Phylogeny of the *Drosophila saltans* Species Group Based on Combined Analysis of Nuclear and Mitochondrial DNA Sequences

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Nucleotide sequences from two nuclear loci, alcohol dehydrogenase and internal transcribed spacer-1 of the nuclear ribosomal DNA repeats, and two mitochondrial genes, cytochrome oxidase I and cytochrome oxidase II, were determined from nine species in the *Drosophila saltans* species group. The partition homogeneity test and partitioned Bremer support were used to measure incongruence between phylogenetic hypotheses generated from individual partitions. Individual loci were generally congruent with each other and consistent with the previously proposed morphological hypothesis, although they differed in level of resolution. Since extreme conflict between partitions did not exist, the data were combined and analyzed simultaneously. The total evidence method gave a more resolved and highly supported phylogeny, as indicated by bootstrap proportions and decay indices, than did any of the individual analyses. The *cordata* and *elliptica* subgroups, considered to have diverged early in the history of the *D. saltans* group, were sister taxa to the remainder of the *saltans* group. The *sturtevanti* subgroup, represented by *D. milleri* and *D. sturtevanti*, occupies an intermediate position in this phylogeny. The *saltans* and *parasaltans* subgroups are sister clades and occupy the most recently derived portion of the phylogeny. As with previous morphological studies, phylogenetic relationships within the *saltans* subgroup were not satisfactorily resolved by the molecular data.

Introduction

The *Drosophila saltans* group is one of four major species groups placed in the subgenus *Sophophora* (Sturtevant 1942). Throckmorton (1975) considered the neotropical *saltans* and *willistoni* species groups to be distinct and derivative lineages within *Sophophora*, clearly separated from the Old World *melanogaster* and *obscura* species groups. The *saltans* species group consists of 21 species which are divided into five subgroups; *cordata, elliptica, parasaltans, saltans,* and *sturtevanti* (table 1) on the basis of a variety of morphological characters (Magalhaes and Bjornberg 1957; Magalhaes 1962; Throckmorton and Magalhaes 1962).

Based on contemporary distribution patterns and geological information, Throckmorton (1975) proposed that the ancestor of the *saltans* species group originated in tropical North America, where the so-called ''primitive'' *cordata* and *elliptica* subgroups are found. This ancestral group colonized the South American continent and the *sturtevanti, saltans,* and *parasaltans* subgroups (the ''derived'' *saltans* subgroups) then diversified prior to the formation of the present day isthmus of Panama. Some members of the *saltans* subgroup, such as *D. saltans* and *D. prosaltans,* have recently diffused back into North America, probably within the past 4.5 Myr (Throckmorton 1975). Within the *saltans* subgroup, species-level relationships are unresolved because of the short time since divergence and conflict between reproductive isolation studies and the chromosome inversion phylogeny (Bicudo 1973*a,* 1973*b*).

Key words: alcohol dehydrogenase, cytochrome oxidase I, cytochrome oxidase II, ITS1, taxonomic and character congruence, *Drosophila saltans* phylogeny.

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This study uses four molecular loci (*Adh, COI, COII,* and ITS1) as well as a morphological data set (Magalhaes 1962) to examine the phylogeny of the *Drosophila saltans* species group. We are interested in estimating the phylogeny of the five major lineages in the *saltans* species group and the species-level relationships within the *saltans* subgroup.

When two or more data partitions are examined in separate phylogenetic analyses, the resultant tree topologies often do not completely agree with one another or with the combined data set (Chippendale and Weins 1994). There are several schools of thought concerning how data partitions, which may be more or less incongruent with one another, should be analyzed (reviewed in de Queiroz, Donoghue, and Kim 1995; Brower, DeSalle, and Vogler 1996). One method is taxonomic congruence, where agreement among well-supported topologies derived from separate analyses of different data sets is presented as a consensus tree (Mikevich 1978; Miyamoto and Fitch 1995). Another method is character congruence, or total evidence (Kluge 1989), in which all data are combined and analyzed simultaneously to increase the descriptive efficiency and explanatory power of the data (Kluge 1989; Barrett, Donoghue, and Sober 1991; Eernisse and Kluge 1993; Jones, Kluge, and Wolf 1993; Kluge and Wolf 1993). A compromise between taxonomic congruence and total evidence, referred to as conditional data combination, or prior agreement, has been proposed by several systematists (de Queiroz 1993; Bull et al. 1993; Huelsenbeck, Bull, and Cunningham 1996). This begins with an analysis of separate data sets, termed process partitions (Bull et al. 1993), followed by a test for heterogeneity between partitions. If significant between-partition heterogeneity does not exist, the data are combined and analyzed simultaneously.

Several methods to test for heterogeneity between data sets have been proposed (Farris et al. 1994, 1995; Huelsenbeck and Bull 1996; Baker and DeSalle 1997). Our work on the *D. saltans* species group offers an op-

Table 1
Taxonomic Relationships and Collection Localities for Species in the *Drosophila saltans* Group

Subgroup	Species	Collection Location	BG Stock Center Number
A. *cordata*	*neocordata*	Minas Gerias, Brazil	14041-0831.0
B. *elliptica*	*emarginata*	Turrialba, Costa Rica	14042-0841.0#
		La Palma, El Salvador	14042-0841.4
		Quito, Ecuador	14042-0841.7
C. *parasaltans*	*subsaltans*	Balem, Brazil	14044-0872.0
D. *saltans*	*austrosaltans*	Pirassununga, Brazil	14045-0881.0
	lusaltans	Petionville, Haiti	14045-0891.0
	prosaltans	Turrialba, Costa Rica	14045-0901.0#
		Leticia, Colombia	14045-0901.4#
	saltans	San Jose, Costa Rica	14045-0911.0
E. *sturtevanti*	*milleri*	El Yunque, Puerto Rico	14043-0861.0
	sturtevanti	Turrialba, Costa Rica	14043-0871.0#
		Volcan Soufriere, Lesser Antilles	14043-0871.2#
		Martinique, West Indies	14043-0871.9
Outgroups	*melanogaster*	See *Materials and Methods*	
	yakuba	See *Materials and Methods*	

portunity to compare several of these measures of heterogeneity and to assess their implications for the methods of taxonomic congruence, total evidence, and prior agreement in reconstructing the phylogeny of the *D. saltans* species group.

Materials and Methods

DNA Sources

Live *Drosophila* stocks were obtained from the National *Drosophila* Species Resource Center in Bowling Green, Ohio. Table 1 shows the taxonomic classifications of the 16 lines used in this study and where each was collected. The following sequences were obtained from the literature: *D. melanogaster* X78384 (*Adh*), J01404 (*COI* and *COII*), M21017 (ITS1); *D. yakuba* X54120 (*Adh*), X03240 (*COI* and *COII*), Z28416 (ITS1). GenBank accession numbers for sequences determined as a result of this study are AF045081–AF045096 (*COII*), AF045097–AF045112 (*COI*), AF045113–AF045126 (*Adh*), and AF045363–AF045371 (ITS1).

Sample Preparation and DNA Sequencing

Genomic DNA was isolated by the method of Gloor and Engels (1992). The four target loci were amplified from each taxon under standard PCR cycling conditions. PCR primers were designed based on the previous studies referred to in table 2. PCR products from the 305-bp fragment of the *COI* gene and the entire *COII* gene (688 bp) were purified by membrane filtration (Millipore) and sequenced directly using a standard dsDNA cycle sequencing protocol (GIBCO-BRL). PCR products from the entire coding region of the *Adh* gene (771 bp) and the entire ITS1 locus (785 aligned positions) were cloned into the TA cloning vector (Invitrogen) or the PCR-Script vector (Stratagene). Single colonies were selected and sequenced using either a

Table 2
Summary of Results from Maximum-Parsimony Analyses

Locus	Size[a]	PI[b]	No. of MPTs[c]	TL[d]	CI[e]	RI[f]	References[g]
COI	305	72	5	191	0.670	0.703	Simon et al. (1994)
COII	688	107	1	341	0.657	0.655	Beckenbach, Wei, and Liu (1993)
Adh	771	98	2	238	0.840	0.822	Russo, Takezaki, and Nei (1995)
ITS1[h]	785	222	6	659	0.898	0.881	Vogler and DeSalle (1994)
Morphology . .	7	5	4	8	0.875	0.875	Magalhaes (1962)
mtDNA[i]	993	179	1	541	0.649	0.655	
nucDNA[j]	1,556	316	1	898	0.881	0.834	
TE[k]	2,549	499	6	1,466	0.785	0.740	

[a] Size of locus (in base pairs).
[b] Number of parsimony-informative sites.
[c] Number of most-parsimonious trees recovered.
[d] Tree length of most-parsimonious trees.
[e] Ensemble consistency index (Kluge and Farris 1969).
[f] Ensemble retention index (Archie 1989*a*, 1989b; Farris 1989).
[g] Selected references used for primer design.
[h] This analysis was performed with fewer taxa than the other individual-locus searches.
[i] Combined mitochondrial analysis (*COI* + *COII*).
[j] Combined nuclear analysis (*Adh* + ITS1).
[k] Total evidence tree.

dsDNA cycle sequencing procedure (GIBCO-BRL) or the Sequenase sequencing kit (Amersham). Nucleotide sequences were determined from between 80% and 100% of both strands of the *Adh, COI,* and *COII* genes. Multiple clones from each species were obtained for the ITS1 locus and the nucleotide sequence of one strand of each clone was determined. Where discrepancies existed between clones from the same species, the differences were verified by consulting the original autoradiograms.

Sequence Alignment

The *COI* and *Adh* coding regions required no gaps to align the species in this study. The *COII* gene required the inclusion of a single gap (positions 673–675) in the outgroup species to align with the *saltans* species group. Any gaps in these analyses were treated as missing data. Because of the noncoding nature of the ITS1 region and notable size variation between species, optimal alignment of this locus was achieved only with the use of appropriate gaps. CLUSTAL W (Thompson, Higgins, and Gibson 1994) and MALIGN, version 2.1 (Wheeler and Gladstein 1994), were used to obtain an optimal alignment of the ITS1 region. The phylogenetic relationships between taxa remained the same when ITS1 was analyzed with and without the gapped positions.

Phylogenetic Analysis of Nucleotide Sequences

All analyses described below were performed using a variety of optimality criteria, including maximum likelihood (ML), neighbor-joining (NJ) and maximum parsimony (MP), to estimate the phylogeny of the *saltans* species group. We present only the MP analyses. All nucleotide partitions were examined both individually and in simultaneous analyses. A variety of weighting schemes (transversions 2× over transitions, transversions 4× over transitions, transversions only) were employed and all gave results congruent with one another. Here, we present unweighted parsimony searches which use the branch-and-bound algorithm implemented in PAUP 4.0d54 (Swofford 1997). Table 2 shows some important aspects of each analysis performed. The level of confidence in each node of all most-parsimonious trees obtained was assessed using bootstrap proportions (Felsenstein 1985, 1988) and decay indices (Bremer 1988; Donoghue et al. 1992). All trees presented are 50% majority-rule consensus phylogenies resulting from 200 bootstrap replicates. Bootstrap proportions are shown above the node and decay indices are shown below the node in each tree. All trees are rooted using two members of the *melanogaster* species group, *D. melanogaster* and *D. yakuba.* MacClade, version 3.0 (Maddison and Maddison 1992), was used for a variety of phylogeny manipulations and character state analyses.

Phylogenetic Analysis of Morphological Data

The morphological data set used in this study was adapted from morphological characters used by Magalhaes (1962). The characters examined include the presence/absence of mesonotal pattern, the presence/absence of subcarinal hairs, a dark versus yellow body color, the presence/absence of sensilla on the first sternite, the presence/absence of sensilla in the seventh sternite of males, and the presence/absence/reduction of vestigial plates of the first sternite of both males and females. Five continuous characters used by Magalhaes (1962) were omitted, because it was difficult to code these characters for parsimony analysis. Maximum-parsimony analyses were performed on these data individually and in combination with the nucleotide data (see table 2). The morphological characters were not used in ML or distance analyses.

Table 3
Results of Partition Homogeneity Test

	Morphology	*COI*	*COII*	*Adh*	ITS1	TE[a]
Morphology . .	—	1.0	0.09*	0.12	0.03*	0.04*
COI		—	0.12	0.77	0.02*	0.97
COII			—	0.31	0.09*	0.33
Adh				—	0.46	0.90
ITS1					—	0.08*
TE						—

[a] Total evidence tree.
* Data partitions which display significant homogeneity when compared.

Phylogenetic Tree Comparisons

We used the partition homogeneity test (as implemented in PAUP 4.0d54; Swofford 1997) to examine differences (1) between each locus and (2) between each locus and the total evidence hypothesis (table 3). We also used partitioned Bremer support (Bremer 1988, 1992; Baker and DeSalle 1997) to measure the amount of support provided by each partition to each node on the total evidence phylogeny.

Partitioned Bremer support (PBS) shows the contribution of each partition to the decay index of every node on the total evidence tree (Baker and DeSalle 1997). To obtain the PBS value for a given node on the total-evidence tree, the length of the partition on the unconstrained total evidence tree is subtracted from the length of a partition on a tree constrained to contain only the node of interest. If the partition supports a relationship represented by a node in the total evidence tree, the constraint tree will be longer, and the the PBS value will be positive. If, on the other hand, a partition supports an alternative relationship, the constraint tree will be shorter, and the PBS value will be negative, indicating incongruence with the simultaneous analysis. The magnitudes of PBS values indicate the level of support for, or incongruence with, a node (Baker and DeSalle 1997). All partition lengths for any given node will always sum to the decay index for that node on the total evidence tree. This method allows us to determine the relative contribution of each partition to the simultaneous analysis tree (table 4).

Results

Phylogenetic Relationships—*Adh*

Figure 1*A* shows the phylogenetic hypothesis for the *saltans* species group based on the alcohol dehydro-

Table 4
Results of Partitioned Bremer Support Analyses

Node	Morphology	*COI*	*COII*	*Adh*	ITS1	TE[a]
1	0	−5.33	2.5	2.33	1.5	1
2	0	10	8	−1	0	17
3	0	5.5	3	1	−2.5	7
4	0	2	9	9	0	20
5	0	10.5	4.5	11	0	26
6	0	6	3.5	0	1.5	11
7	−1.5	−1	1.5	0	5	4
8	0	5	−4	5	0	6
9	0	0	1	2	0	3
10	0	5	19.5	62	−1.5	85
Totals ..	−1.5	37.67	47.5	91.33	4	179

[a] Total evidence tree.

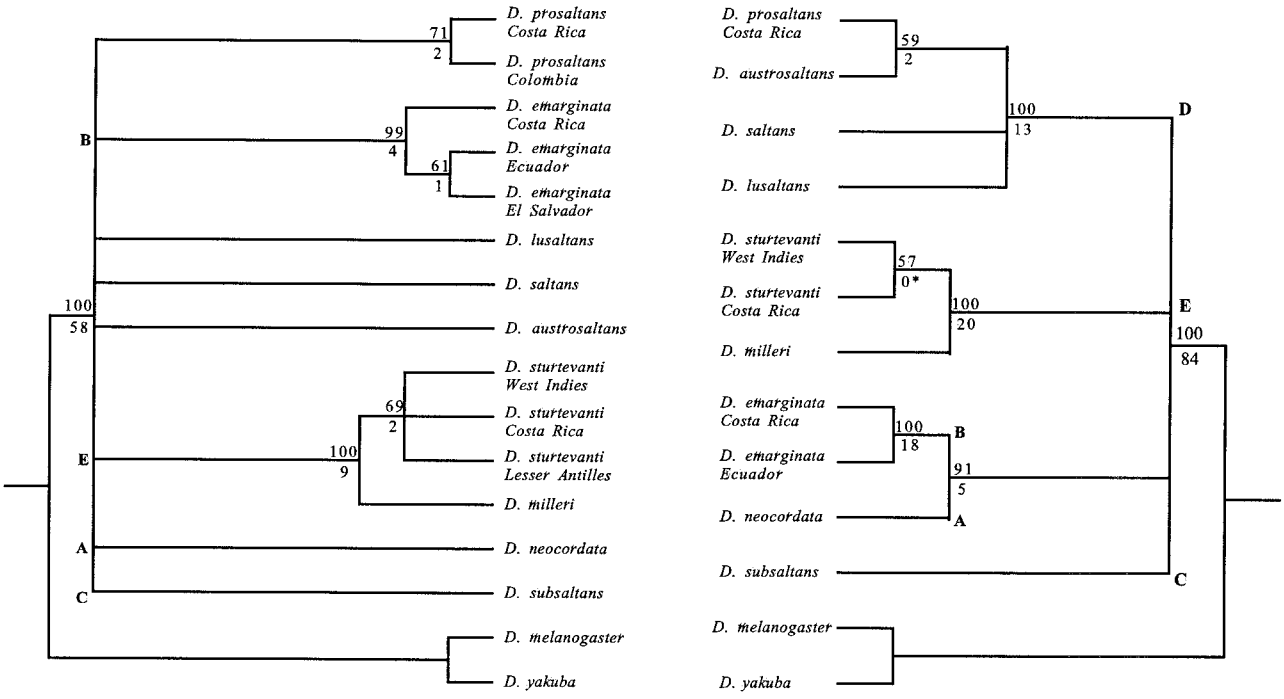
genase gene (see also table 2). This locus was unable to resolve the branching order among any of the five species subgroups. Furthermore, the relationships among the recently diverged species of the *saltans* subgroup were completely unresolved. However, the *Adh* sequence was able to resolve the relationship between *D. milleri* and *D. sturtevanti* in the *sturtevanti* species subgroup (fig. 1*A*, clade E) and between the various geographic isolates of *D. emarginata* (fig. 1*A*, clade B).

Phylogenetic Relationships—ITS1

A phylogeny of the *saltans* species group, based on the ITS1 locus, is presented in figure 1*B* (see also table 2). The ITS1 locus places the *cordata* subgroup (fig. 1*B*, clade A) as a sister taxon to the *elliptica* subgroup (fig. 1*B*, clade B), consistent with morphological studies (Magalhaes 1962). This locus is also able to resolve some relationships among species in the *saltans* (fig. 1*B*, clade D) and *sturtevanti* (fig. 1*B*, clade E) subgroups. However, this sequence provides no information concerning the phylogenetic relationships among most of the subgroups in the *saltans* species group.

Phylogenetic Relationships—*COI*

Figure 2*A* shows a phylogeny based on the mitochondrial cytochrome oxidase I gene. This tree shows much more structure than either the *Adh* or ITS1 tree. It shows that the ''derived'' (*sensu* Throckmorton 1975) members of the *saltans* group (the *parasaltans*, *saltans*, and *sturtevanti* subgroups) are monophyletic. However, the phylogeny cannot reliably determine whether the *parasaltans* or the *sturtevanti* subgroup (fig. 2*A*, clades C and E) is the sister taxon of the *saltans* subgroup (fig. 2*A*, clade D). Within the *saltans* subgroup (fig. 2*A*, clade D), *D. lusaltans* is shown to be the sister taxon to the remainder of the *saltans* subgroup. Interestingly, the *D. prosaltans ''Costa Rica''* is the sister taxon to *D. austrosaltans*, to the exclusion of *D. prosaltans ''Colombia.''* This result is incongruent with both reproductive isolation and chromosome inversion studies (Bicudo 1973*a*, 1973*b*). The *cordata* and *elliptica* subgroups (fig. 2*A*, clades A and B) are placed at the base of the *saltans* species group, in agreement with previous morphological work (Throckmorton and Magalhaes 1962). How-



A **B**

FIG. 1.—*A*, The majority-rule bootstrap phylogeny based on the coding regions of the *Adh* gene. *B*, The majority-rule bootstrap phylogeny based on the ITS1 region. Bootstrap proportions (above) and decay indices (below) are shown at each node. A = *cordata* subgroup; B = *elliptica* subgroup; C = *parasaltans* subgroup; D = *saltans* subgroup; E = *sturtevanti* subgroup.

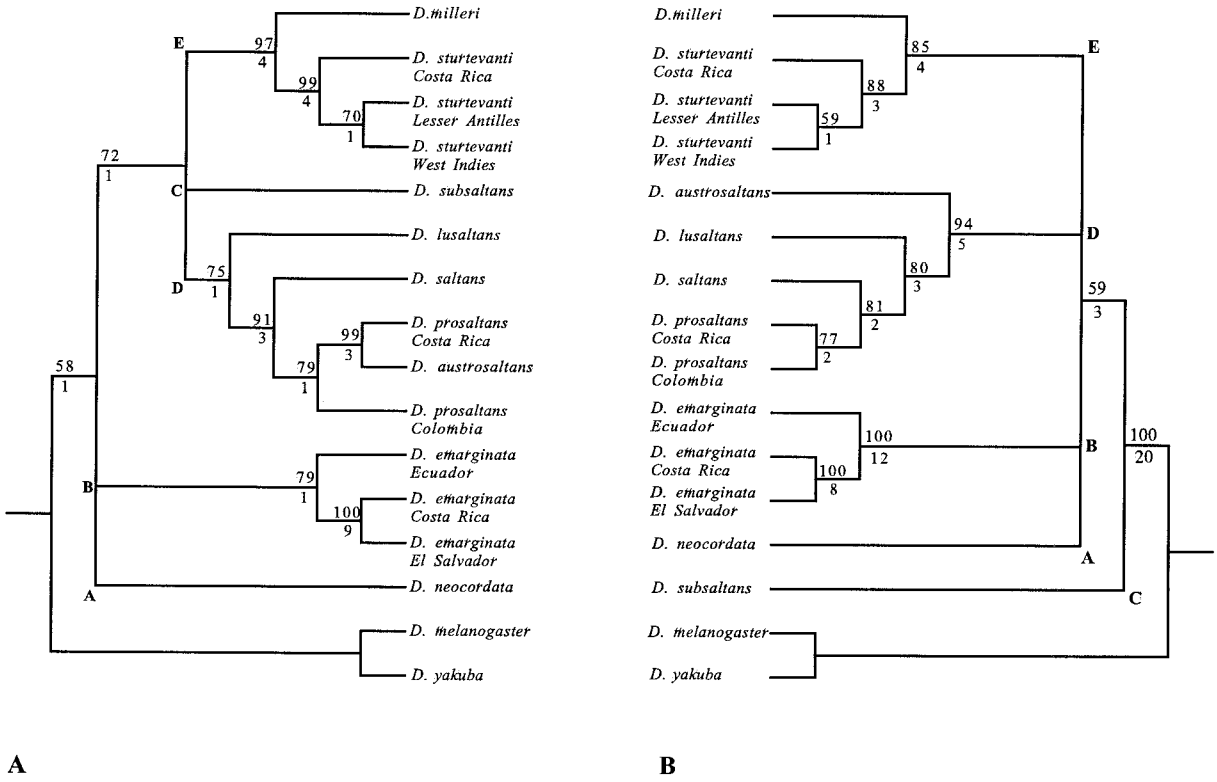


FIG. 2.—*A,* The majority-rule bootstrap phylogeny based on a 305-bp fragment of the mitochondrial *COI* gene. *B,* The majority-rule bootstrap phylogeny based on the complete *COII* gene. Bootstrap proportions (above) and decay indices (below) are shown at each node. A = *cordata* subgroup; B = *elliptica* subgroup; C = *parasaltans* subgroup; D = *saltans* subgroup; E = *sturtevanti* subgroup.

ever, this locus is unable to resolve the deeper branching nodes in the phylogeny.

Phylogenetic Relationships—*COII*

Figure 2*B* shows the mitochondrial cytochrome oxidase II phylogeny (see also table 2). All sequences from the *saltans* species group are distinguished from the outgroup sequences by a single 3-bp deletion located at the 3′ end of the sequence in all *melanogaster* group species. While this mitochondrial locus gives more phylogenetic resolution than the *Adh* or ITS1 sequences, it is not able to resolve the branching order among the *cordata, elliptica, saltans,* and *sturtevanti* subgroups (fig. 2*B,* clades A, B, D, and E). However, within these subgroups, phylogenetic relationships are congruent with the other loci in this study and with previous morphological work. *Drosophila austrosaltans* is shown to be the sister taxon to the remainder of the *saltans* subgroup (fig. 2*B,* clade D), a placement which is consistent with reproductive-isolation studies (Bicudo 1973*a*). *Drosophila lusaltans* is the next species to branch off from this lineage, possibly when it colonized the Caribbean Islands. The closely related species *D. saltans* and *D. prosaltans* form a sibling species cluster. The *COII* phylogeny places the *parasaltans* subgroup (fig. 2*B,* clade C) at the base of the *saltans* phylogeny. Although this placement is congruent with the ITS1 phylogeny (fig. 1*B*), it is incongruent with the *Adh* and *COI* gene trees (figs. 1*A* and 2*A*) and with the traditional view of phylogeny in this group (Throckmorton and Magalhaes 1962).

Phylogenetic Relationships—Morphology

The morphological data set contained eight characters, including body color and pattern, bristle number, and the shapes of a variety of other structures. Only one geographic isolate for each species is analyzed in the original paper (Magalhaes 1962). Furthermore, all taxa not available for nucleotide saquencing were omitted from this search. There is a single most-parsimonious tree (table 2) when the morphological data are analyzed phylogenetically (phylogeny not shown). These data place the *elliptica* and *cordata* subgroups as sister taxa, with the *parasaltans* subgroup being the sister group of the *elliptica-cordata* clade. The *saltans* and *sturtevanti* subgroups are unresolved with respect to one another. They are placed sister to the *elliptica-cordata-parasaltans* clade.

Comparisons Among Data Sets

Table 3 shows the results of the partition homogeneity test. Pairwise comparisons which show significant homogeneity ($P < 0.10$) are indicated. The ITS1 and morphological data sets stand out as being incongruent with most, but not all, of the other partitions in this study. For example, ITS1 shows significant heterogeneity when compared with the morphological and mitochondrial partitions, but not when compared with the other nuclear partition, *Adh.* The morphological partition

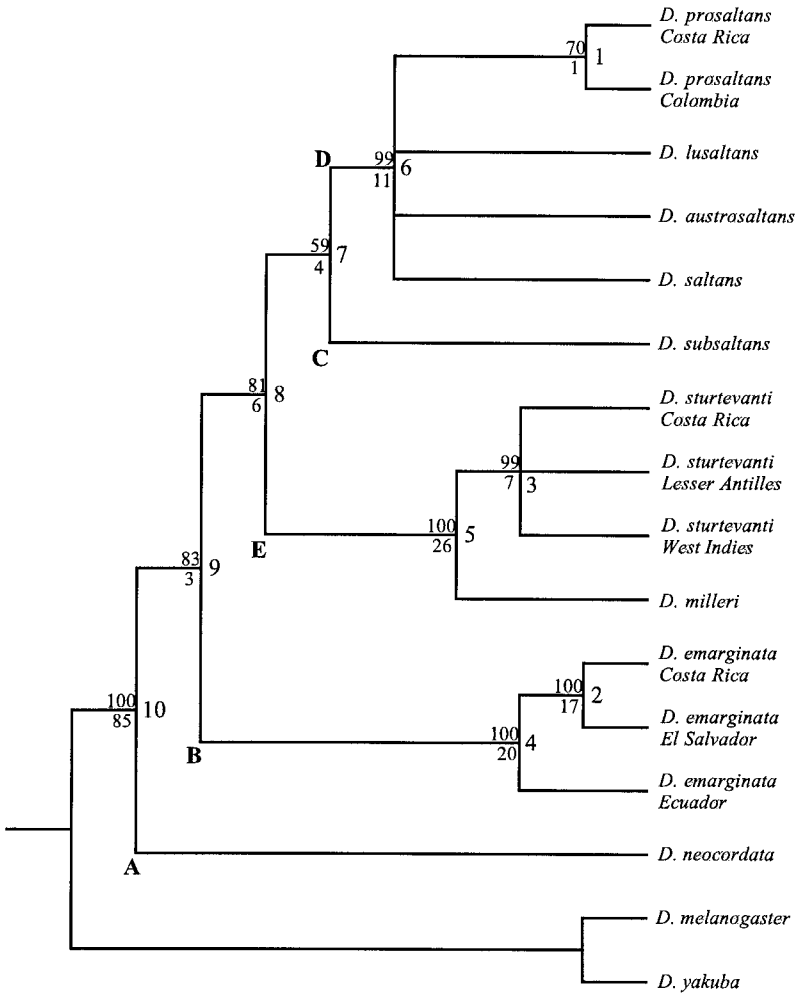


FIG. 3.—The majority-rule bootstrap phylogeny based on total evidence analysis. Bootstrap proportions (above) and decay indices (below) are shown at each node. A = *cordata* subgroup; B = *elliptica* subgroup; C = *parasaltans* subgroup; D = *saltans* subgroup; E = *sturtevanti* subgroup. Numbered nodes (1–10) correspond to table 5.

is incongruent with ITS1 and *COII*, but not with *COI* or *Adh*.

Partitioned Bremer support values were calculated for all nodes, numbered 1–10, on the total evidence tree (table 4). The morphological data set was incongruent with node 7, the *saltans-parasaltans* subgroup relationship (fig. 3), and instead supports grouping the *parasaltans*, *cordata*, and *elliptica* subgroups in a clade. The alcohol dehydrogenase partition was mostly congruent with the total evidence hypothesis, supporting 7 and being equivocal at 2 of 10 nodes. *Adh* conflicted with the total evidence tree only at node 2, which united the Central American populations of *D. emarginata* to the exclusion of the isolate from Ecuador. The ITS1 locus was in agreement with 3 of the 10 nodes and equivocal at half of the nodes on the simultaneous analysis tree. The ITS1 data disagreed at nodes 3 and 10, probably because of lack of resolution present in the individual analysis. The *COI* partition supported 7 of 10 nodes on the total evidence tree but was incongruent in two places, nodes 1 and 7. The *COII* gene was the partition most congruent with the total evidence hypothesis, supporting 9 of the 10 nodes on the total evidence tree. Only node 8, which supports the ''derived'' saltans clade (Throckmorton 1975), was shown to be incongruent.

Phylogenetic Relationships—Total Evidence Analysis

The total evidence phylogeny (fig. 3) includes the morphological data set of Magalhaes (1962) and all four molecular data sets generated in this study (table 2). This phylogeny places the *parasaltans* and *saltans* subgroups as sister taxa (fig. 3, clades C and D). Within the *saltans* subgroup, which has diversified only recently, relationships are mostly unresolved. This is probably due to lack of informative sites and conflicting information from the different sequences used in this study. The *sturtevanti* subgroup (fig. 3, clade E) is the sister taxon to the *saltans-parasaltans* clade. The *cordata* and *elliptica* subgroups (fig. 3, clades A and B) are sister to the ''derived'' *saltans* subgroups, with the *cordata* subgroup representative, *D. neocordata*, being the sister taxon to all other *saltans* group species. The analyses are in agreement with previous taxonomic work on the *saltans* species group (Magalhaes 1962; Throckmorton 1975). However, the molecular data are unable to re-

Table 5
Number of Node on Total-Evidence Tree, Monophyletic Group that it Represents, and Partition that Supports that Monophyletic Group in Individual Analysis

Node	Monophyletic Group	Partition with this Clade
1	*D. prosaltans*	*COII, Adh*
2	Central American *D. emarginata*	*COI, COII*
3	*D. sturtevanti*	*COI, COII, Adh,* ITS1
4	*D. emarginata/elliptica* subgroup	*COI, COII, Adh,* ITS1
5	*sturtevanti* subgroup	*COI, COII, Adh,* ITS1
6	*saltans* subgroup	*COI, COII,* ITS1
7	*saltans/parasaltans* subgroups	—
8	"Derived" *saltans*	*COI*
9	"Derived" *saltans* + *elliptica* subgroup	—
10	All *saltans* species	*COI, COII, Adh,* ITS1

solve the most difficult systematic issue, the branching order within the *saltans* subgroup.

Table 5 shows the partitions that, when analyzed individually, unequivocally support nodes seen in the total evidence tree. Several monophyletic groups are present in all partitions, including those represented by nodes 3, 4, and 5. Interestingly, two nodes that are present in the total evidence tree, 7 and 9, are absent in all individual partition analyses, indicating that these partitions either lack the resolution of the total evidence tree or support an alternative relationship.

Discussion

Comparisons of Phylogenetic Hypotheses

Visual inspection of the phylogenies derived from each partition (figs. 1 and 2) indicates that they differ in (1) their placement of the *parasaltans* subgroup and (2) the branching order within the *saltans* subgroup. The partition homogeneity test does in fact show the morphological and ITS1 data to be incongruent with some other partitions in this study. However, each of these partitions are congruent with at least one other partition. For example, this test cannot reject homogeneity when comparing the ITS1 partition with the *Adh* partition or when comparing the morphological partition with either the *COI* or the *Adh* partition. Therefore, no partition is in conflict with all other partitions. Furthermore, all partitions contribute to PBS values (table 4), indicating that each partition does influence the topology of the total evidence tree. Therefore, if one were employing a prior agreement approach, it would be difficult to determine which data partition to exclude from the analysis. Baker and DeSalle (1997) encountered this same problem in their study of the phylogeny of the Hawaiian *Drosophila.* They concluded that if a partition was homogeneous when compared to at least one other partition, it should be included in the total-evidence analysis. We agree with this conclusion and propose that all partitions in this study be combined in a simultaneous analysis to estimate the *saltans* group phylogeny.

Individual analyses indicate that the different data partitions are incongruent in the placement of the *parasaltans* subgroup, represented by *D. subsaltans.* The *Adh* and ITS1 partitions do not yield any information on the relationships of this taxon to any of the other subgroups. The *COI* partition indicates that *D. subsaltans* is closely related to the *saltans* and *sturtevanti* subgroups, although it is unclear which subgroup is most closely related. The *COII* partition shows weak support for this subgroup being the sister taxon to all other *saltans* species. However, the partition homogeneity test is unable to reject the null hypothesis of homogeneous data for comparisons between the *COI* and *COII* partitions (table 3). Therefore, it would seem that, although the relationships presented in the *COI* and *COII* bootstrap trees are in conflict, this conflict is not statistically significant.

The simultaneous analysis indicates support for a *saltans-parasaltans* clade (fig. 3, node 7), a relationship not seen in any individual analysis. Examining the PBS values (table 3) shows that the ITS1 and *COII* partitions support this relationship and that *COI* and the morphological partitions support alternative relationships. This result is somewhat surprising, since the ITS1 and *COII* partitions alone did not support a *saltans-parasaltans* clade. However, previous studies have demonstrated that combined analyses can uncover phylogenetic affiliations not observed in individual analyses (Chippendale and Weins 1994). It is possible that in the simultaneous analysis, character conflict present in individual partitions is resolved to support the *saltans-parasaltans* clade.

There are also conflicts between partitions when estimating the phylogeny of the *saltans* subgroup. No two gene trees give the same branching order within the *saltans* subgroup, and some partitions, such as *Adh*, yield no information at all concerning these relationships. The *COI* partition is incongruent with all other partitions in that it shows *D. prosaltans* to be paraphyletic with respect to *D. austrosaltans.* The ITS1 and *COI* partitions place *D. austrosaltans* well within the *saltans* subgroup, while the *COII* partition places this species as a sister taxon to all species within this subgroup. Given the recent time of divergence (Throckmorton 1975), large population sizes (Throckmorton 1975), and potential for gene flow between these species (Bicudo 1973*a*), this conflict is not surprising. It is possible that ancestral polymorphisms are incompletely sorted within this subgroup, creating either a lack of resolution or conflict between different partitions.

Phylogeny of the *Drosophila saltans* Species Group

The total evidence tree (fig. 3) is in complete agreement with the proposed morphological phylogeny of the *saltans* group (Magalhaes 1962). Even the *saltans-parasaltans* relationship, which was not clearly seen in the separate molecular analyses, is resolved by the total evidence method. The total evidence analysis shows all of the species subgroups to be monophyletic with respect to each other and the outgroup species. The *cordata* subgroup, represented by *D. neocordata,* is the sister taxon to the rest of the species in the *saltans* group. The *elliptica* group is the next most basal subgroup. The mo-

lecular data agree with morphological and biogeographical studies (Magalhaes 1962; Throckmorton 1975) and place the *sturtevanti* subgroup at an intermediate position as the sister group to the *saltans* and *parasaltans* subgroups. It is interesting to note, however, that the total-evidence phylogeny is not congruent with the phylogenetic reanalysis of a selected group of morphological characters. This is likely due to the fact that the taxonomists who established the various *saltans* subgroups took into account more discrete and continuous characters than were presented in Magalhaes (1962) and likely had a good ''gestalt'' feeling for how the groups were related based on fieldwork, biogeography, and laboratory experiments. The branching order within the *saltans* subgroup is not well defined because of the relatively recent divergence of these species and conflicting information from each locus. The molecular data are therefore unable to resolve the previous conflict between the results of reproductive-isolation studies and the observations on chromosome inversion patterns (Bicudo 1973*a*, 1973*b*). We argue that in the absence of more conclusive data, the phylogenetic relationships of species within the *saltans* subgroup should be presented as unresolved.

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