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Phylogeny of the Subgenus *Sophophora* (Diptera: Drosophilidae) Based on Combined Analysis of Nuclear and Mitochondrial Sequences

P. M. O'Grady,* and M. G. Kidwell,†

*Department of Invertebrate Zoology, American Museum of Natural History, New York, New York 10024; †Program in Genetics and the Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721

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Sequences from the nuclear (nu) alcohol dehydrogenase gene, the nu *28S* ribosomal RNA locus, and the mitochondrial cytochrome oxidase II gene were used both individually and in combined analyses to infer the phylogeny of the subgenus *Sophophora* (Diptera: Drosophilidae). We used several optimality criteria, including maximum likelihood, maximum parsimony, and minimum evolution, to analyze these partitions to test the monophyly of the subgenus *Sophophora* and its four largest species groups, *melanogaster, obscura, saltans,* and *willistoni*. Our results suggest that the *melanogaster* and *obscura* species groups are each monophyletic and form a closely related clade. The Neotropical clade, containing the *saltans* and *willistoni* species groups, is also recovered, as previous studies have suggested. While the *saltans* species group is strongly supported as monophyletic, the results of several analyses indicate that the *willistoni* species group may be paraphyletic with respect to the *saltans* species group.

INTRODUCTION

The subgenus *Sophophora* contains several species, including *Drosophila melanogaster* and *Drosophila pseudoobscura*, which have served as important model systems in the study of genetics, ecology, behavior, evolution, and developmental biology. The group was erected by Sturtevant (1939, 1942) when he subdivided the genus *Drosophila* into subgenera and species groups. The subgenus *Sophophora* was described based on the type species *D. melanogaster* and originally included four species groups, *melanogaster, obscura, saltans*, and *willistoni* (Sturtevant, 1939, 1942). Three additional subgroups, *dispar, fima*, and *dentissima*, were added as more was learned about the Australasian and Afrotropical fauna (Burla, 1954; Mather, 1954; Tsacas, 1979, 1980). Currently, the seven species groups placed in *Sophophora* contain approximately 300 species (Wheeler, 1982, 1986; Lemeunier *et al.*, 1986).

Throckmorton (1975) considered species placed in the subgenus *Sophophora* to be part of a large radiation of flies which also contained the genera *Chymomyza* and *Neotanygastrella*. He considered the "Sophophoran radiation" to be basal to the radiation which gave rise to the remainder of the genus *Drosophila* (Throckmorton, 1975). Recent molecular studies support the notion that *Sophophora* is basal within the Drosophilinae and quite distantly related to the subgenus *Drosophila* (Remsen and DeSalle, 1998; Kwiatowski and Ayala, 1999; Remsen and O'Grady, manuscript in preparation).

Based on biogeographical data, a common ancestral "*protomelanogaster*" lineage gave rise to both the *melanogaster* and the *obscura* species groups in southeast Asia during the mid-Oligocene (Throckmorton, 1975). The *melanogaster* species group consists of predominantly Old World tropical species, although some members are cosmopolitan in distribution (Lemeunier *et al.*, 1986; Lachaise *et al.*, 1988). Although the *obscura* species group is found primarily in the Holarctic region, some members are found in tropical regions (Lakovaara and Saura, 1982; Heed and O'Grady, 2000). The *saltans* and *willistoni* species groups comprise a Neotropical clade of species, closely related to one another, but distinct from all other Sophophoran forms (Throckmorton, 1975). Throckmorton (1975) considered the *saltans* and *willistoni* species groups to be derivative within the subgenus, originating after the divergence of the *melanogaster* and *obscura* species groups from the *protomelanogaster* ancestor.

Morphology, DNA–DNA hybridization, and phylogenetic analysis of a variety of nucleotide sequences indicate that the subgenus *Sophophora* is monophyletic (Throckmorton, 1975; DeSalle, 1992; Thomas and Hunt, 1993; Russo *et al.*, 1995), as are its four largest species groups, *melanogaster, obscura, saltans*, and *willistoni* (reviewed in Powell, 1997). Furthermore, these data also support the monophyly of the Old

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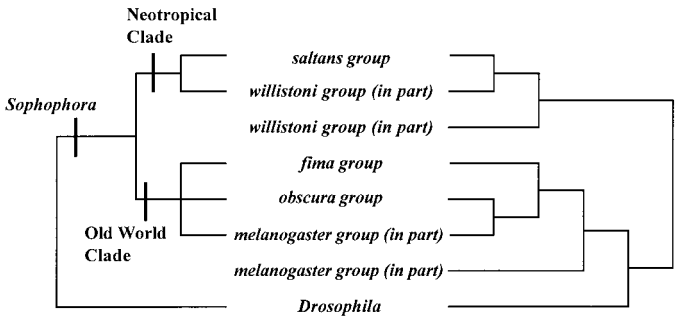

FIG. 1. Proposed evolutionary relationships within the subgenus *Sophophora*. The hypothesis supported by morphology, biogeography, and molecular mt *16S* (DeSalle, 1991) and nu *Adh* (Russo *et al.*, 1995) data is shown on the left. The hypothesis supported by analysis of nu *28S* rRNA (Pelendakis *et al.*, 1991; Pelendakis and Solignac, 1993) is on the right.

World *melanogaster–obscura* and Neotropical *saltans–willistoni* clades (Fig. 1). In these studies, the subgenus *Sophophora* was always monophyletic with respect to both the subgenus *Drosophila* and several genera within the family Drosophilidae.

In contrast to the majority of phylogenetic work (Throckmorton, 1975; Lemeunier *et al.*, 1986; Lachaise *et al.*, 1988; DeSalle, 1992; Thomas and Hunt, 1993; Russo *et al.*, 1995), two studies have suggested that *Sophophora* is not monophyletic. Pelendakis *et al.* (1991) and Pelendakis and Solignac (1993), based on neighbor joining analysis of the *28S* rRNA locus, suggest that the *melanogaster* species group, as traditionally defined, is not a monophyletic lineage. The *ananassae* subgroup, which has traditionally been placed in the *melanogaster* species group, was shown to be the sister taxon of the *obscura* species group (Fig. 1). However, it should be noted that bootstrap support for this relationship is not high. The *fima* species group is the sister group to the *obscura–ananassae* clade (Fig. 1). The *saltans* species group was monophyletic in this study, as was the *saltans–willistoni* clade, but the *willistoni* species group is paraphyletic with respect to the *saltans* species group (Fig. 1). The result that was most incongruent with the previous morphological and molecular studies, however, was the finding that *Sophophora* was paraphyletic with respect to the subgenus *Drosophila*. The *28S* data support the notion that the *obscura–melanogaster* clade is the sister taxon of the subgenus *Drosophila*, not the Neotropical *saltans* and *willistoni* species (Fig. 1).

In order to test the monophyly of *Sophophora*, we have gathered additional molecular sequence data from the mitochondrial (mt) cytochrome oxidase II (COII) and nuclear (nu) alcohol dehydrogenase (*Adh*) genes. We have examined these data, along with previously published data from the *28S* rRNA locus (Pelendakis *et al.*, 1991; Pelendakis and Solignac, 1993), in both individual and combined analyses, to test the monophyly of *Sophophora* and its component groups. Although we were unable to address the monophyly of *Sophophora* relative to *Lordiphosa* (see below), we (1) determined the relationships among the major groups defined within the subgenus *Sophophora* (the *melanogaster*, *obscura*, *saltans*, and *willistoni* species groups; the Neotropical clade; and the *melanogaster–obscura* clade) and (2) tested whether these groups are monophyletic.

MATERIALS AND METHODS

DNA Sources, Isolation, and Sequencing

Live *Drosophila* stocks used in this study were maintained at the National *Drosophila* Species Resource Center in Tucson, Arizona. Cultures of *D. madeirensis* were obtained from D. Sperlich at the University of Tubingen. Specimens of *D. obscura* were obtained from M. Radak at the University of Belgrade. Table 1 shows the taxonomic classification of the species used in this study along with the GenBank accession numbers for each sequence.

Genomic DNA was isolated following the method of Gloor and Engels (1992). The target loci were amplified from each taxon using standard PCR cycling conditions. Oligonucleotides used to amplify the *Adh* and COII genes are described elsewhere (O'Grady *et al.*, 1998; O'Grady, 1999). PCR products from the *Adh* gene were then cloned into the TA cloning vector (Invitrogen). Two colonies were selected at random and sequenced using a dsDNA cycle sequencing procedure (GIBCO BRL). When possible, both DNA strands were sequenced from multiple clones to reduce the effect of polymerase error on the analysis. PCR products from the entire 688-bp COII gene were amplified, purified by membrane filtration (Millipore), and sequenced directly using a standard dsDNA cycle sequencing protocol (GIBCO BRL). Approximately 90% of both DNA strands were sequenced from each PCR product.

Sequence Availability and Alignment

GenBank accession numbers for the sequences examined in this study are listed in Table 1. Alignment of the *28S* locus was exactly as in Pelendakis *et al.* (1991) and Pelendakis and Solignac (1993). The alignment of the COII and *Adh* coding regions was trivial. One gap, following nucleotide 685 at the 3′ end of the COII gene, was required to align the *melanogaster* species group to the other taxa in this study.

Data Analysis

PAUP* 4.0 (Swofford, 2000) was employed for all maximum parsimony, maximum likelihood, and minimum evolution searches. All trees were rooted with representatives of the *D. immigrans* and *D. repleta* species groups (subgenus *Drosophila*). Several individual and combined analyses were also done employing

TABLE 1

Taxonomic Classification and GenBank Accession Numbers of Taxa in the Present Study

Species group	Subgroup	Species	28*S*	*Adh*	*COII*
fima		*D. fima*	X71207	—	—
melanogaster	*ananassae*	*D. ananassae*	X71197	—	AF474077
		D. malerkotliana	X71195	—	AF474078
		D. vallismaia	X71193	—	—
		D. varians	X71199	—	—
	elegans	*D. elegans*	X71183	—	—
	eugracilis	*D. eugracilis*	X71175	AF476922	AF474079
	ficusphila	*D. ficusphila*	X71181	—	AF474080
	melanogaster	*D. erecta*	X71171	X54116	—
		D. mauritiana	X71163	M19264	AF474081
		D. melanogaster	X71159	M17833	J01404
		D. orena	X71173	M37837	—
		D. sechellia	X71165	X04672	—
		D. simulans	X71161	M36581	AF474082
		D. teissieri	X71169	X54118	—
		D. yakuba	X71167	X54120	X03240
	montium	*D. auraria*	—	—	AF474083
		D. bakoue	X71191	—	—
		D. biauraria		AF476923	AF474084
		D. kikkawai	X71185	—	—
		D. malagassya	X71187	—	—
		D. nikananu	—	—	AF474086
		D. quadauraria	—	AF476924	AF474085
		D. serrata	X71189	—	—
		D. triauraria	—	AF476925	AF474087
		D. tsacasi	—	X63954	AF474088
	suzukii	*D. biarmipes*	—	—	AF474094
		D. mimetica	X71179	—	AF474092
		D. pulcherella	—	—	AF474093
	takahashii	*D. lutescens*	—	AF476926	AF474090
		D. prostipennis	—	—	AF474091
		D. takahashii	X71177	—	AF474089
obscura	*affinis*	*D. affinis*	X71207	AF067280	M95140
		D. algonquin	—	—	M95144
		D. athabasca	—	—	M95141
		D. azteca	X71205	—	M95146
		D. helvetica	—	AF067282	—
		D. narragansett	—	—	M95149
		D. tolteca	—	AF081357	M95152
	obscura	*D. ambigua*	*	X54813	M95150
		D. eskoi	—	AF067281	—
		D. hubeiensis	—	U90953	—
		D. imaii	—	U40987	—
		D. sinobscura	—	M55545	—
		D. subsilvestris	—	AF067283	—
		D. tristis	—	U90956	—
		D. tsukabaensis	—	AF067284	—
		D. bifasciata	*	U40986	M95147
		D. obscura	*	U90955	AF081356
	pseudoobscura	*D. lowei*	—	—	M95142
		D. miranda	—	M60998	M95148
		D. persimilis	*	M60997	M95143
		D. pseudoobscura	X71203	M60998	M95145
	subobscura	*D. guanche*	*	X60113	AF081354
		D. madeirensis	—	X60112	AF081355
		D. subobscura	*	M55545	M95151
saltans	*cordata*	*D. neocordata*	X71219	AF045120	AF045088
	elliptica	*D. emarginata*	X71217	AF045124	AF045094
	parasaltans	*D. subsaltans*	—	AF045117	AF045085
	saltans	*D. austrosaltans*	—	AF045123	AF045091
		D. lusaltans	—	AF045122	AF045090
		D. prosaltans	X71213	AF045119	AF045087
		D. saltans	—	AF045113	AF0450081
	sturtevanti	*D. milleri*	—	AF045121	AF045089
		D. sturtevanti	X71215	AF045114	AF045084

TABLE 1—*Continued*

Species group	Subgroup	Species	28*S*	*Adh*	*COII*
willistoni	*subgroup "A"*	*D. capricorni*	—	AF264073	AF474095
		D. equinoxialis	—	AF264074	AF474096
		D. insularis	—	AF264076	AF474098
		D. paulistorum	—	AF264078	AF474100
		D. pavlovskiana	—	AF264079	AF474101
		D. sucinea	—	AF264080	AF474102
		D. tropicalis	—	AF264081	AF474103
		D. willistoni	X71209	L08648	AF474104
	subgroup "B"	*D. fumipennis*	—	AF264075	AF474097
		D. nebulosa	X71211	AF264077	AF474099

* Sequence not present in GenBank.

different taxon sampling strategies. A number of these analyses (not shown here) can be viewed in TreeBASE (http://www.herbaria.harvard.edu/treebase/index.html).

Individual and combined analyses were performed on all 21 taxa which were common to the *28S*, *Adh*, and COII data sets. A combined pairwise analysis was also done on these loci, as well as on all 42 taxa common to the *Adh*+COII genes. Although we also analyzed all other pairwise combinations of taxa, we show only the *Adh*+COII analysis because it is the largest pairwise analysis and contains the highest percentage of newly determined sequences.

Maximum likelihood (ML) analyses were performed as follows: (1) the transition–transversion ratio, proportion of invariant sites, and gamma-shape parameter were estimated for the most parsimonious trees (see below). When more than one equally parsimonious tree was present, the parameters from the tree with the best −ln likelihood score were used. (2) The parameter values estimated above were used in a likelihood search (search type = heuristic, starting trees obtained by random addition, replicates = 100, TBR branch swapping) using PAUP* 4.0 (Swofford, 2000). Parameters estimated using likelihood, such as base frequencies, percentage invariant sites (%1), and a gamma-shape parameter (Γ), are presented in Figs. 2–4. Support for each clade in the ML trees was determined using bootstrap proportions (Felsenstein, 1985, 1988). One hundred replicates were performed for each locus.

Maximum parsimony (MP) searches were performed using either the branch-and-bound (21 taxa) or the heuristic (all other searches) algorithm. Heuristic searches were carried out as follows: starting trees obtained via random stepwise addition, 500 replicates, TBR branch swapping employed. When multiple most parsimonious islands of trees were discovered, strict consensus trees of each island were evaluated (Maddison, 1990). The level of support at each node in all parsimony searches was assessed using decay indices (DI; Bremer, 1988) and bootstrap proportions (BP; Felsenstein, 1985, 1988). Five hundred replicates were performed in each bootstrap search. Partitioned Bremer support (PBS; Baker and DeSalle 1997) was used to measure the amount of support provided by each individual partition to the DI for every node in the combined analysis phylogenies. The sign (either positive or negative) and magnitude of PBS values indicate the level of support for, or incongruence with, a given node. The sum of all partition lengths for any given node will always equal the decay index for that node on the total evidence tree. Using this method allows us to determine the relative contribution of each partition to the different simultaneous analysis trees.

Minimum evolution searches were performed using the log determinant distance measure in PAUP* 4.0 (Swofford, 2000). Stepwise addition was used to construct starting trees (100 random addition sequences). Bootstrap proportions were used to assess support at each node in these analyses.

The results of the maximum parsimony, maximum likelihood, and minimum evolution searches were quite similar, so only selected results are presented here. For individual analyses, maximum likelihood trees are shown. Two bootstrap proportions are shown in the text and figures; the first is based on maximum likelihood, the second on maximum parsimony. Nodes present in the parsimony strict consensus tree(s) also have decay indices.

Partition Homogeneity Test

The partition homogeneity test (PHT; Farris *et al.*, 1994, 1995), as implemented in PAUP* 4.0 (Swofford, 2000), was used to test for incongruence between data sets. The null hypothesis of the PHT is that each pair of loci is as congruent as two randomly generated partitions of equal size. The test compares the length of the most parsimonious tree(s) for the original pair of partitions with a number of randomly generated data sets. One hundred randomly generated data sets were used to create a null distribution to test the statistical significance of tree lengths from the original partitions. We performed pairwise PHTs to test for incongruence on two sets of taxa. Table 2 shows the results of the

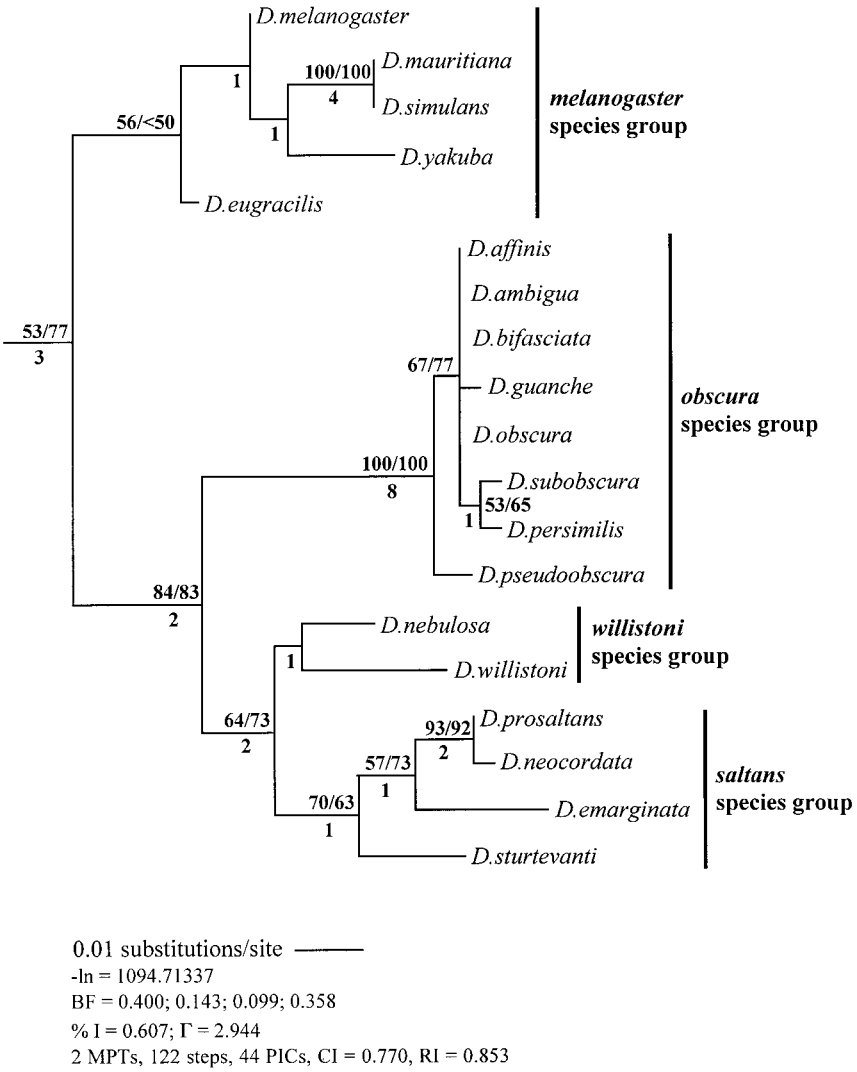

FIG. 2. Phylogeny of the subgenus *Sophophora* based on analysis of the *28S* locus. The tree shown was generated by maximum likelihood analysis and branch lengths are proportional to the number of nucleotide substitutions per site. Bootstrap proportions (ML and MP) are above and decay indices (MP) are below each node. Base frequencies (BF; A, C, G, T), percentage of invariant sites (%I), and gamma-shape parameter (Γ) were estimated using likelihood. The number of most parsimonious trees (MPTs), number of parsimony informative characters (PICs), consistency index (CI), and retention index (RI) are also shown.

PHT on those species used in the small individual and combined analyses. Table 3 shows the PHT results from those taxa used in the pairwise combined analyses.

RESULTS

Individual Analyses

Figure 2 shows the results of the maximum likelihood analysis of the *28S* locus, in which branch length is proportional to the number of substitutions per site. Other optimality criteria (analyses not shown) are, for the most part, in agreement with this tree. All sampled species groups are monophyletic. The Neotropical clade, consisting of the *saltans* and *willistoni* species groups, is supported (BP = 64/73, DI = 2). Unlike previous studies, the *obscura* group is supported (BP = 84/83, DI = 2) as the sister group of the *saltans–willistoni* clade (Fig. 2). Relationships between some of the species (e.g., *D. persimilis* and *D. subobscura*, *D. prosaltans* and *D. neocordata*, and *D. yakuba* as the sister group of the *mauritiana–simulans* clade) are also unconventional. A larger *28S* analysis (not shown), including 39 taxa, is broadly in agreement with these results, although only two of the nodes on this tree were strongly supported (BP > 90, DI > 2).

Figure 3 shows the results of a maximum likelihood analysis based on a fragment of the coding regions of the nu *Adh* gene. Branch lengths are shown proportional to the number of substitutions per site. This

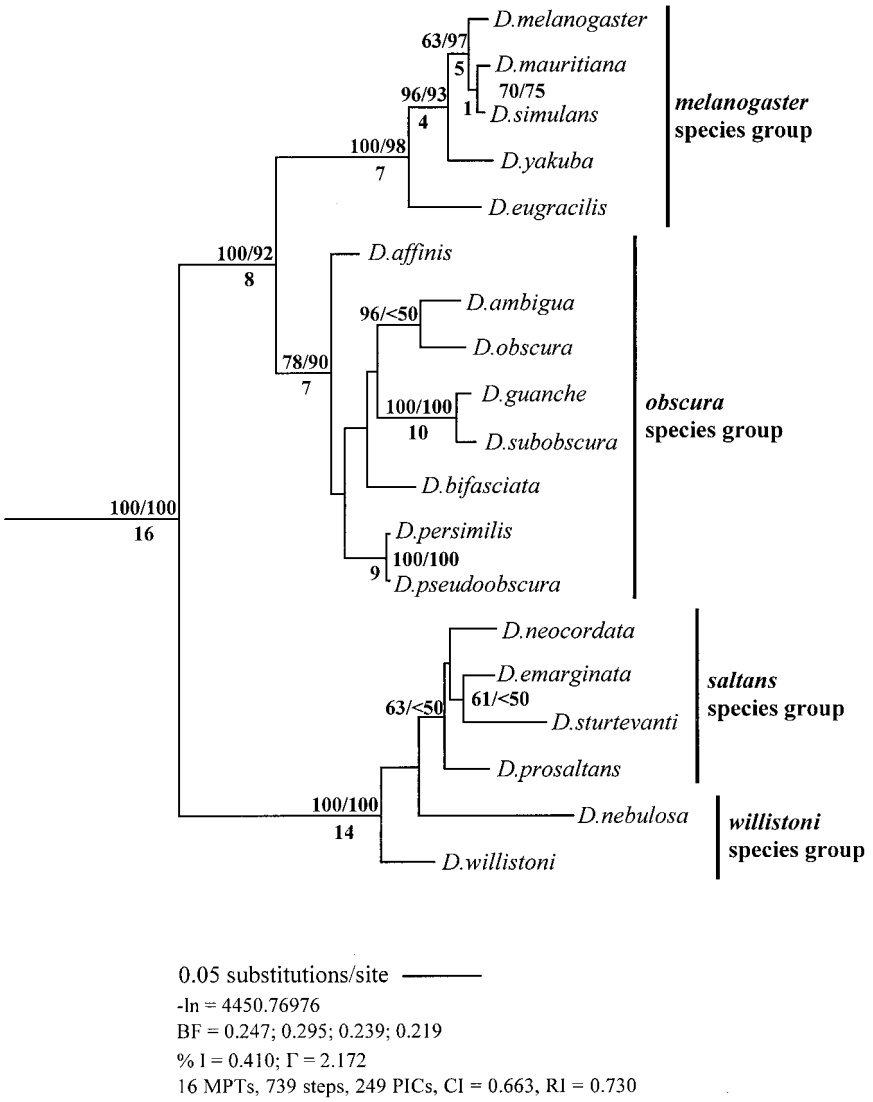

FIG. 3. Phylogeny of the subgenus *Sophophora* based on analysis of the *Adh* locus. The tree shown was generated by maximum likelihood analysis and branch lengths are proportional to the number of nucleotide substitutions per site. Bootstrap proportions (ML and MP) are above and decay indices (MP) are below each node. Base frequencies (BF; A, C, G, T), percentage of invariant sites (%I), and gamma-shape parameter (Γ) were estimated using likelihood. The number of most parsimonious trees (MPTs), number of parsimony informative characters (PICs), consistency index (CI), and retention index (RI) are also shown.

analysis recovers the Neotropical clade (BP = 100/100, DI = 14) with high support, although there is little support for either the 2monophyly of the *saltans* or *willistoni* species groups or the relationships within these groups (Fig. 3). The *melanogaster* and *obscura* species groups are each monophyletic (BP = 100/98, DI = 7 and BP = 78/90, DI = 7, respectively) and are strongly supported as sister taxa (BP = 100/92, DI = 8). Several relationships which have been suggested by previous studies (*melanogaster* subgroup, *subobscura* subgroup, *pseudoobscura* subgroup) are well supported in this study as well (Fig. 3). *Sophophora* is well supported as monophyletic (BP = 100/100, DI = 16) with respect to the outgroup taxa (Fig. 3). A larger analysis, which included 54 taxa, was also performed (results not shown) and is largely in agreement with the smaller search, although some relationships among species groups were less well supported.

The results of the individual maximum likelihood analysis of the mt COII gene are shown in Fig. 4. All branch lengths are drawn proportional to the number of substitutions per site. As in the 28S and *Adh* analyses, the Neotropical clade is recovered, but this group, as well as relationships within it, is not strongly supported (Fig. 4). The *melanogaster* and *obscura* species groups are both monophyletic (BP = 90/84, DI = 4 and BP = 86/66, DI = 3, respectively) and form a weakly supported clade (BP = 60/64, DI = 1). Some previously

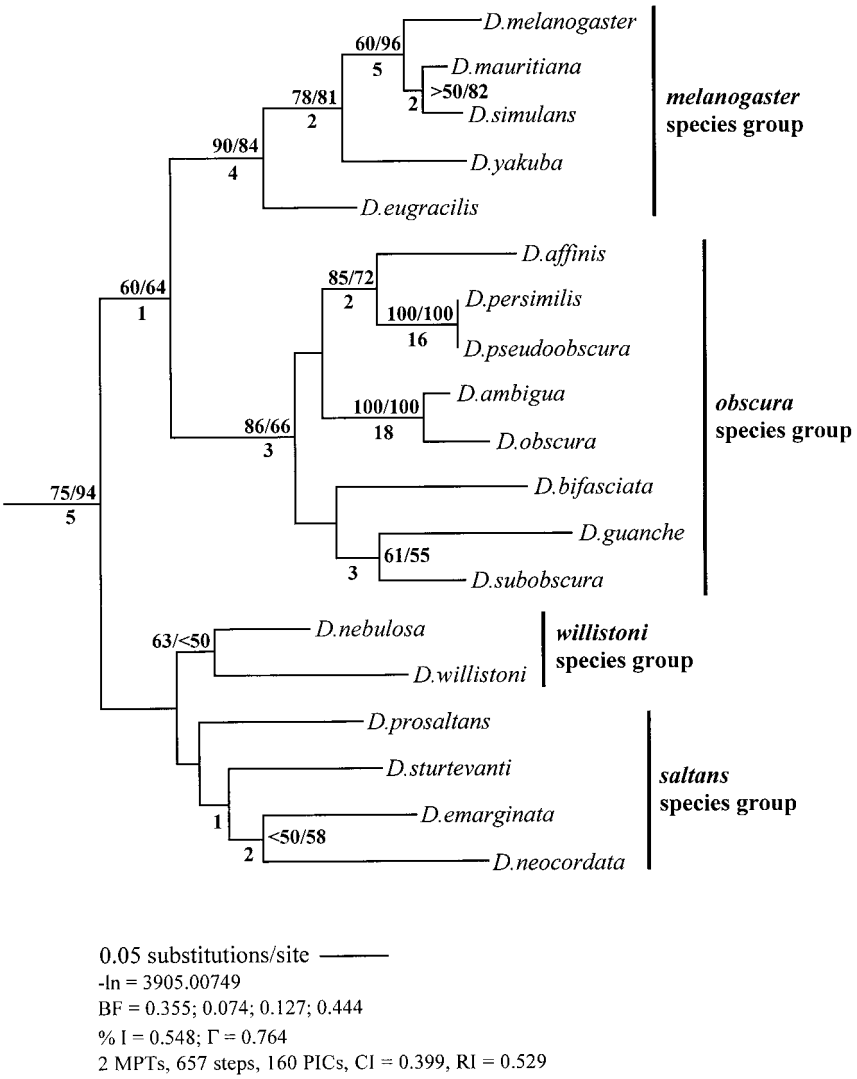

FIG. 4. Phylogeny of the subgenus *Sophophora* based on analysis of the COII locus. The tree shown was generated by maximum likelihood analysis and branch lengths are proportional to the number of nucleotide substitutions per site. Bootstrap proportions (ML and MP) are above and decay indices (MP) are below each node. Base frequencies (BF; A, C, G, T), percentage of invariant sites (%I), and gamma-shape parameter (Γ) were estimated using likelihood. The number of most parsimonious trees (MPTs), number of parsimony informative characters (PICs), consistency index (CI), and retention index (RI) are also shown.

suggested relationships, such as the monophyly of the *melanogaster* subgroup (BP = 78/81, DI = 2) and the *affinis–pseudoobscura* clade (BP = 85/72, DI = 2), are also supported in this analysis (Fig. 4). A maximum parsimony analysis, consisting of 57 COII sequences, was also performed (results not shown) and is in agreement with Fig. 4, although relationships among the species groups are not well resolved.

Combined Analyses

Results of the PHT on data sets containing the 21 taxa common to all three partitions indicate that none of the loci in this analysis were significantly incongruent compared to one another (Table 2). Figure 5 shows the results of a combined maximum parsimony analysis with the amount of support given by each of the three loci partitioned and indicated at each node. Branch lengths in Fig. 5 are for clarity only and are not proportional to change. The Neotropical clade is

TABLE 2

Results of Partition Homogeneity Test (Small Combined Data Sets)

Locus	*28S*	*Adh*	COII
28S	—	0.42	0.57
Adh		—	0.89
COII			—

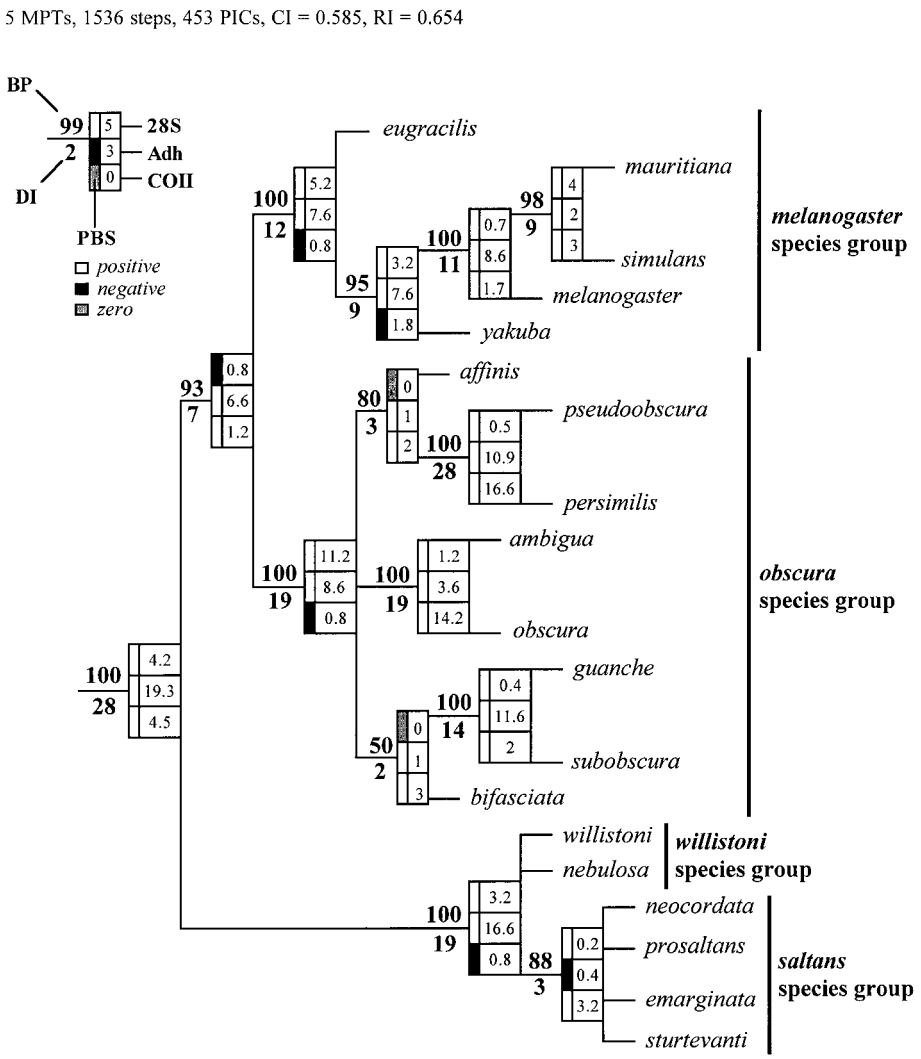

FIG. 5. Phylogeny of the subgenus *Sophophora* based on combined parsimony analysis of the *28S*, *Adh*, and COII loci. Bootstrap proportions (MP only) are above and decay indices are below each node. PBS values (in boxes at node) are indicated. Branch lengths are not proportional to change; they vary only for clarity.

present and well supported (BP = 100, DI = 19; Fig. 5). Within this lineage, the *saltans* species group is monophyletic (BP = 88, DI = 3) but, as in the *willistoni* species group, relationships are not resolved. The PBS values at most nodes are either positive or zero, indicating either support for a given node or lack of conflict at that node (Fig. 5). Negative PBS values are generally weak (<2) and not attributed to any single locus. The Old World *melanogaster* and *obscura* species groups are each monophyletic (BP = 100, DI = 12 and BP = 100, DI = 19, respectively) and sister taxa (BP = 93, DI = 7). This combined analysis indicates that the *melanogaster* subgroup is monophyletic (BP = 95, DI = 9). The *affinis* and *pseudoobscura* subgroups form a clade (BP = 80, DI = 3), although relationships among the *obscura* subgroups are not otherwise resolved.

Pairwise analyses were performed on the largest sets of taxa which had two sequences determined (i.e., *28S*+*Adh*, *28S*+COII, *Adh*+COII). The results of maximum parsimony analysis of the taxa common to both the *Adh* and the COII genes are shown in Fig. 6. Branch lengths are not drawn proportional to change. Interestingly, the COII and *Adh* loci were the only partitions shown to be significantly incongruent (Table 3). Examining the PBS values for the *Adh*+COII pairwise combined analysis (Fig. 6), however, we can see that the areas of most incongruence between these two loci occur within the *saltans* and *willistoni* species groups. This conflict leads to poorly supported relationships in these clades. The Neotropical clade itself, however, is well supported (BP = 100, DI = 23). The *saltans* species group is also monophyletic, with the *cordata* subgroup placed at the base of the group, sister to a clade of the *saltans*, *sturtevanti*, and *parasaltans*

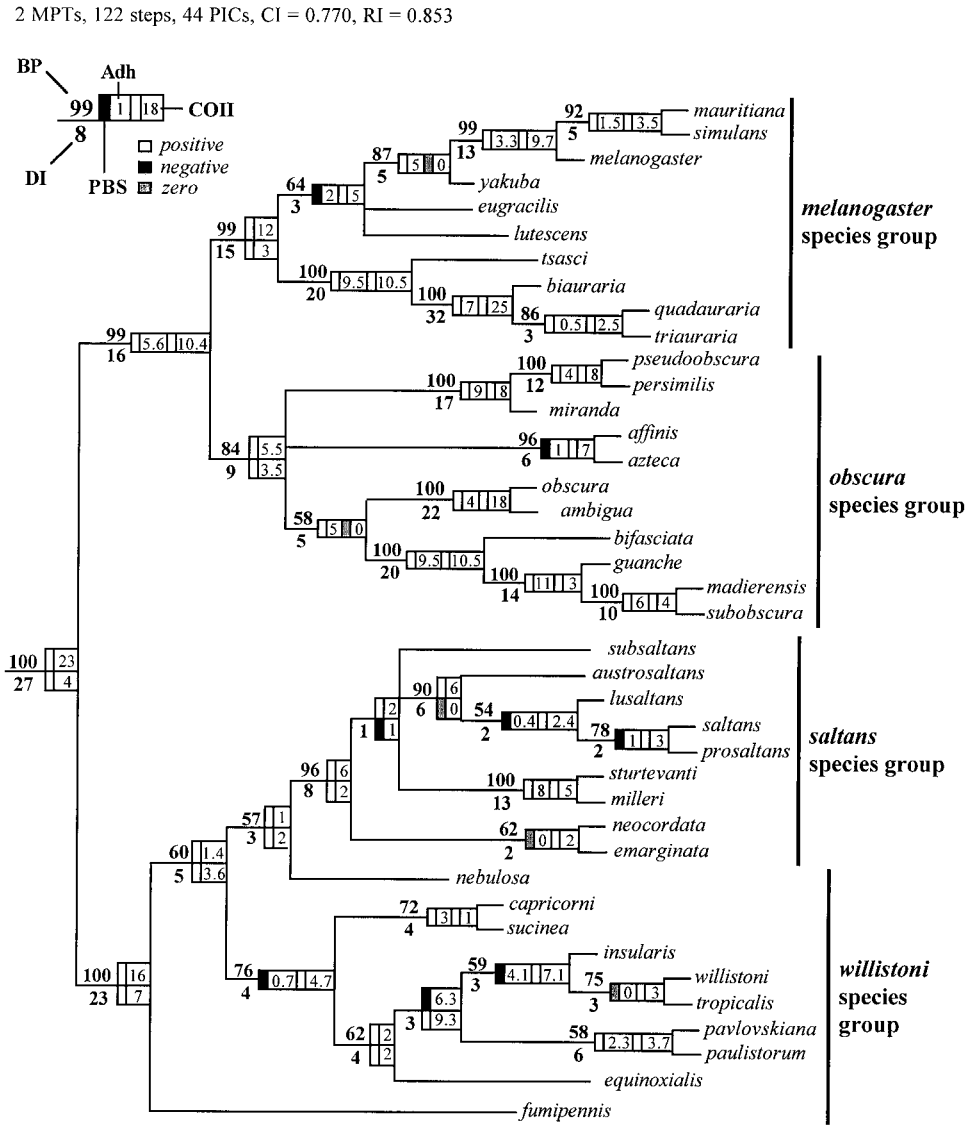

FIG. 6. Phylogeny of the subgenus *Sophophora* based on parsimony analysis of the combined *Adh* and COII loci. Bootstrap proportions (MP only) are above and decay indices are below each node. PBS values (in boxes at node) are indicated. Branch lengths are not proportional to change; they vary only for clarity.

subgroups (Fig. 6). Two *willistoni* species, *D. fumipennis* and *D. nebulosa*, are placed unconventionally, suggesting that the *willistoni* group may not be monophyletic (Fig. 6). The remaining species in the *willistoni* group are resolved, but support for some nodes is weak. This analysis also indicates strong support for the *melanogaster–obscura* clade (BP = 99, DI = 16). Within this clade, both the *melanogaster* and the *obscura* species groups are monophyletic (BP = 99, DI = 15 and BP = 84, DI = 9, respectively) and relationships among species in each group are well resolved and congruent with previously published data (Fig. 6).

TABLE 3

Results of Partition Homogeneity Test (Pairwise Combined Data Sets)

Locus	*28S*	*Adh*	COII
28S	—	0.33	0.87
Adh		—	0.01[a]
COII			—

[a] Data partitions which display significant homogeneity when compared.

DISCUSSION

The relationships of species within the subgenus *Sophophora* have previously been studied using both morphological (Throckmorton, 1975) and molecular

characters (Pelendakis *et al.*, 1991; DeSalle, 1992; Pelendakis and Solignac, 1993; Thomas and Hunt, 1993; Russo *et al.*, 1995; Katoh *et al.*, 2000). The results of some of these studies are incongruent with one another, primarily concerning the monophyly of the subgenus *Sophophora* and the monophyly of some major lineages in *Sophophora*. This may be due to the fact that the *28S* sequences were determined through reverse transcription of RNA. This enzyme, unlike other polymerases, lacks proofreading function. These *28S* sequences may therefore contain incorporation errors that can, in turn, lead to misleading phylogenetic results. The present analyses include representatives from two species groups (*immigrans* and *repleta*) placed in the subgenus *Drosophila* to test specifically the monophyly of the subgenus *Sophophora*. Consistent with the individual analyses, the combined analysis of the *28S*, *Adh*, and COII sequences suggests that *Sophophora* is monophyletic with respect to the subgenus *Drosophila*.

Using *Adh* gene sequences, Katoh *et al.* (2000) have recently suggested that *Sophophora* is paraphyletic with respect to some members of the genus *Lordiphosa*. [Katoh *et al.* (2000) consider *Lordiphosa* to be a subgenus of *Drosophila*, rather than a distinct genus, as suggested by the most recent taxonomic revision of Drosophilidae (Grimaldi, 1990). We will refer to *Lordiphosa* as a genus.] Specifically, they suggest that the *denticeps*, *fenestratum*, and *miki* species groups of *Lordiphosa* are the sister group of the Neotropical *saltans* and *willistoni* species groups. Relationships other than the monophyly of *Sophophora*, such as the *obscura–melanogaster* and *saltans–willistoni* clades, and the monophyly of the species groups are well supported. The analyses we present here obviously cannot address this issue, as we do not have access to any taxa in the genus *Lordiphosa*. Therefore, the monophyly of *Sophophora* should be considered tentative until tested with more extensive taxon sampling, including members of *Lordiphosa*.

The Neotropical *saltans–willistoni* clade is monophyletic in every analysis in the present study, usually with high bootstrap proportions and decay indices. The PBS values calculated for the combined analyses performed indicate that all loci contribute positively to the decay index at the node supporting the monophyly of the *saltans–willistoni* lineage (Figs. 5 and 6). The *saltans* species group is monophyletic in all analyses presented and strong support for the monophyly of this group is seen in several analyses. In terms of PBS values, all loci in most of the combined analyses (Figs. 5 and 6) support the monophyly of the *saltans* group. Relationships among species in the *saltans* group are variable, depending on the gene examined and the taxa included. Often, however, relationships within the *saltans* group are not well supported (Figs. 3–5). The combined analyses which sample extensively within the *saltans* group (Fig. 6) are able to recover monophyletic *saltans* and *sturtevanti* subgroups, but cannot strongly resolve relationships among any of the subgroups. More extensive species and sequence sampling should better resolve the somewhat contentious relationships within this species group (O'Grady *et al.*, 1998; Rodriguez-Trelles *et al.*, 1999). The data in the present study indicate that the *saltans* species group is monophyletic, although relationships beyond the *saltans–parasaltans–sturtevanti* clade (Fig. 6) of this species group should be considered tentative.

Several analyses in this study are unable to recover a monophyletic *willistoni* species group (Figs. 3, 5, and 6). Those analyses, which do suggest that this group is monophyletic, are not well supported. Within the *willistoni* group, relationships are not well resolved in any analyses. Figure 6 supports the close relationships of *D. insularis*, *D. willistoni*, *D. tropicalis*, *D. pavlovskiana*, *D. paulistorum*, and *D. equinoxialis*. This is in agreement with a recent study by Tarrio *et al.* (2000) which suggested, based on *Xdh* sequences, that there were three major lineages within the *willistoni* group, *sucinea–capricorni*, *nebulosa*, and *equinoxialis–paulistorum–willistoni–tropicalis–insularis*. It is also similar to relationships proposed by Gleason and colleagues in several studies which extensively sampled species in the *willistoni* group (Gleason, 1996; Gleason and Powell, 1997; Gleason *et al.*, 1998). The *willistoni* group should tentatively be considered monophyletic, pending more extensive sampling. The three lineages proposed by Gleason (1996) and others are supported as the relationships within this group.

The *obscura–melanogaster* clade (Pelendakis *et al.* 1991; Pelendakis and Solignac, 1993) is monophyletic in all but one of the analyses in the present study. Examining PBS values from combined analyses, we see that the *Adh* and COII genes are giving the most support to the monophyly of the *obscura–melanogaster* clade (Figs. 5 and 6). PBS values for the *28S* locus are zero or greater in all combined analyses (Fig. 5), indicating that while these characters do not strongly support this clade, they do not conflict with it either.

The *melanogaster* species group was monophyletic in all of the analyses in this study. However, in the case of some analyses (Figs. 2–5), many of the *melanogaster* species group sequences examined were from very closely related species, indicating that perhaps the monophyly of the larger group was not tested rigorously enough. More extensive taxon sampling will be required before this can be tested rigorously. Previous studies based on morphology (Bock and Wheeler, 1972), polytene chromosome banding patterns (Ashburner *et al.*, 1984), and nucleotide sequences (Goto and Kimura, 2001) support the notion that the *ananassae* and *montium* species groups diverged from the other subgroups early in the evolution of the *melanogaster* species group. Both the previous studies and our

analyses suggest three clades, (1) *ananassae*, (2) *montium*, and (3) *suzukii–takahashii–ficusphila–melanogaster–eugracilis*.

The *obscura* species group is strongly supported as monophyletic in all analyses in this study. PBS support for the *obscura* group in combined analyses comes equally from each locus (Figs. 5 and 6). Many relationships in this group are congruent with other phylogenetic analyses of the *obscura* species group (Beckenbach *et al.*, 1993; Barrio *et al.*, 1994; Barrio and Ayala, 1997; Gleason *et al.*, 1997; O'Grady, 1999; Katoh *et al.*, 2000).

CONCLUSIONS

The conflicting results concerning the monophyly of *Sophophora* and some of its component groups presented in previous studies (Pelendakis *et al.*, 1991; DeSalle, 1992; Pelendakis and Solignac, 1993; Thomas and Hunt, 1993; Russo *et al.*, 1995) are somewhat reconciled by the analyses presented here. The reanalysis of the *28S* data, along with additional sequences from the *Adh* and COII genes, indicates that *Sophophora* is monophyletic with respect to the subgenus *Drosophila*. In contrast to the analyses of Pelendakis *et al.* (1991), our data do support the monophyly of *Sophophora* relative to the subgenus *Drosophila*.

The *obscura–melanogaster* and Neotropical clades within *Sophophora* are monophyletic. The results presented here also indicate that the *obscura*, *saltans*, and *melanogaster* species groups are monophyletic. However, it seems that the *willistoni* species group is either not or only weakly supported as monophyletic. Additional morphological and molecular data from other species closely related to *D. fumipennis* and *D. nebulosa*, such as the poorly known *alagitans–bocainensis* subgroup (Wheeler, 1949), should be collected and included in an expanded phylogenetic analysis to fully understand how this group evolved.

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