Four independent gene phylogenies confirm the ancient relationships of Madagascar endemic species in the Papilio demoleus group (Lepidoptera, Papilionidae).


Published in:
Evolution

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Download date:14. Dec. 2018
INDEPENDENT GENE PHYLOGENIES AND MORPHOLOGY DEMONSTRATE A MALAGASY ORIGIN FOR A WIDE-RANGING GROUP OF SWALLOWTAIL BUTTERFLIES

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Abstract.—Madagascar is home to numerous endemic species and lineages, but the processes that have contributed to its endangered diversity are still poorly understood. Evidence is accumulating to demonstrate the importance of Tertiary dispersal across varying distances of oceanic barriers, supplementing vicariance relationships dating back to the Cretaceous, but these hypotheses remain tentative in the absence of well-supported phylogenies. In the *Papilio demoleus* group of swallowtail butterflies, three of the five recognized species are restricted to Madagascar, whereas the remaining two species range across the Afrotropical zone and southern Asia plus Australia. We reconstructed phylogenetic relationships for all species in the *P. demoleus* group, as well as 11 outgroup *Papilio* species, using 60 morphological characters and about 4 kb of nucleotide sequences from two mitochondrial (cytochrome oxidase I and II) and two nuclear (wg and EF-1α) genes. Of the three endemic Malagasy species, the two that are formally listed as endangered or at risk represented the most basal divergences in the group, while the more common third endemic was clearly related to African *P. demodocus*. The fifth species, *P. demoleus*, showed little differentiation across southern Asia, but showed divergence from its subspecies *sthenelus* in Australia. Dispersal-vicariance analysis using cladograms derived from morphology and three independent genes indicated a Malagasy diversification of lime swallowtails in the middle Miocene. Thus, diversification processes on the island of Madagascar may have contributed to the origin of common butterflies that now occur throughout much of the Old World tropical and subtemperate regions. An alternative hypothesis, that Madagascar is a refuge for ancient lineages resulting from successive colonizations from Africa, is less parsimonious and does not explain the relatively low continental diversity of the group.

Key words.—Biogeography, dispersal, island colonization, Madagascar, Papilionidae, speciation, vicariance.

Received May 7, 2004. Accepted September 1, 2004.

Islands can play an important role in both generating and conserving biodiversity. Species radiations on island archipelagos are well studied (e.g., Grant and Grant 2002; Gillespie and Roderick 2002; Jordan et al. 2003), although the contribution of such island diversification to surrounding continents remains poorly documented. Under the principles of island biogeography, island size and distance from the mainland are the primary determinants of the accumulation of species that disperse from larger land masses (MacArthur and Wilson 1967). The likelihood that island species will colonize continents seems relatively low, because islands usually contain both fewer species and smaller total populations than mainlands. Furthermore, island species are likely to be at a competitive disadvantage when islands are colonized by continental species (Gargominy et al. 1996; Whittaker 1998; Cowie 2001; O’Dowd et al. 2003). However, the widespread assumption that islands contribute little to continental biotas remains relatively untested, and the development of more rigorous methods of phylogenetic and biogeographic analysis, as well as opportunities for the generation of large molecular datasets that can contribute to more robust reconstructions, have greatly improved the opportunities to assess such biogeographic hypotheses.

The island of Madagascar is widely known for its unique and diverse flora and fauna. It has been isolated from Africa since the late Jurassic and from other Gondwanan elements such as India since the late Cretaceous (Rabinowitz et al. 1983; Storey et al. 1995; Briggs 2003). These conditions have led to the evolution of a very large number of endemic species, although the processes that have contributed to the biotic diversity within Madagascar are still poorly understood. Many endemics represent high-ranking, very distinct groups, such as lemurs and tenrecs. As a result, and because much of the island’s biodiversity is now under threat of extinction, Madagascar is considered one of the world’s 10 most important regions for biodiversity conservation (Myers et al. 2000).

In spite of the proximity of Madagascar to Africa since its separation from that continent about 165 million years ago, the Malagasy flora exhibits remarkably high affinity with Indo-Australo-Malesian floras far to the east (Schatz 1996). Such phytogeographic connections are especially prevalent among eastern humid forest taxa, and in some cases may represent relictual Cretaceous Gondwanan disjunctions, as
well as repeated long-distance or stepping-stone dispersal
events across the Indian Ocean. Although the fauna of Mad-
gaascar shows more connections with Africa (Paulian 1972;
Warren et al. 2003), some Oriental affinities are still present.
For example, several Malagasy birds show clear affinities
with Oriental groups that are unknown in Africa (Dorst 1972).

The presence of some lineages of plants and animals in Mad-
gaascar may be explained by Cretaceous dispersals to the
island from India or South Africa, and floral and faunal ex-
change with the remains of Gondwana via Antarctica during
the time of the initial radiation of the angiosperms (Koechlin
1972; Schatz 1996; Vences et al. 2001). This scenario may
account for the evolution of seven endemic plant families
and three recently extinct ratite species (Cooper et al. 2001).

In addition, recent phylogenetic evidence and fossils sug-

gest that part of the modern vertebrate fauna of Madagascar
had an independent origin from that of the Cretaceous fauna
of Madagascar (Krause et al. 1999). There is growing evi-
dence for nontectonic dispersal to Madagascar (and Africa)
from Laurasia and western Malesia via India along Lemurian
stepping-stones in the western Indian Ocean during the
Eocene-Oligocene (McKenzie and Sclater 1973; Schatz 1996;
Jansa et al. 1999). Late Tertiary introduction by rafting has
been suggested for an endangered endemic tortoise in Mad-
gaascar (Caccone et al. 1999). Neogene colonization of Mad-
gaascar by African ancestors has been reported in Malagasy
oscine songbirds (Cibois et al. 2001) and sunbirds in the
Pliocene (Warren et al. 2003). Yoder et al. (1996) found that
the Malagasy lemurofoms comprise a monophyletic group
that probably dispersed from Africa in the early Tertiary and
a single African Miocene origin is also posited for carnivores
(Yoder et al. 2003). Thus, multiple overseas dispersals to and
from Madagascar have been proposed both for plants and
animals (see also Renvoise 1979; Fisher 1996; Raxworthy et
al. 2002; Treutlein and Wink 2002; Nagy et al. 2003; Vences
et al. 2003, 2004). Nonetheless, such hypotheses of overseas
dispersal remain tentative in the absence of more and better
supported phylogenetic evidence for the relationships of dif-
frent elements of the Malagasy biota.

Complex patterns of vicariance and dispersal, along with
long isolation of the island, account for much of the ende-
mism of the Malagasy biota. At the species level, at least
80% of the Malagasy flora and 75% of its fauna is endemic
(Schatz 2001), and this is also the level of species endemism
for butterflies (Lees et al. 2003). For the conservation of invertebrates, butterflies of the family Papilionidae have been

championed as a flagship group (Collins and Morris 1985).

Thirteen species of the family are found on Madagascar (Pa-
ulian and Viette 1968), of which 10 are endemic to mainland
Madagascar and the Comoros (Ackery et al. 1995). Three of
the endemics are currently classified by the IUCN as threat-
ened (IUCN 2003), and one of these belongs to the Papilio
demoleus group, the subject of this study.

The P. demoleus group, or lime swallowtails, comprises
five butterfly species from the Old World tropics that share a
distinctive black and yellow pattern. Three of them, P.
erithonioideus Grose-Smith, 1891, P. grosesmithi Rothschild,
1926, and P. morondaviana Grose-Smith, 1891, are found only
on Madagascar, while the other two have wide distributions
in the Afrotropical (P. demodocus Esper, 1798) and Indo-
Australian (P. demoleus Linnaeus, 1758) regions.

Papilio demodocus occurs throughout sub-Saharan Africa,
extending into Saudi Arabia, Yemen, and Oman. Its existence
on Madagascar, Mauritius, Réunion, and the four Comoro Islands is likely to be the result of recent and perhaps even
deliberate introductions (Paulian 1951; Paulian and Viette
1968; Turlin 1994), though it is also possible that the species
arrived naturally in Madagascar as two of us (D. C. Lees and
A. Cameron) have observed it occasionally in natural habi-
tats. The species is abundant in anthropogenic habitats and
is not threatened.

The larvae feed mostly on Rutaceae but with no record on
native species and the species is considered to be a pest of
Citrus crops (Paulian and Viette 1968; Collins and Morris
1985; Ackery et al. 1995). Although some populations in
South Africa have switched to larval feeding on Apiaceae
(van Son 1949; Clarke et al. 1963), there is no evidence that
they have done this in Madagascar. Occasional use of An-
cardiaceae, Asteraceae, Pteroxygaleae, and Sapindaceae as
larval host plants has been reported (Ackery et al. 1995),
though these records may represent oviposition mistakes be-
cause larvae of P. demodocus are not known to develop ad-
equately on host plants in these families. Two subspecies are
recognized, P. demodocus demodocus Esper, 1798, from vir-
tually the entire range, and the distinctive P. demodocus ben-
nettii Dixey, 1898, originally described as a separate species
from Socotra Island (Yemen).

The distribution of P. demoleus extends from Iran through
India, Sri Lanka, Malaysia, southern China, and Japan, and
from the Lesser Sunda Islands to mainland New Guinea and
Australia. The species was absent from the Philippines, Su-
matra, Java, and Borneo until its recent invasion of the islands
after their large-scale deforestation by man (Hiura 1973; Cor-
et and Pendlebury 1978, 1992). Most recently, the species
is now established on the northern tip of Sulawesi (Vane-
Wright and de Jong 2003). Six subspecies are currently rec-
ognized: demoleus Linnaeus, 1758 (China through South Asia
and Pakistan to the Arabian Peninsula); malayanus Wallace,
1865 (Malay Peninsula and Sumatra); libanius Fruhstorfer,
1908 (Philippines, Talaud, Sula, Taiwan); sthenelus Macleay,
1826 (Sumba and Australia); novoguineensis Rothschild,
1908 (Papua New Guinea); and sthenelinus Rothschild, 1895
(Flores and Alor). In Papua New Guinea and in Australia,
the larvae develop on wild leguminous plants of the genus
Cullen (Fabaceae; Braby 2000), but no host record is avail-
able for the Lesser Sunda island populations (Parsons 1999).
All Asian subspecies feed on Citrus, which is commonly
planted as a crop or ornament in towns and smaller settle-
ments and probably facilitates range expansion of P. demo-
leus in Asia (Matsumoto 2002).

The rarest of the three Malagasy endemics, P. moronda-
vana, is currently classified as potentially “endangered”
while “data deficient” (IUCN 2003). It is confined to dry
deciduous forests of west-southwestern and northern Mad-
gaascar and is threatened by loss of habitat (Collins and Mor-
is 1985; Conservation International 2003). Our field obser-
vations and museum collections suggest that P. grosesmithi
(lower risk: near threatened) is more commonly encountered
than P. morondaviana. It is found over a very similar geo-
graphic range below about 800 m in dry deciduous and gallery forests of western and northern Madagascar, ranging east as far as Fianarantsoa, and slightly further southwest than *P. morondavana*, while *P. morondavana* is known from the far north. According to field observations in 2001–2002 (A. Cameron), *P. morondavana* can be abundant at the beginning of the wet season at some sites, but appears to be more localized than *P. grosssmithi*.

*Papilio erithonioides* (not classified as threatened) occurs in spiny forests of the far southwestern as well as deciduous and gallery forests in the southwestern, western, and northern parts of the island, also penetrating marginally into northwestern and southwestern rainforests. Our recent observations show it to be by far the most common endemic. Significantly, all three species can be found sympatrically at some sites, notably the unprotected Kirindy Forest near Morondavana. *Papilio demodocus* is found in anthropic habitats throughout the island including the plateau. Further details of the nomenclature, distribution, and bionomics will be provided in a companion publication (C. R. Smith and R. I. Vane-Wright, unpubl. ms.).

Although the life history and geographic variability of the *P. demoleus* species group is relatively well known (at least outside Madagascar), their phylogenetic relationships remain unknown and pose questions about their origin and evolution. Carcasson (1964) considered *P. demodocus* to be the ancestral species, from which the three species endemic in Madagascar were isolated, one after another, as the result of multiple invasions of a mainland form. This model corresponds to the concept of duplex species, in which the rare species, with a more restricted range, owes its origin to isolations and reunions with the broader range of a sister species (Zeuner 1943; Corbet 1944). However, in this case the vicariance that is normally caused by sea-level rise is replaced by dispersal of a relatively mobile species across a fixed sea level gap. In contrast to a sequential colonization scenario, speciation of Malagasy endemics solely on Madagascar, whether in different parts of the island or in contact with each other, remains a possibility for the *P. demoleus* group. Endemic Malagasy radiations have been reported for a variety of taxa such as tortoises, songbirds, butterflies, and colubrid snakes (Caccione et al. 1999; Cibois et al. 2001; Torres et al. 2001; Nagy et al. 2003). However, only a handful of recent studies have suggested that the Malagasy biota has contributed to the surrounding continents.

Here, we report results of phylogenetic analyses based on morphology and nucleotide sequences of two mitochondrial genes, cytochrome oxidase subunit I (COI), cytochrome oxidase subunit II (COII), and two nuclear genes, wingless (wg), and elongation factor 1a (EF-1a). We use inferred phylogenies combined with estimated divergence times to reconstruct ancestral areas for the *P. demoleus* species group and to identify patterns of species dispersal that have contributed to the enigmatic biodiversity of Madagascar. Moreover, any improvement in our understanding of the systematics of these butterflies provides a better foundation for appropriate conservation action (Vane-Wright 2003).

### Materials and Methods

#### Sampling Strategy

Sampled species and GenBank accession numbers are given in Table 1. Ingroup taxa included all five recognized species of the *P. demoleus* group, with sampling across species ranges where possible. Four out of six described subspecies of *P. demoleus* were sampled; *P. d. novoguineensis* and *P. d. sthenelinus* were not available for this study. Both subspecies of *P. demodocus* were sampled, including *P. d. demodocus* and *P. d. bennetti* (a taxon restricted to Socotra Island, Yemen). However, only two legs from two old pinned specimens collected in 1967 (British Museum of Natural History Entomology Department, specimen register nos. 220150 and 220151) were available for DNA extraction for *P. d. bennetti*.

Eleven outgroup taxa were chosen, representing major monophyletic lineages within the genus based on a recent reconstruction of *Papilio* phylogeny (Zakharov et al. 2004) to test the monophyly of the *P. demoleus* group and to relate its phylogenetic position within *Papilio*. Trees were rooted with a single neotropical species, *Papilio thoas*. The original data and tree files are available from www.treebase.org (accession number SN1870).

#### Morphology

A total of 60 characters (Fig. 1, Appendix 1) were scored by C. R. Smith from wing patterns and male and female genitalia for the five species of lime swallowtails (including both subspecies of *P. demodocus*) and all outgroup taxa. Where material was available, male and female genitalia were examined in several specimens of each species. Dissections were made using standard techniques, after abdomens were soaked in cold 10% KOH solution overnight and subsequently stored in glycerol. Voucher information for specimens scored for morphological characters is available as supplementary online materials at http://dx.doi.org/10.1554/04-293.1.s1.

Although all species of the *P. demoleus* group share the distinctive wing pattern of the group, some problems in morphology assessment of wing pattern elements were encountered in scoring outgroup species. Certain elements of wing pattern in some species of *Papilio* are fused or lost completely, making the wing pattern very different from a generalized swallowtail groundplan of black and yellow stripes, spots, and patches. To help hypothesize homology, we evaluated the position of pattern elements on the wing with respect to wing venation and examined series of related species. Some genitalic homologies were likewise problematic. Further details of the wing patterns and male and female genital anatomy, and the character hypotheses derived from them, will be provided in a companion publication (C. R. Smith and R. I. Vane-Wright, unpubl. ms.).

#### Molecular Techniques

Selection of genes for molecular phylogenetic reconstruction was based on previous successful studies in Lepidoptera (Sperling 2003) and other insects (Caterino et al. 2000). The two most commonly used mitochondrial DNA protein-coding genes, *COI* and *COII*, which are generally most useful in
<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Date</th>
<th>Voucher ID</th>
<th>Haplotype</th>
<th>COI, COII</th>
<th>EF-1a</th>
<th>Wingless</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. <em>Papilio demoleus malayanus</em></td>
<td>Thailand: Chiang Mai</td>
<td>May 10, 2001</td>
<td>NP:DL01-Q676</td>
<td>DL.1</td>
<td>AY569056</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. <em>Papilio demoleus libanius</em></td>
<td>Taiwan</td>
<td>September 2002</td>
<td>SHY:1856</td>
<td>DL.1</td>
<td>AY569059</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Continued.

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<th>Species</th>
<th>Locality</th>
<th>Date</th>
<th>Voucher ID</th>
<th>Haplotype</th>
<th>COI, COII</th>
<th>EF-1α</th>
<th>Wingless</th>
</tr>
</thead>
<tbody>
<tr>
<td>49. <em>Papilio morondavana</em></td>
<td>Madagascar: Kirindy</td>
<td>November 30, 2001</td>
<td>CAS:8000801</td>
<td>M1</td>
<td></td>
<td>AY5690936</td>
<td>AY569097</td>
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</table>

Outgroup taxa

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<th>Species</th>
<th>Locality</th>
<th>Date</th>
<th>Voucher ID</th>
<th>Haplotype</th>
<th>COI, COII</th>
<th>EF-1α</th>
<th>Wingless</th>
</tr>
</thead>
<tbody>
<tr>
<td>54. <em>Papilio paris</em></td>
<td>China: Guandong Province</td>
<td>June 20, 2001</td>
<td>UAFS:1572</td>
<td></td>
<td></td>
<td>AY4575745</td>
<td>AY4576055</td>
</tr>
<tr>
<td>55. <em>Papilio constantinus</em></td>
<td>Kenya (ex pupa)</td>
<td>1989</td>
<td>CU: Lot No. 1204</td>
<td></td>
<td></td>
<td>AY4575855</td>
<td>AY569117</td>
</tr>
<tr>
<td>60. <em>Papilio machaon</em></td>
<td>France: Coudoux (ex pupa)</td>
<td>February 18, 1988</td>
<td>CU: Lot No. 1204</td>
<td></td>
<td></td>
<td>AY4575925</td>
<td>AY569120</td>
</tr>
</tbody>
</table>

1 Voucher locations are indicated as: CU, insect collection (see Caterino and Sperling 1999) of the Department of Entomology at Cornell University, Ithaca, NY; UAFS, DNA and tissue collection in F.A.H.S. Laboratory at the University of Alberta, Edmonton, AB; BMNH (E), Department of Entomology at Natural History Museum, London, U.K.; CASENT, Department of Entomology at California Academy of Sciences, Golden Gate Park, San Francisco, CA; SHY, specimens from private collection of Shen-Horn Yen; NP, DNA and tissue collection in Naomi Pierce lab at Harvard University, Cambridge, MA.
2 A 245-bp fragment was sequenced to extend 3’ end of previously published sequence of EF-1α (Reed and Sperling 1999).
3 (Caterino and Sperling 1999).
4 (Reed and Sperling 1999).
5 (Zakharov et al. 2004).
6 Specimens were sequenced for full 2.3 kb of COI + COII in addition to 825-bp fragment at 3’ end of COI.
Fig. 1. Edited digital images showing morphological characters, with circled numbers referring to numbered character states (Appendix 1). (A) Upper and undersides of Papilio demoleus demoleus. Ellipses on wings indicate the position of character traits. (B) Male genitalia of P. demodocus bennetti. (C) Female genitalia of P. erithonioides. All character states shown are coded as (1) unless indicated otherwise.

delineating taxa at the species level (Sperling 2003; but see Wahlberg et al. 2003), also represent the maternal history of speciation in the P. demoleus species-group. Two other loci, wg and EF-1α, are the most commonly used protein-coding nuclear genes in phylogenetic studies in Lepidoptera and are usually considered useful at higher taxonomic levels due to their lower substitution rates (Sperling 2003). Both nuclear genes appear to have only a single copy in lepidopteran genome and have either no introns (EF-1α) or a 450-bp exon between introns (wg).

Mitochondrial genes COI, tRNA-leu, COII and nuclear genes wg and EF-1α were sequenced new by E. V. Zakharov or retrieved from GenBank for all 11 outgroups and two specimens per species (10 total) in the ingroup. For EF-1α, an additional fragment of about 245 bp was sequenced from the 3'-end to extend previously published sequences that were incomplete for the gene (Reed and Sperling 1999). An 825-bp fragment comprising the 3'-end of the COI gene was sequenced for an additional 40 ingroup individuals, except the two specimens of P. demodocus bennetti, which produced only highly degraded DNA.

Material of varying quality was used for DNA extraction, ranging from alcohol-preserved freshly caught samples to dried nonrelaxed specimens and legs of museum pinned but-
terflies. Total genomic DNA was extracted either by standard phenol-chloroform procedure as in Sperling and Harrison (1994) or using a Qiagen (Valencia, CA) DNeasy tissue kit. Polymerase chain reactions (PCR) were performed in Biomетra (Goettingen, Germany) TGradient or TPersonal thermal cyclers using reaction and cycling conditions described previously (Brower and DeSalle 1998; Caterino et al. 2001). PCR products were cleaned using a Qiagen QIAquick PCR purification kit when only a single DNA band was visible in a gel or, when more than one band was observed, a combination of gel-separation and subsequent purification with a Qiagen QIAEX II gel extraction kit. Purified PCR products were directly sequenced using DYEnamic<sup>TM</sup> ET terminator cycle sequencing (Amersham Pharmacia Biotech, Cleveland, OH) or Applied Biosystems (ABI, Foster City, CA) Big Dye terminator cycle sequencing, under manufacturer’s recommendations. Fluorescently labeled sequencing products were filtered through Sephadex-packed columns, dried, resuspended, and fractionated on an ABI 377 automated sequencer. All fragments were sequenced in both directions using the same primers that were used for PCR (Appendix 2, available online at http://dx.doi.org/10.1554/04-293.1.s2). Sequences were assembled into contiguous arrays using Sequencher, version 4.1 (GeneCode Corp., Ann Arbor, MI).

**Phylogenetic Analysis**

For morphological data, most parsimonious cladograms were inferred from the equally weighted and unordered data matrix using a branch-and-bound search in PAUP* version 4.0b10 (Swofford 1998). Bootstrap analysis was done in PAUP using 1000 replicates and used heuristic searches with tree bisection-reconnection (TBR) branch swapping with no additional random replicates. The results of maximum parsimony analysis were checked using a heuristic search algorithm (with 10 random additional replicates and TBR branch swapping) of NONA 2.0 (Goloboff 1999) spawned with the aid of WinClada (Nixon 2002). Bayesian analysis of morphological dataset was performed in MrBayes version 3.04b (Huelsenbeck and Ronquist 2001) with the matrix treating all characters as standard binary characters (DATATYPE = STANDARD) of morphological dataset was performed in MrBayes version 3.04b (Huelsenbeck and Ronquist 2001) with the matrix treating all characters as standard binary characters (DATATYPE = STANDARD). We ran four chains simultaneously, three heated and one cold. Each Markov chain was started from a random tree and run for 1.0 x 10<sup>6</sup> generations, sampling the chains every 100th cycle. The log-likelihood scores of sample points were plotted against generation time to determine when the chain became stationary. The first half of the sampled trees was discarded as burn-in samples. We ran each analysis three times, each beginning with different random starting trees, and compared their apparent stationarity levels for convergence (Huelsenbeck and Bollback 2001). Data remaining after discarding burn-in samples were used to generate a majority-rule consensus tree, where percentage of samples recovering any particular clade represented the clade’s posterior probability (Huelsenbeck and Ronquist 2001). Probabilities of 95% or higher were considered as significant support. The mean, variance, and 95% credibility interval were calculated from the set of substitution parameters.

**Dispersal-Vicariance Analysis**

To reconstruct the distribution history of the *P. demoleus* group we used the dispersal-vicariance approach implemented in program DIVA (Ronquist 1996). In DIVA a fully bifurcated phylogeny is used to optimize the distribution of ancestral species with parsimony. The method is based on optimization of a three-dimensional cost matrix derived from a simple biogeographic model. Distributions are described in terms of a set of unit areas, and speciation is assumed to divide ancestral distributions allopatrically into mutually exclusive sets of unit areas. DIVA finds the optimal distributions of ancestral species by minimizing the number of dispersal and extinction events. Unlike other methods in historical biogeography, areas are not required to be hierarchically related, and DIVA does not make any assumptions about the shape or existence of general patterns of area relationships.

We used the trees obtained from bootstrap analysis of our combined and partitioned datasets. The distribution of each species was classified as present/absent in four different areas. In the optimization we allowed a maximum of two geographical areas per species, under the assumption that ancestral populations had limited geographical distributions.

**Results**

**Data Description**

Among 60 morphological characters scored for the *P. demoleus* group, 16 characters in one or more outgroup species were scored missing or inapplicable being dependent on a character itself scored as absent (0; Table 2). Two characters...
were scored as doubtful, and four characters were polymorphic. Across all species, two characters were nonvariable and eight variable characters were parsimony uninformative, leaving 50 parsimony informative characters for the whole dataset. For the ingroup alone, the number of nonvariable characters increased to 11 and number of informative characters decreased to 31.

The final alignment of DNA sequences numbered 3929 nucleotides in the combined dataset, including 1532 bp of COI, 68 bp of tRNA-leu, 685 bp of COII, 404 bp of wg, and 1240 bp of EF-1α. GenBank accession numbers for sequences are given in Table 1. Statistics for the ingroup and for all taxa are given in Table 3. Within the ingroup, COI and COII had the highest proportion of informative characters (10.9% and 10.3%, respectively), while wg had 5.9% and EF-1α only 3.9% parsimony informative characters. The tRNA-leu sequences had only one variable nucleotide in the ingroup, and for that reason this fragment was excluded from all further analyses except the combined dataset with all outgroup taxa. The number of informative characters varied over genes and nucleotide positions and, as is common in such data (Reed and Sperling 1999), most substitutions were observed in third codon positions and second codon positions were the most conservative in all genes. Very few or no substitutions were registered in first and second codon positions in nuclear genes in the ingroup. Relatively low levels of homoplasy were registered in all genes for the ingroup, while the inclusion of outgroups increased homoplasy in nucleotide data as seen from CI and RI values (Table 3). The same was true for saturation levels, with transition/transversion ratios declining in the total dataset compared to the ingroup.

Plotting p-distances versus maximum-likelihood corrected distances under GTR + G + I revealed higher levels of saturation in COII and COI and lower amounts in nuclear datasets (data not shown). The average percent sequence divergence (p-distance) for the ingroup ranged from 1.7% in EF-1α to 2.4% in wg and 5.1% both in COI and COII, while adding outgroups increased the amount of divergence to 4.4% in EF-1α, 7.7% in wg, 8.0% in COII, and 8.4% in COI.

Phylogenetic Analyses

A branch-and-bound search in PAUP retained six most parsimonious trees for morphological data (140 steps, CI = 0.429, RI = 0.529). Alternative topologies for both ingroup and outgroup relationships were recovered and no resolution was obtained in the strict consensus tree for any outgroups except pari + helenus and machaon + xuthus (Fig. 2). The strict consensus also had an unresolved trichotomy between P. erithonioides, P. demodocus and (P. demoleus, P. grossmithi, P. morondavana). Levels of support on the morphological tree were very low, and did not allow confidence about phylogenetic relationships between species in the P. demoleus species-group. However, the bootstrap consensus tree gave a basal position for P. morondavana. Also, when the most distant outgroups (based on highest pairwise sequence divergences from the ingroup), P. thoas and P. canadensis, were removed from the maximum parsimony analysis and P. anactus was used as an outgroup as the next most distant relative of lime swallowtails (Zakharov et al. 2004), the strict consensus of four most parsimonious trees also showed a basal position for P. morondavana. NONA heuristic searches of the morphological data recovered the same six trees that were found by branch-and-bound algorithm in PAUP.

Heuristic searches of molecular data with the full set of outgroup species resulted in a single tree (1157 steps) for COI data, five trees for COII (469 steps), one tree for COI + COII (1641 steps), one tree for wg (267 steps), one tree for EF-1α (476), four trees for wg + EF-1α (757 steps), and one tree (2415 steps) for all genes combined (Fig. 3, outgroup taxa are not shown). Two most parsimonious trees (2571 steps) were found for all data including molecular and morphological characters. When more than one most parsimonious tree was found for a particular partition, the ingroup
topologies were identical among trees found for all data except for the wg + EF-1α dataset. Each gene partition produced alternative groupings for the *P. demoleus* group. However, only COI, COI + COII, and all combined data produced relatively robust trees.

Mitochondrial DNA data suggest that *P. morondavana* is the most basal species in the group, while nuclear genes indicate *P. grosssmithi*. All partitions confirm the monophyly of the *P. demoleus* species-group with 91–100% bootstrap proportions and decay indices of 6–12, except for morphological data that had a 76% bootstrap proportion and decay index of 4. Combining data increased support both for monophyly of the *P. demoleus* species-group and intragroup relationships.

Bayesian analyses of mitochondrial DNA genes and partitioned wg and EF-1α revealed the same patterns of relationship among ingroup taxa that were obtained in corresponding maximum parsimony heuristic searches. Bayesian analysis of combined nuclear genes converged on the tree topology recovered for the wg dataset. As in maximum parsimony searches of molecular data, the same tree topology was found for COI, COI + COII and all four genes combined. Combining all four genes and morphological data in Markov chain Monte Carlo analyses with all substitution model parameters being estimated individually for gene partitions resulted in the highest levels of ingroup node support.

The trees shown in Figure 4 illustrate the cladograms obtained for all data combined, for both the *P. demoleus* group and representative *Papilio* outgroups. Maximum parsimony and Bayesian analyses recovered trees with an almost identical pattern of relationships, with the only difference in the position of *P. anactus*. Both maximum parsimony and Bayesian analyses showed *P. helenus* as the sister taxon to the *P. demoleus* group; however, support for this relationship was rather low. Partitioned Bremer support (Baker and DeSalle 1997) was calculated for each node of the combined maximum parsimony tree to provide a measure of how individual gene data contribute to the total decay indices. Most relationships among outgroups had low bootstrap proportions and conflicting phylogenetic signals from independent gene partitions. However, our main concern is the relationship within the ingroup, where all genes gave high support to monophyly of the *P. demoleus* group. The gene wg demonstrates the lowest support for a sister relationship between *P. demodocus* and *P. erithonioides* and is in greatest conflict with grouping *P. demoleus* with these two species. This partition also has negative support for allying *P. grosssmithi* with ((*P. demodocus*, *P. erithonioides*), *P. demoleus*). Data from COII also demonstrate visible conflict with the grouping of *P. demoleus* with *P. demodocus* + *P. erithonioides*.

The COI gene appears to have the most stable and reliable phylogenetic signal among closely related species and so the 3′ half of COI was selected for investigation of intraspecific variability within the *P. demoleus* species-group. A total of 27 unique haplotypes were identified from 50 ingroup individuals (Fig. 5). We found seven haplotypes for *P. demoleus*, with the most common haplotype (DL1) shared between populations of *P. d. malayanus* and *P. d. libanius*. None of these populations contained haplotypes of *P. d. demoleus* (DL5) or *P. d. sthenelus* (DL6 and DL7). *Papilio demodocus* revealed 14 haplotypes with no haplotypes shared between different localities in Africa. Also, none of the haplotypes of *P. demodocus* from Africa were found in Madagascar. *Papilio grosssmithi* revealed only four haplotypes, two of those (G2 and G3) were found in more than one collecting site. All four specimens of *P. morondavana*, collected in different years from three distant localities, had the same COI haplotype. No intraspecific variation was revealed within *P. erithonioides*, with all eight analyzed specimens from a wide range of sampled sites in southern and western Madagascar producing identical sequence for the 3′ half of COI.

Bayesian estimation of phylogenetic relationships of the 27 haplotypes identified for the five species of the *P. demoleus* group and 11 outgroup species of *Papilio* is shown in Figure 6. Very little nucleotide variability was found in *P. grosssmithi* with zero to two substitutions (*p* = 0–0.242%) in the 825-bp fragment. The degree of sequence divergence between two subspecies of *P. demoleus* (ssp. malayanus and ssp. libanius) was also low, with *p* ranging from 0% to 0.242%. Average *p* between these two subspecies and *P. demoleus* demoleus was only about 1%, whereas the Australian subspecies *P. demoleus* sthenelus had a 3.8% sequence divergence from other subspecies of *P. demoleus*. *Papilio demodocus* samples from Africa did not form a monophyletic lineage. All Malagasy specimens of *P. demodocus* differed from each other by zero to two substitutions (*p* = 0–0.242%) and grouped into a monophyletic assemblage (88% posterior probability) characterized by a single synapomorphic substitution. Number of substitutions among African *P. demodocus* varied from one to 10 (*p* = 0.121–1.212%). The average distance between *P. demodocus* from Africa and from Madagascar was 0.78%, ranging from 0.36% to 1.212%. All species of the *P. demoleus* group are well diverged, as indicated by the amount of sequence divergence ranging from 4.9% to 6.5%.

**Biogeography and Ancestral Areas**

Dispersal-vicariance analysis (Ronquist 1996) was applied to the inferred alternative cladograms (Fig. 7) with the distribution of each species classified as present/absent in four different areas. There were several alternative optimizations but in all cases the most optimal reconstruction required three dispersal events (four if *P. demodocus* arrived in Madagascar naturally) with different sequences of vicariance events. Based on this form of analysis, the ancestral area of the group was inferred to be Madagascar.

**DISCUSSION**

**Conflicting Phylogenetic Signals in Mitochondrial DNA, Nuclear Genes, and Morphology**

Except for the most basal relationships within the *P. demoleus* species-group, as well as outgroups, we obtained largely congruent evidence from the multiple sources of data, including nucleotide sequences from three independent gene regions (COI + COII, wg, EF-1α) and morphology. We observed more discordances between gene partitions and morphological data but here, as for mitochondrial versus nuclear genes, the conflict stemmed primarily from disagreement about basal relationships. Tests for incongruence between
Ingroup partitions revealed that COI and COII were the most congruent genes in our data, as should be expected because mitochondrial DNA genes are generally not considered to recombine due to almost exclusively maternal inheritance of mitochondrial DNA genes (with few exceptions, e.g., Kondo et al. 1990; Kaneda et al. 1995) and therefore to represent a single history. The observed disagreement between mitochondrial DNA genes and nuclear genes, the alternative phylogenetic hypotheses inferred from nuclear data (the partition with the lowest number of informative characters) had the lowest average bootstrap support for the wg genes were not very robust. Phylogeny estimation based on alternative phylogenetic hypotheses inferred from nuclear genes were not very robust. Phylogeny estimation based on mitochondrial DNA, allozymes, and morphology, demonstrated much higher hybrid incompatibility (Sperling et al. 1997). Häuser et al. (2002) place the lime swallowtails within the subgenus Papilio (Princes), a group of 19 species that, with the single exception of P. demoleus, is restricted to Africa and Madagascar.

The most recent evidence from a more comprehensive molecular phylogeny of Papilio (Zakharov et al. 2004) indicates that Princes itself is not monophyletic and lime swallowtails occupy a phylogenetic position between two Papilio subvisions, Menelaides and Achillides, which are recognized by Häuser et al. (2002) as subgenera, with good support for Menelaides as the sister taxon to the P. demoleus group. Our data continue to support this relationship, as P. (Menelaides) helenus was found to be the closest outgroup to the P. demoleus species-group. Papilio lornieri, from the P. menestheus group, is one outgroup species that was added to the current study to evaluate Hancock’s (1983) hypothesis that this species is closely related to the P. demoleus group. However, this species was shown to have a stronger relationship with P. delalandei and P. constantinus, which are relatively distantly related to the P. demoleus group.

Our results leave no doubt as to the monophyly of the P. demoleus species-group, and give strong resolution of intragroup relationships in the combined analyses. Our data do not agree with traditional assumptions about phylogenetic relationships within the P. demoleus group, in which P. demodocus or P. demoleus are considered the most basal species (Carcasson 1964). None of the analyses supports a basal position for P. demodocus and this species instead appears to be relatively derived. The strongest evidence indicates a clear sister relationship between P. demodocus and P. erithonioides, with P. demoleus likely being their sister taxon. There is good support for P. grosssmithi as the sister taxon of these three species, and a basal position for P. morondavana. Thus, the endemic species whose conservation status is of most concern, P. grosssmithi and P. morondavana, appear to represent the oldest

**Phylogenetic Relationships**

Hancock (1983) considered lime swallowtails to belong to the subgenus Princes (Princes), with the Papilio menestheus species-group as their sister-group. Based on experimental hybridization of P. demoleus with 13 other Papilio species, Ae (1979) considered P. dardanus and P. phorcas to be part of the P. demoleus group. However, there is little correlation between hybridization success and genetic similarity of swallowtail species (Zakharov et al. 2004), and hybridization data can be misleading. A similar situation was reported for water-striders in the genus Limnoporus, where one species that was clearly closely related to others, as was shown by mitochondrial DNA, allozymes, and morphology, demonstrated much higher hybrid incompatibility (Sperling et al. 1997). Häuser et al. (2002) place the lime swallowtails within the subgenus Papilio (Princes), a group of 19 species that, with the single exception of P. demoleus, is restricted to Africa and Madagascar.

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lineages among lime swallowtails. These relic species speak for additional protection being given to the dry deciduous forests of Madagascar as a conservation priority (Vane-Wright et al. 1991; Stiassny and de Pinna 1994).

Another interesting finding is the degree of genetic variation among and within species of the *P. demoleus* group. Although percent sequence divergence is quite variable among closely related Lepidoptera and is not necessarily a reliable indicator of species status, there is an obvious trend with overall sequence divergence increasing at higher taxonomic levels. Almost 98% of species recognized through prior morphological studies are reported to fit a species level threshold value of 3% divergence in COI proposed by Hebert et al. (2003). However, the amount of sequence divergence in COI at the interspecific level within lepidopteran species complexes ranges from 0.1–0.6% (0.4% average) in ermine moths *Yponomeuta* (Sperling et al. 1995), 0–3.6% (2.1% average) in *Choristoneura* (Sperling and Hickey 1994), and 0.1–3.7% (2.2% average) in *Feltia* (Sperling et al. 1996). Divergences of less than 1% and up to 2.5% divergence in COI was reported for sister species of torticid moths of the genus *Archips* (Kruse and Sperling 2001). Higher levels of variation in interspecific mitochondrial DNA divergences was demonstrated for the one of the most well studied species groups of the genus *Papilio*, the *P. machaon* complex, with values ranging from <1% to 8% (Sperling and Harrison 1994).

In lime swallowtails, intraspecific mitochondrial DNA divergences ranged from nothing within *P. morondavana* and *P. erithonioides* to less than 0.5% between closely related subspecies of *P. demoleus*, but up to almost 4% sequence divergence between more distant subspecies of *P. demoleus*. At the same time, the species themselves were separated by nucleotide differences of 5–6%, which leaves no doubt that all lime swallowtail species are very distinct by any comparison to other Lepidoptera species.

Lack of variation in the COI gene in *P. erithonioides* and *P. morondavana*, sampled in different localities and time series, requires further investigation. While *P. erithonioides* may still be too evolutionarily young to have accumulated much variation, the latter species is probably a relic. Lack of mitochondrial DNA variation in *P. morondavana* might indicate that the ancestral population has gone through one or more recent bottlenecks.
Fig. 3. Phylogenetic relationships for species of the *Papilio demoleus* group inferred from partitioned and combined molecular and morphological data using maximum parsimony (MP) and Bayesian analyses. Trees represent ingroup relationships from MP bootstrap consensus trees that were identical to ingroup topologies of MP trees in each analysis that included full set of outgroups (except F and H) and were identical to all Bayesian reconstructions (except H). For F (wg + EF-1a), two MP trees had the given ingroup topology; two other trees showed sister relationships for *P. grosesmithi* with *P. demoleus* + *P. morondavana*. For H (morphological data), the MP bootstrap consensus is on the left, and Bayesian reconstruction is on the right. Numbers above nodes show bootstrap proportions and Bremer support; numbers under nodes indicate Bayesian posterior probabilities.

**Biogeography**

The approximate age of divergence of the *P. demoleus* species-group from other *Papilio* is estimated to be about 16.8 ± 6.7 million years (Zakharov et al. 2004). This date excludes any possibility for older vicariance via Cretaceous plate tectonics. Recent reports of invasion of *P. demoleus* into islands in Southeast Asia where the species was originally absent indicate the ability of lime swallowtails to expand their range, whether through active flight or with the aid of man and *Citrus* cultivation (Hiura 1973; Corbet and Pendlebury 1978, 1992; Vane-Wright and de Jong 1993).

Phylogenetic reconstruction clearly indicates a basal position for *P. morondavana* and *P. grosesmithi* within the *P. demoleus* species-group. Based on estimated substitution rates for COI and COII genes (Zakharov et al. 2004), the ancestors for *P. morondavana* and the rest of lime swallowtails diverged between approximately 14.1 and 10.6 million years ago, and the split of the ancestor for *P. grosesmithi* from its sister group is dated at between 13.5 and 10.3 million years ago. Dispersal-vicariance analysis based on these relationships suggests their origin and speciation within Madagascar (Fig. 7), followed by dispersal of lime swallowtails into Australia and Asia (becoming *P. demoleus*) around 10.1 to 7.8 million years ago, and Africa (becoming *P. demodocus*) around 7.3 to 5.6 million years ago.

Due to weakly supported outgroup relationships, our data are insufficient to resolve with confidence how the ancestor of lime swallowtails came to Madagascar. Based on a currently available molecular phylogeny for the genus *Papilio* (Zakharov et al. 2004), the *P. demoleus* species group had a...
common ancestor with an Oriental lineage of *Papilio*, the subgenus *Menelaides*. Together they are the sister group to *Papilio* (*Achillides*), another Oriental subgenus. This suggests that an Asian ancestor of the *P. demoleus* species-group may have first reached Madagascar in a manner similar to the hypothesized origin of the Madagascan mycalesine butterflies (Lees 1997; Torres et al. 2001). This scenario is also supported by a close approach to Madagascar of two lineages of an Indo-Australian genus *Euploea* (*Lepidoptera: Nymphalidae*) to the Seychelles and Mascarene Islands (Ackery and Vane-Wright 1984).

Colonization of Madagascar would have been followed by speciation of Malagasy endemics, succeeded by dispersal of the ancestors of each of *P. demodocus* and *P. demoleus* to Africa and back to the Oriental region and Australia. Under the best-supported parsimony reconstructions, *P. moronda-vana*, *P. grosssmithii*, and *P. erithonioides* would have diverged from each other on Madagascar itself, whether on different parts of the island or by a sympatric process where they were in contact with each other.

The probability of dispersal across the Indian Ocean (c. 4000 km) may seem relatively low compared to a scenario involving multiple colonizations of Madagascar from Africa (separated by just c. 400 km). Nonetheless, a similar hypothesis has also been proposed for the origin of other representatives of the Malagasy fauna. Jansa et al. (1999) suggested a single invasion of native rodents (*Muridae: Nesomyinae*) to Madagascar from Asia followed by secondary invasion from Madagascar into Africa (but see Jansa and Weksler 2004). Lees (1997) and Torres et al. (2001) also suggested that the Malagasy mycalesine butterfly radiation may have originated from India rather than Africa, and that...
a dispersal event from India was followed by subsequent colonization of Africa by founders from Madagascar.

Long-distance dispersal has been proposed to explain similar patterns of relationships in chameleons, where the oldest lineages are distributed in Madagascar, and more recently derived forms that are found in Africa, the Seychelles, the Comoros, and India are believed to be the result of several dispersal events across the Indian Ocean (Raxworthy et al. 2002). Both the oldest fossil records that are available for chameleons and geological age of the volcanic islands of Comoros are substantially younger than the time of Cretaceous vicariance (see Raxworthy et al. 2002). The Comoros archipelago has never been in contact with larger land masses since its formation within the last 5.4 million years (Emerick and Duncan 1982); thus, the only possible way the ancestor of the endemic chameleons endemic to these islands could have arrived there is by transmarine dispersal.

Oceanic dispersal of terrestrial animals to and from Madagascar and other islands in the western Indian Ocean has been favored by many other studies that hypothesized post-Gondwanan transmarine migration by rafting on tangles of vegetation (e.g., Fisher 1996; Yoder et al. 1996; Nagy et al. 2003; Vences et al. 2003). Dispersal across an uninhabitable space is even more plausible when a taxon possesses a significant degree of vagility, as in actively flying Lepidoptera and lime swallowtails in particular.

It also appears that the Malagasy biota has provided sources for multiple radiations that have contributed to the biodiversity of the surrounding continents and islands. For example, reconstruction of island radiations in extinct and extant geckos using ancient and recent DNA by Austin et al. (2004) suggested that at least four different archipelagos in the Indian Ocean have been colonized independently by dispersals from Madagascar. According to Warren et al. (2003), Madagascar has given rise to two independent sunbird lineages in the Aldabra archipelago.

Complex seasonal systems of oceanic currents and winds in the Indian Ocean provide conditions for long-distance transmarine dispersals (see Fig. 5). The currents of the northern Indian Ocean are influenced by the Asiatic monsoon while the currents of the southern Indian Ocean are influenced by anticyclonic circulation in the atmosphere (Walter 1970). The northwest monsoon stimulates the North Equatorial Current, which flows from east to west and upon reaching the east coast of Africa the largest portion turns southward and eventually becomes the Mozambique Current. The Mozambique Current flows south along the east coast of Africa and at about 35°S merges with the Alguhas Stream, which flows...
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FIG. 6. Bayesian estimation of the phylogeny of 27 unique haplotypes (from 50 ingroup samples) and 11 outgroups inferred from 825 bp from the 3' end fragment of COI (10^6 cycles, each 100th sample, contype = halfcompat, burnin = 5000). Numbers above nodes indicate clade posterior probabilities, and under nodes indicate bootstrap proportions from maximum parsimony analyses. For haplotype definitions and species list, refer to Table 1. For geographic distribution of haplotypes, see Figure 5.

westward along the southern coast of Madagascar and the east African coast toward the Cape of Good Hope, where it partly joins the West Wind Drift Current, which then carries its waters toward southwest Australia. During the southwest monsoon in August and September, the North Equatorial Current reverses direction and flows west to east as the Monsoon Current.

Thus, a westward dispersal of lime swallowtails across the northern part of the Mozambique Channel is more probable based on prevailing winds and has been inferred to occur on several occasions in Lepidoptera (Pierre 1992; Torres et al. 2001), though on more tenuous phylogenetic grounds. Prevailing easterly trade winds and ocean currents in the Indian Ocean also increase the probability of long-distance dispersal from Asia to the western Indian Ocean (Donque 1972; Renvoise 1979). At the same time, dispersal from southern Madagascar and southern Africa can be facilitated by West Wind Drifts, as has been hypothesized for some sea stars (Waters and Roy 2004).

Alternatively, the assumptions of dispersal-vicariance analysis may simply be wrong, in that the distribution of these butterflies may not be parsimonious. For example, ex-
tinction of intermediate or ancestral taxa would complicate and obscure reconstruction of dispersal, refuting an indigenous origin of the group within Madagascar. Under a scenario of dispersal from Africa, there could have been a number of successive arrivals to Madagascar from an anagenetically evolving stem lineage, as suggested by Carcasson (1964).

Additional uncertainty is associated with the origin of *P. demodocus* in Madagascar. It is reported that the presence of *P. demodocus* on the island is the result of introduction by man (Paulian 1951; Paulian and Viette 1968; Turlin 1994). The monophyly of mitochondrial DNA haplotypes found in Madagascan samples of *P. demodocus* relative to African individuals may be a consequence of founder effect from a single introduction. However, the fact that the mitochondrial lineage already displays moderate genetic variability (four haplotypes of seven samples) suggests that colonization of Madagascar by this lineage may have occurred thousands of years ago or even longer, prior to island colonization by man about 2000 years ago (Dewar 1997).

The origin of *P. demoleus* in Saudi Arabia and Iran is also
believed to be the result of introduction along with the first importation of Citrus in the 10th century (Wiltshire 1945; Larsen 1977). The 1% sequence divergence between our sample of P. demoleus from Iran and our samples from Southeast Asia and eastern Asia indicates that this introduction would probably have come from closer sources, such as the Indian subcontinent (from which we were unable to obtain samples), which already had a moderate amount of divergence from populations farther to the east. Interestingly, all Asian individuals of P. demoleus had very similar or identical haplotypes, supporting the hypothesis of recent range expansion in this region. In contrast, there was a striking difference in mitochondrial DNA between Oriental subspecies and the Australian subspecies P. d. sthenelus. Taking into account host use differences between this and other subspecies of P. demoleus, these haplotypes appear to be well-diverged lineages that might be considered separate species (Hebert et al. 2003). At minimum, it appears likely that ancestral populations of P. demoleus in the Oriental and the Australian regions have been isolated for a long time, from 3.24 to 4.24 million years ago based on substitution rates estimated for mitochondrial DNA in Papilio (Zakharov et al. 2004). Thus P. d. sthenelus may represent the oldest lineage in P. demoleus. More detailed sampling across its range is required to reconstruct the evolutionary history of this species on both sides of Wallace’s line.

Conclusion

Biogeographic scenarios are strongly dependent on the selection of phylogenetic hypotheses for a particular group of organisms, while phylogenetic reconstructions themselves can be subject to different biases in data. Thus, selection of informative loci for a given taxonomic level remains key to understanding the evolution and biogeography of any group of organisms. In our reconstructions of the phylogeny of species in the P. demoleus group, nucleotide sequences have provided a useful source of data in addition to traditional morphology. Comparison of four genes representing three independent loci supports the utility of the COI gene in reconstructing the phylogeny of closely related species, such as in the P. demoleus group.

Our results provide strong evidence for the basal relationships of Malagasy endemic species in the P. demoleus group. Relationships among lime swallowtails on Madagascar include elements similar to those reported in nymphalid butterflies and even chameleons, indicating not only that post-Cretaceous dispersal across large oceanic barriers has contributed to the diversity of Madagascar, but that the Malagasy biota has speciated actively on the island, serving as an incubator for radiations that have contributed to the diversity of surrounding continents on all sides of the Indian Ocean. Our study may be the best documented case of this pattern to date, despite the fact that morphology does not yet seem to provide enough resolution both for ingroup lime swallowtails and outgroup species of Papilio, as it is supported by multiple independent genes.

Of more immediate importance, our data support a recommendation that further protection should be given to the dry forests of Madagascar (generally underrepresented within the protected area system; Dupuy and Moat 1996) to ensure survival of the endemic species of lime swallowtails in Madagascar. These large and charismatic butterflies give new insight into the complex history of the unique and ancient biodiversity of this island, as well as add weight to the urgency of arresting the rapid clearance of dry deciduous forests on the island via creation of new protected areas, for the mutual benefit of other species restricted to these ecosystems.

ACKNOWLEDGMENTS

We are grateful to the following people for help in providing specimens for this study: N. Pierce, D. Lohman, and M. Cornwall from Harvard University (Cambridge, MA, USA); N. Tatarnik and V. Nazari from the University of Alberta (Edmonton, AB, Canada); H. F. Wong from Deco Enterprise (Taiping, Malaysia); S. Schoeman from ARC, Institute for Tropical and Subtropical Crops (South Africa); M. G. Wright from the University of Hawaii (Honolulu, HI, USA); D. Goh from the Penang Butterfly Farm (Penang, Malaysia); S. A. Ae (Gifu, Japan); S. H. Yen from The Natural History Museum (London, United Kingdom); and J. Demay from SNC Ornithoptera (Agny, France). This study was funded by an NSERC grant to FAHS. Collections from Madagascar were partially supported by grant DEB-0072713 from the National Science Foundation to B. L. Fisher and C. E. Griswold. Fieldwork that provided the basis for this work could not have been completed without the gracious support of the Malagasy people. We are grateful for comments on an earlier version of this manuscript by A. Yoder, N. Wahlberg, and an anonymous reviewer.

LITERATURE CITED


Morphological characters and states used in the cladistic analysis of five species of the Papilio demoleus species group and eleven outgroup species of Papilio. Characters are illustrated in Figure 1 and will be fully discussed in C. R. Smith and R. I. Vane-Wright (unpubl. ms.).

APPENDIX 1

Wing pattern

Forewing upperside
1. Discal cell with pale scales: absent = 0; present = 1.
2. Discal cell scales (patterning): random = 0; organized = 1.
3. Cell R 4 marginal cell scale pattern: longitudinal stripes = 0; transverse bands = 1.
4. Discal cell with an array of three distal marks: absent = 0; present = 1.
5. Cell R 2 with postdiscal mark: absent = 0; present = 1.
7. Cell R 2 postdiscal mark: absent = 0; present = 1.
9. Cell 1A with both a postdiscal and a submarginal mark: absent = 0; present = 1.

Forewing underside
10. Cell R 2 with apical mark: absent = 0; present = 1.
11. Cell M 1 with postdiscal mark: absent = 0; present = 1.

Hindwing upperside
12. Cell R 1 with an eyespot: absent = 0; present = 1.
15. Cell M 1 with postdiscal spot: absent = 0; present = 1.
17. Cell M 2 postdiscal spot: absent = 0; present = 1.

Hindwing underside
18. Cells R 1 and discal cell with pale basal band: absent = 0; present = 1.
19. Basal band in cells R 1 and discal cell: narrow = 0; broad = 1.
20. Cells R 2 to CuA 1 with a pattern of blue and orange bands proximal to the submarginal marks: absent = 0; present = 1.
22. Cell R 2 with discal mark reaching root of M 1: absent = 0; present = 1.
23. Cell M 1 with marginal mark subdivided into two large marks: absent = 0; present = 1.
24. Cell M 1 with marginal mark subdivided into two large marks: absent = 0; present = 1.
25. Hindwing tail (size): absent or rudimentary = 0; short = 1; medium = 2; long = 3.
26. Hindwing tail with club: absent = 0; present = 1.
27. Upperside surface of male forewing with androconial scales: absent = 0; present = 1.

Antennae
28. Antennal color: unicolorous = 0; with pale mark on one surface near tip = 1.

Male genitalia
29. Valve rim with postero-ventral expansion: absent = 0; present = 1.
30. Valve rim with terminal notch: absent = 0; present = 1.
31. Harpe with vertical projection: absent = 0; present = 1.
32. Vertical projection of harpe: free = 0; fused = 1.
33. Vertical projection of harpe: simple = 0; double = 1.
34. Vertical projection of harpe: contiguous = 0; detached = 1.
35. Harpe reaching or surpassing valve rim: absent = 0; present = 1.
36. Harpe with ventral flange serrate: absent = 0; present = 1.
37. Pseuduncus width: narrow = 0; broad = 1.
38. Pseuduncus strongly declivous: absent = 0; present = 1.
39. Uncus with prominent, terminal ridge or projection: absent = 0; present = 1.
40. Uncus with prominent sub-terminal swelling: absent = 0; present = 1.
41. Uncus with prominent serrations: absent = 0; present = 1.
42. Saccus: absent = 0; present = 1.

Female genitalia
43. Vestibulum with pocket: absent = 0; present = 1.
44. External genitalic pocket: shallow = 0; deep = 1.
45. Pocket with ventral lining of pocket: rugose = 0; lanose = 1.
46. Ostium bursae position: anterior = 0; central/posterior = 1.
47. Ostium bursae opening on sagittal ridge: absent = 0; present = 1.
48. Peripheral vestibular plates: absent = 0; present = 1.
49. Peripheral vestibular plates extending around ostium: absent = 0; present = 1.
50. Peripheral vestibular plates joining anteriorly: absent = 0; present = 1.
51. Peripheral vestibular plates with posterior expansion: absent = 0; present = 1.
52. Peripheral vestibular plates with processes: absent = 0; present = 1.
53. Lateral ostial plates: absent = 0; present = 1.
54. Lateral ostial plates extended posteriorly: absent = 0; present = 1.
55. Lateral ostial plates with lateral ridges: absent = 0; present = 1.
56. Lateral ostial plates with peripheral lamella: absent = 0; present = 1.
57. Lateral ostial plates with joining anteriorly: absent = 0; present = 1.
58. Tonguelike process: absent = 0; present = 1.
59. Tonguelike process with posterior transverse ridge: absent = 0; present = 1.
60. Posterior central cup: absent = 0; present = 1.