (Molecular Research Center, Inc., Cincinnati, OH) (Chomczynski et al. 1997). Following the drying of the precipitated DNA from each extract, DNA was returned to solution with 100–300 μl of H_2O. These solutions were used in polymerase chain reactions (PCR) employing the primers EVA (5′ GAG ACC ATT ACT TGC TTT CAG TCA TCT 3′) and STROM (5′ TAA TTT GAA CTA TYT TAC CNG CA 3′), yielding a ≈ 470 bp polymerase chain reaction product of the mtDNA cytochrome oxidase subunit II (COII) region (Caterino and Sperling 1999). Amplifications were carried out using the following thermocycler protocol: 94°C for 2 min; 35 to 40 cycles of 94°C for 1 min; 45° to 56°C for 45 sec; 72°C for 1.5 min; and a final elongation step at 72°C for 10 min. This portion of the COII region of the mtDNA genome is homologous to approximately position 3289 through 3782 of the *Drosophila yakuba* reference sequence (Clary and Wolstenholme 1985). Each amplified PCR product was sequenced in both directions on an ABI 377 automated sequencer at the DNA sequencing facility, Division of Biological Sciences, University of California, Davis. From these sequences, 422 bp could be reliably read for all specimens. Sequences were aligned by eye and by using the SeqEd (vers. 1.0.3, Applied Biosystems, Foster City,CA) computer program.

Analysis

Two strategies were employed to infer the phylogeographic history of *B. philenor* from mtDNA variation. Nested clade analysis was used to separate historical events from contemporary processes (Templeton et al. 1995, Templeton 1998) and analysis of molecular variance (AMOVA) was used to evaluate the amount of genetic variation explained by partitioning this variation among broadly defined populations and subspecific designations (Excoffier et al. 1992).

Nested clade analysis tests the null hypothesis of no geographical association among haplotypes. Two distance measures are calculated for each clade at each nesting level. The clade distance, D_C, is the average distance of that clade's haplotypes from their geographical center. The nested clade distance, D_N, is the average distance of a given clade from the geographical center of all other clades within a nesting group (Templeton et al. 1995). Random permutations of the haplotypes among localities are used to measure the distribution of these distances under the null hypothesis of no geographical association. Analyses of significantly large or small D_C or D_N values are used to distinguish among various models of population structure and historical events following the most recent version (24 October 2001) of Templeton's (1998) inference key (available at http://InBio.byu.edu/Faculty/kac/crandall_lab/geodis.htm). To implement nested clade analysis, the computer program TCS (Clement et al. 2000) was used to estimate the haplotype network using the method of Templeton et al. (1992) and to group haplotypes into hierarchical clades following the nesting rules of Templeton et al. (1992). Nesting by hand gave the same results. The root probabilities were also calculated in TCS using the method of Castelloe and Templeton (1994), which applies neutral coalescent theory to provide a probability that a given hap-

lotype is the most likely outgroup for a network. These probabilities are correlated with age, providing some indictation of the relative ages of haplotypes in the network. Rooting the network by either *B. polydamas* or *B. devilliers*, the two most closely related species to *B. philenor*, was problematic. Sequences from these species are between 26 and 30 steps removed and consequently beyond the limits of parsimony. Nested clade analysis was performed using GEODIS version 2.0 (Posada et al. 2000). Significance levels were estimated from a distribution generated from 10000 random permutations of the data.

Analysis of molecular variance (Excoffier et al. 1992) was used as a tool to investigate the amount of genetic variation and structure among populations of *B. philenor*. Six populations were defined for this analysis: Florida, Virginia (inclusive of Virginia and West Virginia populations), Texas, Arizona, California, and Mexico. Individuals from Tennessee and Illinois were excluded from these analyses because of insufficient sample sizes from each of these locales. The computer program ARLEQUIN version 2.0 (Schneider et al. 2000) was used to partition the total sum of squares into among individuals within populations, among populations within groups, and among group components. Two separate AMOVA analyses were conducted. In the first, each population was considered independently. In the second AMOVA, *B. philenor* were grouped by nominal subspecies, specifically *B. philenor hirsuta* (California) and *B. philenor philenor* (Florida, Virginia, Texas, and Arizona). The Mexico individuals were excluded from this analysis because of ambiguities in subspecific designations and low sample sizes for the subspecies that were identifiable based on collection locality. As an additional way to describe these broadly defined populations, ARLEQUIN version 2.0 was used to calculate two diversity indices, gene diversity (*h*) (Nei 1987) and the mean number of pairwise differences (π) (Tajima 1983).

RESULTS

Nineteen haplotypes were detected for the 422-bp mtDNA sequences (Genbank accession nos. AF513726–AF513744). Mean A-T content was high (≈75%), as is commonly observed for insect mtDNA (Simon et al. 1994). Of the 422 nucleotides examined, 19 were variable. Uncorrected sequence divergence among the haplotypes ranged between 0.2 and 1.2%. The most common haplotype, designated haplotype A, was found in 37 of the 74 individuals. This common haplotype was found throughout *B. philenor*'s range, and was nearly fixed in California and throughout Mexico. Table 1 shows the haplotypes present from each sampling locale. The previously published sequence (Caterino et al. 2001) was found in four additional individuals (haplotype Q).

The haplotype network and hierarchical nested groups provided by TCS is illustrated in Figure 3. The tip–interior status of the two-step clades was not determinable because there are three potential homoplasious linkages. Haplotypes A and K, A and Q, and K and L are separated by one step, creating a loop at this level. This generates three equally parsimonious nesting schemes. If we break the linkage between haplotype K and L, then A becomes the interior. However, breaking the loop between A and K or A and Q results in haplotype

Q or K becoming interior, respectively. Haplotype A has the highest root probability, supporting clade 2-1 as the interior at this level, however, Haplotype K and Haplotype Q have root probabilities that are only slightly lower. We conducted nested clade analysis on each of the three possible network schemes. Results of the nested clade analysis of geographical distance for haplotypes with clade 2-1 as interior are presented in Figure 4, as well as results that are qualitatively different under the alternative network schemes. The inference chain for each clade based upon the most recent version of Templeton's (1998) key for each of the three network schemes are provided in Table 2. Clade 1-1, which includes eight haplotypes found throughout *B. philenor*'s range, indicates contiguous range expansion based upon the significantly large clade distance observed for haplotype B. Similarly, Clade 1-5, which includes three haplotypes found only in Florida, Texas, and West Virginia, also indicates range expansion. The inference for Clade 2-3, which is confined to the eastern portion of North America, depends on the network scheme used. When Clade 2-1 is considered as interior, Clade 2-3 signals contiguous range expansion. However, when Clade 2-2 is designated as interior we were unable to discriminate between past fragmentation and isolation by distance because of inadequate sampling between the southern locales (Florida and Texas) and the northeastern locales (Illinois and Virginia). Inferences for the total cladogram indicate either restricted gene flow with isolation by distance (Clade 2-1 as interior) or contiguous range expansion (Clade 2-2 or 2-3 as interiors).

Haplotypes were grouped as broadly defined populations as a means to characterize the genetic variation observed throughout *B. philenor*'s range. Measures of gene diversity (*h*) and mean number of pairwise differences (π) show that both the California and Mexico populations are less diverse than other North American populations (Table 3). These large areas of low genetic diversity, especially true for the broadly defined Mexico population, conform to the predictions of Ibrahim et al. (1996) regarding leptokurtic range expansion (i.e., lower genetic diversity at the leading edge of a range expansion). Thus, they support the range expansion inferences of nested clade analysis described above.

Only 14.38% of the total molecular variance was explained by partitioning it among the six defined populations (Table 4a). When genetic variance was partitioned between *B. philenor hirsuta* and *B. philenor philenor*, only 3.08% of the variance was explained by subspecific designations (Table 4b). The small amount of variance explained by populations or groups of populations as described by AMOVA indicate little genetic structure of *B. philenor* throughout its range. This is largely the result of the frequency and widespread occurrence of the most common haplotype (A).

DISCUSSION

History

The inferences based upon nested clade analysis, the minimal amount of detectable genetic structure throughout the range of *B. philenor*, and the lower genetic diversity observed in California and Mexico support the hypothesis of a recent range expansion into these regions. The range expansion de-
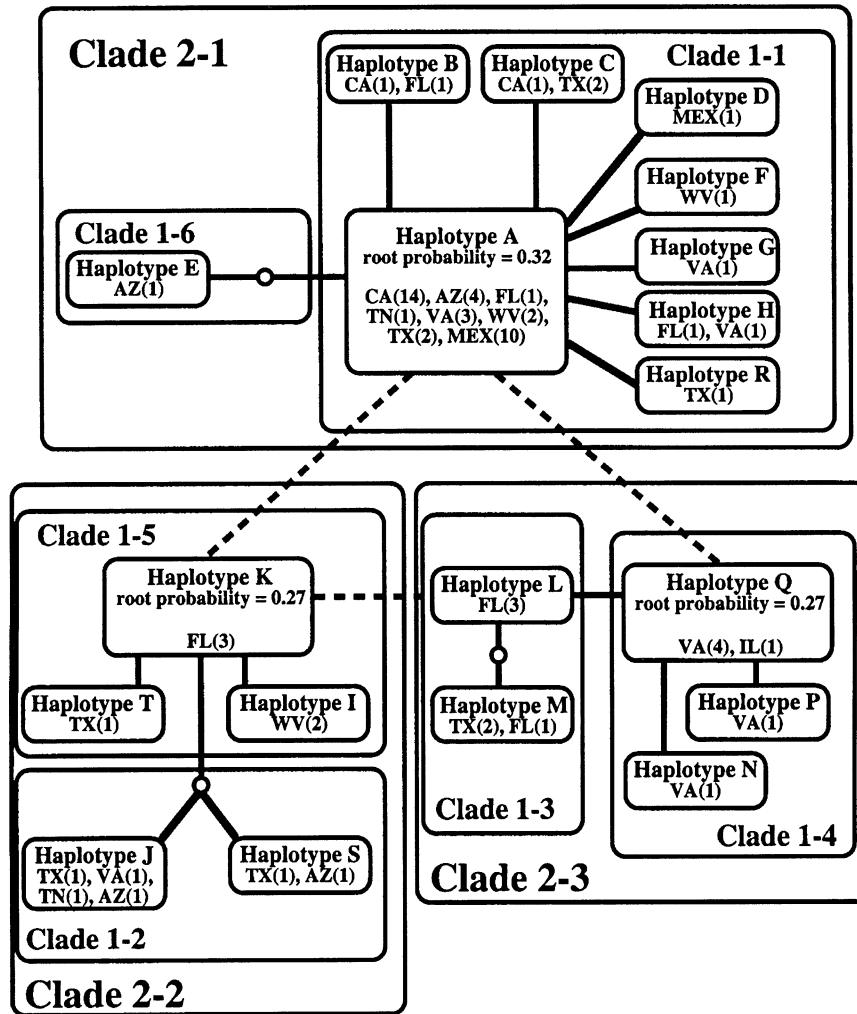
FIG. 3. Nested haplotype network of *Battus philenor* used for nested clade analysis. Dotted lines indicate equally parsimonious network connections between two-step clades creating a loop at this level (see text for details). Locality (two-letter state abbreviations; MEX, Mexico) for each haplotype is provided and the number of individuals possessing a haplotype from each location is provided in parentheses.

tected for clade 1-1, which includes all of the haplotypes detected in California and Mexico, is based solely on the large clade distance detected for haplotype B. Thus, if we had not detected haplotype B in either Florida or California the result of the nested clade analysis for clade 1-1 would have been inconclusive. Templeton (1998) acknowledges the difficulty of detecting range expansion using nested clade analysis, especially when the colonization event is recent enough so that few new mutations have arisen in the newly colonized region. In our view, Templeton's (1998) inference key arrives at range expansion when geographic association of haplotypes is detected (step 1 of the inference key) and models of gene flow and fragmentation are excluded (step 2 of the inference key). Once these more theoretically supported inferences are excluded, steps 11 and 12 of the key are used to distinguish between the patterns predicted from range expansion (either contiguous expansion or long-range colonization) and inconclusive patterns (but see Knowles and Maddison 2002). Consequently, even though range expansion is difficult to detect, the inference key appears to avoid erroneous inferences in this case. For *B. philenor*, the inference

of range expansion provided by nested clade analysis is supported by the low haplotype diversity detected in California and Mexico and agrees with the expectations of Ibrahim et al. (1996) and Hewitt (1996) that newly colonized regions should have lower genetic diversity (Table 3).

Recent range expansion may explain the exceedingly low phenotypic variation observed for this species throughout its range (Scott 1986). Interestingly, *B. philenor* appears to have been confined to the southeastern portion of the continental United States. We assume that its restricted range was a consequence of Pleistocene climate conditions. The maximum sequence divergence we observed for *B. philenor* was 1.4%, and though no molecular clock currently exist for the Papilionidae, a generic clock of 2% divergence between two lineages per million years (Avise et al. 1987) places the current variation observed clearly within the past two million years (i.e., the Pleistocene). Whether more than one refuge supported populations in this southeastern United States that later came into secondary contact is unclear. Evidence of multiple refugia and historical allopatry has been demonstrated for numerous species throughout the southeastern

0-step	D_C	D_N	1-step	D_C	D_N	2-step	D_C	D_N

2-1 interior

	0-step	D_C	D_N
A		1350	1347
B		1842L	1992
C		1097	1378
D		0	777
F		0	2294
G		0	2273
H		425	2191L
R		0	597
I vs T		639	-327

E

1-1	1366	1367
1-6	0	817
I vs T	1366	550

	D_C	D_N
Q	277	307
N	0	496
P	0	496
I vs T	277	-189

L, M

1-4	329S	451S
1-3	679	974
I vs T	-350	-523S

	D_C	D_N
K	0S	492S
T	0	1416
I	0	509
I vs T	0	-320S

J, S

1-5	616	959
1-2	859	816

	D_C	D_N
2-1	1362	1381
2-3	601S	1493
2-2	877S	1300
I vs T	629L	-19

2-2 interior

1-4	329S	451S
1-3	679	974L
I vs T	350	523L

	D_C	D_N
2-1	1362	1381
2-2	877S	1295
2-3	601S	1493
I vs T	-405	-190

2-3 interior

1-4	329S	451S
1-3	679	974L
I vs T		

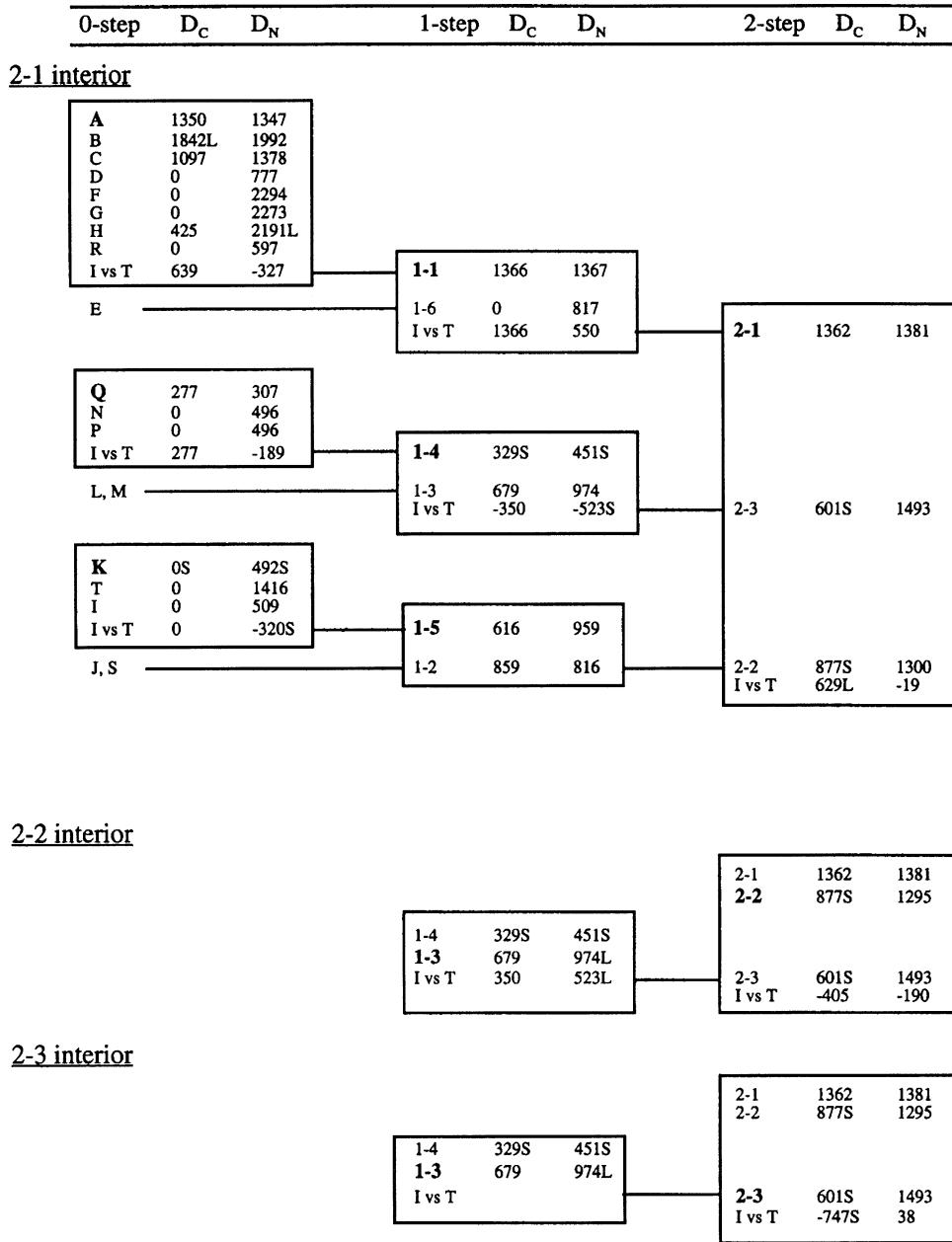|	D_C	D_N
2-1	1362	1381
2-2	877S	1295
2-3	601S	1493
I vs T	-747S	38

FIG. 4. Results of the nested clade analysis of geographical distance for mtDNA haplotypes of *Battus philenor*. Nested design, haplotype, and clade designations are given in Figure 3. Values of clade distance (D_C) and nested clade distance (D_N) follow the name of haplotypes or clade number. I vs T refers to the average difference between interior versus tip clades for both distance measures. L and S following distance values designate significantly large or small values, respectively, at $\alpha = 0.05$. Inferences based upon Templeton 1998 are presented in Table 2. Tip versus interior status of two-step clades is ambiguous and results for each of the three possible network configurations are included (see text for details). For 2–2 interior and 2–3 interior networks, only values that are quantitatively different from 2–1 interior are presented.

United States (for review see Avise 1995). Our data cannot distinguish between the hypothesis of multiple refugia (past fragmentation) or isolation by distance due to the lack of fine scale sampling in this region. However, our data indicate that the current population occupying California is of recent origin, as is that in Mexico. Both regions have the lowest genetic diversity and are nearly fixed for the same common haplotype (A), with the remaining observed haplotypes being only one step removed.

This recent colonization of California and Mexico is curious because *Aristolochia* host plants were likely available in both regions during the Pleistocene. Mexico currently boasts the greatest number of *Aristolochia* species in the Americas (Pfeiffer 1966, 1970). Phylogeographic studies of other *Aristolochia* specialists that currently occupy Mexico, such as *B. polydamas*, might be informative concerning the historical habitability of this region for the genus *Battus*. It is possible that *B. philenor* populations did occupy these re-

TABLE 2. Inferences based upon nested clade analysis for clades with significant D_C and/or D_N values. Inference chain refers the sequence followed in the inference key of Templeton 1998. Network scheme refers to tip/interior status of the two-step clades. Inferences from one-step clades remain unchanged among each nesting scheme.

Network scheme	Clade	Inference chain	Inferred pattern
2-1 interior	1-1	1, 2, 11, 12 (no)	Contiguous range expansion
	1-5	1, 2, 11, 12 (no)	Contiguous range expansion
	2-3	1, 2, 11, 12 (no)	Contiguous range expansion
	Total Cladogram	1, 2, 3, 4 (no)	Restricted gene flow with isolation by distance
2-2 interior	2-3	1, 2, 3, 4, 9, 10 (no)	Unable to discriminate between past fragmentation and isolation by distance
	Total Cladogram	1, 2, 11, 12 (no)	Contiguous range expansion
2-3 interior	2-3	1, 2	Tip/interior status cannot be determined. Inconclusive outcome
	Total Cladogram	1, 2, 11, 12 (no)	Contiguous range expansion

gions during the Pleistocene, but subsequently went extinct or maintained extremely small populations whose signature was not detected in our study. Alternatively, the currently observed haplotype diversity could reflect a recent fixation of a selectively advantageous haplotype (i.e., a selective sweep). Genetic evidence based upon nuclear markers would be a means to test these alternative hypotheses.

These results are in contrast to many other phylogeographic studies of Lepidoptera in North America, which have found some genetic structure on a continental scale. Sperling et al. (1998) found that mtDNA sequence variation of the hemlock looper, *Lambdina fiscellaria* (Geometridae), revealed two major lineages that were associated with either eastern or western populations. Similarly, Sperling and Harrison (1994) found geographic structure for mtDNA variation in *Papilio zelicaon*. Recent studies by Nice and Shapiro (1999, 2001) detected distinct western clades in both the *Oeneis* (Satyridae) and *Lycaeides* (Lycaenidae) species complexes. However, it should be noted that all of the preceding examples are species whose extant range is limited to cooler environments, either high latitude or high altitude. Thus, it is not surprising, that these species might be effected differently by climate change, presumably during the Pleistocene, than would a tropical species, such as *Battus*.

Host Plant Association and Oviposition Behavior

The ecological interactions between *B. philenor* and its *Aristolochia* host plants can now be viewed from the historical perspective provided by this study. The evidence of recent arrival of *B. philenor* to California allows us to interpret the relationship between it and the California host plant in this historical light. *Battus philenor* larvae are the only herbivores commonly observed using *A. californica* as a food-

plant (J. A. Fordyce pers. obs.). It is possible that this California endemic persisted for an extended period of time with no pressure from specialist herbivores. The evolution or maintenance of traits effective against specialists would not be under selection in this long-lived perennial in the absence of specialist herbivore pressure. Daehler and Strong (1997) suggest that loss of specialist herbivores can result in the relaxation of defensive traits in plants, even over a short period of time. Although the presence of aristolochic acids is an effective defense against most generalist herbivores (Chen and Zhu 1987; Park et al. 1997), the aristolochic acids found in *A. californica* have no detectable adverse effect on *B. philenor* (Fordyce 2001). Thus, upon its arrival in California, *B. philenor* may have encountered a naive host plant that could be exploited using novel strategies, such as increased clutch size, to enhance larval performance (Fordyce and Agrawal 2001; Fordyce 2003) and increase the realized fecundity of ovipositing females. Conversely, the *Aristolochia* species encountered in Mexico may not have been as vulnerable to *B. philenor* upon its arrival because other *Aristolochia*-specialists co-occur with these species, including at least five other species of *Battus* and nine species of *Parides* (Papilionidae) (Tyler et al. 1994).

Compared to populations observed elsewhere, the population of *B. philenor* in California lays larger clutches of eggs, in tight clusters. Interestingly, Racheli and Oliverio (1993) commented in their phylogenetic analysis of *Battus* that the ''*philenor*''-clade, which included *B. philenor* and five other species, all laid eggs singly or in small clusters. The average clutch size in California is 13.3 ± 0.3 (mean $\pm$ SE, $n = 766$ clutches; J. A. Fordyce, unpubl. data), with a range of 1–69. In Texas, Rausher observed an average clutch size of 2.2 with a range of 1–20 (Rausher 1980). In Mexico, Rausher (1979) observed clusters of one to nine eggs per plant, and Spade et al. (1988) observed four to six *B. philenor* eggs per host plant, although these were not arranged in tight clusters and may not have been from the same female. Pilson and Rausher (1988) observed that the Texas population of *B. philenor* adjusts clutch size depending upon the suitability of the host plant. Larger clutches were observed on plants that maximized the growth of developing larvae before they were forced to leave and search for a new host plant. However, in California females do not appear to adjust clutch size based upon plant quality (Tatar 1991). Thus, there appears to be

TABLE 3. Genetic diversity indices ($\pm$ sampling variance) for six broadly defined *B. philenor* populations (see text for details).

Population	n	Gene diversity (h)	Average pairwise difference (π)
Florida	10	0.87 ($\pm$0.08)	1.8 ($\pm$1.1)
Virginia	17	0.88 ($\pm$0.06)	1.8 ($\pm$1.1)
Texas	10	0.93 ($\pm$0.06)	3.0 ($\pm$1.7)
Arizona	7	0.71 ($\pm$0.18)	2.1 ($\pm$1.3)
California	16	0.24 ($\pm$0.14)	0.2 ($\pm$0.3)
Mexico	11	0.18 ($\pm$0.14)	0.2 ($\pm$0.3)

TABLE 4.   Analysis of molecular variance results using two different grouping schemes.

Source	Variance	% total	P	φ statistics
A. Each population independently				
Among populations	0.1168	14.38	<0.001	
Within populations	0.6950	85.62	<0.001	$\phi_{ST} = 0.1439$
B. Populations grouped by nominal subspecies				
Among groups	0.0289	3.08	0.210	$\phi_{CT} = 0.0308$
Among populations within groups	0.1048	11.17	0.001	$\phi_{SC} = 0.1152$
Within populations	0.8049	85.75	<0.001	$\phi_{ST} = 0.1425$

geographic variation in oviposition behavior as observed for these populations (Pilson and Rausher 1989; Tatar 1989).

Large groups of larvae are more effective than small groups of larvae at overcoming the dense trichome defenses of the California host plant and larger groups develop at an increased rate in California (Fordyce and Agrawal 2001; Fordyce 2003). Increased growth rate and larger size is beneficial to larvae in California because larger larvae are less susceptible to many predators and more resilient to periods of cool weather (Fordyce and Agrawal 2001; Fordyce and Shapiro 2003). Whether growth rate increases with group size in other populations feeding on alternative host plants is unknown. In California, larger clutches may always be advantageous, whereas females in Texas may maximize offspring survival by allocating the number of eggs per clutch dependent upon host plant quality. The large clutch size observed in California may reflect an adaptation to *A. californica*, which suggests that clutch size can respond rapidly to selection.

This study provides a historical context for past and future studies on geographic variation in *B. philenor*. For example, Papaj (1986) investigated interpopulation differences in oviposition behavior and learning between a Texas and Virginia population. Texas populations, unlike those in Virginia, are required to shift between two species of *Aristolochia* over the course of a single season. This host-plant discrimination is based upon leaf shape (Rausher 1980). Papaj (1986) reported that, although there was interpopulation differences in search behavior, there was surprisingly no difference between populations in the ability of females to learn. Papaj (1986) speculated that the ability to learn leaf shape may be a conserved trait, constrained by the selection for other types of discrimination behavior. This study provides at least one alternative hypothesis. The low amount of population genetic structure and substantial haplotype sharing between these populations indicates some contemporary gene flow or that they share a common recent history. Thus, one possibility is that learning ability may respond more slowly to selection than search behaviors.

Mimicry

The genetic evidence presented in this study supports *B. philenor*'s recent arrival to California. This may explain the lack of *B. philenor* mimics in California despite its unpalatability (Codella and Lederhouse 1989; Fordyce 2000). Mimicking *B. philenor* is ineffectual when it is absent (Waldbauer and Sternburg 1987) and the lack of mimics in California may reflect its historical absence from the California fauna. Many likely candidates for a mimetic relationship currently are sympatric with *B. philenor* in California. Mimetic

melanism has independently evolved numerous times in the genus *Papilio* (Sperling and Harrison 1994; Vane-Wright et al. 1999). It seems unlikely that there is an intrinsic constraint on the evolution of melanic mimicry in the nonmimetic western *Papilio* species. However, *Papilio rutulus* and *P. multicaudatus* are sympatric with California *B. philenor*, yet do not exploit a mimetic strategy. Both of these mostly yellow swallowtails are closely related to *P. glaucus* (Caterino and Sperling 1999), whose females effectively mimic *B. philenor* where sympatric with the model in the southeastern United States (Brower 1958). One prediction of this hypothesis is that the current distribution of *P. multicaudatus*, which extends into Central America and is sympatric with a number of potential models such as *B. polydamas*, reflects a recent range expansion. A phylogeographic study of *P. multicaudatus* may elucidate whether the lack of melanism of this candidate mimic reflects recent sympatry with the model, or genetic or ecological constraints on the evolution of this defensive strategy.

The yellow and black colored *P. zelicaon* occupies western North America and does not mimic *B. philenor*. However, it is the sister species to *P. polyxenes* (Caterino and Sperling 1999) which ranges from South America through the eastern portion of North America, is mostly black, and is widely believed to be a *B. philenor* mimic. Interestingly, Sperling and Harrison (1994) observed that *P. polyxenes* was fixed for a single haplotype throughout its range in the United States and Canada and that the center of haplotype diversity was in Ecuador. They interpreted this as a recent range expansion into North America. This raises the possibility that the mimetic melanism observed in *P. polyxenes* evolved in isolation from *B. philenor*. Thus, it is possible that dark coloration was initially favored by selection to mimic other unpalatable models, including other species of *Battus*. Conversely, the spicebush swallowtail, *P. troilus*, is believed to be a *B. philenor* mimic and is restricted to eastern North America and does not extend into Central America. Thus, it may represent a ''true'' *B. philenor* mimic, in the sense that prolonged sympatry with *B. philenor* may have led to its mimetic melanism. Future phylogeographic studies of *P. troilus* and other butterflies that exhibit mimetic melanism will contribute to our understanding of the evolution of this defensive strategy. It is evident that the current ranges of the model and at least some of its mimics may not reflect a long historical association.

Conclusion

Phylogeographic studies, such as the one presented here, provide a temporal perspective of a species' range, thereby

adding an additional dimension in which ecological and evolutionary investigations can be placed. The current distribution of *B. philenor* in California and Mexico appears to reflect a recent expansion of its range. Thus, it is a recent component of the ecological communities it occupies throughout a large portion of its range. Geographical comparisons of mimetic relationships, behavior, and host plant associations can now be placed in this historical context. Studies aimed at comparing the ecologies of different populations benefit when placed in the context of such historical information.

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