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

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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*	

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).

CO1mJalsIo3 4.9 0 e1isP .X9647On 4.9 0l34nqj /F5 1 Tf 1.932nrr6r4p9/8nsXbT 4.9 0 0 4.5 143oo5 142 2eiA6r494.9 0lis9Tm u4.i7gth-0871.3-0871.9
238
659
1Method87-htlle.....nd

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.

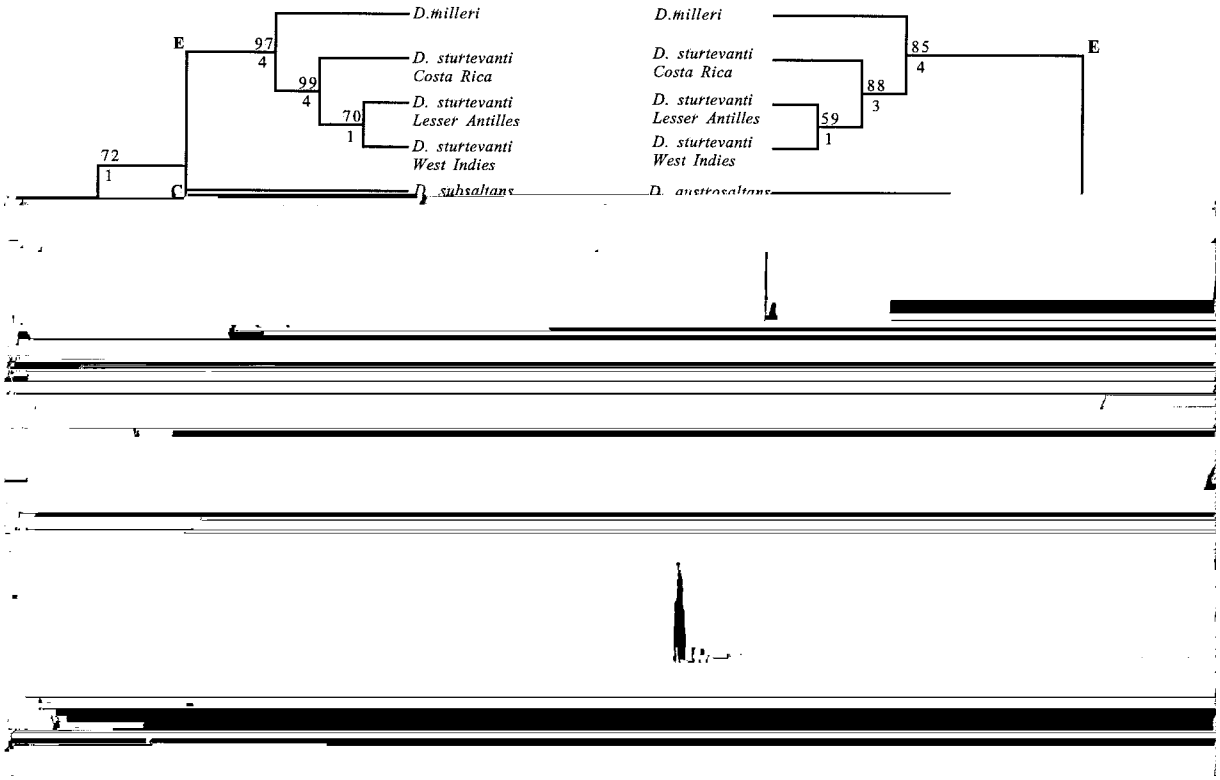

FIG. 2.—*A,* The majority-rule bootstrap phylogeny based on a 305-bp fragment of the mitochondrial *COI* gene. *B,* The majority-rule bootstrap phylogeny based on the complete *COII* gene. Bootstrap proportions (above) and decay indices (below) are shown at each node. A = *cordata* subgroup; B = *elliptica*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

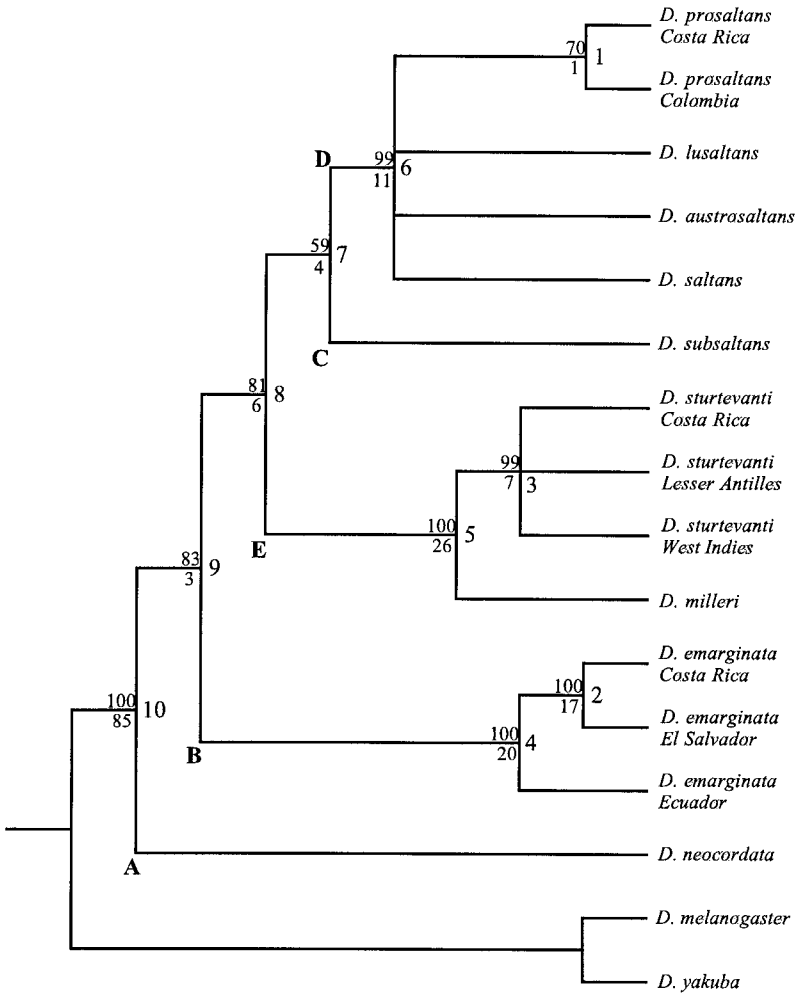

FIG. 3.—The majority-rule bootstrap phylogeny based on total evidence analysis. Bootstrap proportions (above) and decay indices (below) are shown at each node. A = *cordata* subgroup; B = *elliptica* subgroup; C = *parasaltans* subgroup; D = *saltans* subgroup; E = *sturtevanti* subgroup. Numbered nodes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

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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