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MULTIPLE SOURCES OF CHARACTER INFORMATION AND THE PHYLOGENY OF HAWAIIAN DROSOPHILIDS

RICHARD H. BAKER[1,2] AND ROB DESALLE[1]

[1]*Department of Entomology, American Museum of Natural History, 79th Street at Central Park West, New York, New York 10024, USA; E-mail: baker@amnh.org (R.B.), desalle@amnh.org (R.D.)*
[2]*Department of Biology, Yale University, New Haven, Connecticut 06520, USA*

Abstract.—Relationships among representatives of the five major Hawaiian *Drosophila* species groups were examined using data from eight different gene regions. A simultaneous analysis of these data resulted in a single most-parsimonious tree that (1) places the *adiastola* picture-winged subgroup as sister taxon to the other picture-winged subgroups, (2) unites the modified-tarsus species group with flies from the *Antopocerus* species group, and (3) places the white-tip scutellum species group as the most basal taxon. Because of the different gene sources used in this study, numerous process partitions can be erected within this data set. We examined the incongruence among these various partitions and the ramifications of these data for the taxonomic consensus, prior agreement, and simultaneous analysis approaches to phylogenetic reconstruction. Separate analyses and taxonomic consensus appear to be inadequate methods for dealing with the partitions in this study. Although detection of incongruence is possible and helps elucidate particular areas of disagreement among data sets, separation of partitions on the basis of incongruence is problematic for many reasons. First, analyzing all genes separately and then either presenting them all as possible hypotheses or taking their consensus provides virtually no information concerning the relationships among these flies. Second, despite some evidence of incongruence, there are no clear delineations among the various gene partitions that separate only heterogeneous data. Third, to the extent that problematic genes can be identified, these genes have nearly the same information content, within a combined analysis framework, as the remaining nonproblematic genes. Our data suggest that significant incongruence among data partitions may be isolated to specific relationships and the "false" signal creating this incongruence is most likely to be overcome by a simultaneous analysis. We present a new method, partitioned Bremer support, for examining the contribution of a particular data partition to the topological support of the simultaneous analysis tree. [Hawaiian *Drosophila*; partitioned Bremer support; process partitions; simultaneous analysis.]

The Hawaiian drosophilids include over 500 described species and have long been recognized as one of the most prominent examples of adaptive radiation and rapid speciation in nature (Carson, 1987; Kaneshiro and Boake, 1987). Because of their remarkable diversity, these flies have been the subject of numerous systematic studies at various taxonomic levels (Spieth, 1966; Takada, 1966; Throckmorton, 1966; Carson, 1970, 1982; Stalker, 1972; Hunt and Carson, 1983; Beverley and Wilson, 1985; DeSalle and Giddings, 1986; DeSalle et al., 1987; Grimaldi, 1990; DeSalle, 1992, 1995; Thomas and Hunt, 1993; Kambysellis et al., 1995). Despite this attention, many questions concerning the evolutionary relationships among Hawaiian drosophilids remain obscure. In the present study, we addressed a few of these areas by examining the monophyly of the various morphological assemblages that have been established for this large group of flies and the relationships among these assemblages. In particular, we addressed (1) whether the picture-winged *Drosophila* are monophyletic and (2) which of the five major morphologically defined species groups (picture-winged, modified mouthparts, modified tarsi, white tip scutellum, and *Antopocerus*) are basal in the phylogeny. Overlain on these important organismal questions concerning Hawaiian flies are controversial issues of data analysis that also require attention.

Picture-winged Drosophila Monophyly

Since Carson's (1970) seminal work on Hawaiian *Drosophila* systematics, the question of picture-winged monophyly has remained a matter of debate. His chromosomal phylogeny and the more recent yolk protein DNA sequencing study (Kambysellis et al., 1995) suggested that the three major

clades of picture-winged *Drosophila* (*adiastola, grimshawi, planitibia*) are monophyletic to the exclusion of flies in the group with modified mouthparts. Two other molecular studies, however, suggested that the *adiastola* subgroup is not closely related to the other two picture-winged groups. An immunoprecipitation study (Beverley and Wilson, 1985) placed the group with modified mouthparts as the closest relative of the *planitibia* subgroup–*grimshawi* subgroup clade. DeSalle et al. (1987) found additional support for this relationship using mitochondrial DNA sequence data.

Basal Relationships of Species Groups

Determining the most basal taxon or taxa in this group is important in light of comparative studies of ecologically interesting characters. Polarization of character states for ecological and life history traits, such as breeding substrate and ovarian morphology, is needed to complete schemes of character transformation and to infer the direction of evolution for these presumably adaptive features (Kambysellis et al., 1995). Previous systematic work on Hawaiian *Drosophila* suggests that the white-tip scutellum species group may be the most basal taxon, but this result requires further attention.

The early morphological work of Takada (1966) and Throckmorton (1966) addressed the phylogenetic placement of a few of the Hawaiian *Drosophila* species groups. Although both studies (Figs. 1a, 1b) placed the white-tip scutellum flies as the most basal taxon, several of the other species groups were not included in the analyses. Spieth (1966) presented behavioral data that can be used as character state information for taxa from a majority of the species groups. Because he did not produce a phylogeny for these flies, we analyzed his data using only those characters that were coded unambiguously (Spieth, 1966: table 1) and that are also phylogenetically informative. By this criterion, his original 30 behavioral characters reduces to 13. We have chosen one representative from each of the five major species groups examined in our study to give an overall picture of the relationships from behavioral characters. Figure 1c shows the topology of a tree based on the behavioral data and hypothesizes a basal position in the phylogeny for the white-tip scutellum flies.

The most recent morphological treatment of these flies can be found in Grimaldi's (1990) monograph on the family Drosophilidae, for which he examined several representatives of Hawaiian drosophilids (Grimaldi, 1990: fig. 543; Fig. 1d). Although a white-tip scutellum fly emerged as one of the basal species in his consensus tree, virtually none of the species groups are monophyletic. This result casts doubts on whether any given species group can be designated as basal.

Analytical Approaches

Because we present sequence data from various gene sources, there are numerous process partitions (Bull et al., 1993; Kluge and Wolf, 1993; Miyamoto and Fitch, 1995) that can be recognized within our data set. A controversy involving different philosophical approaches to phylogenetic hypothesis testing has led to many suggestions concerning how distinct process partitions should be analyzed (for reviews of this debate, see Kluge and Wolf, 1993; de Queiroz et al., 1995; Miyomoto and Fitch, 1995; Hueselbeck et al., 1996; Nixon and Carpenter, 1996). The primary focus of this discussion concerns the suggestion that partitioning allows one to assess the equivalence of phylogenetic information derived from different sources and that combining heterogeneous data violates certain assumptions of phylogenetic analysis (Bull et al., 1993; de Queiroz, 1993). This approach has been referred to as the prior agreement approach (Chippindale and Wiens, 1994). Some authors have even suggested that partitioning is a requisite to phylogenetic analysis and that the signals inherent in the various data matrices need to be considered separately (Miyamoto and Fitch, 1995) in a taxonomic congruence framework (Mickevich and Farris, 1981; Swofford, 1991). Alternatively, others have argued that data should be combined in all cases (Kluge, 1989; Barrett et al., 1991; Eer-

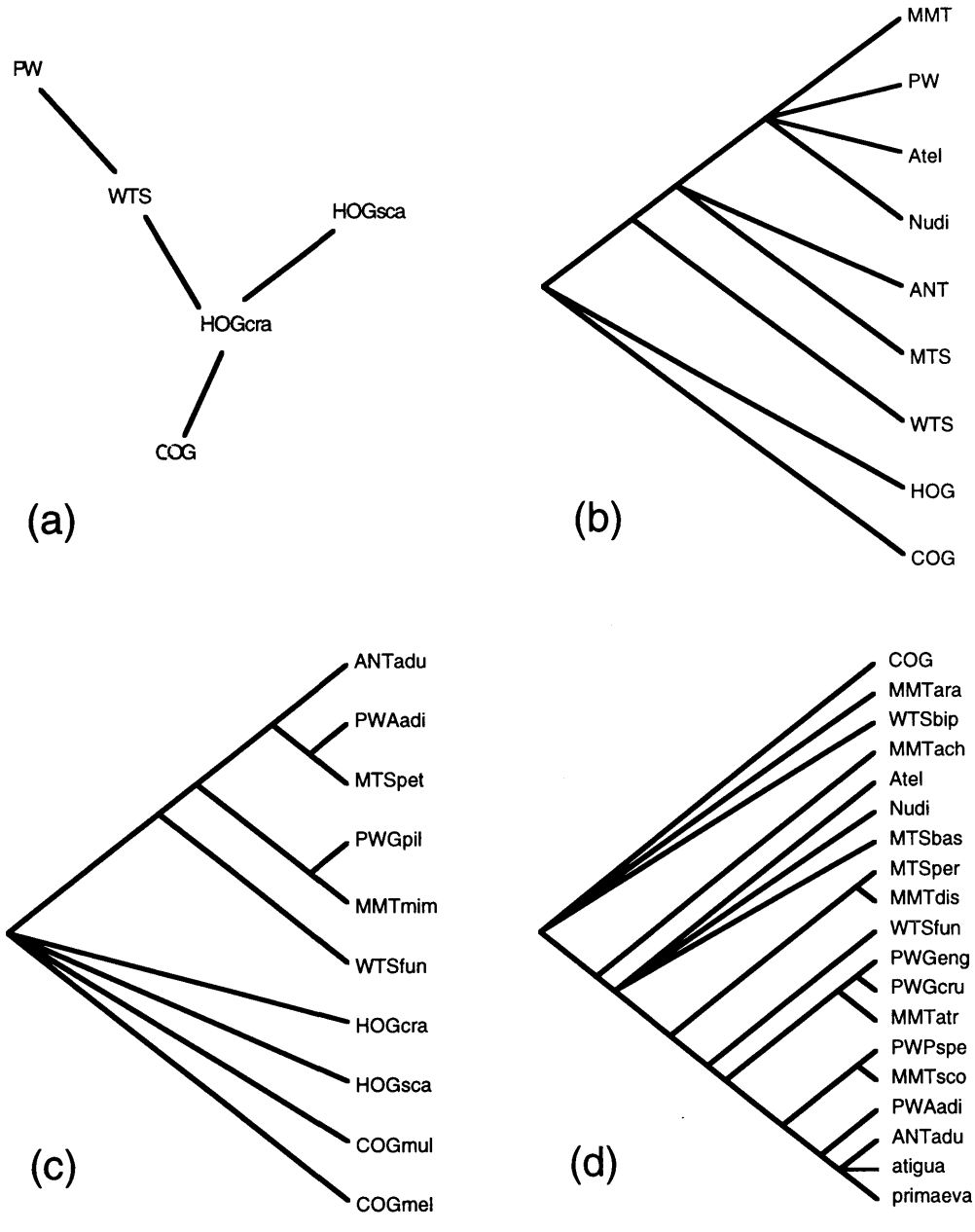

FIGURE 1. Relationships proposed for Hawaiian *Drosophila* groups. MMTara = *D. araiotrichia*; WTSbip = *D. bipolita*; MMTach = *D. achyla*; Atel = *Atelidrosophila*; Nudi = *Nudidrosophila*; MTSbas = *D. basimacula*; MTSper = *D. perissopoda*; MMTdis = *D. dissita*; WTSfun = *D. fungiperda*; PWGeng = *D. engyochracea*; PWGcru = *D. crucigera*; MMTatr = *D. atroscutellata*; PWPspe = *D. spectabilis*; MMTsco = *D. scolostoma*; PWAadi = *D. adiastola*; ANTadu = *D. adunca* (*Antopocerus*). Species group abbreviations are as in Table 1. (a) Redrawn from Takada's (1966) analysis of genitalia. (b) Redrawn from Throckmorton's (1966) morphological analysis. (c) Taken from Spieth's (1966) behavioral data. This tree is a single parsimony tree obtained by exhaustive search with PAUP (steps = 22, consistency index = 0.58, retention index = 0.53). (d) Redrawn from Grimaldi's (1990) cladistic analysis of morphology.

TABLE 1. The five species groups of Hawaiian *Drosophila* used in this study.

Species group	No. species in group (approx.)	Species used	Abbreviations
Antopocerus	18	*D. adunca*	ANTadu
		D. tanythrix	ANTtan
Modified tarsus	150	*D. dasycnemia*	MTSdas
		D. petalopeza	MTSpet
Modified mouthpart	100	*D. mimica*	MMTmim
		D. soonae	MMTsoo
White-tip scutellum	100	*D. iki*	WTSiki
		D. sp.[a]	WTSlon
Picture-winged	100		
planitibia	27	*D. silvestris*	PWPsil
		D. cyrtoloma	PWPcyr
grimshawi	15	*D. disjuncta*	PWGdis
		D. lineosetae	PWGlin
adiastola	15	*D. adiastola*	PWAadi

[a] White-tip scutellum fly currently being described by Dr. Ken Kaneshiro.

nisse and Kluge, 1992; Kluge and Wolfe, 1993; Nixon and Carpenter, 1996) based on the rationale that the best explanation will include all of the relevant data and that a consensus approach implies arbitrary character weighting (Cracraft and Mindell, 1989). Much of this debate, however, has been conducted on theoretical grounds, so it is important to examine actual data sets to fully understand the implications of each approach. Our study includes data from eight gene regions and, therefore, offers a unique opportunity for this type of detailed examination.

MATERIALS AND METHODS

Flies

Drosophilids from the Hawaiian Islands were collected in the wild by standard techniques, identified, and archived with voucher information by Dr. Kenneth Kaneshiro. The flies slated for molecular analysis were mailed alive to the American Museum of Natural History and Yale University, where upon receipt they were frozen at −70°C. We attempted to use the same DNA from a single specimen as template for all DNA amplifications using PCR. When DNA from a particular individual was depleted, we used DNA from another individual of the same species collected at the same site.

Exemplars

Five major species groups were analyzed in this study (Table 1). *Atelidrosophila* and *Nudidrosophila* (two very small genera of endemic Hawaiian flies) were not collected and so were not examined. At least two species from each of the five major species groups were analyzed for all of the genes in this study. One group that required special attention, and therefore included more representatives, is the picture-winged (PW) group. The PW group has 103 members, arranged by Carson (1970) into three major subgroupings; the *planitibia* subgroup (PWP), the *adiastola* subgroup (PWA), and the combined monophyletic subgroup of *grimshawi*, *fasciculasetae*, and *hawaiiensis* (PWG). Previous immunological data (Beverley and Wilson, 1985) and a preliminary mitochondrial DNA (mtDNA) sequencing study (DeSalle et al., 1987) suggested that the *adiastola* subgroup is basal to all drosophilids from Hawaii. Carson's (1970) chromosomal data showed the PW flies as monophyletic with respect to one of his outgroups (*D. mimica*, a member of the modified mouthpart species group).

Outgroups

Many of the genes used in this study are evolving rapidly; hence, the choice of outgroup is critical in establishing polarity of

TABLE 2. Summary of the different gene regions used in this study. Primers for some of the gene regions are provided in the references listed.

Gene	Abbrev.[a]	No. characters		Reference/primer
		Total	PI[b]	
Mitochondrial	mt	1,103	196	
16S rDNA	16S	360	42	DeSalle, 1992
NADH dehydrogenase I	*ND1*	148	19	DeSalle, 1992; Vogler et al., 1993
Cytochrome oxidase III	*COIII*	229	58	Simon et al., 1994
Cytochrome oxidase II	*COII*	366	77	Brower, 1994
Nuclear	nuc	1,447	301	
Alcohol dehydrogenase	*ADH*	222	58	Thomas and Hunt, 1993
Acetylcholinesterase	*ACHE*	323	60	
				5'-GAGATCTGGAATCCCAA-3'
Wingless	*wg*	429	66	5'-CCCACTCGRTACTGAAACGA-3'
				5'-GGAGTTCAAGAAGAGTGTCTTTGA-3'
				5'-GGATTCGATGGCGCCACACGCGTCCA-3'
Hunchback	*hb*	473	117	5'-GAGCAGCACAAYGCNTGGTA-3'
				5'-GGCCATGTACTTCATRTCYTC-3'

[a] Gene abbreviation used throughout the paper.
[b] Number of phylogenetically informative characters.

relationships among these flies. DeSalle (1992) and Thomas and Hunt (1993) suggested that the Hawaiian scaptomyzids (subgenera *Scaptomyza* and *Engiscaptomyza*) are the sister taxa to the Hawaiian drosophilids. Consequently, we chose a member of each of the two scaptomyzid subgenera as two of our outgroups: *Scaptomyza albovittata* (HOGsca) and *Engiscaptomyza crassifemur* (HOGcra). In addition, representatives of the two clades most closely related to all the Hawaiian taxa were also included. Based on DeSalle's (1992) study, these are the continental subgenera *Drosophila* and *Sophophora*. The species chosen from these subgenera were *D. mulleri* (*Drosophila*, COGmul) and *D. melanogaster* (*Sophophora*, COGmel).

DNA Isolation and Manipulation

DNA was isolated from single flies using the small scale preparation outlined by DeSalle et al. (1993). A single fly usually yields enough template DNA for at least 20–30 PCR reactions using this approach. PCR primers designed for three mitochondrial gene regions, cytochrome oxidase II, cytochrome oxidase III, and 16S mitochondrial ribosomal RNA plus NADH dehydrogenase I, were used to compile the mitochondrial component of the data matrix. PCR primers for four unlinked nuclear gene regions, hunchback, wingless, acetylcholinesterase, and alcohol dehydrogenase, were also designed and used to compile the nuclear gene region component of the data matrix. Information on the length of gene fragments and primer sequences is presented in Table 2, and accession numbers for sequence data deposited in GenBank are listed in Table 3. All sequence data for *D. melanogaster* and the *ADH* sequences for *D. mimica*, *D.mulleri*, *D. adiastola*, *D. crassifemur*, *D. silvestris*, and *S. albovittata* were acquired from GenBank. For two of the nuclear genes, *hb* and *wg*, sequence data were not obtained for a middle portion of the PCR product.

PCR conditions differed for the different primer pairs, but in general all PCR reactions were a variation on the general profile of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, for a total of 35 cycles. Annealing temperature and number of cycles were usually varied independently to optimize the conditions for each primer pair. Amplified DNA was sequenced using one of three methods: (1) direct sequencing of the double-stranded PCR product using manual dideoxy sequencing and ^{35}S, (2) use of the ABI 373 automated sequencer to obtain sequences using flourescent dideoxy terminator mix, and (3) use of the TA vector to clone the amplified product. Cloned products were

TABLE 3. GenBank accession numbers for new *Drosophila* and *Scaptomyza* sequence data generated in study.

Species	Genes							
	hb	*wg*	*ACHE*	*ADH*[a]	*COIII*	16S[b]	*ND1*[b]	*COII*
D. adiastola	U92996, U92997	U94553, U94554	U94267		U94225			U94209
D. adunca	U92998, U92999	U94555, U94556	U94268	U94194	U94226	U94241	U94254	U94210
D. crassifemur	U93000, U93001	U94557, U94558	U94269		U94227			U94211
D. cyrtoloma	U93002, U93003	U94559, U94560	U94270	U94196	U94228	U94242	U94255	U94212
D. disjuncta	U93004, U93005	U94561, U94562	U94271	U94197	U94229	U94243	U94256	U94213
D. iki	U93006, U93007	U94563, U94564	U94272	U94198	U94230	U94244	U94257	U94214
D. lineosetae	U93008, U93009	U94565, U94566	U94273	U94199	U94231	U94245	U94258	U94215
D. sp.[c]	U93010, U93011	U94567, U94568	U94274	U94200	U94232	U94246	U94259	U94216
D. mimica	U93012, U93013	U94569, U94570	U94275		U94233			U94217
D. mulleri	U93014, U93015	U94571, U94572	U94276		U94234	U94247	U94260	U94218
D. petalopeza	U93016, U93017	U94573, U94574	U94277	U94203	U94235	U94248	U94261	U94219
S. albovittata	U93018, U93019	U94575, U94576	U94278		U94236	U94249	U94262	U94220
D. silvestris	U93020, U93021	U94577, U94578	U94279		U94237	U94250	U94263	U94221
D. soonae	U93022, U93023	U94579, U94580	U94280	U94206	U94238	U94251	U94264	U94222
D. tanythrix	U93024, U93025	U94581, U94582	U94281	U94207	U94239	U94252	U94265	U94223
D. dasycnemia	U93026, U93027	U94583, U94584	U94282	U94208	U94240	U94253	U94266	U94224

[a] Species with no accession number were listed by Thomas and Hunt (1993), except *D. silvestris* (M63291).
[b] Species with no accession number were listed by DeSalle (1992).
[c] White-tip scutellum fly currently being described by Dr. Ken Kaneshiro.

then sequenced using the double-stranded manual sequencing protocol with ^{35}S. Manual sequences were read into the program MacVector and verified using this software. Automated sequences were transferred into MacClade and verified by visual inspection of the chromatographs produced from each sequencing run.

Three aspects of the data collected in previous Hawaiian *Drosophila* studies prevent us from combining this information with our molecular characters. In two studies (Takada, 1966; Throckmorton, 1966), the anatomical data were not coded as character state information. For the other studies in which the data were coded as characters, there is either limited overlap of species between their studies and ours (Spieth, 1966; Grimaldi, 1990) or incomplete sampling of the various species groups (Carson, 1970; Kambysellis et al., 1995). The third problem concerns the basic incompatibility of distance data, in particular the immunoprecipitation data of Beverley and Wilson (1985), with character-based data. The inherent difference in the nature of distance data and character-based data precludes the simultaneous analysis and assessment incongruence among these data (Brower et al., 1996).

Data Analysis

Alignment.—Mitochondrial ribosomal RNA sequences were aligned using MALIGN (Wheeler and Gladstein, 1994). The alignments obtained were trivial because very few indels occur in the 16S sequences that we obtained, as previously reported by DeSalle (1992). Alignment of protein-coding regions was done by translating the DNA sequences into amino acid sequences and aligning the amino acid sequences in MEGALIGN (DNASTAR, version 1.02), using the Clustal algorithm. For six of the protein-coding genes (*wg, ACHE, ADH, COIII, COII, ND1*), these alignments were trivial because no more than a single amino acid indel was hypothesized in any of these Clustal alignments. The nucleotide sequences of these six genes were aligned by codon to the amino acid alignments, and these aligned nucleotide sequences were used in the data matrix. The seventh protein-coding gene (*hb*) has several hypervariable-length regions where amino acids are repeated multiple times, and it was not possible to obtain unambiguous alignment for these regions. Consequently, we modified the CULL (Gatesy et al., 1994) procedure to determine alignment-ambig-

uous and -unambiguous regions of the amino acid sequences. We performed three alignments on the *hb* amino acid sequences using gap-to-change costs of 10, 20, and 40. The alignments were examined for ambiguous regions (Gatesy et al., 1994), and these regions were eliminated from the matrix using the rationale that alignment ambiguity indicates failure to establish topographical identity (sensu Brower and Schawaroch, 1996). The aligned amino acid sequences minus the alignment-ambiguous regions were then aligned with the nucleotide sequences, eliminating triplets of nucleotides that correspond to the eliminated amino acids. The nucleotide sequences obtained in this manner were then added to the other nucleotide data from the seven other gene regions. The alignments are available at http://www.utexas.edu/depts/systbiol/.

Phylogenetic analysis.—All tree-generating analyses were accomplished using PAUP 3.1 (Swofford, 1993) with all characters equally weighted. The justification for this weighting approach as a first approximation to phylogeny estimation was described by Brower and DeSalle (1994) and Nixon and Carpenter (1996). The number of taxa and the size of the data matrix precluded branch-and-bound searches, so we implemented the heuristic search option using 20 random-addition searches for each search attempted. Clade stability was estimated using two different methods: bootstrap analysis (Felsenstein, 1985) and Bremer support analysis (Bremer, 1988, 1994). Bootstrap values were generated in PAUP from 200 replicates of simple-addition heuristic searches. For Bremer support indices, we searched in PAUP for trees 1–10 steps longer than the most-parsimonious tree. Many of the nodes on the simultaneous analysis tree, however, had support >10. For these cases, we identified the individual nodes and then ran constrained heuristic searches to find the most-parsimonious trees not containing these nodes.

Partitions.—To examine the difference in phylogenetic signal between gene partitions, incongruence length differences (ILD) were calculated as described by Mickevich and Farris (1981) and Farris et al. (1994, 1995). This index measures the amount of extra homoplasy that results from the combination of two or more data partitions. We used the ARNIE program (Siddall, 1995) in the Random Cladistics software package to assess the significance of the ILDs. This program tests the null hypothesis that the data partitions do not differ by taking a data matrix comprised of several partitions and randomly reorganizing the character columns into new partitions that correspond to the sizes of the original partitions. An ILD measurement is then made for these randomized data. Several repetitions of this procedure serve as a null distribution for assessing the statistical significance of the ILD index of the original partitions. In all cases, 999 permutations were executed to generate the null distributions.

We initially partitioned along the eight gene boundaries, performing three types of congruence analyses for this partitioning scheme: (1) examining congruence across the entire data set by setting ARNIE to generate artificial partitions the same size as all eight gene partitions, (2) examining all possible pairwise gene comparisons, which resulted in 28 separate ARNIE runs, and (3) examining incongruence between single gene partitions and the rest of the data matrix combined. We also partitioned along the boundary between nuclear and mitochondrial genes, and for all the protein-coding genes combined we assessed the congruence between third codon position characters and the combination of first and second codon position characters.

Partitioned Bremer support.—Because data from different sources are often combined, it is of interest to know how each of these data sources contributes to the branch support of the simultaneous analysis cladogram. This information is usually obtained by comparing the topologies for data sets analyzed separately with the topology of the simultaneous analysis cladogram. When data are combined, however, relationships often emerge that are present in

none of the separate analyses (see Chippindale and Wiens [1994] for a list of empirical examples). These cases highlight the fact that support for relationships on a simultaneous analysis tree may be provided by data sets that do not exhibit this support in individual analyses.

Therefore, given that support from data partitions may change when combined with other character information, we present a method that summarizes the topological support provided by data from different sources within the context of all the included evidence. This method partitions the Bremer support (Bremer, 1988, 1994) on the simultaneous analysis tree among the various data sets used to construct it. Bremer support measures the number of extra steps in tree length required before a node collapses. Comparisons are made between the length of the most-parsimonious tree(s) and the length of the shortest tree(s) not containing a given node. The partitioned Bremer support uses this same information by comparing the length of a data partition on the most-parsimonious tree(s) with the length of the partition on the most-parsimonious tree(s) not containing the specified node. The difference in these two lengths provides the contribution of a given partition to the simultaneous analysis support at that node. Positive values indicate that within the simultaneous analysis framework a given gene provides support for that particular relationship over the alternative relationships specified in the tree(s) without the given node. The amount of support is given by the magnitude of the value and can be thought of as the Bremer support for that gene at that node. Negative values indicate that, again within the simultaneous analysis framework, the length of that gene is less on the topology of the alternative tree(s) and, thus, provides contradictory evidence for the relationship found on the simultaneous analysis tree. The sum of the partitioned Bremer support for all data sources at a node will always equal the total support for that node on the simultaneous analysis tree. In this way we can examine how the various gene partitions influence the topological support of the simultaneous analysis tree.

For this study, partitioned Bremer support was calculated in PAUP by saving all the most-parsimonious trees found in a heuristic search constrained not to include one of the nodes on the simultaneous analysis tree. For each node, 10 random-addition constrained searches were performed. The length of a gene partition was then calculated on the simultaneous analysis tree and the most-parsimonious constrained tree(s). The length of the gene partition on the constrained tree(s) minus the length of the gene partition on the simultaneous analysis tree gives the partitioned Bremer support for that gene at the node specified to be excluded from the constrained tree(s). If more than one most-parsimonious tree was found during the 10 random constrained searches, the average gene partition length for all these trees was used.

RESULTS

Simultaneous Analysis

The combined analysis of the four nuclear and four mitochondrial genes (Table 2) is shown in Figure 2. A single tree of length 1,895, with a consistency index (CI) of 0.50 (all CIs reported in this paper are calculated excluding uninformative characters) and a retention index (RI) of 0.49, was obtained in the parsimony search. Nodes that define the various morphological species groups or subgroups are generally supported by high bootstrap values (six of seven groups have bootstrap values >88%) and by large Bremer support values (5–34). The PW clade including the *adiastola* representative is supported by a bootstrap value of 71% and a Bremer support value of 5. Other points of significance in the overall topology of the tree are that (1) the modified-mouthpart species are seen as the sister group to all picture-winged flies (bootstrap = 58%, Bremer support = 5); (2) the *Antopocerus* and modified-tarsus flies are hypothesized as a monophyletic group (99%, 11); and (3) the fungus feeder group of white-tip scutel-

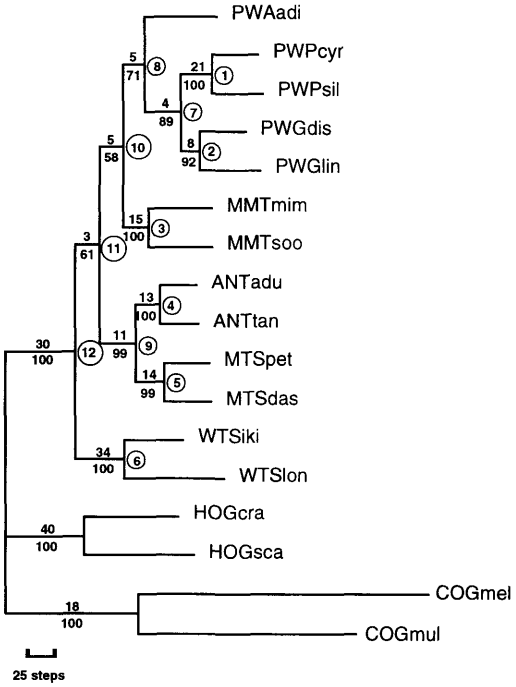

FIGURE 2. Phylogram resulting from the simultaneous analysis of all eight gene-by-gene partitions. Taxon abbreviations are as in Table 1. Character optimization was done by ACCTRAN, and the lengths of the branches are proportional (see scale bar). Bremer support values are presented above each branch and bootstrap values are presented below each branch. Circled numbers at each node designate clades referred to throughout the text.

lum flies is hypothesized as the most basal group of the cladogram (61%, 3).

Partitioning and Congruence

There are numerous intuitive partitions in this data matrix that can be established along the boundaries of the eight genes used in this study. Other ways to subdivide the data include the partition between nuclear and mitochondrial genes and third position sites versus first and second position sites.

Gene by gene.—We first partitioned the data matrix by gene boundaries because it is more likely that sequences within genes rather than multiple unlinked genes evolve as units. Figure 3 shows the eight trees constructed from each gene analyzed separately. All gene trees show some degree of resolution, but topologies differ among gene trees, and no two gene trees have the same topology. We next assessed the degree of incongruence among these various gene trees using the ILD index (Farris et al., 1994, 1995) and the ARNIE program. The ILD for the entire data set analyzed simultaneously by partitions is 0.05 ($P < 0.01$). Table 4 shows the matrix of ILD measures for each pairwise gene-by-gene comparison.

The eight genes can be arranged by the degree of significant incongruence each has with the other seven in the following way, where the number of incongruent measurements for each gene partition is given in parentheses: *ND1* (2) < *COII* = *ACHE* (3) < *hb* = *wg* = 16S (4) < *ADH* = *COIII* (6). We also examined the congruence of each gene individually with the rest of the data combined (Table 4). This analysis shows that four of the genes, *ADH*, *COIII*, 16S, and *COII*, are significantly incongruent with the rest of the data matrix.

Several asymmetries in the pattern of ILD values lead to problems in identifying a boundary that clearly separates heterogeneous data. For instance, although *COIII* is incongruent with most of the other partitions it is still congruent with *COII*, which in turn is incongruent with three other genes. Overall, there is no clear way to cluster the eight process partitions into definitive congruent sets or incongruent sets using a gene-by-gene set of partitions.

The suggestion that taxonomic congruence be used as a way of extracting phylogenetic information can also be examined. Because none of the individual gene trees are entirely congruent (i.e., share the same topology), much resolution in tree topology is lost when taxonomic congruence is used. In fact, a strict consensus of the over 100 fundamental parsimony trees that were used to generate the single gene trees in Figure 3 is totally unresolved.

Mitochondrial versus nuclear.—The potential difference in evolutionary history between maternally inherited mtDNA and the biparentally inherited nuclear DNA presents another obvious rationale for data

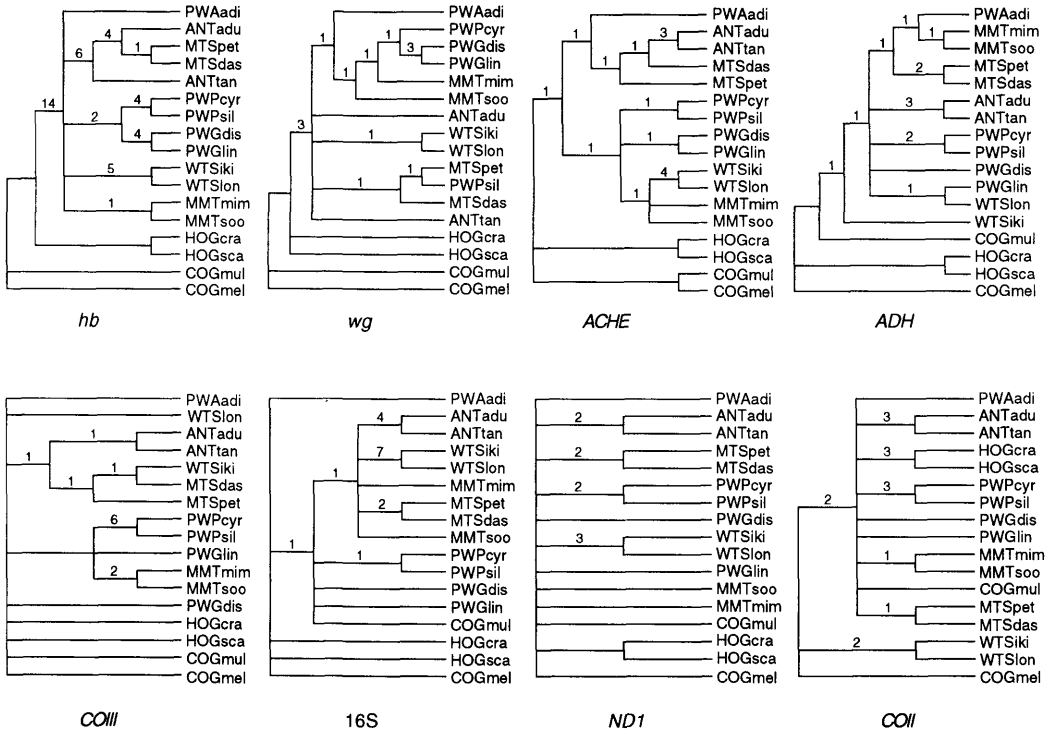

FIGURE 3. The eight genealogies generated from the individual analysis of each gene partition. Taxon abbreviations are as given in Table 1. All trees are a strict consensus of equally most-parsimonious trees. The numbers above each branch are Bremer support values. The *hb* tree is a consensus of 13 trees, each with CI = 0.64, RI = 0.68, and length = 390. The *wg* tree is a consensus of three trees, each with CI = 0.52, RI = 0.54, and length = 247. The *ACHE* tree is a consensus of four trees, each with CI = 0.58, RI = 0.53, and length = 248. The *ADH* tree is a consensus of six trees, each with CI = 0.57, RI = 0.57, and length = 204. The *COIII* tree is a consensus of five trees, each with CI = 0.41, RI = 0.45, and length = 223. The 16S ribosomal RNA tree is a consensus of 33 trees, each with CI = 0.45, RI = 0.63, and length = 126. The *ND1* tree is a consensus of 26 trees, each with CI = 0.56, RI = 0.64, and length = 76. The *COII* tree is a consensus of 12 trees, each with CI = 0.41, RI = 0.37, and length = 316.

TABLE 4. Incongruent length differences (ILDs) for the pairwise comparison of gene partitions. Numbers in the top of the matrix are the number of extra steps introduced by combining partitions (Mickevich and Farris, 1981; Farris et al., 1994, 1995). Numbers in the bottom of the matrix are the ILD measurements normalized by the length of the most-parsimonious tree(s) for the combined data of each pairwise gene comparison. The bottom row provides ILD measurements for each gene individually compared with the rest of the data combined. * = statistical significance of the ILD at $P < 0.05$.

Genes	*hb*	*wg*	*ACHE*	*ADH*	*COIII*	16S	*ND1*	*COII*
hb	—	5	4	11*	17*	12*	5	15*
wg	0.01	—	8	12*	17*	13*	8	15*
ACHE	0.01	0.02	—	10*	17*	9	3	13*
ADH	0.02	0.03	0.02	—	17*	14*	10*	13
COIII	0.03	0.03	0.03	0.04	—	19*	16*	17
16S	0.02	0.03	0.02	0.04	0.05	—	9	10
ND1	0.01	0.02	0.01	0.04	0.05	0.04	—	9
COII	0.02	0.03	0.02	0.03	0.03	0.02	0.02	—
Total	5	4	4	9*	14*	12*	5	12*

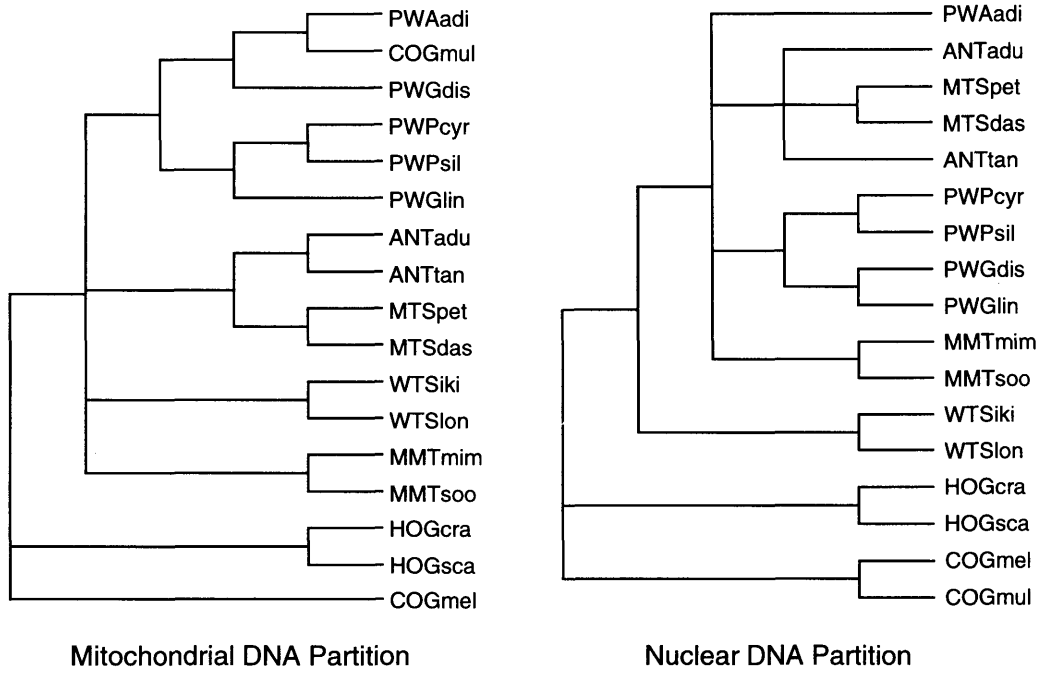

FIGURE 4. The two parsimony trees generated by partitioning between nuclear and mitochondrial genes. Taxon abbreviations are as in Table 1. The mitochondrial partition tree is a consensus of three equally parsimonious trees: 778 steps, CI = 0.42, RI = 0.42. The nuclear partition tree is a consensus of three equally most-parsimonious trees: 1,111 steps, CI = 0.57, RI = 0.57.

partitioning. Partitioning the data matrix by mitochondrial and nuclear genes results in the two cladograms shown in Figure 4. Partitioning in this manner gives an ILD of 0.003, which is not significant. This result suggests that there is no empirical basis for discriminating between the process partition of mtDNA and nuclear DNA for these flies.

Third positions versus first and second positions.—Because most third position nucleotide substitutions do not affect amino acid composition, there are clearly different selective/functional constraints operating on these sites than are operating on first and second positions. The difference in evolutionary processes affecting these sites has led numerous authors to down-weight all or some of the third position character transformations (Normark et al., 1991; Arnason and Gullberg, 1994; Brown et al., 1994; Lara et al., 1996; Russo et al., 1996). Measurement of saturation for either all third position sites or only third position transitions has been the traditional means for assessing the reliability of these characters. Alternatively, however, character congruence can be used to compare the difference in phylogenetic information provided by third position versus first and second position sites. Therefore, for the seven protein-coding genes sequenced in this study, we tested the degree of incongruence between all third positions combined and all first and second positions combined.

There is very little incongruence between these two data partitions. The third position data yield two equally most-parsimonious trees (Fig. 5), and the first and second position data produce four equally most-parsimonious trees (Fig. 5). The combined data produce a tree only three steps longer than the sum of the two separately analyzed data sets, for an ILD of 0.002, which is not significant.

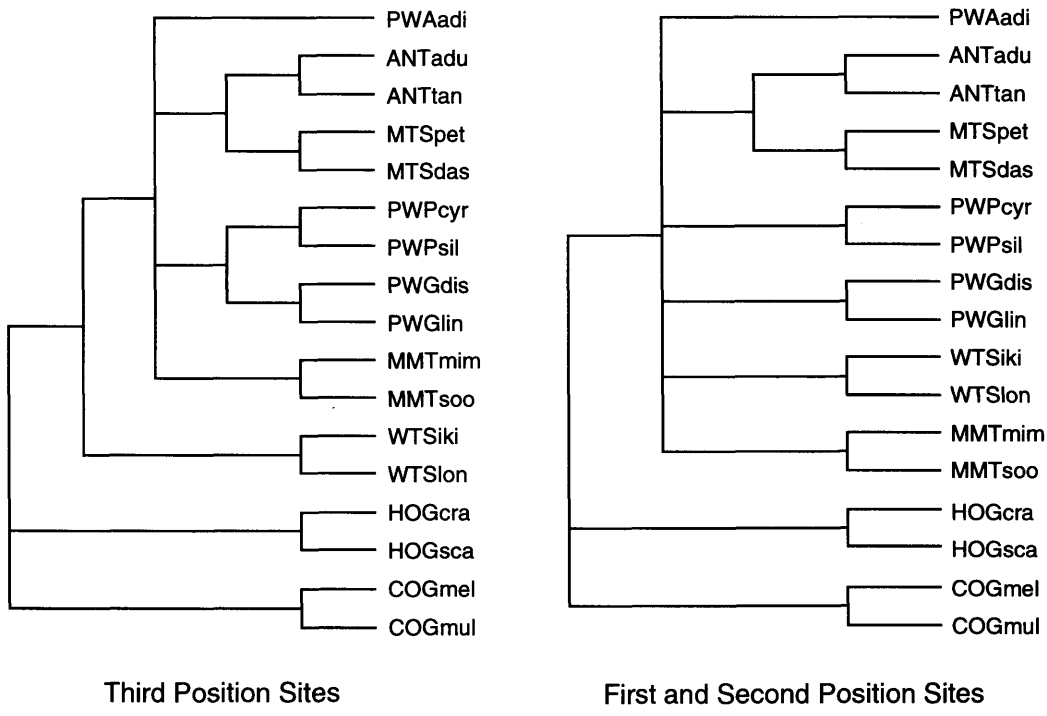

FIGURE 5. The two parsimony trees generated by partitioning between third codon position sites and a combination of first and second position sites. Taxon abbreviations are as in Table 1. Third position characters yield two most-parsimonious trees: 1,224 steps, CI = 0.49, RI = 0.48. The first and second position tree is a strict consensus of four equally parsimonious trees: 529 steps, CI = 0.54, RI = 0.53.

DISCUSSION

Simultaneous Analysis

One of the potential benefits of combining data is that relationships will emerge in a simultaneous analysis that exist in few, if any, of the individual partition trees (Kluge, 1989; Barrett et al., 1991; Chippindale and Wiens, 1994; Olmstead and Sweere, 1994; de Queiroz et al., 1995; Nixon and Carpenter, 1996). In these cases, true but weak phylogenetic signal may be present in most of the partitions but is only amplified over the noise when data are combined. In a phylogenetic study of the Solanaceae using three different data sources, Olmstead and Sweere (1994) showed that relationships found in only one of the three individual partition analyses were recovered by the other two partitions when they were combined. Similarly, for the Hawaiian flies in this analysis, six of the nodes (2, 7, 8, 9, 10, 11) on the simultaneous analysis tree appear in at most only three of the individual gene trees (Table 5), and three of these nodes (8, 10, 11) are unique to the simultaneous analysis tree. When new relationships emerge in a simultaneous analysis, there is no guarantee these relationships reflect the true history of the group. However, methods for assessing branch support (e.g., bootstrapping and Bremer support), provide some means for assessing the strength of evidence for relationships on the simultaneous analysis topology. In this study, for several of the nodes that appear in at most only three of the individual gene trees, there exists reasonably strong support on the simultaneous analysis tree. One node (9), in fact, has a bootstrap value of 99% and Bremer support of 11. In order of decreasing support, the other relationships have values of 92% and 8 (node 2), 89% and 4 (7), 71% and 5 (8), 61% and 3 (11), and 58% and 5 (10). Although any of these

TABLE 5. Data partitions that support in separate analysis the various relationships in the simultaneous analysis tree (Fig. 2).

Monophyletic group	Node	Partition
PWP	1	*hb, ACHE, ADH, COIII,* 16S, *ND1, COII*
PWG	2	*hb, wg, ACHE*
MMT	3	*hb, ADH, COIII, COII*
ANT	4	*ACHE, ADH, COIII,* 16S, *ND1, COII*
MTS	5	*hb, ADH,* 16S, *ND1, COII*
WTS	6	*hb, wg,* 16S, *ND1, COII, ACHE*
PWP, PWG	7	*hb*
PWP, PWG, PWA	8	
ANT, MTS	9	*hb, ACHE*
PW, MMT	10	
PW, MMT, ANT, MTS	11	
PW, MMT, ANT, MTS, WTS	12	*hb, wg, ACHE, ADH*

nodes (10 and 11 in particular) may be overturned with the addition of more data, the simultaneous analysis topology appears to be the best available estimate of the evolutionary relationships among these flies. Furthermore, it seems preferable to any alternative approach that does not combine all the data, either a completely unresolved consensus tree or eight individual partition trees with vastly different biological implications (e.g., in four of the eight trees the ingroup is not monophyletic).

Given the relative strength of the simultaneous analysis topology, comparisons of the actual information found in each partition are best examined in the context of all the data combined. By comparing the number of nodes that are in both the simultaneous analysis tree and the parsimony trees of the eight process partitions, we can get some idea of the contribution of each partition to the simultaneous analysis tree. In Table 6, the *hb* tree stands apart in having a relatively high number of nodes (eight) in agreement with the simultaneous analysis tree. As mentioned earlier, another means for assessing the evidential impact of individual genes on the simultaneous analysis topology is provided by the partitioned Bremer support.

TABLE 6. Number (percentage) of nodes in the process partition trees (Fig. 3) that are also found in the simultaneous analysis tree (Fig. 2). Twelve ingroup nodes were resolved in the simultaneous analysis tree.

Gene partition	No. nodes (%)
hb	8 (66)
wg	3 (25)
ACHE	6 (50)
ADH	5 (42)
COIII	3 (25)
16S	4 (33)
ND1	4 (33)
COII	5 (42)
mt	6 (50)
nuc	9 (75)

Table 7 provides the partitioned Bremer support for each gene summed across the entire simultaneous analysis tree. The differential influence of the *hb* data is also evident using this information. Nearly 1/3 (47.5/163) of the tree's total support is provided by the *hb* data, whereas very little (8 and 9.75) is provided by either *ACHE* or *ADH*. Whether this phenomenon is particular to these taxa or whether *hb* is a consistently more useful phylogenetic indicator than other genes must await future studies using numerous data sources. For other genes in this study, the partitioned Bremer support provides a slightly different picture of its impact on the simultaneous analysis tree than is indicated by separate analyses. For instance, both the *COIII* and the *wg* topologies (Fig. 3) have the fewest number of nodes (three) in common with the simultaneous analysis tree (Table 6), suggesting that these genes may provide little useful information for the final hypothesis. The partitioned Bremer support, however, indi-

TABLE 7. Partitioned Bremer support for the simultaneous analysis tree (Fig. 2). Each gene column gives the contribution of that gene to the Bremer support at the corresponding nodes.

Node no.[a]	Bremer support	Gene partitions							
		hb	*wg*	*ACHE*	*ADH*	*COIII*	16S	*ND1*	*COII*
1	21	4	4	0	2	8	1	1	1
2	8	4	2	2	0	1	0	0	−1
3	15	5	1	2	2	4	−3	1	3
4	13	−4	0	2	2	3	4	2	4
5	14	1	3.5	0	1.5	3.5	2	2	0.5
6	34	10	4	2.5	−2	1.5	8.5	4	5.5
7	4	3	2	0	1	0	0	0	−2
8	5	2	1	−1	−2	3	4	−2	0
9	11	6	2	−1	1	3	−1	1	0
10	5	1	2.25	−1	−0.25	3.5	−0.75	0	0.25
11	3	−1	3	0	2	0	−2	0	1
12	30	16.5	4	2.5	2.5	0	3	1	0.5
Total	163	47.5	28.75	8	9.75	30.5	15.75	10	12.75

[a] As specified in the simultaneous analysis tree (Fig. 2).

cates that after *hb*, *COIII* and *wg* have the largest influence on the simultaneous analysis tree's total support. In addition, the difference in total support among genes is not simply a function of their size. *ND1*, which has only 19 informative characters, has a total support of 10, whereas *COII* has a total support of only 12.75, despite 77 informative characters. A Spearman rank correlation of total support for each gene and number of informative characters is not significant (r_s = 0.375). This evidence contradicts the argument that data partitions with many characters will likely overwhelm data partitions with fewer characters (Miyamoto, 1985; Hillis, 1987; Doyle, 1992; Lanyon, 1993).

Using the partitioned Bremer support to assess the utility of different genes clearly depends on the topology of the simultaneous analysis hypothesis and, therefore, may be sensitive to the addition of a few characters that change the topology of the most-parsimonious and the near most-parsimonious trees. However, just as our belief in a particular set of phylogenetic relationships is continually susceptible to revision, so must, to some extent, our assessment of a data source's utility depend on its current contribution in the context of all the other available evidence. Comparing a data set's topology against a "known" phylogeny (Friedlander et al., 1994; Graybeal, 1994; Russo et al., 1996) or examining aspects of a gene's molecular evolution (e.g., G + C content, saturation) provide only limited means for determining a gene's phylogenetic utility. Partitioned Bremer support provides an alternative, if similarly limited, method for assessing phylogenetic utility, but with the advantage over taxonomic congruence that secondary signal below the surface of a data set's most-parsimonious tree may be accounted for within a strict parsimony framework.

Data Partitions

Despite the extensive theoretical discussion concerning combined and separate analyses, few of the practical issues pertinent to this debate have been explored, primarily because of the previous lack of appropriate statistical tests for identifying incongruence. Several tests, however, have recently been developed (Farris et al., 1994, 1995; Larson, 1994; Huelsenback and Bull, 1996), facilitating a more empirical examination of these issues. Here we explore some of the difficulties that emerge if we adopt a prior agreement approach to the data matrix in this study.

Although statistically significant incongruence is detected in 57% of the gene-by-gene pairwise comparisons (Table 4), there is no clear way to interpret these data as a decision-making criterion for whether or

not partitions should be combined because of the asymmetrical pattern of the ILD tests. For instance, *COIII* is incongruent with the rest of the characters combined (Table 4) and also shows the largest number of significant pairwise incongruences (six) with all other partitions (Table 4), but this gene is not significantly incongruent with *COII*. Similarly, *COII* is congruent with *ADH*, *COIII*, and 16S, but none of these genes are congruent with each other. This pattern, however, is not simply a result of the *COII* sequence lacking signal, because this gene is incongruent with *hb*, *wg*, and *ACHE*. Overall, any separation made among these data sets necessitates the exclusion of data not significantly incongruent with each other. More studies are needed to see if this pattern is common in data sets consisting of many genes.

One of the major philosophical disagreements between advocates of combined analysis and advocates of separate analyses concerns the ultimate goal of phylogenetic studies. Proponents of the combined approach argue that maximizing explanatory power is their primary objective (Kluge and Wolf, 1993; Nixon and Carpenter, 1996), whereas discovering the "true tree" is the primary goal for those in favor of the prior agreement approach (Bull et al., 1993; de Queiroz, 1993; de Queiroz et al., 1995). Under the prior agreement approach, separate analyses are preferred for heterogeneous data because combining these data can give misleading results (Bull et al., 1993). Although the inclusion of false signal may clearly result in erroneous topologies, there is no guarantee that even with heterogeneous data separate analyses will mislead less often than combining data. For the data in this study, the prior agreement approach seems unlikely to accomplish its intended goal of reducing the probability of accepting misleading results.

Despite the asymmetry in the pattern of pairwise gene comparisons, there is evidence suggesting certain genes, *ADH*, *COIII*, 16S, and *COII*, are more problematic than the others: (1) only these four genes are individually incongruent with the rest of the data combined (Table 4); (2) all the pairwise comparisons that are significantly different in Table 4 involve at least one of these genes; and (3) a pairwise matrix consisting of the remaining nonproblematic genes (*hb*, *wg*, *ACHE*, *ND1*) shows no significant ILDs. Therefore, it is worthwhile exploring the basis of these gene's differences, the effect that these partitions have on the simultaneous analysis, and the ramifications of their exclusion.

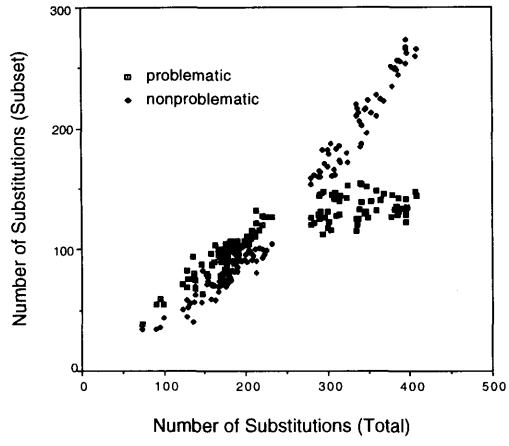

FIGURE 6. Relative substitution rates for two subsets of the data matrix. Genes in the problematic subset are *ADH*, *COIII*, 16S, and *COII*; those in the nonproblematic subset are *hb*, *wg*, *ADH*, and *ND1*. Genes were divided on the basis of the statistical significance of their ILD measurements, with the problematic genes exhibiting more disagreement across the various ILD comparisons.

Reasons for conflicting phylogenic signal among gene partitions are many. For characters that share the same history, however, incongruence in phylogenetic information is likely to result from differences in rates of evolution. When substitution rates are particularly high, phylogenetic signal may diminish because of multiple changes that erase history and create homoplasy (DeSalle et al., 1987). Figure 6 presents the relative substitution rates for all the problematic genes combined and the nonproblematic genes combined. There is a sharp contrast in the behavior of the two sets of genes. The problematic data exhibit a clear asymptote, suggesting saturation in the data. It seems

likely, therefore, that the substantial incongruence associated with the problematic genes is a result of homoplastic substitutions confounding the true historical relationships.

Given that this saturation appears for only the most divergent comparisons of taxa, if this phenomenon is the principal cause of the conflicting phylogenetic signal among genes then the incongruence should be confined primarily to the basal taxa in the phylogeny. To examine this possibility, we constrained the monophyly of the white-tip scutellum taxa and all the nodes associated with the outgroup taxa and calculated the subsequent ILD measurements for each problematic gene individually compared with the rest of the data combined. For three of the four genes, there is a sharp decline in the ILDs when these basal nodes are constrained. The *ADH* ILD drops from 9 extra steps to 4 extra steps, *COIII* drops from 14 to 1, and *COII* drops from 12 to 4. Although 16S only drops from 12 to 9, there is an extra decrease of 5 steps for this gene when the monophyly of all the ingroup taxa except the white-tip scutellum flies (i.e., node 11 in Fig. 2) is specified as an additional constraint. Overall, this analysis suggests that most of the incongruence associated with the four problematic genes involves the more basal taxa in the phylogeny and has little impact on the relationships among the picture-winged, modified mouthpart, modified tarsus, and *Antopocerus* taxa. This information provides additional evidence that the saturation depicted in Figure 6 is a major cause of the conflicting phylogenetic signal characterizing the problematic genes.

What, then, is the relationship between the saturated nature of the problematic characters and their information content within a combined analysis framework? Advocates of prior agreement would likely argue that including this information in a simultaneous analysis will produce misleading results. However, there is evidence that, when combined, the information provided by the problematic data is nearly identical to that provided by the nonproblematic data. Figure 7 shows the single most-parsimonious tree for all the problematic data combined. With the exception of the placement of PWGdis, the topology of this tree for the ingroup taxa is identical to that of the simultaneous analysis tree presented in Figure 2 (in addition, one of the most-parsimonious trees for all the nonproblematic data combined is also the tree in Fig. 2). Therefore, despite the extensive incongruence and saturation characterizing the problematic genes, there exists a common signal similar to that of the other genes. Furthermore, in the simultaneous analysis tree the percentage of total support provided by the problematic data (42%) is comparable to the proportion of phylogenetically informative characters (47%) comprised by these genes, indicating that the contribution to the total support of the tree is similar for the problematic and nonproblematic genes.

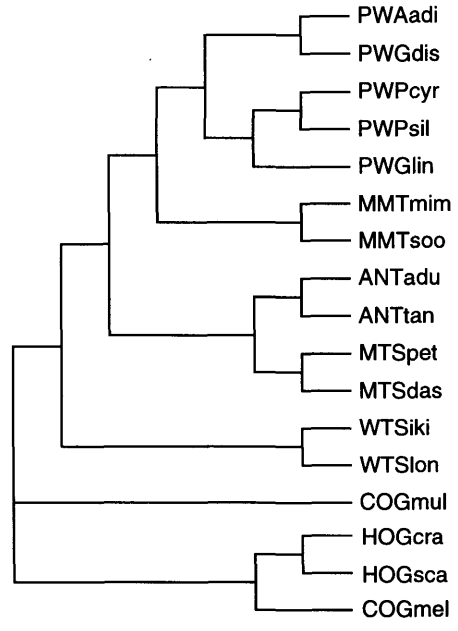

FIGURE 7. The most-parsimonious tree for all the problematic genes (*ADH, COIII,* 16S, *COII*) combined: length = 910 steps, CI = 0.44, RI = 0.42. Taxon abbreviations are as in Table 1.

Overall, this analysis suggests a tenuous relationship between the means available for assessing problematic data and the like-

lihood that these data will bias a combined analysis of the data. The theoretical justifications associated with arguments in favor of separate analyses (Bull et al., 1993; de Queiroz et al., 1995; Hueselbeck et al., 1996) have only demonstrated that combining incongruent data may produce erroneous results without specifying how likely this is, given the type of data used in systematic studies. Alternatively, advocates of simultaneous analysis (Kluge, 1989; Barrett et al., 1991; Chippindale and Wiens, 1994; Nixon and Carpenter, 1996) have stressed the importance of common phylogenetic signal that emerges when data are combined. To some extent, the relative importance of these two alternatives is an empirical question that (despite the obvious limitation that we can never be sure we have the truth) can be examined by data sets comprised of numerous partitions. For the data presented here, the fact that the information content of significantly heterogeneous data, when combined, is equivalent to that of homogeneous data seems to verify the advantages associated with combining information.

In addition, an examination of the effects of separate analyses for this data matrix suggests that the consequences of removing genes from the simultaneous analysis are undesirable given the objectives of the prior agreement approach. For instance, the *COIII* data appear to be the most problematic. The information from this gene is incongruent in the most number (six) of pairwise comparisons and has the highest ILD (14 extra steps) of any gene compared with the rest of the data combined. This conflict is restricted to the basal taxa in the simultaneous analysis tree. Specifically, the monophyly of the continental outgroups and the white-tip scutellum taxa are the main areas of incongruence. When these nodes are constrained to be monophyletic, the ILD between *COIII* and the rest of the data drops from 14 to 2. This phenomenon, referred to here as local incongruence, creates problems when using data heterogeneity as a justification for separating data.

First, the inclusion of the *COIII* data influences the topology of the simultaneous analysis tree (Fig. 8). The change in resolution, however, results from the combination of data not representing the source of incongruence. When the *COIII* sequences for the WTS and COG taxa are excluded from a simultaneous analysis that includes the rest of the *COIII* sequences, the same relationships emerge that existed in the original simultaneous analysis tree (Fig. 2), suggesting the source of incongruence is not critical to the change in topology. Overall, the separation of *COIII* from the rest of the data prevents the inference of new relationships among taxa in clades that are not influenced by data heterogeneity. Presently, the prior agreement approach is not sensitive to such local effects and is unlikely to account for this information.

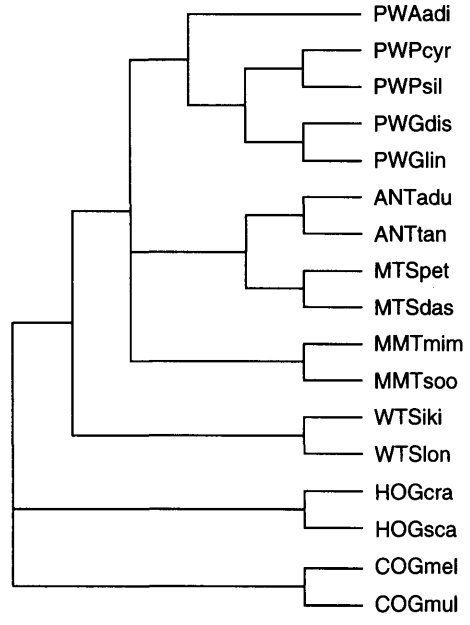

FIGURE 8. Consensus of the two most-parsimonious trees generated from the combination of all the gene sources excluding *COIII*. Taxon abbreviations are as in Table 1.

Second, the nodes specifying the monophyly of the COG and WTS taxa are among the most strongly supported relationships (Bremer supports of 18 and 34, respectively) in the simultaneous analysis tree. Therefore, by excluding *COIII* from a combined analysis we would prevent this

gene from exerting its influence on the final topology in order to be conservative about a disagreement (i.e., the differential placement of basal taxa) that is overwhelmingly supported in one direction. In general, the potential problems concerning local incongruence seem likely to increase as systematic studies incorporate both more taxa and more data partitions. As the number of taxa increases, incongruence will more likely be isolated to specific relationships (for recent examples, see Mason-Gamer and Kellogg, 1996; Poe, 1996) and the "false" signal creating this incongruence is more likely to be overwhelmed as the number of different data sources increases.

Implications for Hawaiian Drosophila Phylogeny

The hypothesis obtained from our simultaneous analysis (Fig. 2) addresses several important questions concerning the phylogeny of these flies, including the monophyly of the various species groups, the affinity of the *adiastola* subgroup and the modified-mouthpart species group to other Hawaiian flies, and the identity of the most basal species group of Hawaiian *Drosophila*. The most extensive study pertinent to Hawaiian *Drosophila* species group relationships is Grimaldi's (1990) analysis of morphological characters. He found a widespread lack of monophyly at the species group level for these flies. The molecular data presented here strongly suggest monophyly for each of the five species groups that we included in our analysis (Fig. 2), but not enough taxa may have been sampled from each group to address the monophyly problem that Grimaldi's (1990) cladogram suggests. It is encouraging, however, that four of the five species groups examined in this study (the picture-winged taxa being the lone exception) are supported by bootstrap values of 99–100% and Bremer support >10.

Previous molecular studies (Beverley and Wilson, 1985; DeSalle et al., 1987) have suggested that the *adiastola* PW subgroup is not closely related to the other PW flies. In fact, both studies presented the *adiastola* subgroup as the most basal Hawaiian taxon examined. Our simultaneous analysis topology (Fig. 2), however, places the *adiastola* subgroup with the other PW flies and is completely congruent with Carson's (1982) chromosomal hypothesis based on polytene banding patterns. The chromosomal data and Throckmorton's (1966) hypothesis indicate a sister-group relationship of the PW flies with the modified-mouthpart flies, and this relationship is also supported by our analysis. An unexpected result of our study is the implied sister-group relationship between the *Antopocerus* species group and the modified-tarsus species group. None of the previous morphological studies detected this sister-group scenario (but see Heed, 1971). This result warrants further examination in light of the modified forelegs that are characteristic of both groups.

Of the numerous studies of these species groups, three (Spieth, 1966; Takada, 1966; Throckmorton, 1966) suggested that the white-tip scutellum group is the most basal taxon. Our data also indicate this relationship, although the branch support indices for this node are not very strong. The placement of the white-tip scutellum flies in this position may have important implications for the evolution of ecological characters (Kambysellis et al., 1995).

Conclusion

The best available hypothesis for the relationships of these flies appears to reside in the simultaneous analysis of all the data (Fig. 2). The notion that empirical data possess the most explanatory power when they are not subjected to arbitrary weighting schemes and that maximization of explanatory power is desirable has been previously discussed (Farris, 1983; Kluge, 1989; Kluge and Wolf, 1993; Nixon and Carpenter, 1996), although some may disagree with this viewpoint (de Queiroz et al., 1995; Miyamoto and Fitch, 1995; Huelsenbeck et al., 1996). We have presented an empirical demonstration of the effects of numerous partitions on the prior agreement (Bull et al., 1993) and taxonomic congruence (Miyamoto and Fitch, 1995)

approaches to phylogenetic hypothesis testing. As the types and numbers of data partitions diversify and increase in systematic studies, the incongruence patterns that can be defined will be less clear and the problems associated with keeping data separate will become more pronounced. In addition, we emphasize that combination of partitions in our study led to a more resolved and highly reasonable hypothesis of relationships for these flies.

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