Syst. Biol. 50(1):106–127, 2001

A Partitioned Likelihood Analysis of Swallowtail Butterfly Phylogeny (Lepidoptera: Papilionidae)

MICHAEL S. CATERINO,[1,3] ROBERT D. REED,[2] MAY M. KUO,[1]
AND FELIX A. H. SPERLING[1,4]

[1]*Division of Insect Biology, 201 Wellman Hall, University of California, Berkeley, CA 94720-3112, USA*
[2]*Department of Molecular and Cellular Biology, University of Arizona, Life Science South 444, Tucson, AZ 85721, USA*

Abstract.—Although it is widely agreed that data from multiple sources are necessary to confidently resolve phylogenetic relationships, procedures for accommodating and incorporating heterogeneity in such data remain underdeveloped. We explored the use of partitioned, model-based analyses of heterogeneous molecular data in the context of a phylogenetic study of swallowtail butterflies (Lepidoptera: Papilionidae). Despite substantial basic and applied study, phylogenetic relationships among the major lineages of this prominent group remain contentious. We sequenced 3.3 kb of mitochondrial and nuclear DNA (2.3 kb of cytochrome oxidase I and II and 1.0 kb of elongation factor-1α, respectively) from 22 swallowtails, including representatives of Baroniinae, Parnassiinae, and Papilioninae, and from several moth and butterfly outgroups. Using parsimony, we encountered considerable difficulty in resolving the deepest splits among these taxa. We therefore chose two outgroups with undisputed relationships to each other and to Papilionidae and undertook detailed likelihood analyses of alternative topologies. Following from previous studies that have demonstrated substantial heterogeneity in the evolutionary dynamics among process partitions of these genes, we estimated evolutionary parameters separately for gene-based and codon-based partitions. These values were then used as the basis for examining the likelihoods of possible resolutions and rootings under several partitioned and unpartitioned likelihood models. Partitioned models gave markedly better fits to the data than did unpartitioned models and supported different topologies. However, the most likely topology varied from model to model. The most likely ingroup topology under the best-fitting, six-partition GTR + Γ model favors a paraphyletic Parnassiinae. However, when examining the likelihoods of alternative rootings of this tree relative to rootings of the classical hypothesis, two rootings of the latter emerge as most likely. Of these two, the most likely rooting is within the Papilioninae, although a rooting between *Baronia* and the remaining Papilionidae is only nonsignificantly less likely. [Data partitioning; heterogeneity; likelihood; process partitions.]

Phylogeny reconstruction is one of the most dynamic and challenging pursuits in modern biology. With recent computational advances, phylogeneticists are increasingly able to incorporate knowledge of molecular evolutionary dynamics in the estimation of organismal phylogenies. This becomes particularly important when examining deeper branches of the tree of life, because with sufficient time, molecular evolution tends to overwrite its own signal, thereby obscuring much phylogenetic information. Maximum likelihood methods, which incorporate models of molecular evolution, can compensate for unobserved substitutions and thus offer a practical solution to this problem. Developing a sound phylogenetic hypothesis generally necessitates sampling multiple independent sources of data (e.g., molecules and morphology, multiple unlinked loci). However, the evolutionary dynamics of independent data may vary widely (Bull et al., 1993; Reed and Sperling, 1999), such that a single evolutionary model might be inappropriate for such heterogeneous data sets. Rather, invoking several models may be advantageous, each one closely matching the dynamics of one or more of the particular process partitions of the data (Liò and Goldman, 1998; Amrine and Springer, 1999; DeBry, 1999). In this study we examine the performance of a partitioned likelihood analysis in reconstructing phylogenetic relationships among the subfamilies and tribes of papilionid butterflies.

Swallowtail butterflies, in the family Papilionidae, are among the best known insects. Besides serving as the flagships of invertebrate conservation (Collins and Morris, 1985), swallowtails have been well-studied taxonomically and ecologically and have been popular as paradigm systems

[3]Present address (and address for correspondence): Department of Entomology, The Natural History Museum, Cromwell Rd., London SW7 5BD, UK; E-mail: histerid@nhm.ac.uk

[4]Present address: Department of Biological Sciences, CW-405 Biological Sciences Center, University of Alberta, Edmonton, Alberta T6J 2E9, Canada.

for illustrating numerous biological phenomena, including mimicry (Clarke and Sheppard, 1963), coevolution (Ehrlich and Raven, 1964), and key adaptations (Berenbaum et al., 1996). A thorough understanding of these evolutionary phenomena requires a reasonable estimate of phylogeny. For example, much of the continued debate regarding insect/plant coevolution (e.g., Miller, 1987a; Pellmyr et al., 1996; Brower, 1997; Farrell and Mitter, 1998) rests on disagreements over phylogenetic details. Recent studies have made progress in understanding relationships within limited groups of Papilionidae (Troidini: Miller, 1987b; Weintraub, 1995; Morinaka et al., 1999; *Battus*: Racheli and Oliverio, 1993; *Ornithoptera*: Parsons, 1996; Papilionini: Aubert et al., 1999; Caterino and Sperling, 1999; Reed and Sperling, 1999). However, the higher-level relationships of swallowtails remain equivocal (Rothschild and Jordan, 1906; Ford, 1944; Ehrlich, 1958; Munroe, 1961; Hancock, 1983; Miller, 1987b; Brown et al., 1995; Yagi et al., 1999).

The Papilionidae contains three subfamilies: the Baroniinae, Parnassiinae, and Papilioninae. The monophyly of the family is undisputed and is supported by several synapomorphies (see Kristensen, 1976; Hancock, 1983; Miller, 1987b), most convincingly, the larval osmeterium, an eversible, forked gland in the thorax that produces and advertises defensive chemicals (Eisner and Meinwald, 1965). The phylogeny of Hancock (1983; our Fig. 1), although not universally accepted in all of its details, represents the prevailing hypothesis of subfamilial and tribal relationships, and we refer to it hereafter as the "classical" hypothesis. The position of the family within the Papilionoidea has been controversial. A sister group relationship between Papilionidae and Pieridae has long been favored (e.g., Ehrlich, 1958; Scott, 1986). However, placing the Papilionidae as the sister lineage to all other Papilionoidea is gaining favor (de Jong et al., 1996; Weller et al., 1996).

The Baroniinae contains only *Baronia brevicornis*. Populations of this butterfly occur across southern Mexico in deciduous scrub forest where its sole host plant, *Acacia cochliacantha* (Fabaceae), occurs (Tyler et al., 1994). On the basis of morphology, *Baronia* has been suggested to be the sister lineage to all other Papilionidae, and some have referred to it as a "living fossil" (Collins and Morris, 1985). The position of *Baronia* as basal within Papilionidae seemed assured (Munroe, 1961; Hancock, 1983), but the comprehensive morphological analysis of butterfly phylogeny by de Jong et al. (1996) has suggested, instead, that *Parnassius* might occupy this position. *Baronia* does resemble one of the oldest known fossil butterflies, *Praepapilio colorado* (Eocene: 48 million years before the present (MaBP); Durdon and Rose, 1978). However, the resemblance offers no evidence of its phylogenetic placement. Even the interpretation of *Praepapilio* as a true papilionid has not been universally accepted (Scott, 1986). Furthermore, some authorities place the divergence of the major swallowtail groups before the Gondwanan breakup (i.e., ~90 MaBP; Tyler et al., 1994), well before the time of *Praepapilio*. These inconsistencies remain to be reconciled. The phylogenetic placement of *Baronia* has important implications for understanding much of butterfly evolution, in particular, whether its use of a leguminous host represents the plesiotypic butterfly condition (Scott, 1986).

The subfamily Parnassiinae contains ~48 species in two tribes: the Parnassiini, containing the extant genera *Archon, Hypermnestra*, and *Parnassius* (containing 32 of the 48 species of Parnassiinae); and the Zerynthiini, with *Sericinus, Allancastria, Zerynthia, Bhutanitis*, and *Luehdorfia*. Häuser (1993) pointed out several weaknesses in the hypothesis of parnassiine monophyly, emphasizing that several uniting features of the subfamily actually vary substantially among the genera, with *Hypermnestra*, in particular, lacking many parnassiine apomorphies. Häuser (1993) also noted that the production of an elaborate sphragis (a mating plug, produced by the male, but observed on mated females) does not correspond to the current tribal division, being found only in *Parnassius, Bhutanitis*, and *Luehdorfia*. Häuser concluded that even the removal of the obviously controversial *Hypermnestra* from Parnassiinae would yield a "nonmonophyletic taxon," a view supported by the morphological studies of de Jong et al. (1996) and by the work of Yagi et al. (1999) on mitochondrial NADH dehydrogenase subunit 5 (ND5) sequences.

The Papilioninae is by far the largest subfamily of Papilionidae, with >500 species (Collins and Morris, 1985). Although most

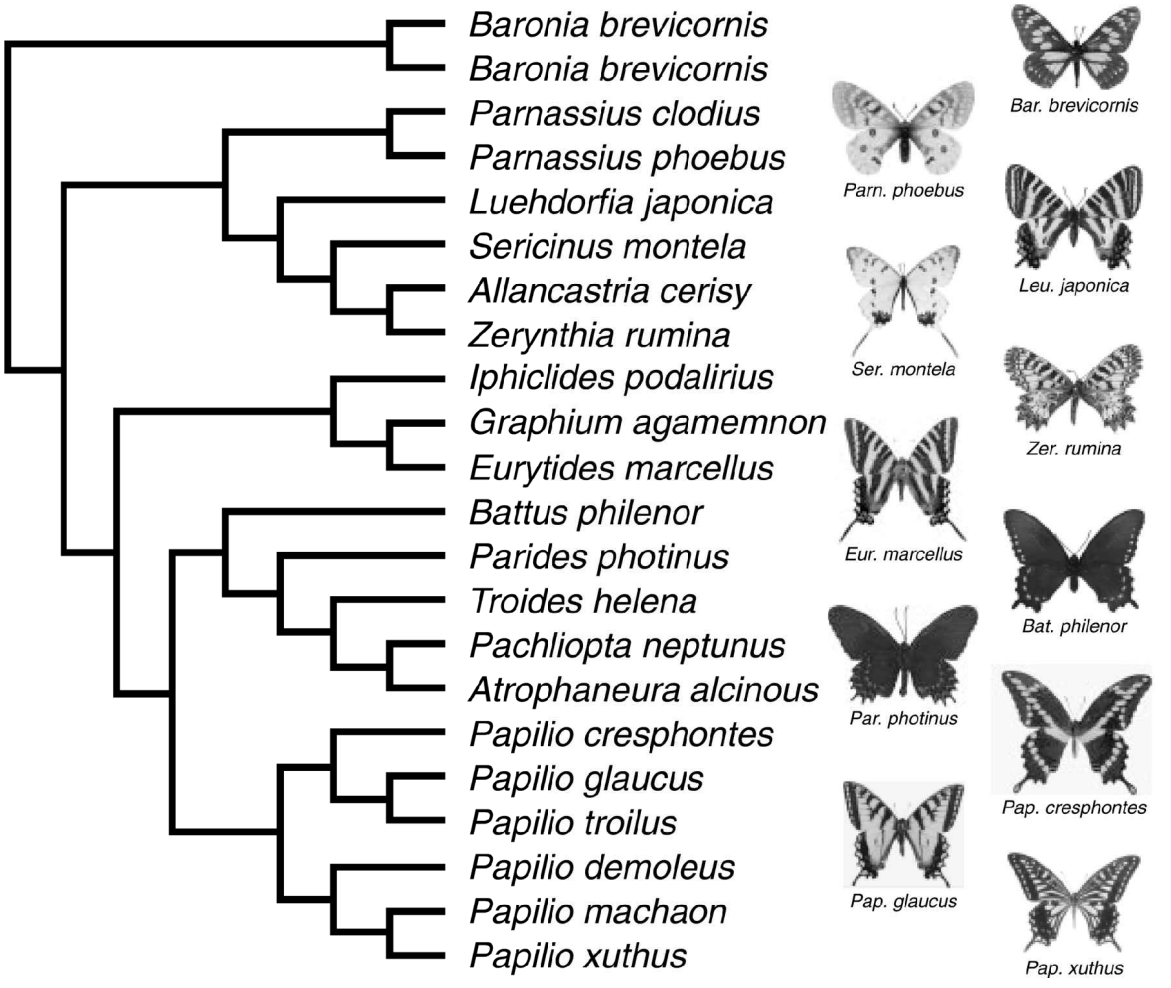


FIGURE 1. The "classical" hypothesis of swallowtail higher relationships. This phylogeny is essentially that of Hancock (1983), as represented by the species in this study. Relationships within Papilionini differ slightly from Hancock's, following Caterino and Sperling (1999) and Reed and Sperling (1999).

authors agree on its monophyly the relationships of the three main tribes (Graphiini [=Leptocircini], Troidini, and Papilionini) have been the subject of considerable controversy. (The enigmatic Teinopalpini is generally placed in Papilioninae as well, but because we have not been able to sample this group, their relationships are not discussed here.) Most authors also have agreed on the "primitive" nature of the Graphiini, although they have represented this by several different cladistic hypotheses. Munroe and Ehrlich (1960) suggested that the Graphiini might be paraphyletic with respect to both the Papilionini and the Troidini. Hancock (1983) appeared to propose a sister group relationship between Troidini and Papilionini (a relationship weakly supported by Caterino and Sperling, 1999). However, although Graphiini appears monophyletic in Hancock's phylogeny, his text suggests that it is paraphyletic with respect to a Troidini + Papilionini lineage (Hancock, 1983:12). Two recent molecular studies have reached conclusions at odds with either of these hypotheses. Yagi et al. (1999) found a sister group relationship between Graphiini and Troidini by using ND5, whereas Morinaka et al. (1999), using the same gene, found *Battus* to be more closely related to Graphiini than to the remaining Troidini and considered Graphiini + *Battus* + Papilionini together to constitute the sister group to the Troidini.

The resolution of relationships among the tribes of Papilioninae will have direct bearing on the reconstruction of several intriguing morphological and behavioral similarities shared by the Parnassiinae and Troidini. All Troidini and most genera of Parnassiinae feed exclusively on Aristolochiaceae, storing and using aristolochic acids as defensive chemicals (von Euw et al., 1968; Rothschild, 1972; Nishida et al., 1993). This mode of feeding and defense is correlated with the presence, in the larva, of raised, frequently red, tubercles. Ehrlich and Raven (1964) suggested that Aristolochiaceae feeding is plesiomorphic within the family (or at least for a common ancestor of Parnassiinae and Papilioninae). Igarashi (1984) proposed a direct ancestry of an *Aristolochia*-feeding parnassiine (*Sericinus*) to the entire Troidini in the clearest hypothesis of homology of this habit. Although this conclusion has been disputed (Miller, 1987a, 1987b; Weintraub, 1995), troidines and parnassiines share several other seemingly independent characteristics including, in at least some species, asymmetrical tarsal claws, the secretion of a large, visible mating plug (sphragis) by the male, and an elongate sclerotized aedeagus (Häuser, 1993). All of these features have been treated variously as symplesiomorphies or convergences, and the issue, as noted by Häuser (1993), is as yet unresolved.

In this study we have sampled members of most currently recognized papilionid subfamilies and tribes (following Miller, 1987b) in an effort to resolve these issues. Although we were unable to sample a few interesting genera (i.e., *Teinopalpus, Meandrusa, Hypermnestra*), their absence should not substantially affect our efforts to examine major phylogenetic events in the family. Using nuclear (elongation factor-1α [EF-1α]) and mitochondrial (cytochrome oxidase I and II [COI and COII]) protein-coding DNA sequences, we have attempted to determine the higher phylogeny of the Papilionidae. The data collected also permit some assessment of the relationship of Papilionidae to other butterflies.

Given the broad range of divergences involved in this problem, we recognized from the outset that a strict parsimony approach with the selected genes might prove inadequate to resolve the deeper nodes. In a previous study on species-level relationships within *Papilio*, Reed and Sperling (1999) examined the relative phylogenetic performance of these loci. The COI and COII data were found to compromise the resolving power of EF-1α for the deeper nodes of the tree because of homoplasy in the mitochondrial third codon positions (downweighting of the putatively homoplasious positions improved bootstrap support for these deeper branches). This assertion was further supported by estimating the rates of evolution of gene- and codon-based process partitions (sensu Bull et al., 1993) by maximum likelihood; rates among the codon positions of the different genes varied as much as 22-fold (see Table 3 in Reed and Sperling, 1999). For the purposes of phylogenetic analysis, their results suggest that applying a single evolutionary model across all the data would lead to biased estimates of the expected divergence for much of the data. This problem would cause particular difficulty in the reconstruction of deep nodes, where the

accurate estimation of nucleotide divergence is especially troublesome. DeBry (1999) has recently demonstrated that partitioned models may fit heterogeneous data better than unpartitioned models and may, in addition, support alternative topologies. In this study we therefore have undertaken analyses designed to accommodate evolutionary heterogeneity observed among subsets of the data by using partitioned likelihood analyses.

Materials and Methods

Ingroup Taxa

Our sampled taxa include multiple representatives of all of the major tribes of Papilionidae (Table 1) At the tribal level, we lack only representatives of the Teinopalpini (Papilioninae; generally considered to contain two genera, *Teinopalpus* and *Meandrusa*). The subfamily Baroniinae includes only a single species, of which we have examined two individuals. From the Parnassiinae we lack representatives of *Hypermnestra*, *Archon*, and *Bhutanitis*; however, the last of these is considered closely related to *Luehdorfia* (Hancock, 1983), which is examined here. From the Papilionini we have included three genera of Graphiini, five genera of Troidini, and six species from widely separated species groups of *Papilio* (Papilionini.) All of the sequences of Papilionini and one each of the Troidini and Graphiini were used in the previous studies of Caterino and Sperling (1999) and Reed and Sperling (1999).

Outgroup Taxa

Because the root of the Papilionidae is unclear, we sequenced a wide variety of Lepidopteran outgroups. The general consensus has been that the Pieridae is the closest relative of the Papilionidae (e.g., Scott, 1985), and we thus include one member each of two pierid subfamilies. However, recent morphological (de Jong et al., 1996) and combined data (Weller et al., 1996) studies have suggested that Papilionidae may be the sister group of the remaining Papilionoidea. According to this view, any other Papilionoidea might serve as appropriate outgroups; therefore, we also included sequences from one member of each of Nymphalidae, Satyridae, Riodinidae, and Lycaenidae. We also included several taxa from outside of the Papilionoidea. The Hesperiidae (skippers) are widely held to be the sister group of the Papilionoidea, or true butterflies, and we have sequenced representatives of two different subfamilies. We finally included representatives of five moth families as well as one representative of the enigmatic Hedylidae, long considered a geometroid moth but now postulated to be a basal butterfly (Scoble and Aiello, 1990).

Genes

We have sequenced the entire mitochondrial COI and COII genes and ~1,000 bp of the nuclear protein-coding EF-1α gene, for a total of 3,328 bp. The deepest papilionid divergences are thought to date to >50 MaBP (Miller, 1987b) with the divergence among butterfly families dating to perhaps 80 MaBP or earlier (Scott, 1986). Both of these estimates are based on the few fossil butterflies known in concert with the biogeography of extant species. There is little consensus regarding appropriate genes for this range of divergences. Two factors have led to our selecting the mitochondrial genes. First, because a substantial database of lepidopteran sequences already exists for these genes, these data are a valuable asset to studies of the evolution of these genes as well as to the prospect of a global lepidopteran phylogeny. And second, though COI and COII are considered to be relatively quickly evolving at the nucleotide level, and therefore may contain substantial homoplasy, we find compelling Hillis's (1996) suggestion that sufficient sampling density can overcome this problem. The nuclear protein-coding gene EF-1α has been evaluated by Cho et al. (1995) and Mitchell et al. (1997), who demonstrated informativeness of synonymous nucleotide substitutions up to divergences of 60 million years (main branches of Noctuoidea) and postulated deeper resolution with increasingly dense taxon sampling.

Molecular Techniques

Total genomic DNA was extracted as in Sperling and Harrison (1994) or using a Qiagen QIAamp tissue kit. Polymerase chain reaction (PCR) amplifications were performed with either an Ericomp TwinBlock EasyCycler or an MJ Research PTC-200 DNA Engine and using a hot start: Taq was added at the

TABLE 1. Species sampled, with localities and GenBank accession numbers.

Species	Locality	GenBank accession no. COI-COII	GenBank accession no. EF-1α
Noctuidae			
[a]*Feltia jaculifera (pheromone type A)*	CAN: AB	U60990	AF173390
Geometridae			
[b]*Lambdina fiscellaria*	CAN: NF	AF064521	AF173391
Sphingidae			
Proserpinus clarkiae	USA: CA	AF170855	AF173394
Saturniidae			
Hemileuca electra	USA: CA	AF170856	AF173395
Hedylidae			
Macrosoma sp.	Costa Rica	AF170854	AF173393
Pyralidae			
Ostrinia nubilalis	CAN: ON	AF170853	AF173392
Hesperiidae			
Pyrginae			
Erynnis tristis	USA: CA	AF170858	AF173397
Pyrgus communis	USA: CA	AF170857	AF173396
Hesperiinae			
Hylephila phyleus	USA: CA	AF170859	AF173398
Satyridae			
Coenonympha tullia	USA: CA	AF170860	AF173399
Nymphalidae			
Boloria epithore	USA: CA	AF170862	AF173402
Riodinidae			
Apodemia mormo	USA: CA	AF170863	AF173403
Lycaenidae			
Euphilotes bernardino	USA: CA	AF170864	AF173404
Pieridae			
[c]*Colias eurytheme*	CAN: ON	AF044024	AF173400
Pieris napi	USA: CA	AF170861	AF173401
Papilionidae			
Baroniinae			
Baronia brevicornis (two specimens)	Mexico	AF170865	AF173405
		AF170866	AF173406
Parnassiinae			
Parnassiini			
Parnassius clodius (*simo* group)	USA: WA	AF170871	AF173411
Parnassius phoebus (*apollo* group)	CAN: AB	AF170872	AF173412
Zerynthiini			
Allancastria cerisy	Greece	AF170869	AF173409
Luehdorfia japonica	Japan: Kanazawa	AF170867	AF173407
Zerynthia rumina	Spain: Malaga	AF170870	AF173410
Sericinus montela	Japan: near Tokyo	AF170868	AF173408
Papilioninae			
Graphiini			
Graphium (Graphium) agamemnon	SE Asia	AF170874	AF173414
Iphiclides podalirius	France	AF170873	AF173413
[c]*Eurytides (Protesilaus) marcellus*	USA: FL	AF044022	AF044815
Troidini			
Troides (Troides) helena	Malaysia	AF170878	AF173418
Battus philenor	USA: VA	AF170875	AF173415
Atrophaneura alcinous	Japan: Okura	AF170876	AF173416
Parides photinus	Costa Rica	AF170877	AF173417
[c]*Pachliopta neptunus*	Malaysia	AF044023	AF044829
Papilionini			
[c]*Papilio (Pterourus) glaucus*	USA: MD	AF044013	AF044826
[c]*Papilio (Pterourus) troilus*	USA: FL	AF044017	AF044820
[c]*Papilio (Papilio) machaon*	France: Coudoux	AF044006	AF044819
[c]*Papilio (Heraclides) cresphontes*	USA: WI	AF044004	AF044832
[c]*Papilio (Princeps) xuthus*	Japan: Tokyo	AF043999	AF044838
[c]*Papilio (Princeps) demoleus*	Malaysia	AF044000	AF055825

[a]Sperling et al., 1996.
[b]Sperling et al., 1999.
[c]Caterino and Sperling (1999), Reed and Sperling (1999).

end of an initial denaturation at 94°C; this was followed by 35 cycles of 1 min at 94°C, 1 min at 45°C, 1.5 min at 72°C and a subsequent 5-min final extension at 72°C. PCR products were cleaned by using a Qiagen PCR Purification Kit and then were cycle-sequenced with Perkin-Elmer/ABI Dye Terminator Cycle Sequencing Kit with Amplitaq FS on an MJ Research PTC-200 according to Perkin-Elmer's suggested thermal profile. Sequenced products were filtered through Sephadex-packed columns and dried. Sequencing was performed with an ABI 377 automated sequencer. All fragments were sequenced in both directions. Sequences were aligned manually to the sequences of *Drosophila yakuba* (COI/COII; Clary and Wolstenholme, 1985) or *Heliothodes diminutivus* (EF-1α; Cho et al., 1995). Most primers used are published in Caterino and Sperling (1999) and Reed and Sperling (1999). Additional primers used are given in Appendix 1.

Phylogenetic Analysis

DNA sequences were aligned by eye, with use of translated amino acid sequences in the few instances of length variation. All phylogenetic analyses were performed with beta test versions of PAUP* (4b2–4b4a; Swofford, 1999). At the outset, we partitioned the nucleotide and amino acid sequence data into mitochondrial and nuclear subsets and examined them for incongruence, using the Incongruence Length Difference test (ILD; Farris et al., 1994) in PAUP*. It has been suggested that the ILD test is an overly conservative estimator of combinability (Cunningham, 1997). Therefore, despite some indications of incongruence (see Results, below), parsimony analyses were performed on the entire nucleotide data set as well as on the separate genes. These preliminary results indicated good resolving power for relationships within Papilionidae but limited informativeness with respect to outgroups. We treated the ingroup separately for most analyses and considered the problem of rooting the ingroup tree in subsequent analyses.

Ingroup analyses proceeded from heuristic parsimony searches (100 random taxon addition replicates, TBR branch-swapping, gaps scored as missing data) with use of equally weighted separate and combined nucleotide data sets. We also examined the effects of weighting based on a priori (codon positions and transition/transversion weighting) and a posteriori (reweighting by rescaled consistency indices [RCI]) criteria. Weighting ultimately made only small differences for papilionid resolution (see below). Support for branches under parsimony was assessed by bootstrap analyses (1,000 replicates starting with simple stepwise addition trees, TBR branch swapping). Decay indices were also calculated for selected analyses. Minimum evolution trees were constructed by using Jukes–Cantor (JC; Jukes and Cantor, 1969), Kimura two-parameter (K2P; Kimura, 1980), Hasegawa–Kishino-Yano (HKY85; Hasegawa et al., 1985), and LogDet (Steel, 1994) distances.

Given the best-supported ingroup topologies derived from the preceding analyses, we examined likelihoods of alternative hypotheses under several models. Likelihoods were calculated under the JC, K2P, HKY85, HKY85 + Γ, and General Time Reversible (GTR; Lanave et al., 1984) + Γ models over the entire unpartitioned data set. The necessary model parameters were estimated over each topology for each model. We also calculated likelihoods under three of these models—the JC, HKY85 + Γ, and GTR + Γ—over a six-partition data set. The designated partitions were (1) COI/COII first codon positions, (2) COI/COII second codon positions, (3) COI/COII third codon positions, (4) EF-1α first codon positions, (5) EF-1α second codon positions, and (6) EF-1α third codon positions. The low number of variable sites for the first and second codon positions of EF-1α may pose problems for parameter estimation (high estimate variance). However, given the low rates of change at these positions, they are expected to provide important information for basal relationships, and we have chosen to maintain them as separate partitions. The tRNA-leucine and intergene spacers of the mitochondrial sequences were excluded from likelihood calculations (because existing likelihood models do not accommodate gaps well). To obtain log-likelihoods for partitioned models, log-likelihoods were calculated for each partition independently and then summed. Model parameters for partitioned models (α for a four-category approximation to a gamma distribution, transition/transversion ratios, and substitution rate matrices) were independently estimated and optimized on fixed

topologies for each model and partition. In all cases, the observed nucleotide frequencies for each individual partition were used. Actual parameter values may be obtained from the senior author. The Kishino–Hasegawa test (Kishino and Hasegawa, 1989), as implemented in PAUP*, was used to test for significant differences in likelihood among topologies for each unpartitioned model, although the confidence intervals for this test may be too narrow for comparing more than two topologies (Shimodaira and Hasegawa, 1999). For partitioned models it was only possible to directly test for differences among topologies within a particular partition.

An important advantage of likelihood methods is that they can be used to examine the assumptions underlying the evolutionary models used (Goldman, 1993; Huelsenbeck and Crandall, 1997; Huelsenbeck and Rannala, 1997). We used likelihood ratio tests (LRTs) to test for statistically significant differences in model fit for models with increasing complexity. Given that likelihoods were calculated over fixed topologies, the models used may be treated as nested hypotheses and the distribution of the LRT statistic (twice the difference between the two likelihoods) is expected to approximate a χ^2 distribution (but see Goldman, 1993; Whelan and Goldman, 1999). The appropriate degree of freedom for the test is then the difference in the number of free parameters between the models being compared (Felsenstein, 1981; Huelsenbeck and Rannala, 1997).

Under parsimony, alternative outgroups yielded widely differing ingroup rootings, with no taxa better supported as a papilionid sister group than any other. Therefore, for the purposes of rooting the ingroup tree, we proceeded from the assumption that all butterfly outgroups were approximately equally informative (given a model that can accurately account for unobserved substitutions, any outgroup *should* be approximately as useful as any other outgroup). We selected two unrooted ingroup topologies for examining rooted likelihoods: the classical hypothesis, and that favored by the most parameter-rich model examined here (six-partition GTR + Γ). We then grafted two outgroup taxa—one from the Pieridae (*Pieris*), frequently considered the sister group to Papilionidae, and one from the Hesperiidae (*Pyrgus*), which is clearly outside of the Papilionoidea—to several possible branches and calculated the likelihoods under the unpartitioned and partitioned HKY85 + Γ and GTR + Γ models. All model parameters were again estimated and optimized for each model and partition. Likelihood differences among topologies and among partitions were tested with Kishino–Hasegawa tests.

RESULTS

Data Properties

The final nucleotide data set contained 3,328 positions (2,333 mitochondrial, 995 nuclear; gaps are observed at 68 positions), translating to 1,069 amino acids (740 mitochondrial, 329 nuclear). Basic variability statistics for all sequences are presented in Table 2. These data present a remarkable range of divergences among genes and codon positions. The low extreme is represented by EF-1α second positions, which had a maximum pairwise divergence of <3%, whereas nearly all third positions exhibited

TABLE 2. Nucleotide variability over genes and codon position partitions, assessed by parsimony reconstruction on one of two most-parsimonious combined data ingroup-only topologies.

	All sites		Codon pos. 1		Codon pos. 2		Codon pos. 3		Amino acids	
	mt	EF	mt	EF	mt	EF	mt	EF	mt	EF
No. characters	2333	995	738	332	738	331	738	332	738	331
No. invariant	1513	701	559	307	679	326	178	68	581	314
No. variable	820	294	179	25	59	5	560	264	159	15
No. informative	632	242	136	17	39	3	446	222	106	8
Autapomorphies	188	52	43	8	20	2	114	42	53	7
CI	0.407	0.455	0.431	0.448	0.647	0.500	0.384	0.454	0.634	0.444
RI	0.392	0.537	0.479	0.529	0.700	0.286	0.349	0.539	0.647	0.444
Ti/Tv	0.893	2.716	2.641	4.500	0.902	1.25	0.681	2.868	-	

divergences >20%. First and second codon positions of all protein-coding genes fall largely within reliable ranges, with mitochondrial first positions showing the greatest divergence, at ~10%. Divergences at the first two codon positions are approximately twice as high in the mitochondrial data as in EF-1α, indicating greater rates of protein evolution as well as nucleotide evolution. The range of third position divergences is greater in EF-1α, ranging from ~8% to nearly 50%, whereas few mitochondrial comparisons exceed 30%. This is almost certainly a result of the highly skewed AT bias of insect mitochondria (% A/C/G/T for all positions: COI and COII: = 32/14/12/42; EF-1α = 27/25/25/23).

ILD testing between mitochondrial and nuclear partitions yielded significant incongruence between partitions based on nucleotides ($P = 0.01$) but not, however, between amino acid partitions ($P = 0.189$). One interpretation of this result is that homoplasy in the nucleotide data may be obscuring the phylogenetic signal. However, only 13 of the EF1-α amino acids are informative under parsimony (considering outgroup + ingroup), and congruence between amino acid partitions may result in part from low resolution in the EF-1α partition. We also conducted ILD tests with only ingroup taxa (Papilionidae), resulting in nonsignificant differences for nucleotide ($P = 0.300$) and amino acid partitions ($P = 0.120$). We suggest that in this case homoplasy among the more distant comparisons is mimicking the effects of incongruence as assessed with ILD. We accept the result of the more restricted (ingroup only) test and combine the mitochondrial and nuclear partitions.

Parsimony Analyses

Parsimony searches over the full data set with all nucleotides equally weighted resulted in two equally parsimonious trees (7,180 steps; CI = 0.280; RI = 0.352). The strict consensus (Fig. 2) of these reveals that deeper nodes are poorly resolved; most notably, neither Papilionoidea nor Papilionidae was found to be monophyletic. *Baronia* appears more closely related to two nonswallowtail butterflies and to *Hemileuca*, a saturniid moth, than to other swallowtails. Distance transformations (including K2P, HKY85, and LogDet, which compensate for multiple substitutions or compositional biases, or both) and parsimony weighting schemes (simultaneously downweighting third positions and transitions by one-half, third position transitions thereby being weighted by one-fourth) resulted in a monophyletic Papilionidae (results not shown), though relationships among the outgroup butterflies and moths still exhibited improbable relationships (e.g., [[Papilionidae[Hesperiidae + remaining Papilionoidea]]). These analyses support the idea that the distant comparisons are hindered by severe homoplasy. Thus, taking papilionid monophyly as supported, based on corrected analyses, we pruned the outgroups and undertook analyses of papilionid taxa alone.

Parsimony searches over the combined equally-weighted nucleotide data for ingroup taxa yielded two equally parsimonious trees (3,613 steps; CI = 0.4204; RI = 0.4419) (Figs. 3a, 3b). Analyses of the separate data sets resulted in two trees for the mitochondrial data (Fig. 3c, 3d) (2,715 steps; CI = 0.3568; RI = 0.3925) and five trees for the EF-1α data (Figs. 3e–3i) (878 steps; CI = 0.4715; RI = 0.5789), all of which were distinct. A strict consensus of these nine trees is presented in Figure 4. The groups supported in all of these (numbered as in Fig. 4) are (1) Baroniinae, (2) Parnassiini (two *Parnassius* species), (3) Zerynthiini without *Luehdorfia* (*Sericinus* + *Allancastria* + *Zerynthia*), (4) Papilioninae, (5) Graphiini, (6) Troidini without *Battus*, and (7) Papilionini. Our main concerns here are relationships among these major clades, and relationships within them will not be addressed further.

Parnassiine monophyly is consistent with one of two combined-data trees (Fig. 3b) although bootstrap support is <70% (even after RCI reweighting). Mitochondrial nucleotides alone suggest that the Zerynthiini (minus *Luehdorfia*) may be the sister group of the remaining Parnassiinae and Papilioninae (Figs. 3c, 3d), whereas EF-1α nucleotides suggest that Zerynthiini is more closely related to Papilioninae than to Parnassiini (Figs. 3d–3h), although, again, bootstrap support is weak (<50%). As with the analysis of the complete ingroup plus outgroup data, weighting schemes (downweighting faster-evolving positions and nucleotides) and distance analyses (all models examined [JC, K2P, HKY85, GTR, LogDet]) designed to compensate for multiple hits

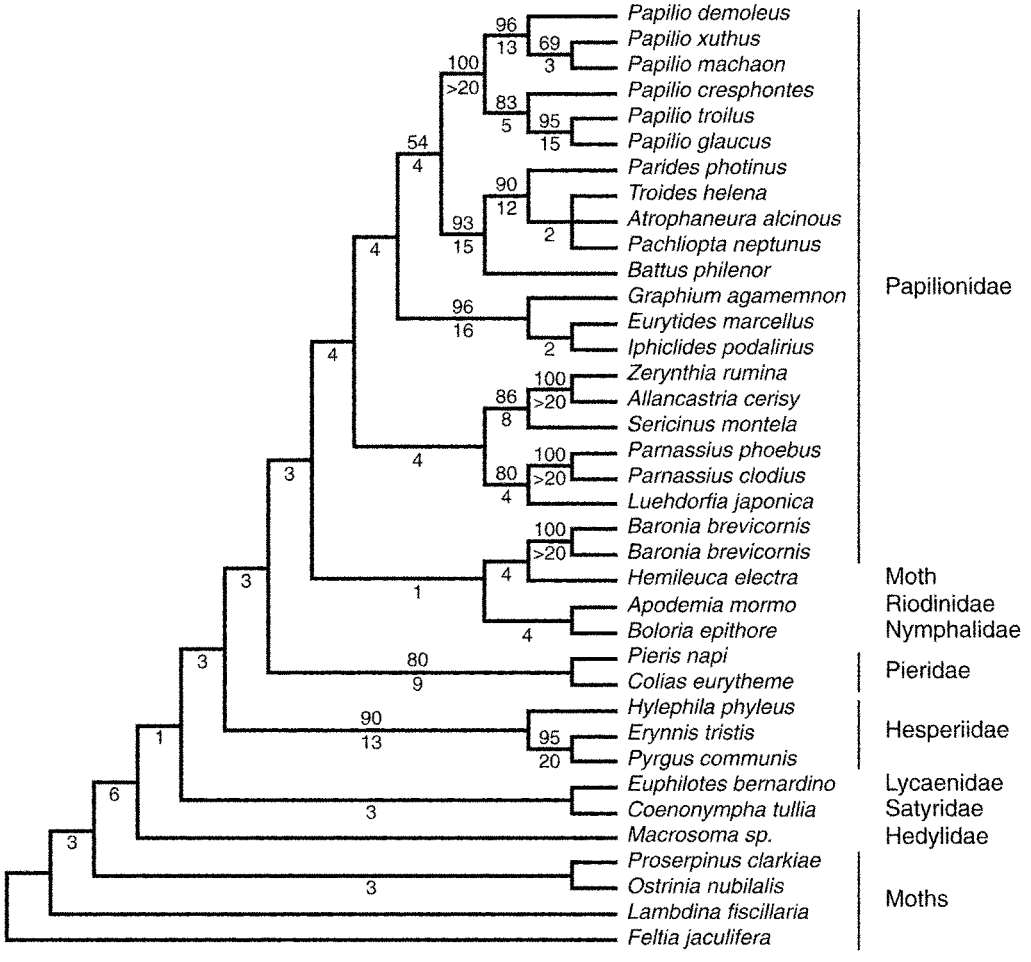


FIGURE 2. Unrooted strict consensus of two equally parsimonious trees based on all unweighted nucleotides over all ingroup + outgroup taxa only (7,180 steps; CI = 0.2797; RI = 0.3520). Values above the branches indicate bootstrap support (where these exceed 50%) and those below branches indicate Bremer support.

nearly all support a monophyletic Parnassiinae (results not shown). The placement of *Luehdorfia* is equivocal. Based on combined analysis (Figs. 3a, 3b) and mitochondrial data alone (Figs. 3c, 3d), the genus is resolved as being more closely related to *Parnassius* than to Zerynthiini, although without strong bootstrap support. EF-1α, however, supports the placement of *Luehdorfia* with Zerynthiini (Figs. 3e–3i). This resolution is only weakly supported by bootstrapping (56%), but that increases to 73% when transitions are downweighted by one-half, and to 81% when EF-1α nucleotides are reweighted by their CI values. Within Papilionini, the most frequent result is the classical resolution of the tribes, with a monophyletic Graphiini sister to monophyletic Troidini + Papilionini (Figs. 3a–3g), and all relevant branches have >85% bootstrap support for combined nucleotides. However, two of five EF-1α trees are inconsistent with this resolution, placing the Papilionini as sister to Graphiini + Troidini in one (Fig. 3h), and with *Battus* split from the remaining Troidini in another (Fig. 3i). Although the combined data results seem sufficient to reject these alternatives, we reexamined these relationships using likelihood analyses.

Likelihood Analyses

The parsimony-based analyses offer a variety of possible resolutions of the major swallowtail lineages, with little basis for choosing among them. Likelihood analysis offers a means of distinguishing among this

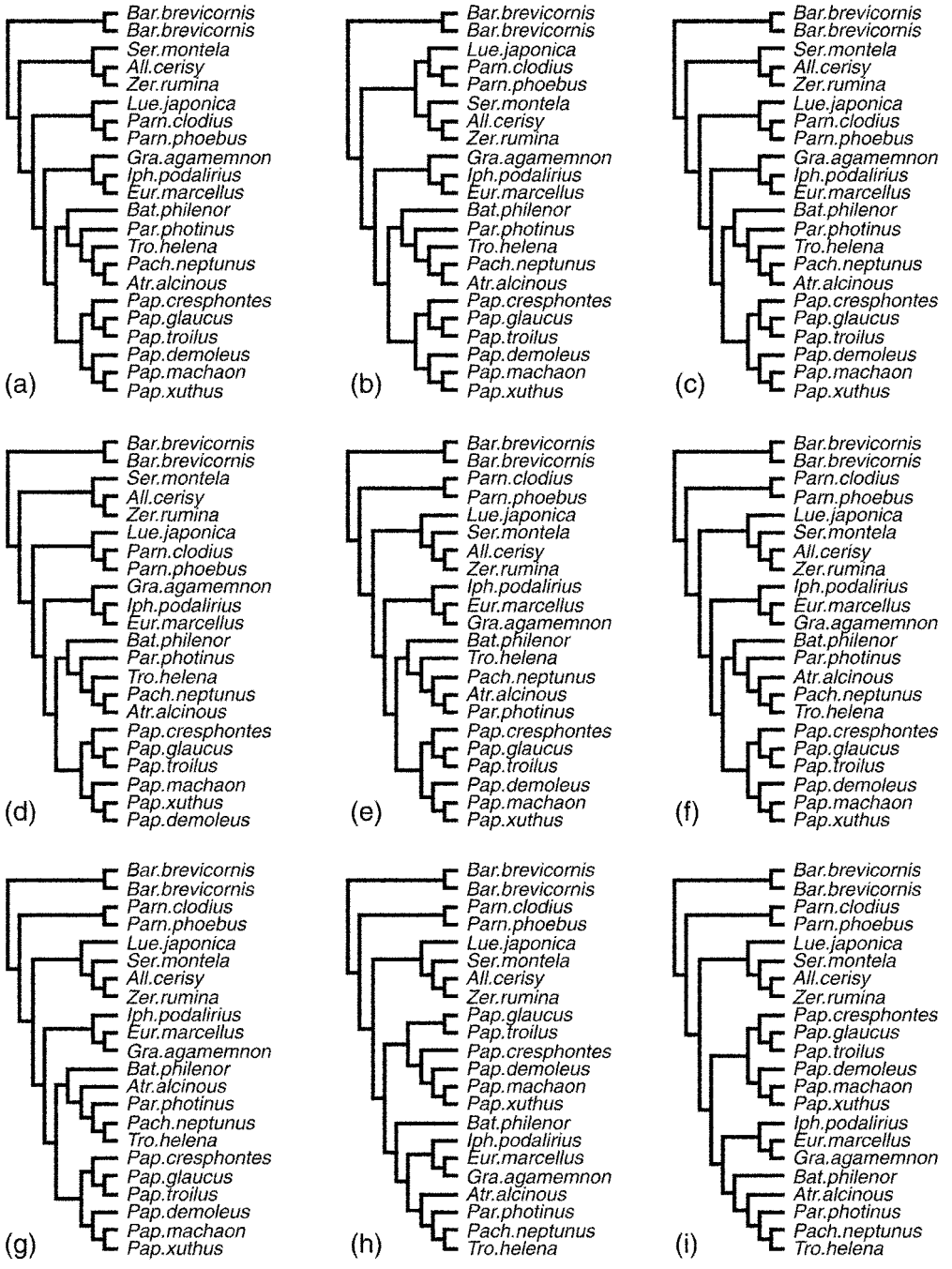


FIGURE 3. Most-parsimonious trees derived from equally weighted separate and combined analyses: (a, b) All nucleotides included; (c, d) mitochondrial data alone; (e–i) EF-1α data alone.

array. Log-likelihoods were calculated over all parsimony trees (Figs. 3a–3i) plus two additional trees not found among them: the classical hypothesis (Fig. 1) and a tree consistent with a monophyletic Parnassiinae but with *Luehdorfia* at the base of the Parnassiini rather than with the Zerynthiini. Results are presented in Table 3, with models arranged roughly in order of increasing complexity from left to right. The goodness-of-fit

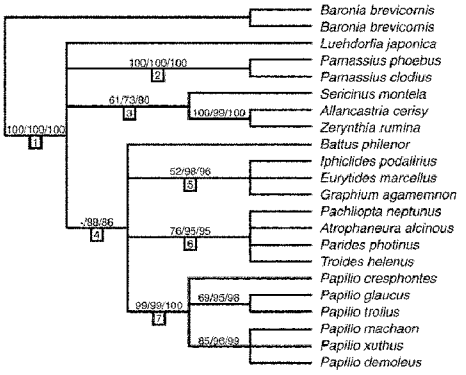


FIGURE 4. Strict consensus of the nine trees in Figure 3, showing groups found in all equally weighted separate and combined analyses. Numbers above branches indicate bootstrap support (where >50%) for the mitochondrial/nuclear/combined data. Numbers below the branches designate clades referred to in the text.

of these models improves substantially with increasing parameter-richness; LRTs support all comparisons as highly significant (results not shown). Particularly large improvements in model fit are seen both with the incorporation of gamma-distributed rate heterogeneity and with data partitioning. The favored topology varies widely among models. The simplest unpartitioned models favor a topology (Fig. 3b) that differs from the classical hypothesis with regard to both the position of *Luehdorfia* (with the Parnassiini) and the resolution of the three graphiine taxa. When gamma-distributed rate heterogeneity is incorporated into the unpartitioned HKY85 and GTR models, the classical hypothesis (Fig. 1) is favored in both cases. However, when the data are partitioned and the model parameters are estimated separately for each partition, this favored topology shifts, first back to the same combined data hypothesis as was supported by simple unpartitioned models (Fig. 3b), and then to one in which the Parnassiinae appear paraphyletic with respect to the Papilioninae (Fig. 3f). Although the log-likelihoods results are not significantly different from the classical hypothesis for any partition, the "likelihood advantage" (see DeBry, 1999) for the best-scoring topology increases slightly with improved model fit (this is despite a decrease in the total range of the estimates). Combined with the nearly identical rankings of topologies under the partitioned HKY85 + Γ and GTR + Γ models, the topology EF-1α 2 clearly best fits these models, and this therefore is the tree we carried forward as the "favored" ingroup topology for rooting purposes.

The difficulty of assessing significance under some models must render any conclusions based on our likelihood analyses tentative; nonetheless, a couple of questions deserve deeper examination. Firstly, the aberrant resolutions of the tribes of Papilioninae found in some most-parsimonious EF-1α topologies (Figs. 3h, 3i) are found here to be least likely under nearly all models, and the paraphyly of Troidini can be rejected with statistical confidence. An additional important issue regards the placement of *Luehdorfia* within Parnassiinae. Although the parsimony results are equivocal, with only the EF-1α trees favoring the classical placement with Zerynthiini, trees containing this resolution are favored under the best-fitting HKY85 + Γ and GTR + Γ models, both unpartitioned and partitioned, and this is the hypothesis we favor. The monophyly of the Parnassiinae as a whole is more difficult to establish. The only trees to contain this group (classical and "combined data 2" [Figs. 1 and 3b, respectively]) rank first in unpartitioned analyses and in the partitioned JC analysis. Yet, under the most realistic partitioned models, paraphyly of Parnassiinae appears more likely, and the question cannot be considered resolved.

Two topologies were used for likelihood analyses of root placement: the classical hypothesis, and that favored by our best-fitting likelihood model (EF-1α 2). The selected outgroups, *Pyrgus communis* (Hesperiidae) and *Pieris napi* (Pieridae), were grafted onto the trees on the branches numbered in Figure 5. The rootings examined include the classical *Baronia*-basal tree (rootings 1 and 8), the *Parnassius*-basal rooting supported by the morphological data of de Jong et al. (1996; rootings 5 and 10), and a range of others to provide a context for evaluating the likelihood scores. Some, such as those within Papilionini, were expected to be quite unlikely at the outset.

The calculated likelihoods for the rooted topologies are shown in Table 4 (with scores for individual partitions in Appendix 2). As with the ingroup-only calculations, improvements in model fit, as assessed with LRTs, are all highly significant (results not shown). Interestingly, two rootings of the

TABLE 3. Log likelihoods of alternative ingroup topologies under various models. The classical tree is that shown in Figure 1. The *Lueh.* with Parn. tree is the classical tree with *Luehdorfia* moved to the base of the Parnassiini. The remaining topologies are shown in Figure 3 and represent all of the most-parsimonious topologies based on equally weighted, separate and combined analyses. The best likelihood scores under each model are shown in boldface type. The number of model parameters for each model is calculated as [data partitions (branch lengths + rate parameter + ti/tv ratio + nucleotide frequencies + relative substitution rate parameters + rate heterogeneity parameter)]. The likelihoods for individual partitions are given in Appendix 2.

	Unpartitioned					Partitioned		
Unrooted topology	JC	K2P	HKY	HKY + Γ	GTR + Γ	JC	HKY + Γ	GTR + Γ
Classical tree	−23697.227	−23483.111	−22971.759	**−20499.228**	**−19939.540**	−21377.254	−18182.537	−17948.142
Lueh. with Parn.	−23699.752	−23484.180	−22969.076	−20500.050	−19941.197	−21373.563	−18188.387	−17951.960
Combined data 1	−23692.902	−23471.435	−22957.391	−20505.934	−19951.141	−21374.504	−18198.853[b]	−17958.293
Combined data 2	**−23685.511**	**−23466.516**	**−22953.883**	−20502.020	−19947.497	**−21367.962**	−18194.649	−17957.609
Mitochondrial 1	−23692.902	−23471.435	−22957.391	−20505.934	−19951.141	−21374.504	−18198.853[b]	−17958.293
Mitochondrial 2	−23703.700	−23480.973	−22965.791	−20509.133	−19957.023	−21378.782	−18201.374[b]	−17962.788[b]
EF-1α 1	−23722.898	−23511.356	−22994.403	−20510.722	−19946.083	−21407.856[c]	−18193.996	−17954.377
EF-1α 2	−23707.488	−23493.396	−22978.475	−20504.673	−19940.100	−21384.098[c]	−18177.096	−17941.079
EF-1α 3	−23717.193	−23503.570	−22987.113	−20505.314	−19941.070	−21404.683[c]	−18187.896	−17948.779
EF-1α 4	−23831.073[a]	−23617.916[a]	−23095.653[a]	−20563.750[a]	−19995.600[a]	−21501.398[c]	−18225.053[c]	−18014.822[c]
EF-1α 5	−23752.553	−23540.541	−23019.986	−20522.631	−19957.592	−21431.992[c]	−18197.657	−17969.078
Range of lnL estimates	145.562	151.400	141.770	64.522	56.060	133.436	47.957	73.743
Free parameters	42	43	46	47	53	252	282	318

[a]Significantly less likely (at $\alpha = 0.05$) as determined by Kishino–Hasegawa test.
[b]One or more nuclear partitions fit this topology significantly worse (at $\alpha = 0.05$) as determined by Kishino–Hasegawa test (see Appendix 2).
[c]One or more mitochondrial partitions fit this topology significantly worse (at $\alpha = 0.05$) as determined by Kishino-Hasegawa test (see Appendix 2).

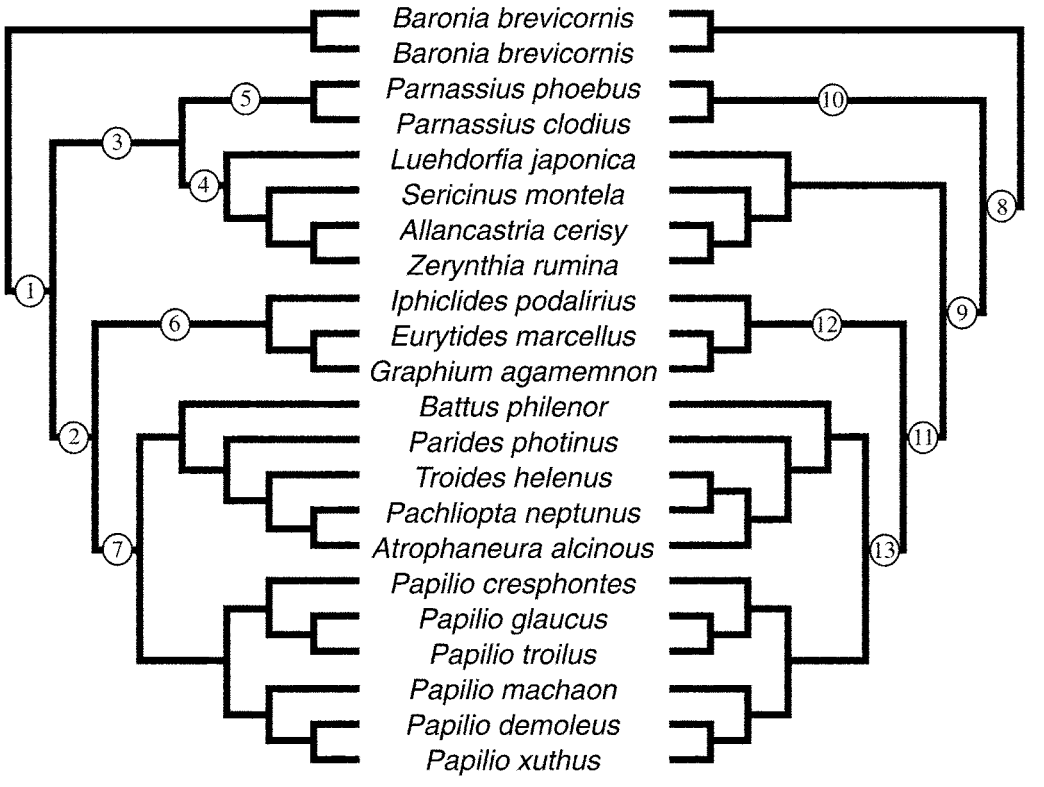


FIGURE 5. Selected ingroup topologies. The topology on the left is the classical hypothesis. The one on the right is the hypothesis favored by likelihood analyses of ingroup taxa. Candidate rooting points used for calculating rooted likelihoods are numbered as in Table 4.

classical hypothesis scored better than any rootings of the topology favored by ingroup-only likelihood analyses, underscoring the persistent uncertainty regarding ingroup relationships. Of the two best-scoring rooted topologies, one of the rootings within Papilioninae was unexpectedly found to be most likely—under the better fitting of the two unpartitioned models and under both partitioned models. However, the *Baronia*-basal rooting of the classical hypothesis ranks first under the unpartitioned HKY85 + Γ model, and is a close second by all others. Neither of these best-scoring topologies is statistically distinguishable from most other topologies, for combined data or for any individual partitions.

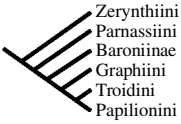
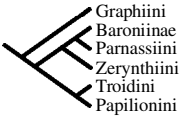
CONCLUSIONS

Our analyses have spanned a wide range of tree reconstruction and evaluation strategies. In the end, we are left with an array of possible resolutions—some suggesting very surprising topologies—with scant available criteria for differentiating them. Although parsimony encountered problems early on, presumably attributable to homoplasy, we anticipated that likelihood analysis would be able to satisfactorily compensate for it to resolve the deeper relationships accurately. The demonstrated heterogeneity in evolutionary dynamics of process partitions of these data offered an opportunity to examine the performance of partitioned models, for which evolutionary parameters could be separately optimized. Given this heterogeneity, the models developed in this paper are both intuitively appealing and seemingly accurate. Previous studies have noted that simple models frequently identify the same most-likely topology as complex ones do (e.g., Cunningham, 1997; DeBry, 1999), the implication being that choice of model, surprisingly, is not as critical as it would seem. However, our results indicate that this idea requires further evaluation. Although some consistency was apparent across a range of more or less simple

TABLE 4. Log likelihoods of alternative rootings of the two selected ingroup topologies (Fig. 5). Ingroup trees were rooted with the outgroups *Pyrgus* (Hesperiidae) and *Pieris* (Pieridae). Tree numbers refer to the rooting points in Figure 5. Parsimony scores and log-likelihoods were calculated with outgroups attached, though they are not shown in these schematics. The scores for the best trees are shown in boldface type and those that differ at $\alpha = 0.05$ are marked with asterisks (for partitioned likelihoods, the asterisks indicate significance for one or more partitions). Likelihoods for individual partitions are given in Appendix 3.

			Unpartitioned		Partitioned	
Tree no.	Rooted topology	MP score	HKY85 + Γ	GTR + Γ	HKY85 + Γ	GTR + Γ
7	Parnassiinae Baroniinae Graphiini Troidini Papilionini	4141	−22521.982	**−21882.310**	**−20018.460**	**−19762.630**
1	Baroniinae Parnassiinae Graphiini Troidini Papilionini	**4134**	**−22521.360**	−21884.347	−20020.255	−19763.633
13	Baroniinae Parnassiini Zerynthiini Graphiini Troidini Papilionini	4143	−22529.120	−21884.157	−20028.634	−19767.901
2	Parnassiinae Baroniinae Graphiini Troidini Papilionini	4137	−22523.847	−21885.585	−20024.323	−19768.668
3	Parnassiinae Baroniinae Graphiini Troidini Papilionini	4143	−22525.187	−21886.598	−20040.472	−19769.883
6	Graphiini Parnassiinae Baroniinae Troidini Papilionini	4140	−22530.564	−21891.242	−20029.770	−19774.098
8	Baroniinae Parnassiini Zerynthiini Graphiini Troidini Papilionini	4145	−22536.031	−21892.718	−20036.998	−19774.680
11	Baroniinae Parnassiini Zerynthiini Graphiini Troidini Papilionini	4143	−22531.601	−21888.889	−20035.160	−19775.292
9	Baroniinae Parnassiini Zerynthiini Graphiini Troidini Papilionini	4148*	−22536.826	−21892.935	−20036.916	−19776.165
10	Parnassiini Baroniinae Zerynthiini Graphiini Troidini Papilionini	4150	−22537.972	−21893.609	−20040.043	−19777.839
5	Parnassiini Zerynthiini Baroniinae Graphiini Troidini Papilionini	4144	−22537.077	−21896.544	−20056.224	−19782.922

TABLE 4. Continued.

Tree no.	Rooted topology	MP score	Unpartitioned		Partitioned	
			HKY85 + Γ	GTR + Γ	HKY85 + Γ	GTR + Γ
4	 Zerynthiini Parnassiini Baroniinae Graphiini Troidini Papilionini	4148	−22540.145*	−21899.139	−20042.945	−19784.296
12	 Graphiini Baroniinae Parnassiini Zerynthiini Troidini Papilionini	4142	−22537.420	−21892.946	−20039.141	−19805.977*

models with regard to favored topology, increasing model complexity caused the preferred topology to shift, especially in the case of the rooted analysis, to a strikingly different phylogeny.

The major shortcoming of the approach presented here is the inability to make any statistical statements regarding the partitioned analyses. In a few cases, individual partitions show considerable conflict with particular topologies. However, the differences required for significance under Kishino–Hasegawa tests for unpartitioned models (which would be expected to have less variance than the partitioned estimates) suggest that few, if any partitioned comparisons would be significantly different. This appears to indicate either that the Kishino–Hasegawa test is insufficiently sensitive for detecting real likelihood differences or that the likelihoods of these trees do not actually differ. In fact, we believe that both of these factors apply to our results. The close agreement in the rankings of topologies across a wide selection of models argues against their likelihoods differing only through random variation in estimates. Parametric bootstrapping may be necessary to adequately establish the variance of these estimates. Nonetheless, many likelihoods for these topologies probably would not differ significantly by any conceivable test. In the case of the two most-likely rooted topologies, (Table 4), which differ by at most two log-likelihood units, the topologies suggest radically different evolutionary scenarios, and this problem merits serious consideration. The apparent increase in likelihood advantage, at least for ingroup-only analyses, suggests that additional model improvements would yield additional power to discriminate among topologies. However, in this study, this value might also be related to the overall range of likelihood estimates.

Both the changes in preferred topology and the possibility of increased discriminatory power point to yet more complex models as a possible salvation. However, this conclusion would not be entirely warranted. Partitioning the data decreases the number of variable characters in each partition, which leads to higher variances of parameter and likelihood estimates and thence to potentially spurious results (Swofford et al., 1996). The six-partition model presented here may suffer from this difficulty for some partitions; for example, the first and second codon positions of EF-1α offer 25 and 5 variable sites, respectively. In fact, the partitioned analyses are not able to extract any phylogenetic information whatsoever from these second codon positions (see Appendices 2, 3), and the information presented by the EF-1α first positions may be similarly suspect. (However, a four-partition analysis with all EF-1α data combined, carried out at the suggestion of one reviewer, resulted in the same rankings of trees.) Possibly additional partitioning of highly variable partitions (e.g., mitochondrial third positions) according to functional regions, amino acid properties, codon biases, and so forth, would extract additional information from these data. Further explorations to determine optimal models are needed.

With respect to swallowtail relationships, our findings concur broadly with accepted ideas. However, despite sampling from all major taxa of the swallowtail family (and outgroups), and extensive sequencing from loci that seem appropriate, we obtained

relatively few results that can be highlighted as incontrovertible. Nonetheless, with two possible exceptions, we believe that our analyses best support a tree congruent with traditional classification. Most important, our ingroup analyses suggest that the Parnassiinae is not monophyletic. A monophyletic Parnassiinae is found in only one of nine parsimony trees and is not supported by the partitioned likelihood analysis. Instead, our ingroup analyses favor a sister group relationship between Zerynthiini (including *Luehdorfia*) and the Papilioninae, and morphology would not contradict such a relationship. This resolution was also found by Yagi et al. (1999), using ND5. Secondly, our analysis cannot confidently establish the root of the swallowtail tree. Although in this case morphology would conflict, a rooting within the Papilioninae is as likely as the classical *Baronia*-basal rooting based on our analyses (including "corrected" parsimony analyses of the full outgroup + ingroup dataset.) With regard to previous workers' hypotheses, we find no support for Munroe and Ehrlich's (1960) suggestion of a closer relationship between Graphiini and Papilionini than between Troidini and Papilionini. A sister group relationship between Troidini and Graphiini, as suggested by Yagi et al. (1999), was found in some initial parsimony trees but is strongly rejected by likelihood analysis. The hypothesis of troidine polyphyly suggested by Morinaka et al. (1999) is not supported by any of our analyses. Before any of these issues can be considered settled, however, substantial phylogenetic work remains to be done. Several problematic genera need to be examined, most notably the parnassiines *Archon* and *Hypermnestra* and the papilionines *Teinopalpus* and *Meandrusa*. Evaluation of relationships at this level might also benefit from the examination of a nuclear ribosomal locus, such as 18S.

A thorough exploration of the origin and evolution of Aristolochiaceae-feeding and its associated morphologies and behaviors is outside the scope of this study. However, a single origin of this feeding mode does map most-parsimoniously onto either the classical hypothesis (as represented by the taxa included here) or the Parnassiinae-paraphyletic tree favored by our likelihood analysis (coding each taxon for its known host plant family or families, following Hancock, 1983). This result would probably not be affected by the addition of the parnassiines *Archon* (which is also an *Aristolochia*-feeder) and *Hypermnestra* (for which its Zygophyllaceae-feeding would be reconstructed as autapomorphic, wherever the taxon belongs on the cladogram). Additional Papilioninae have the greatest potential to provide a new perspective on Aristolochiaceae-feeding. In particular, if basal Papilionini and basal Graphiini are found to share similar host-plant families (for the taxa here there is no overlap), Aristolochiaceae-feeding in the Troidini will have to be viewed as an autapomorphic departure from some ancestral Papilioninae habit. On the other hand, depending on its phylogenetic placement, Aristolochiaceae-feeding in the *asius* group of *Protesilaus* (Graphiini) could potentially reinforce the single origin hypothesis. In any event, too few phylogenetic data are available to draw any serious conclusions regarding host-plant evolution.

In briefly summarizing the behavior of these markers over the range of divergences examined in this study, the most noteworthy point is that, contrary to the widely-held view that the COI/COII loci are mainly useful for species-level studies, they are in fact much more widely applicable. As has been observed previously, the third codon positions of EF-1α do offer information at deeper levels and over a greater range than do those of COI/COII (Reed and Sperling, 1999). However, due to the differences in amino acid variability, the first and second codon positions of the mitochondrial data offer far more informative sites than do those of a comparable amount of EF-1α sequence at the phylogenetic levels examined here. Indeed, EF-1α amino acid sequences have been found useful at far deeper interclass levels in Arthropoda (Regier and Schultz, 1997). Our initial hope was that by partitioning these data and estimating phylogeny by using maximum likelihood, our analysis might extend the utility of the observed variation. That we have been successful, however, is not clear. The likelihood analysis has conclusively settled few of the ambiguities presented by the parsimony analysis. Certainly our arsenal of loci would benefit from the development of additional single-copy nuclear genes for which the amino acid sequences evolved at a higher rate than that of EF-1a. Particularly

promising candidates are dopa decarboxylase (Fang et al., 1997; Friedlander et al., 1998) and phospho*enol*pyruvate carboxykinase (Friedlander et al., 1996; see also Friedlander et al., 1992.)

Phylogeny reconstruction remains a difficult task. Whereas by most accounts denser taxon sampling should lead to greater phylogenetic accuracy (Hillis, 1996, 1998; Graybeal, 1998), perhaps an unanticipated consequence is that as taxa are added and branch lengths decrease, confidence in particular branches of these complex trees will be more difficult to assess by conventional means. Our results suggest that more-complex evolutionary models may be better able to discern differences between similar topologies. Phylogenetics therefore stands to benefit from the continued development of evolutionary models that can account for the vagaries of heterogeneous data. This goal requires both detailed examinations of the evolutionary dynamics of process partitions of popular phylogenetic markers and the elaboration of methods for applying simultaneous partitioned analyses in software packages for phylogenetic analysis. Perhaps the most important remaining question is the degree to which data should be partitioned to optimally extract information. No recommendation beyond finding an undefinable balance between complexity and statistical practicality can be offered at this point. Future work would profitably focus on developing criteria for identifying this optimal balance for a given phylogenetic problem.

ACKNOWLEDGMENTS

In addition to collectors acknowledged in our previous papers, we thank R. Kelson, J. Powell, and D. Rubinoff for providing specimens. We also thank A. Engberg and N. Vane for laboratory assistance. This manuscript was much improved by the comments of A. Cognato, R. DeBry, A. Mitchell, R. Olmstead, H. Trueman, and one anonymous reviewer. This study was supported in part by California Agricultural Experiment Station and NSF-PEET grants to F.A.H.S.

REFERENCES

AMRINE, H. M., AND M. S. SPRINGER. 1999. Maximum-likelihood analysis of the Tethythere hypothesis based on a multigene data set and a comparison of different models of sequence evolution. J. Mamm. Evol. 6:161–176.

AUBERT, J., L. LEGAL, H. DESCIMON, AND F. MICHEL. 1999. Molecular phylogeny of swallowtail butterflies of the tribe Papilionini. Mol. Phylogenet. Evol. 12:156–167.

BERENBAUM, M. R., C. FAVRET, AND M. A. SCHULER. 1996. On defining "key innovations" in an adaptive radiation: Cytochrome P450s and Papilionidae. Am. Nat. 148:S139–S155.

BROWER, A. V. Z. 1997. The evolution of ecologically important characters in *Heliconius* butterflies (Lepidoptera: Nymphalidae): A cladistic review. Zool. J. Linn. Soc. 119:457–472.

BROWN, K. S., C. F. KLITZKE, C. BERLINGERI, AND P. EUZÉBIO RUBBO DOS SANTOS. 1995. Neotropical swallowtails: Chemistry of food plant relationships, population ecology and biosystematics. Pages 405–446 *in* Swallowtail butterflies: Their ecology and evolutionary biology (J. M. Scriber, Y. Tsubaki, and R. C. Lederhouse, eds.). Scientific Publishers, Gainesville, Florida.

BULL, J. J., J. P. HUELSENBECK, C. W. CUNNINGHAM, D. L. SWOFFORD, AND P. J. WADDELL. 1993. Partitioning and combining data in phylogenetic analysis. Syst. Biol. 42:384–397.

CATERINO, M. S., AND F. A. H. SPERLING. 1999. *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. Mol. Phylogenet. Evol. 11:122–137.

CHO, S., A. MITCHELL, J. C. REGIER, C. MITTER, R. W. POOLE, T. P. FRIEDLANDER, AND S. ZHAO. 1995. A highly conserved gene for low level phylogenetics: Elongation factor-1 alpha recovers morphology-based tree for heliothine moths. Mol. Biol. Evol. 12:650–656.

CLARKE, C. A., AND P. M. SHEPPARD. 1963. Interactions between major genes and polygenes in the determination of the mimetic patterns of *Papilio dardanus*. Evolution 17:404–413.

CLARY, D. O., AND D. R. WOLSTENHOLME. 1985. The mitochondrial DNA molecule of *Drosophila yakuba*: Nucleotide sequence, gene organization and genetic code. J. Mol. Evol. 22:252–271.

COLLINS, N. M., AND M. G. MORRIS. 1985. Threatened swallowtail butterflies of the world. IUCN, Gland and Cambridge.

CUNNINGHAM, C. W. 1997. Is congruence between data partitions a reliable predictor of phylogenetic accuracy? Empirically testing an iterative procedure for choosing among phylogenetic methods. Syst. Biol. 46:464–478.

DEBRY, R. W. 1999. Maximum likelihood analysis of gene–based and structure–based process partitions, using mammalian mitochondrial genomes. Syst. Biol. 48:286–299.

DE JONG, R., R. I. VANE-WRIGHT, AND P. R. ACKERY. 1996. The higher classification of butterflies (Lepidoptera): Problems and prospects. Entomol. Scand. 27:65–101.

DURDON, C. J., AND H. ROSE. 1978. Butterflies from the middle Eocene: The earliest occurrence of fossil Papilionidae. Pearce-Sellards Ser. Tex. Mem. Mus. 29:1–25.

EHRLICH, P. R. 1958. The comparative morphology, phylogeny and higher classification of the butterflies. Univ. Kansas Sci. Bull. 39:305–370.

EHRLICH, P. R., AND P. H. RAVEN. 1964. Butterflies and plants: A study in coevolution. Evolution 18:586–608.

EISNER, T., AND Y. C. MEINWALD. 1965. Defensive secretion of a caterpillar (*Papilio*). Science 150:1733–1735.

FANG, Q. Q., S. CHO, J. C. REGIER, C. MITTER, M. MATTHEWS, R. W. POOLE, T. P. FRIEDLANDER, AND S. ZHAO. 1997. A new nuclear gene for insect phylogenetics: DOPA decarboxylase is informative of relationships within Heliothinae (Lepidoptera: Noctuidae). Syst. Biol. 46:269–283.

FARRELL, B. D., AND C. MITTER. 1998. The timing of insect-plant diversification: Might *Tetraopes* (Coleoptera: Cerambycidae) and *Asclepias* (Asclepiadaceae) have co-evolved? Biol. J. Linn. Soc. 63:553–577.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. Cladistics 10:315–319.

FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. J. Mol. Evol. 17:368–376.

FORD, E. B. 1944. Studies in the chemistry of pigments in the Lepidoptera, with reference to their bearing on systematics. 4. The classification of the Papilionidae. Trans. R. Entomol. Soc. London 94:201–223.

FRIEDLANDER, T. P., R. HORST-KURT, J. C. REGIER, C. MITTER, R. S. PEIGLER, AND Q. Q. FANG. 1998. Two nuclear genes yield concordant relationships within Attacini (Lepidoptera: Saturniidae). Mol. Phylogenet. Evol. 9:131–140.

FRIEDLANDER, T. P., J. C. REGIER, AND C. MITTER. 1992. Nuclear gene sequences for higher level phylogenetic analysis: 14 promising candidates. Syst. Biol. 41:483–490.

FRIEDLANDER, T. P., J. C. REGIER, C. MITTER, AND D. L. WAGNER. 1996. A nuclear gene for higher level phylogenetics: Phospho*enol*pyruvate carboxykinase tracks Mesozoic-age divergences within Lepidoptera. Mol. Biol. Evol. 13:594–604.

GOLDMAN, N. 1993. Statistical tests of models of DNA substitution. J. Mol. Evol. 36:182–198.

GRAYBEAL, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Syst. Biol. 47:9–17.

HANCOCK, D. L. 1983. Classification of the Papilionidae: A phylogenetic approach. Smithersia. 2:1–48.

HASEGAWA, M., H. KISHINO, AND T. YANO. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22:160–174.

HÄUSER, C. L. 1993. Critical comments on the phylogenetic relationships within the family Papilionidae. Nota Lepid. 16:34–43.

HILLIS, D. M. 1996. Inferring complex phylogenies. Nature 383:130–131.

HILLIS, D. M. 1998. Taxonomic sampling, phylogenetic accuracy, and investigator bias. Syst. Biol. 47:3–8.

HUELSENBECK, J. P., AND K. A. CRANDALL. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annu. Rev. Ecol. Syst. 28:437–466.

HUELSENBECK, J. P., AND B. RANNALA. 1997. Phylogenetic methods come of age: Testing hypotheses in an evolutionary context. Science 276:227–232.

IGARASHI, S. 1984. The classification of the Papilionidae mainly based on the morphology of their immature stages. Tyo To Ga 34:41–96.

JUKES, T. H., AND C. R. CANTOR. 1969. Evolution of protein molecules. Pages 21–132 *in* Mammalian protein metabolism (H. N. Munro, ed.). Academic Press, New York.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120.

KISHINO, H., AND M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J. Mol. Evol. 29:170–179.

KRISTENSEN, N. P. 1976. Remarks on the family-level phylogeny of butterflies. Z. Zool. Syst. Evol. 14:25–33.

LANAVE, C., G. PREPARATA, C. SACCONE, AND G. SERIO. 1984. A new method for calculating evolutionary substitution rates. J. Mol. Evol. 20:86–93.

LIÒ, P., AND N. GOLDMAN. 1998. Models of molecular evolution and phylogeny. Genome Res. 8:1233–1244.

MILLER, J. S. 1987a. Host–plant relationships in the Papilionidae (Lepidoptera): Parallel cladogenesis or colonization? Cladistics 3:105–120.

MILLER, J. S. 1987b. Phylogenetic studies in the Papilioninae (Lepidoptera: Papilionidae). Bull. Am. Mus. Nat. Hist. 186:365–512.

MITCHELL, A., S. CHO, J. C. REGIER, C. MITTER, R. W. POOLE, AND M. MATTHEWS. 1997. Phylogenetic utility of Elongation factor-1 alpha in Noctuoidea (Insecta: Lepidoptera): The limits of synonymous substitution. Mol. Biol. Evol. 14:381–390.

MORINAKA, S., T. MAEYAMA, K. MAEKAWA, D. ERNIWATI, S. N. PRIJONO, I. K. GINARSA, T. NAKAZAWA, AND T. HIDAKA. 1999. Molecular phylogeny of birdwing butterflies based on the representatives in most genera of the tribe Troidini. Entomol. Sci. 2:347–358.

MUNROE, E. 1961. The classification of the Papilionidae. Can. Entomol. Suppl. 17:1–51.

MUNROE, E., AND P. R. EHRLICH. 1960. Harmonization of concepts of higher classification of the Papilionidae. J. Lepid. Soc. 14:169–175.

NISHIDA, R., J. D. WEINTRAUB, P. FEENY, AND H. FUKAMI. 1993. Aristolochic acids from *Thottea* spp. (Aristolochiaceae) and the osmeterial secretions of *Thottea*-feeding troidine swallowtail larvae (Papilionidae.) J. Chem. Ecol. 19:1587–1594.

PARSONS, M. J. 1996. A phylogenetic reappraisal of the birdwing genus *Ornithoptera* (Lepidoptera: Papilionidae: Troidini) and a new theory of its evolution in relation to Gondwanan vicariance biogeography. J. Nat. Hist. 30:1707–1736.

PELLMYR, O., J. N. THOMPSON, J. M. BROWN, AND R. G. HARRISON. 1996. Evolution of pollination and mutualism in the yucca moth lineage. Am. Nat. 148:827–847.

RACHELI, T., AND M. OLIVERIO. 1993. Biogeographical patterns of the neotropical genus *Battus* Scopoli (Lepidoptera: Papilionidae.) Trop. Zool. 6:55–65.

REED, R. D., AND F. A. H. SPERLING. 1999. Interaction of process partitions in phylogenetic analysis: An example from the swallowtail butterfly genus *Papilio*. Mol. Biol. Evol. 16:286–297.

REGIER, J. C., AND J. W. SHULTZ. 1997. Molecular phylogeny of the major arthropod groups indicates polyphyly of crustaceans and a new hypothesis for the origin of hexapods. Mol. Biol. Evol. 14:902–913.

ROTHSCHILD, M. 1972. Secondary plant substances and warning colouration in insects. Pages 59–83 *in* Insect/plant relationships (H. F. van Emden, ed.). R. Entomol. Soc. London Symp. 6.

ROTHSCHILD, W., AND K. JORDAN. 1906. A revision of the American *Papilios*. Novit Zool. 13:412–752.

SCOBLE, M. J., AND A. AEILLO. 1990. Moth-like butterflies (Hedylidae: Lepidoptera): A summary, with comments on the egg. J. Nat. Hist. 24:159–164.

SCOTT, J. A. 1986. The butterflies of North America: A natural history and field guide. Stanford University Press: Stanford, California.

SHIMODAIRA, H., AND M. HASEGAWA. 1999. Multiple comparisons of log–likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16:1114–1116.

SIMON, C., F. FRATI, A. BECKENBACH, B. CRESPI, H. LIU, AND P. FLOOK. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87:651–701.

SPERLING, F. A. H., R. BYERS, AND D. HICKEY. 1996. Mitochondrial DNA sequence variation among pheromotypes of the dingy cutworm, *Feltia jaculifera* (Gn.) (Lepidoptera: Noctuidae). Can. J. Zool. 74:2109–2117.

SPERLING, F. A. H., AND R. G. HARRISON. 1994. Mitochondrial DNA variation within and between species of the *Papilio machaon* group of swallowtail butterflies. Evolution 48:408–422.

SPERLING, F. A. H., A. G. RASKE, AND I. S. OTVOS. 1999. Mitochondrial DNA sequence variation among populations and host races of *Lambdina fiscellaria* (Gn.) (Lepidoptera: Geometridae). Insect Mol. Biol. 8:1–10.

STEEL, M. 1994. Recovering a tree from the Markov leaf colourations it generates under a Markov model. Appl. Math. Lett. 7:19–23.

SWOFFORD, D. L. 1999. PAUP*: Phylogenetic Analysis Using Parsimony, ver. 4.0b2a. Sinauer Associates, Sunderland, Massachusetts.

SWOFFORD, D. L., G. J. OLSEN, P. J. WADDELL, AND D. M. HILLIS. 1996. Phylogenetic Inference. Pages 407–514 *in* Molecular systematics (D. M. Hillis, C. Moritz, and B. K. Mable, eds.). Sinauer Associates, Sunderland, Massachusetts.

TYLER, H., K. S. BROWN, AND K. WILSON. 1994. Swallowtail butterflies of the Americas: A study in biological dynamics, ecological diversity, biosystematics and conservation. Scientific Publishers, Gainesville, Florida.

VON EUW, J., T. REICHSTEIN, AND M. ROTHSCHILD. 1968. Aristolochic acid in the swallowtail butterfly *Pachlioptera aristolochiae*. Isr. J. Chem. 6:659–670.

WEINTRAUB, J. D. 1995. Host plant association patterns and phylogeny in the tribe Troidini (Lepidoptera: Papilionidae). Pages 307–316 *in* Swallowtail butterflies: Their ecology and evolutionary biology. (Scriber, J. M., Tsubaki, Y., and R. C. Lederhouse, eds.). Scientific Publishers, Gainesville, Florida.

WELLER, S. J., D. P. PASHLEY, AND J. A. MARTIN. 1996. Reassessment of butterfly family relationships using independent genes and morphology. Ann. Entomol. Soc. Am. 89:184–192.

WHELAN, S., AND N. GOLDMAN. 1999. Distributions of statistics used for the comparison of models of sequence evolution in phylogenetics. Mol. Biol. Evol. 16:1292–1299.

YAGI, T., G. SASAKI, AND H. TAKEBE. 1999. Phylogeny of Japanese papilionid butterflies inferred from nucleotide sequences of the mitochondrial ND5 gene. J. Mol Evol. 48:42–48.

Received 29 October 1999; accepted 13 December 1999
Associate Editor: R. Olmstead

APPENDIX 1.

In addition to primers listed in Caterino and Sperling (1999), Reed and Sperling (1999), and Cho et al. (1995), we used or designed the following primers for this study. Most are minor variants of existing primers. Mitochondrial location numbers refer to *Drosophila yakuba* (Clary and Wolstenholme, 1985). Nuclear location numbers refer to *Heliothodes diminutivus* (Cho et al., 1995). See Simon et al. (1994) for additional mitochondrial primers at these and other sites.

Gene	F/R	Location	Sequence
COI	R	1751	GGA GCT CCA GAT ATA GCT TTC CC
	R	1840	TGG GGG GTA TAC TGT TCA (T/A) CC
	R	2329	ACA GTA AAT ATA TGA TGT GCT CA
	R	2329	ACT GTG AAT ATG TGA TGG GCT CA
	R	2329	ACA GTA AAT ATA TGA TGA GCC CA
	F	2495	CCT CTA TAC TTT GAA GAT TAG G
	F	2495	CAT CAA TT(C/T) TAT GAA GAT TAG G
	F	2495	CCT CAA TTT TAT GAA GAT TAG G
	R	3014	TCA TTG CAT TTA TCT GCC ACA TTA
COII	F	3038	CTA ATA TGG CAG ATT ATA TCT AAT GGA
EF-1α	F	453	AAC TGA GCC ACC TTA CAG TGA GAG
	R	551	GGA GAC AAC ATG CTG GAC TCC A
	R	572	CTC CTT ACG CTC AAC ATT CC
	R	1048	AAC CGT TTG AGA TTT GAC CAG GG

APPENDIX 2.

Log likelihoods of unrooted ingroup-only topologies for individual data partitions under three separate models. The best likelihoods under each partition and model are shown in boldface type. Asterisks indicate a likelihood significantly worse than the best for that partition (as determined by Kishino–Hasegawa test; $\alpha = 0.05$).

	COI/II			EF-1α			
Topology	Pos1	Pos2	Pos3	Pos1	Pos2	Pos3	Sum
Jukes–Cantor							
Standard tree	−3979.349	−1747.648	−10119.523	−855.248	−528.567	−4146.912	−21377.254
Lueh. with Parn.	−3983.291	**−1746.031**	−10107.738	−855.401	−528.567	−4152.535	−21373.563
Combined data 1	−3984.145	−1755.120	**−10093.330**	−852.967	−528.567	−4160.373	−21374.504
Combined data 2	**−3975.927**	−1748.974	−10096.152	−858.531	−528.567	−4159.811	**−21367.960**
Mitochondrial 1	−3984.145	−1755.120	−10093.333	−852.967	−528.567	−4160.373	−21374.504
Mitochondrial 2	−3983.551	−1755.120	−10096.576	−846.540	−528.567	−4168.427*	−21378.782
EF-1α 1	−4003.487	−1758.311	−10128.958*	−850.714	−528.567	−4137.820	−21407.856
EF-1α 2	−3984.633	−1752.328	−10130.972*	−849.996	−528.567	−4137.602	−21384.098
EF-1α 3	−4001.549	−1758.311	−10129.517*	−850.079	−528.567	**−4136.659**	−21404.683
EF-1α 4	−4038.044*	−1777.139*	−10171.629*	**−840.429**	−528.567	−4145.589	−21501.398
EF-1α 5	−4014.354*	−1762.293	−10146.479*	−842.938	−528.567	−4137.361	−21431.992
HKY85 + Γ							
Standard tree	−3396.144	−1632.758	−8057.197	−775.884	−514.509	−3806.043	−18182.537
Lueh. with Parn.	−3397.046	**−1631.942**	−8055.900	−775.884	−514.509	−3813.105	−18188.387
Combined data 1	−3397.408	−1636.982	−8054.574	−774.388	−514.509	−3820.991*	−18198.853
Combined data 2	**−3395.443**	−1632.800	−8054.867	−777.851	−514.509	−3819.179	−18194.649
Mitochondrial 1	−3397.408	−1636.982	**−8054.574**	−774.388	−514.509	−3820.991*	−18198.853
Mitochondrial 2	−3397.079	−1636.982	−8054.181	−770.915	−514.509	−3827.707*	−18201.374
EF-1α 1	−3404.945	−1640.792	−8059.500	−771.581	−514.509	−3802.668	−18193.996
EF-1α 2	−3398.060	−1636.012	−8058.535	−769.807	−514.509	**−3800.173**	**−18177.100**
EF-1α 3	−3404.040	−1640.792	−8058.574	−769.807	−514.509	**−3800.173**	−18187.896
EF-1α 4	−3414.287*	−1651.695*	−8066.857	**−767.046**	−514.509	−3810.659	−18225.053
EF-1α 5	−3408.670	−1642.468	−8061.169	−768.287	−514.509	−3802.553	−18197.657
GTR + Γ							
Standard tree	−3309.198	−1615.683	−8034.699	−735.677	−505.767	−3747.118	−17948.142
Lueh. with Parn.	−3310.100	**−1615.033**	−8033.377	−735.705	−505.767	−3751.977	−17951.960
Combined data 1	−3309.150	−1619.865	−8031.845	−733.616	−505.767	−3758.049	−17958.293
Combined data 2	**−3308.092**	−1615.913	−8032.476	−737.683	−505.767	−3757.677	−17957.609
Mitochondrial 1	−3309.150	−1619.865	−8031.845	−733.616	−505.767	−3758.049	−17958.293
Mitochondrial 2	−3309.595	−1619.865	**−8031.762**	−731.543	−505.767	−3764.255*	−17962.788
EF-1α 1	−3314.284	−1622.761	−8036.635	−730.416	−505.767	−3744.514	−17954.377
EF-1α 2	−3309.775	−1618.871	−8035.885	**−728.511**	−505.767	**−3742.269**	**−17941.080**
EF-1α 3	−3313.578	−1622.761	−8035.893	**−728.511**	−505.767	**−3742.269**	−17948.779
EF-1α 4	−3323.765	−1633.834*	−8067.418*	−729.948	−505.767	−3754.100	−18014.822
EF-1α 5	−3317.822	−1624.589	−8044.044	−731.392	−505.767	−3745.463	−17969.078

APPENDIX 3.

Log likelihoods of rooted topologies for individual data partitions under two separate models. The best likelihoods under each partition and model are shown in boldface type. Asterisks indicate a likelihood significantly worse than the best for that partition (as determined by Kishino–Hasegawa test; $\alpha = 0.05$).

	COI/II			EF-1α			
Rooting	Pos1	Pos2	Pos3	Pos1	Pos2	Pos3	Sum
HKY85 + Γ							
1	**−3719.239**	−1774.295	**−8884.742**	−815.482	−534.946	−4291.550	−20020.254
2	−3722.491	−1774.746	**−8884.742**	−815.678	−534.946	−4291.720	−20024.323
3	−3722.516	−1775.784	−8899.366	−815.678	−534.946	−4292.183	−20040.471
4	−3727.380	−1782.155	−8884.770	−815.678	−534.946	−4298.015	−20042.945
5	−3727.877	−1780.440	−8899.420	−815.678	−534.946	−4297.864	−20056.224
6	−3724.059	−1774.746	−8888.654	−815.678	−534.946	−4291.687	−20029.770
7	−3722.561	**−1772.379**	−8888.654	−808.371	−534.946	−4291.553	**−20018.460**
8	−3728.412	−1785.538	−8886.796	−808.143	−534.946	−4293.163	−20036.998
9	−3731.059	−1785.745	−8886.796	−808.177	−534.946	−4290.193	−20036.916
10	−3731.059	−1785.384	−8886.796	−808.177	−534.946	−4293.681	−20040.042
11	−3732.306	−1782.511	−8886.796	−812.805	−534.946	**−4285.796**	−20035.160
12	−3733.034	−1782.511	−8890.734	−812.021	−534.946	−4285.896	−20039.141
13	−3731.450	−1780.305	−8890.734	**−805.289**	−534.946	−4285.909	−20028.634
GTR + Γ							
1	**−3623.634**	−1755.207	**−8862.620**	−774.578	−522.942	−4224.652	−19763.633
2	−3626.978	−1756.235	**−8862.620**	−775.259	−522.942	−4224.634	−19768.668
3	−3626.978	−1756.635	−8862.620	−775.259	−522.942	−4225.449	−19769.883
4	−3630.080	−1762.618	−8862.655	−775.259	−522.942	−4230.743	−19784.296
5	−3630.557	−1760.820	−8862.654	−775.259	−522.942	−4230.690	−19782.922
6	−3628.259	−1756.235	−8866.699	−775.259	−522.942	−4224.704	−19774.098
7	−3626.406	**−1753.676**	−8866.699	−768.329	−522.942	−4224.574	**−19762.630**
8	−3628.800	−1765.304	−8864.384	−767.315	−522.942	−4225.935	−19774.679
9	−3630.403	−1766.265	−8864.384	−767.995	−522.942	−4224.176	−19776.165
10	−3630.701	−1764.990	−8864.384	−767.995	−522.942	−4226.826	−19777.839
11	−3632.574	−1763.144	−8864.384	−772.586	−522.942	**−4219.662**	−19775.292
12	−3632.835	−1763.144	−8895.457*	−771.796	−522.942	−4219.803	−19805.977
13	−3631.280	−1760.744	−8868.476	**−764.754**	−522.942	−4219.705	−19767.901