Phylogeny of Drosophila and Related Genera: Conflict between Molecular and Anatomical Analyses¹

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INTRODUCTION

The family Drosophilidae is among the most diverse of the Diptera, encompassing more than 2500 species (Wheeler, 1986). Species of this family are often used in many areas of contemporary biological research. Understanding the phylogenetic relationships among the species is crucial to many of these studies. However, despite extensive investigations, the taxonomy of the Drosophilidae remains controversial. Wheeler’s (1981, 1986) standard classification is inconsistent with the

1 The sequences reported in this paper have been submitted to the GenBank/EMBL Data Libraries under Accession Nos. AF021825–AF021832 and AF022218.

2 Abbreviations used: Adh, alcohol dehydrogenase gene; Gpdh, glycerol-3-phosphate dehydrogenase gene; ML, maximum likelihood; MP, maximum parsimony; NJ, neighbor joining; nt, nucleotide; s.g., subgenus; Sod, superoxide dismutase gene.

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Key Words: Chymomyza; Drosophila; Hawaiian Drosophila; Scaptodrosophila; molecular systematics; Adh; Gpdh; Sod

Drosophila species are extensively used in biological research; yet, important phylogenetic relationships within the genus and with related genera remain unresolved. The combined data for three genes (Adh, Sod, and Gpdh) statistically resolves outstanding issues. We define the genus Drosophila inclusively so as to include Scaptomyza and Zaprionus (considered distinct genera in the taxonomy of Wheeler, 1981) but excluding Scaptodrosophila. The species of this family are often used in biological research; yet, important phylogenetic relationships among the species, whether the inferences are based on morphology (Throckmorton, 1975; Grimaldi, 1990) or molecular data (DeSalle, 1992a,b; Kwiatowski et al., 1994, 1997; Pélandakis and Solignac, 1993; Powell, 1997; Renssen and DeSalle, 1998; Russo et al., 1995; Tatarenkov et al., 1999; Thomas and Hunt, 1993). Throckmorton’s (1975) conclusion, based on morphological, behavioral, and biogeographical data, that the standard taxonomy implies numerous paraphyletic relationships but he did not advance a new taxonomy. Grimaldi’s (1990) analysis is the proposal that the subgenera Drosophila and Sphophora are sister clades and the exclusion from the genus Drosophila of the previous subgenus Hirtodrosophila, the Hawaiian species of Drosophila (which he classified as the genus Idiomyia), and the genera Zaprionus and Scaptomyza. Grimaldi’s (1990) proposal contrasts with Throckmorton’s (1975), who proposed an early divergence of the subgenus Sphophora, which would be the sister group to a complex of taxa that included the subgenus Drosophila, as well as Hirtodrosophila, Dorsilopha, Zaprionus, Scaptomyza, and the Hawaiian Drosophila.

Introduction of molecular data into taxonomic discourse should help in resolving some perplexing questions but has so far left unresolved some key relationships. Generally, molecular data (Kwiatowski et al., 1994, 1997; Pélandakis and Solignac, 1993; Renssen and DeSalle, 1998; Tatarenkov et al., 1999; Thomas and Hunt, 1993) contradict in very many important aspects Grimaldi’s revisions of the Drosophila phylogeny, while they often support Throckmorton’s hypothesis. The molecular studies have, however, paid relatively less attention to several taxa, including Zaprionus, Scaptomyza, Hirtodrosophila, and Dorsilopha. We have obtained the nucleotide sequence of two genes, Cu,Zn superoxide dismutase (Sod)² and alcohol dehydrogenase (Adh) in several critical species. We have combined our data with preexisting sequences of the same
two genes, as well as of glycerol-3-phosphate dehydrogenase (Gpdh), seeking to resolve the phylogeny of these and other taxa within the Drosophilidae.

MATERIALS AND METHODS

Species

Table 1 lists the 29 Drosophilidae species that we have investigated. Strains of Chymomyza procnemis, D. mimica, D. immigrans, D. (Hirtodrosophila) pictiventris, D. (Scaptomyza) adusta, and D. (Zaprinus) tuberculatus were obtained from the National Drosophila Species Stock Center at Bowling Green, Ohio; the rest were from cultures available in our laboratory. We list Scaptomyza and Zaprinus, classified as genera by Wheeler (1981), as well as Hirtodrosophila, Dorsilopha, and Engiscaptomyza as Drosophila subgenera, following Tatarenkov et al. (1999), but Scaptodrosophila as a genus, following Grimaldi (1990) and Tatarenkov et al. (1999; see also Kwiatowski et al., 1994, 1997; Remsen and DeSalle, 1998; Russo et al., 1995).

DNA Preparation, Amplification, Cloning, and Sequencing

Genomic DNA from about 10 to 20 flies was prepared following the method of Kawasaki (1990). The amplification, cloning, and sequencing of Sod fragments used for most species are described elsewhere (Kwiatowski et al., 1994). Sod fragments from D. teissieri, D. paulistorum, D. nebulosa, and D. immigrans were amplified with primers N (5'-CCTCTAGAAATGGTGGTTAAAGCTGTNTGCGT-3') and O (5'-ACGGAATCTAGAAGG-

| Family       | Genus       | Subgenus | Group | Species         | Adh   | Sod    | Gpdh  |
|--------------|-------------|----------|-------|-----------------|-------|--------|-------|
| Drosophilida | Drosophila  | Sophophora | melanogaster | melanogaster | X78384 | X13780 | X14179 |
|              |             |          | simulans | simulans        |       |        |       |
|              |             |          | tassieri | tassieri        | X54118 |        | AF021828 |
|              |             |          | yakuba   | yakuba          | X54120 |        | U47809 |
|              |             |          | obscura  | subobscura      | X55545 | U38233 | U47877 |
|              |             |          |         | pseudoobscura   | X62181 | U47871 | L41249 |
|              |             |          |         | ambiguа         | X54813 | U47868 | U47880 |
|              |             |          | willistoni | willistoni    | L08648 | L13281 | L37038 |
|              |             |          |         | paulistorum    |       |        | AF021829 |
|              |             |          |         | nebulosa        |       |        | L14648 |
|              |             |          | saltans  | saltans         |       |        | L14250 |
|              | Drosophila  | virilis  |         | virilis         | U26846 | X13831 | D10697 |
|              |             | repleta  |         | hydei           | X58694 | U37714 | L41650 |
|              |             |         | mulleri  | mulleri         | X03048 |        |        |
|              |             | immigrans |         | immigrans      | M97638 | AF021825 |
|              |             | quinaria |         | guttifera       |       |        | AF021826 |
| Hawaiian     |             |         |         | mimica          | M60792 | AF022218 |
|              |             |         |         | nigra           | M60793 |          |
|              |             |         |         | differens       | M36785 |          |
|              |             |         |         | heteroneura     | M36781 |          |
|              |             |         |         | picticornis     | M63392 |          |
|              |             |         |         | albovittata     | M80925 |          |
| Scaptomyza   |             |         |         | adusta          |       |        | AF021827 |
| Engiscaptomyza |         |         |         |                 |       |        |       |
| Hirtodrosophila |         |         |         |                 |       |        |       |
| Dorsilopha   |             |         |         |                 |       |        |       |
| Zaprinus     |             |         |         |                 |       |        |       |
| Scaptodrosophila |         |         |         |                 |       |        |       |
| Chymomyza    |             |         |         |                 |       |        |       |
| Tephritidae  | Ceratitis   |         |         |                 |       |        |       |

Note. Sequences newly obtained in this study are underlined. The busckii and lebanonensis Gpdh sequences are courtesy of Dr. Wells and available on-line (see Materials and Methods).

a Scaptodrosophila was classified by Wheeler (1981) as a subgenus of Drosophila but has been raised to genus by Grimaldi (1990). Scaptomyza and Zaprinus are classified as genera by Wheeler (1981); in this paper we shall refer to them, as well as to Engiscaptomyza, Hirtodrosophila and Dorsilopha, as subgenera within the genus Drosophila.

b The Hawaiian Drosophila groups are: modified mouthparts (mimica); white tip scutellum (nigra); and picture wing (differens, heteroneura, picticornis).
GCTTTTGCGCTTTGCCACCTG-3'), resulting in 441 nt of coding sequence. The Sod of D. (S.) adusta was obtained with the previous N primer and a C primer, (5'-CTTGTGCTAGCTGTGTCACCACCCCTGCCCAGATCATC-3'), resulting in 345 nt of coding sequence.

The Adh gene fragments were amplified by PCR, cloned into the pCRII vector from the Invitrogen TA-cloning kit, and sequenced using standard methods (Ausubel et al., 1987), as previously described (Kwiatowski et al., 1994). The 576-nt-long coding fragment from C. procnemis was obtained with primers L3 (5'-GAACTGAAGGCAAT(CT)AATCC(AC)AA-3'), derived from a conserved protein region ELKAINPK, corresponding to the beginning of exon 2, and R1 (5'-TTAGATGCC(GC)GA(AG)TCCCA(AG)TG(TC)T-TGGTCCA-3'), coming from the end of exon 3 WSKHWDSGI(STOP). The fragment of D. pictiventris was obtained with a single R2 primer, shorter than R1 by six bases from the 3'-end, by adjusting the temperature to 48°C, which produced a fragment starting from the end of intron 1 and ending at the end of exon 3 (642 nt of coding DNA). For sequencing, in addition to the PCR primers, standard M13 Uni and Rev primers were used, as well as primers specific for Adh IL (5'-GTGAC(CT)GG(CT)TT(CT)ATGCCAT-3') and IR (5'-ATGGCATT(AG)AA(AG)CC(AG)GTCAC-3').

Sequence Analysis

Neighbor-joining (NJ) trees (Saitou and Nei, 1987) were obtained with the MEGA 1.0 program (Kumar et al., 1993). Trees of maximum parsimony (MP) (Fitch, 1971) and maximum likelihood (ML) (Felsenstein, 1981) were obtained and tested according to Templeton (1983) and Kimura and Hasegawa (1989), respectively, using the PHYLIP 3.572c package programs DNAPARS and DNAML (Felsenstein, 1989). We give equal weights to all sites in MP trees and use empirical base frequencies and a transition/transversion ratio of 2 in the ML calculations. No saturation was observed for the third codon position for Adh and Sod genes (Tatarenkov et al., 1999) or for synonymous nucleotide replacement in the Gpdh gene (Ayala et al., 1996). Kimura's (1980) two-parameter distances were used for constructing NJ trees. Using other distance measures for NJ trees or changing the transition/transversion ratio in ML trees did not affect the topologies in any substantial way. Our alignments of the coding sequences are available online at http://www.bot.uw.edu.pl/~jmkwiat/aln.html or upon request from the first author. The bootstrap values are based on 1000 replications. The SEQBOOT, DNAPARS, and CONSENSE programs of PHYLIP were used to achieve this for the MP trees. We consider Scaptodrosophila as an outgroup for the Drosophila species used in this paper. There is ample evidence, both molecular (Kwiatowski et al., 1994, 1997; Remsen and DeSalle, 1998; Russo et al., 1995; Tatarenkov et al., 1999) and morphological (Grimaldi, 1990), that this is the case with respect to Drosophila but not to Chymomyza.

RESULTS

Figure 1 shows a neighbor-joining (NJ) tree of Adh, which includes sequences available in the literature

![NJ tree with Kimura two-parameter distances for 21 Adh sequences. Numbers above branches are percentage bootstrap values based on 1000 replications.](http://www.bot.uw.edu.pl/~jmkwiat/aln.html)
plus those of D. (Hirtodrosophila) pictiventris and Chymomyza procnemis obtained in this study. This tree is essentially congruent with the tree obtained previously for 42 Drosophilidae species using similar methods (Russo et al., 1995). As shown in Fig. 1, Scaptodrosophila and Chymomyza are sister clades to a complex clade that includes all other Drosophilidae. The early divergence of Chymomyza and Scaptodrosophila is not herein robust but it has been confirmed in previous molecular analyses (Kwiatowski et al., 1994, 1997; Remsen and DeSalle, 1998; Tatarenkov et al., 1999). The rest of the species split into two clades, one of which includes all species of the subgenus Sophophora (groups melanogaster, obscura, and willistoni). The other clade is composed of the subgenus Drosophila, including the Hawaiian Drosophila (Idiomyia, sensu Grimaldi, 1990), as well as Scaptomyza, Zaprinus, and Hirtodrosophila (D. pictiventris). The bootstrap value supporting this clade is 76%. The maximum likelihood and maximum parsimony methods give identical trees, which differ from the tree in Fig. 1 only in that Zaprinus splits off first and then D. immigrans and D. pictiventris branch off as a pair from the rest of the clade. The monophyly of the clade containing Zaprinus, Hirtodrosophila, Scaptomyza, and the subgenus Drosophila is supported in the MP analysis by 66% occurrences. Our results differ from those of an analysis of a shorter fragment of the Adh gene with fewer species (DeSalle, 1992a), which places Hirtodrosophila as a sister clade to the complex of the Sophophora and Drosophila subgenera. This shorter Adh fragment is not available in GenBank, although it has, apparently, been used in a later analysis that places Hirtodrosophila together with Drosophila s.g. (sensu lato), leaving Sophophora outside (Remsen and DeSalle, 1998, Fig. 2A). Therefore, despite some claims (DeSalle and Grimaldi, 1992; Powell and DeSalle, 1995), the only molecular evidence supporting the position of Hirtodrosophila outside the Drosophila genus seems to come from only one set of mitochondrial sequences (DeSalle, 1992a). The early divergence of the Sophophora subgenus from the rest of the genus Drosophila is, nevertheless, consistent with other results (Kwiatowski et al., 1997; Pelandakis and Solignac, 1993; Remsen and DeSalle, 1998; Russo et al., 1995). This has been, moreover, recently corroborated by the occurrence of a single-codon deletion in Ddc (dopa-decarboxylase) in all Sophophora species but not in any other Drosophila species or Drosophilidae genera (Tatarenkov et al., 1999).

A controversial relationship revealed by the Adh phylogeny is the close association of all Hawaiian Drosophila (Idiomyia), Scaptomyza, and Engiscaptomyza (D. crassifemur) with the rest of the Drosophila subgenus (Thomas and Hunt, 1993; Russo et al., 1995). This particular association is favored by Throckmorton (1975) and by a mtDNA phylogeny (DeSalle, 1992a,b) but has been challenged by Grimaldi (1990). DeSalle (1992a), however, favors the monophyly of the subgenus Drosophila as sister clade to the Hawaiian Drosophila, contrary to Throckmorton (1975) and to the phylogeny in Fig. 1, which shows D. immigrans outside a clade that includes most other Drosophila s.g. species as well as the Hawaiian Drosophila. We have obtained Sod sequences of the two Hawaiian species, D. (S.) adusta and D. mima, as well as D. immigrans, seeking additional evidence on these issues (see below).

Figure 2 displays an NJ tree based on Sod sequences. This tree is very similar to the tree in Fig. 1 and it is identical to the Adh trees obtained with the ML and MP methods, with respect to the species being represented. The Sod tree confirms a close association of Scaptomyza with the rest of the Hawaiian Drosophila (represented here by D. mimica) as well as the association of Hirtodrosophila (D. pictiventris) with D. immigrans, which together with Zaprinus form a clade with the Hawaiian Drosophila, Scaptomyza, and the other species of the subgenus Drosophila. However, the bootstrap values supporting these particular relationships are not satisfactory, which is the same situation obtained with 28S rRNA (Pelandakis and Solignac, 1993) and Gpdh (Kwiatowski et al., 1997). The Sod ML tree differs from the NJ tree in that Zaprinus splits after (D. guttifera (D. immigrans, D. pictiventris)). The MP tree is almost identical to the NJ tree in Fig. 2 but instead of the cluster (D. guttifera (D. immigrans, D. pictiventris)), the MP tree has ((D. guttifera, D. immigrans) D. pictiventris). However, the bootstrap values are even lower than in the NJ tree.

Figure 3 is an NJ tree obtained with the combined sequences. A potential benefit of combining data from several loci when testing phylogenetic hypotheses is that the phylogenetic signal weakly present in some genes becomes amplified (Baker and DeSalle, 1997). This approach improved the recovery of monophyletic groups within Drosophilidae even when incongruent data were combined (Remsen and DeSalle, 1998). The trees in Figs. 1 and 2 are very similar. Moreover, G + C content, codon usage, and Kimura's distances of Sophophora and Drosophila subgenera. This shorter Adh fragment is not available in GenBank, although it has, apparently, been used in a later analysis that places Hirtodrosophila together with Drosophila s.g. (sensu lato), leaving Sophophora outside (Remsen and DeSalle, 1998, Fig. 2A). Therefore, despite some claims (DeSalle and Grimaldi, 1992; Powell and DeSalle, 1995), the only molecular evidence supporting the position of Hirtodrosophila outside the Drosophila genus seems to come from only one set of mitochondrial sequences (DeSalle, 1992a). The early divergence of the Sophophora subgenus from the rest of the genus Drosophila is, nevertheless, consistent with other results (Kwiatowski et al., 1997; Pelandakis and Solignac, 1993; Remsen and DeSalle, 1998; Russo et al., 1995). This has been, moreover, recently corroborated by the occurrence of a single-codon deletion in Ddc (dopa-decarboxylase) in all Sophophora species but not in any other Drosophila species or Drosophilidae genera (Tatarenkov et al., 1999).

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eral similar hypotheses (Fig. 4, Trees 1–3) are, on the basis of the Sod and Adh sequences, equal by the Templeton test criteria, whether the Adh and Sod data are used separately or combined (Table 2). Two other hypotheses with Drosophila s.g. closer to Zaprionus and Hirtodrosophila than to Sophophora have been proposed by Remsen and DeSalle (1998) (Fig. 4, Tree 4) and by Throckmorton (1975) (Fig. 4, Tree 5). The former (Tree 4) is best for the Adh data but is rejected by

FIG. 2. NJ tree with Kimura two-parameter distances for 22 Sod sequences. Bootstrap values based on 1000 replications are above the branches.

FIG. 3. NJ tree with Kimura two-parameter distances for Adh and Sod sequences combined for 15 species. Scaptomyza refers to adusta for Sod but albovittata for Adh. Chymomyza is C. amoena. Bootstrap values based on 1000 replications are above the branches.
Similarly, the phylogenies having *Sophophora* closer to the *Drosophila* s.g. than *Zaprionus* and *Hirtodrosophila*, proposed by Grimaldi (1990), DeSalle (1992a), and Powell and DeSalle (1995) (Fig. 4, Trees 6–8), are all rejected by the Templeton test using the Adh and Sod data either separately or combined (Table 2). The ML tests give similar results.

We further assess the position of *Zaprionus* and *Hirtodrosophila* relative to *Sophophora* and *Drosophila* s.g. using DNA sequences of glycerol-3-phosphate dehydrogenase. Although this protein evolves in an erratic way in the Drosophilidae family, the Kimura genetic distance of nucleotide sequences changes monotonically with time and the phylogeny based on Gpdh sequences (Kwitowski et al., 1997) produces a topology very similar to those of the Sod and Adh trees (Figs. 1–3), although Hawaiian Drosophila, Sceptomyza, and *D. immigrans* are not represented in the Gpdh data set. However, similar to the Sod tree (Fig. 2), the separation of the Hirtodrosophila, *Zaprionus*, and *Drosophila* s.g. cluster from *Sophophora* is not supported by high bootstrap values (Kwitowski et al., 1997).
Figure 5 shows an NJ tree obtained with the combined sequences of Sod and Gpdh. The set of species belonging to the s.g. Drosophila, Zaprionus, Dorsilopha (D. busckii), and Hirtodrosophila (D. pictiventris) form a well-supported clade (86%), much better than when the Sod (Fig. 2, 49%) or Gpdh (Kwiatowski et al., 1997) (54%) sequences are used separately. The monophyly of the s.g. Sophophora is not well supported, which may be attributed to the deep division between the willistoni group and the melanogaster + subobscura group of species (Kwiatowski et al., 1994, 1997; Tatarenkov et al., 1999). However, the outgroup status of Chymomyza and Scaptodrosophila relative to the rest of Drosophilidae is very well supported (95%), although the sequence of branching of the two genera remains unresolved. Although the sequences of two Adh genes are known for the medfly Ceratitis, they are very distinct from the Drosophila Adh. We, therefore, have not used herein the Ceratitis Adh genes as outgroups that might resolve the branching sequence of Chymomyza and Scaptodrosophila (Fig. 6). However, combining the Adh, Sod, and Gpdh sequences increases bootstrap support for a clade composed of Zaprionus, Hirtodrosophila, and Drosophila s.g., represented here by the virilis/repleta group, to a very high value of 97%. Because not all gene sequences are available for all Drosophila species, combining the data results in a decrease of the species of the Drosophila s.g. in the sample. Additional work is therefore required in order to establish the position of Zaprionus and Hirtodrosophila relative to other Drosophila s.g. species. However, in the light of evidence presented here, the sister status of Sophophora relative to a clade composed of Zaprionus, Hirtodrosophila, and Drosophila s.g. seems to be firmly established.

**DISCUSSION**

The Adh sequences that we have analyzed manifest a deep split between two sets of taxa within the genus Drosophila. One clade includes the subgenus Sophophora (melanogaster, obscura, and willistoni groups). The second clade includes the other Drosophila subgenus but also species traditionally classified in separate genera, namely Scaptomyza and Zaprionus. Within this second clade there are three taxa that split first from the rest of the clade. These taxa are Zaprionus, Hirtodrosophila (D. pictiventris), and D. immigants. This last species is traditionally included with the subgenus Drosophila, which is in the Adh phylogeny shown to be paraphyletic (as had been proposed by Throckmorton, 1975). The remainder of the species in the second clade form a well-defined monophyletic group consisting in turn of two well-defined clades. One clade includes the Hawaiian Drosophila (the group's modified mouthparts, white tip scutellum, and picture wing, as well as Engiscaptomyza) and Scaptomyza, which is thought to have originated in Hawaii, although it includes species endemic elsewhere. The other set includes two species groups traditionally included within the subgenus Drosophila, namely virilis and repleta. It has been proposed that the Adh gene is particularly useful in providing robust Drosophilidae phylogenies (Thomas and Hunt, 1993; Russo et al., 1995; Powell, 1997). This is corroborated by our analysis.

There are a variety of reasons why a gene phylogeny, whether robust or not, may not well represent a species phylogeny (Brower et al., 1996). Confidence, however, will tend to increase when separate unrelated genes yield similar phylogenies. The two genes that we have investigated, Sod (Fig. 2) and Gpdh (Kwiatowski et al., 1997), separately or in combination with each other (Fig. 5) or with Adh (Figs. 3 and 6), consistently resolve certain phylogenetic relationships: (1) the outgroup status of Chymomyza and Scaptodrosophila relative to other Drosophilids (which are included within the genus Drosophila—sensu lato, Table 1); (2) the subgenus Sophophora as a sister clade to all other Drosophila taxa; (3) the early branching of Zaprionus, Hirtodrosophila, and D. immigants relative to the remainder of the Drosophila clade; (4) the monophyly of all Hawaiian Drosophila, including the Hawaii-originated Scapto-
myza; and (5) the monophyly of the repleta and virilis groups of the subgenus Drosophila.

The monophyly of the clade encompassing all Drosophila other than Sophophora (i.e., the lineages comprised by (3)–(5) above) is supported by other studies, such as Kwiatowski et al. (1994, Sod), Kwiatowski et al. (1997, Gpdh), and by other genes, such as Ddc (Tatarenkow et al., 1999) and domains D1 and D2 of the 28S rRNA (Pélandakis and Solignac, 1993). The clade Hirto-
drosophila/ D. immigrans ((3) above), which had been proposed by Throckmorton (1975), is not statistically supported by Adh alone but it also appears in the Sod phylogeny (Fig. 2) and is strongly supported (88%) by Adh and Sod combined (Fig. 3). The combination of Sod and Gpdh (Fig. 5) provides strong statistical support (86%) for the monophyly of the complex clade that

![Figure 5](image_url)

**FIG. 5.** NJ tree with Kimura two-parameter distances for Gpdh and Sod sequences combined from 18 species. Bootstrap values based on 1000 replications are above the branches.

![Figure 6](image_url)

**FIG. 6.** NJ tree with Kimura two-parameter distances for Adh, Gpdh, and Sod sequences combined from 11 species.
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includes all the subgenera of Drosophila (sensu lato) other than Sophophora, as well as the monophyly of the whole genus (95%) in the inclusive sense that we are using in this paper.

The Adh and Sod data alone, as well as both combined, are sufficient to reject several phylogenetic hypotheses that have been proposed, namely those of Throckmorton (1975), Grimaldi (1990), DeSalle (1992), and Powell and DeSalle (1995); see Fig. 4 and Table 2. The hypothesis is not rejected by low margin by Adh data but is rejected by Sod and Adh + Sod data. The first three topologies shown in Fig. 4, however, are not statistically differentiable. Figure 7 shows a consensus tree that summarizes the data available for all three genes, Adh, Sod, and Gpdh (Dorsilopha is not represented in the Adh data, nor Hawaiian Drosophila, Scaptomyza and D. immigrans in the Gpdh data, Table 1). This phylogeny is consistent with the "total evidence" hypothesis of Remsen and DeSalle (1998) (Fig. 4, Tree 4), with one exception. The Remsen and DeSalle (1998) hypothesis places Hirtodrosophila (D. pictiventris) as a sister clade to (s. g. Drosophila, Hawaiian Drosophila), whereas we show D. immigrans as monophyletic with Hirtodrosophila, an association that has 88% bootstrap support when the Adh and Sod data are combined (Fig. 3). The remaining ambiguities in Fig. 7 concern the order of divergence between Chymomyza and Scaptodrosophila and between some Drosophila clades.

The phylogeny of Fig. 7 is also consistent with the results of Thomas and Hunt (1993), Peλandakis and Solignac (1993), Kwiatkowski et al. (1994, 1997), Russo et al. (1995), and Tatarenkov et al. (1999) but not with mitochondrial DNA phylogenies (DeSalle, 1992a,b). It has been argued that mitochondrial DNA better reflects species phylogeny than nuclear genes (DeSalle and Giddings, 1986; Moore, 1995). The results of this study do not warrant such claims, at least regarding Drosophila phylogeny, since the weight of evidence suggests otherwise.

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