

A supertree analysis and literature review of the genus *Drosophila* and closely related genera (Diptera, Drosophilidae)

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In the 17 years since the last familywide taxonomic analysis of the Drosophilidae, many studies dealing with a limited number of species or groups have been published. Most of these studies were based on molecular data, but morphological and chromosomal data also continue to be accumulated. Here, we review more than 120 recent studies and use many of those in a supertree analysis to construct a new phylogenetic hypothesis for the genus *Drosophila* and related genera. Our knowledge about the phylogeny of the genus *Drosophila* and related genera has greatly improved over the past two decades, and many clades are now firmly supported by many independent studies. The genus *Drosophila* is paraphyletic and comprises four major clades interspersed with at least five other genera, warranting a revision of the genus. Despite this progress, many relationships remain unresolved. Much phylogenetic work on this important family remains to be done.

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Introduction

The genus *Drosophila* is of great interest to biologists because of our vast knowledge of the biology of many of its members (~1500 species; Bächli 1999-2008). Much of this knowledge stems from the century-long study of the model organism *Drosophila melanogaster*; but a large number of other drosophilid taxa have been subjects of genetic, evolutionary, and ecological research. The interpretation of this wealth of information is greatly aided by modern comparative methods (see, e.g., Pitnick et al. 1999; Kopp & True 2002; Zhang et al. 2003b; Prud'homme et al. 2006). The quality of the conclusions that can be drawn from comparative analyses depends on having a good estimate of the evolutionary history of the taxa involved. Unfortunately, even a cursory examination of the literature reveals that many aspects of drosophilid phylogeny are controversial or poorly

studied (Ashburner et al. 2005; Markow & O'Grady 2006).

Grimaldi's (1990) phylogeny, based entirely on morphological characters, is the most recent comprehensive family-wide treatment. An important competing phylogenetic hypothesis is that of Throckmorton (1975), which differs from it in many respects. Throckmorton's work was clearly based on many sources of evidence (see, e.g., Throckmorton 1962, 1965, 1966), but he failed to make the basis for much of his classification sufficiently explicit. More recently, many fragmentary phylogenetic hypotheses based on molecular data have been published (see Table 1 for the most important studies). Some aspects of the phylogeny, such as relationships within the *melanogaster* species subgroup (see Coyne et al. 2004; Pollard et al. 2006), now seem robustly supported by analysis of large molecular data sets, but comparing

Table 1. Studies particularly important in forming the conclusions presented here. The order of citations is based on the part of the tree about which the study is most informative.

Study	Number of genera ^a			Number of species ^a			Completeness ^c	Method	Variance in rate?	Confidence
	Groups within Siphophora ^a			Number of species ^a						
	Groups within virilis-repleta ^a			Genes ^b						
	Groups within immigrans-tripunctata ^a			Groups within Idiomyia ^a						
	Groups within Siphophora ^a			Number of species ^a						
(1) Molecular studies that cover a wide range of genera, subgenera, and species groups										
Davis et al. (2000)	8	3	1	3	19	fru	full	NJ	no	no
DeSalle (1992)	8	3	3	3	0	EcoRI	full	MP	yes	no
Da Lage et al. (2007)	12	4	9	9	2	Amyrel	full	NJ, MP, Bayesian	yes	bootstrap, posterior
Gailey et al. (2000)	8	3	1	1	3	fru	full	NJ	no	no
Kwiatowski et al. (1997)	7	4	2	0	0	Gpdl	full	NJ, JC, (MP, ML)	no	bootstrap
Kwiatowski & Ayala (1999)	10	4	2	2	3	Adh, Gpdl, Sod	23, 17, 23	NJ, K2P, MP equal, ML HKY	no	bootstrap
Pélandakis & Solignac (1993)	11	5	10	8	0	28S	full	NJ, MP	no	bootstrap
Remsen & DeSalle (1998)	9	3	4	3	2	16S, 28S, Adh, Sod, Mor	?	MP	no	bootstrap, Bremer
Remsen & O'Grady (2002) ^d	20	4	7	13	2	46 16S, 28S, Adh, COII, Sod, Mor	46, 46, 24, 36, 24, 33	MP	no	bootstrap, Bremer
Russo et al. (1995)	7	3	1	1	3	Adh	full	NJ, MP, ME	no	bootstrap, CP
Tarrio et al. (2001)	8	3	2	0	1	13 Adh, Ddc, Gpdl, Sod, Xdh	10, 11, 10, 11, 11	NJ T92+Γ+GC; NJ GTR+Γ	yes	bootstrap, Likelihood of trees
Tatarenkov et al. (1999)	12	3	1	2	1	23 Adh, Ddc, Gpdl, Sod	12, 23, 10, 13	NJ, JC, MP	no, but partition positions	bootstrap
Tatarenkov et al. (2001)	11	3	3	5	2	32 Adh, amd, Ddc, Sod	18, 29, 21, 13	NJ, JC, MP	no, but partition positions	bootstrap
Thomas & Hunt (1993)	5	2	1	0	3	11 Adh	full	MP, ML (unnamed)	yes	bootstrap
(2) <i>Lordiphosa/Diclaetophora</i> studies										
Hu & Toda (2001)	13	2	3	3	0	41 Morphological	full	MP	N/A	bootstrap
Katoh et al. (2000)	15	4	4	3	3	81 Adh	full	ME (JC), MP	No	bootstrap
(3) Subgenus <i>Siphophora</i>										
O'Grady & Kidwell (2002)	1	5	0	0	0	75 28S, Adh, COII	37, 52, 55	ML GTR(?)H+Γ, MO, ME	yes	bootstrap

Author	Year	1	4	0	0	0	79	not-specified	N/A	MP, ML GTR(?) + Γ, Bayesian	yes	bootstrap, posterior
Prud'homme et al.	(2006)	1	4	0	0	0	17	Xdh	full	ML GTR + C (NJ, MP)	yes	Quartet—puzzling support values
(4) Subgenus <i>Sophophora</i>: <i>melanogaster</i> group												
Coyne et al.	(2004)	1	1	0	0	0	9	Review of <i>melanogaster</i> subgroup	full	NJ, MP	yes	bootstrap
Goto et al.	(2000)	1	2	0	0	0	14	COI, Gpdh	full	NJ logDet, MP, ML HKY + Γ	yes	bootstrap
Goto & Kimura	(2001)	1	2	0	0	0	22	COI, Gpdh	full	NJ, Fitch Margoliash, MP, ML	no	bootstrap
Inomata et al.	(1997)	2	2	1	0	0	23	Amy	full	MP equal, Bayesian	yes	bootstrap
Kastanis et al.	(2003)	1	1	0	0	0	18	ND1	full	ML GTR + Γ	yes	Bayesian
Kopp & True	(2002)	1	1	0	0	0	21	28S, Amy, COI, Gpdh, kl3, ND1	14, 16, 18, 18, 8, 12	Only Amy showed different codon usage	bootstrap	
Kopp	(2006)	1	2	0	0	0	12	Adh, Amy, Amyrel, COI, full COII, Ddc, esc, Gpdh, H2s, hb, ksr, Pgi, Tpi, Xdh	ML GTR + Γ	yes	bootstrap	posterior
Lewis et al.	(2005)	1	3	0	0	0	43	COI, COII	41, 38	ML GTR + Γ	yes	bootstrap
Schawaroch	(2002)	1	2	0	0	0	49	Adh, COII, hb	full	MP	no	bootstrap, Bremer
Yang et al.	(2004)	1	2	0	0	0	38	H2A-H2B	full	NJ, MP, ML GTR + Γ	yes	bootstrap
Zhang et al.	(2003)	2	2	1	0	0	48	Amy	full	ML K81uf + Γ, Bayesian GTR + Γ + I	no	bootstrap
(5) Subgenus <i>Sophophora</i>: <i>obscura</i> group												
Barrio & Ayala	(1997)	1	2	0	0	0	15	Gpdh, Sod	15, 14	NJ, MP, ML	no	bootstrap
Gao et al.	(2007)	1	2	0	0	0	29	ND2, COI, COII, Cyt-b, Adh, 28S	23, 24, 29, 25, 24, 24	MP, ML GTR + Γ + I, Bayesian	yes	bootstrap
Gleason et al.	(1997)	1	2	0	0	0	18	16S, COI, COII, cyt-b, ND1, ND5	16, 17, 11, 16, 16, 16	NJ, MP	yes	posterior
O'Grady	(1999)	1	2	0	0	0	24	16S, 28S, 5S, Adh, COI, COII, cyt-b, Gpdh, ND1, ND5, Sod	16, 14, 10, 13, 16, 18, 16, 15, 16, 16, 14	NJ, MP, ML GTR(?) + Γ	yes	bootstrap, Bremer
(6) Subgenus <i>Sophophora</i>: <i>saltans</i> group												
O'Grady et al.	(1998)	1	2	0	0	0	11	Adh, COI, COII, ITS1	full	NJ, MP, ML, majority rule	yes	bootstrap, Bremer
Rodriguez-Trelles et al.	(1999)	1	3	0	0	0	9	28S, Adh, COI, COII, ITS1, Xdh	6, 9, 7, 7, 7, 9	ML GTR + CdG _{rak} , various others (NJ)	yes	bootstrap

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Table 2. Studies used for the supertree analysis. Semicolons separate individuals trees; multiple genes combined in one tree are combined with +'s. Weighting factors are equivalent to the number of genes in the tree, unless indicated between parentheses. Abbreviations as in Table 1.

Baker & DeSalle 1997	16S; ACHE; Adh; COII; COIII; hb; ND1; wg
Beverly & Wilson 1982	LHP
Bonacum 2001 (<i>Scaptomyza</i>)	16S + Adh + COI + COII + Gpdh
Bonacum 2001 (Hawaiian overview)	16S + Adh + COI + COII + Gpdh
Brncic et al. 1971	Chromosome
Carrasco et al. 2003	COII
Clark et al. 2007	Many (5)
Da Lage et al. 2007	Amyrel
Diniz & Sene 2004	Chromosome
Durando et al. 2000	16S; COII; Ef1 α ; hb; ND2
Flores et al. 2008	cac + sc; COI + COII
Gailey et al. 2000	Fru
Gao et al. 2007	ND2 + COI + COII + Cyt b + Adh + 28S
Gleason & Powell 1997	per
Gleason et al. 1997	COI + COII + cyt b + ND1 + ND5 + 16S
Gleason et al. 1998	COI
Goto & Kimura 2001	COI; Gpdh
Haring et al. 1998	P-element + Adh
Harr et al. 2000	microsatellite
Hu & Toda 2001	Morph
Kambysellis et al. 1995	yp1
Kaneshiro et al. 1995	chromosome
Kastanis et al. 2003	ctb + tRNA-Leu + tRNA-ser + NADH + 16S
Katoh et al. 2000	Adh
Katoh et al. 2007 ^a	Adh; Gpdh
Ko et al. 2003	Adh + Adhr + Gld + ry
Kopp 2006	COII; esc; H2s; hb; ksr; Pgi; Tpi; Xdh
Kopp & True 2002	28S; Amy; COI; Gpdh; kl3; ND1
Lakovaara & Saura 1982	Gpdh
Lathe & Eickbush 1997	R2
Lee & Song 1991	morphology
Lee et al. 1990	morphology
Lewis et al. 2005	COI + COII
Manfrin et al. 2001	COI
Morán & Fontdevila 2007	COI + COII + COIII + Xdh
Narayanan 1973	chromosome
O'Grady 1999	Sod; Gpdh; 16S; 28S; Adh; COII ; cyt-b; ND1; ND5
O'Grady & Kidwell 2002	28S; Adh; COII
O'Grady & Zilversmit 2004	COII + sia + glass + l(2)not-1 + Marf + Rpt4 + ITS-1 + snf; morphology
Oliveira et al. 2005	Nadh2 + COI + COII + 16S/12S (1) ^b
Pélandakis & Solignac 1993	28S D1 + 28S D2
Perlman et al. 2003	COI/COII/COIII
Pissios & Scouras 1993	mtDNA
Prud'homme et al. 2006	Various (6)
Remsen & DeSalle 1998	COII
Remsen & DeSalle 1998	Sod
Remsen & O'Grady 2002	16S; Adh; Mor (Grimaldi reanalysed)
Robe et al. 2005	Amd; COII
Rodriguez-Trelles et al. 2000a	COI; COII; COIII; Xdh
Rodriguez-Trelles et al. 2000b	Xdh
Rodriguez-Trelles et al. 2000c	Sod
Russo et al. 1995	Adh
Schawaroch 2002	Adh + hb + COII

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Silva & Kidwell 2000	Adh
Silva-Bernardi et al. 2006	COI
Pitnick et al. 1999 (repleta)	Chromosomal data of Wassermann and sequences (28S and CO?)
Stalker 1966; Stalker 1972	Chromosomal
Tamura et al. 1996	Adh
Tarrio et al. 2000	Xdh
Tarrio et al. 2001	Adh + Ddc + Gpdh + Sod + Xdh
Tatarenkov & Ayala 2001	amd; Ddc
Tatarenkov et al. 2001	amd + Ddc + Adh + Sod
van der Linde et al. accepted (flexa)	Adh + Amyrel + COI + COII + COIII + per + 16S + Ddc + Sod + yp1 + 28Sd1 + 28Sd2 + 28Sd8 (5)
van der Linde et al. accepted (quadrilineata)	Adh + Amyrel + COI + COII + COIII + per + 16S + Ddc + Sod + yp1 + 28Sd1 + 28Sd2 + 28Sd8 (5)
Wang et al. 2006	Adh; ND2 + COI
Yang et al. 2004	H2A-H2B
Yotoko et al. 2003	COII
Zhang et al. 2003a	Amy1; Amy3

^aDr. Katoh kindly provided the trees for both genes.

^bWeighted 1 because of the substantial overlap with other studies.

other results of these studies shows that they differ in many, perhaps even most, key aspects.

The many available partial studies contain a wealth of information on the phylogenetic history of the genus *Drosophila* and related genera. Our objective is to review primarily those studies that have been produced since the last family-wide phylogeny, by Grimaldi (1990), and to summarize the relevant information. Summaries for many groups have been provided by Markow & O'Grady (2006), but they do not synthesize the phylogenetic relationships at the higher levels that will be the focus of this review. Although this is the era of molecular studies, our review is not limited to molecular studies; cladistic studies using morphological characteristics or chromosomal data are often remarkably consistent with those based on molecular data (O'Grady et al. 2001a; Diniz & Sene 2004). Bringing together so many studies will provide an up-to-date overview of our knowledge on the phylogenetic history of the genus *Drosophila* and related genera. We realize that, despite the large amount of data currently available, ours will be far from the last word on all aspects of the phylogeny of the family Drosophilidae.

Here, we present an overview of the phylogenetic literature with an emphasis on the recent publications. We used a formal algorithmic approach, called the supertree method, to obtain a consensus phylogeny. In a supertree analysis, the trees obtained from previous analyses (source

trees) are themselves analysed (see Sanderson et al. 1998 for an introduction). The supertree method takes the existence of a particular clade in a published source, rather than the character states, as the data for analysis. Our supertree analysis of 117 trees (Table 2), covering 624 species, resulted in a well-resolved phylogenetic tree.

General problems

We note at the outset several problems with the available literature. Virtually all workers agree that the genus *Drosophila* is paraphyletic, yet most of the taxonomic literature is focused on the species currently included in the genus. As a result, many potential members of the more inclusive clade that includes *Drosophila*, such as *Hirtodrosophila* and *Zaprionus*, are rarely included in phylogenetic studies. Different studies tend to use different, very small, and arbitrary sets of species to represent established genera, species groups, or other taxonomic levels. Evidence suggests that several other traditional taxa are paraphyletic or polyphyletic as well, including *Hirtodrosophila* and *Scaptodrosophila* (Bächli et al. 2004).

We must also address some terminology. The terms species group and species subgroup have no official status among taxonomists but have had a long tradition of use among *Drosophila* taxonomists since they were erected over 50 years ago (Hsu 1949; see chapter 33 of Ashburner et al. 2005 for more discussion). In some cases, these groupings do not stand up to modern analysis, but we

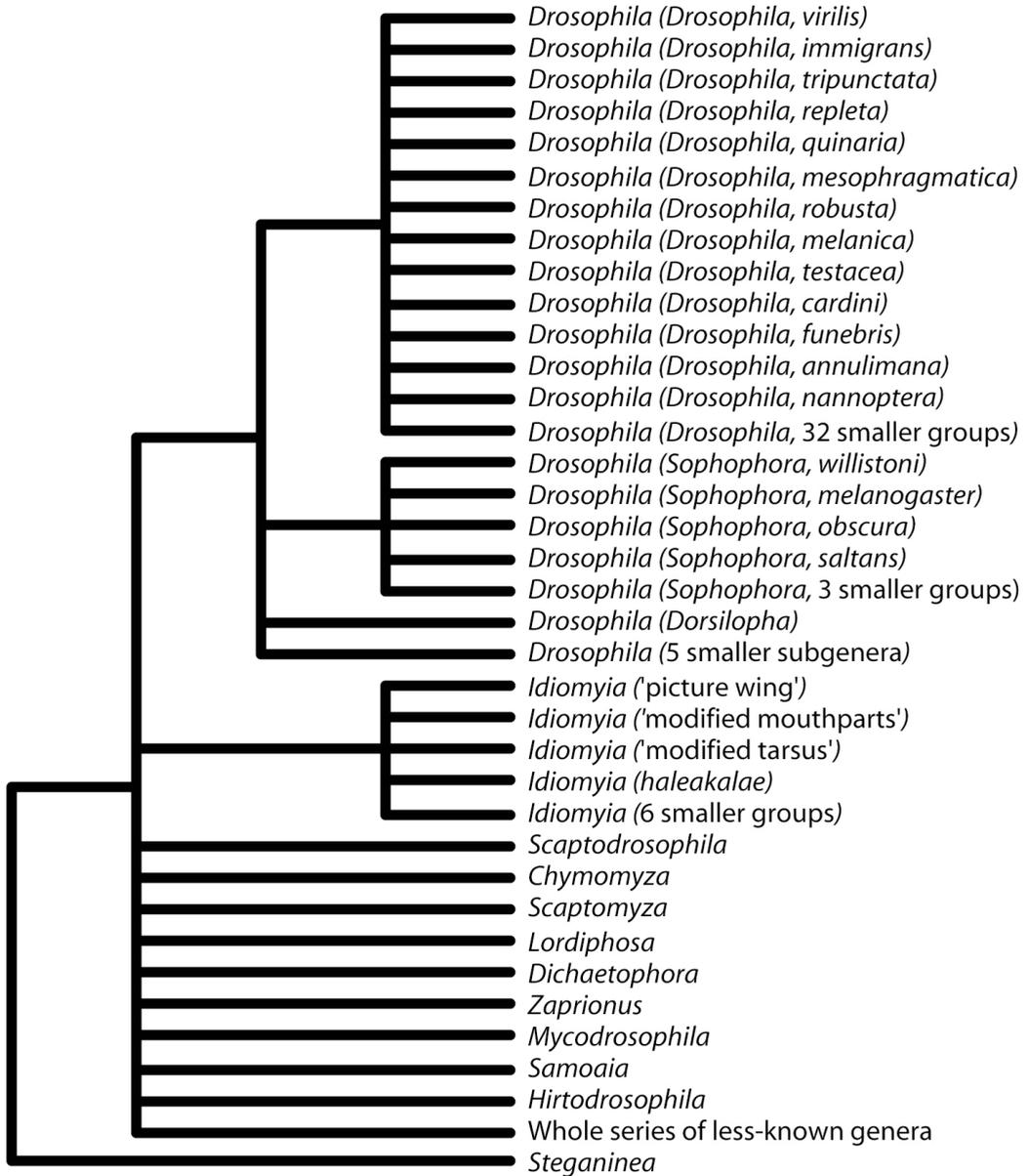


Fig. 1. A tree of the genera, subgenera, and major species groups under consideration in the present article. The taxonomic organization in this figure is based on the database of Bächli (1999-2008, <http://taxodros.unizh.ch>), which presents a full overview of the current taxonomic status of the family Drosophilidae. The levels represent subfamilies, genera, subgenera, and species groups. Subdivisions within the genera appear in parentheses; the first term indicates the subgenus, the second the species group (cf. International Commission on Zoological Nomenclature 1999).

will use the terms group and subgroup as a convenient shorthand for reference to clades. In doing so, we do not mean to imply the acceptance of any

particular classification. Fig. 1 gives a summary of the phylogeny implied by the current classification (Bächli 1999-2008), but the classification in

TaxoDros is conservative and has only changed when it has stabilized (G. Bächli, pers. comm.). Table 1 is a list of the most important articles we have reviewed, plus a summary of the data and methods the authors used. The number of species we list in the text for a group is based on the online database of Bächli (1999–2008) unless marked otherwise. Taxonomic authorities are listed in Bächli's (1999–2008, <http://taxodros.unizh.ch>) up-to-date database of the Drosophilidae of the world.

Molecular phylogenetics has been very dynamic (see Swofford et al. 1996 for a detailed introduction), and methods have evolved greatly over the last decades, partly as a result of the availability of stronger desktop computers, which permit use of more computational intensive methods. The earlier studies we review used less computer-intensive methods based on distance or parsimony, whereas more recent studies have tended to use maximum-likelihood or Bayesian methods with complex models of nucleotide substitution. Variation between clades in the nucleotide substitution rates can result in incorrect topologies when a single model is assumed to be correct across the tree (Steel et al. 1993; Lockhart et al. 1994; Galtier & Gouy 1995; Tourasse & Li 1999). Nucleotide variation is present within at least some genes within the family Drosophilidae (e.g., Clark et al. 2007). When nucleotide variation is present in the data, addressing the variation can lead to different conclusions about the topology (see, e.g., Moriyama & Hartl 1993; Tarrío et al. 2001).

Supertree methods

Our goal was to construct a supertree for the genus *Drosophila* and related genera. For that purpose, we collected 117 source trees (Table 2). Several potential pitfalls limit the use of the supertree method (see, e.g., Gatesy et al. 2002; de Queiroz & Gatesy 2007). Several of these are particularly problematic for the literature on the Drosophilidae. First, most studies depend on reanalysis of some of the same data as previous studies, a violation of the assumption of independence. Second, most studies have relatively poor and haphazard taxon sampling, so overlap (use of the same taxon) between the species sampled in different studies is often narrow or nonexistent, a circumstance under which supertree (as well as supermatrix) methods perform poorly (Bininda-Emonds & Sanderson 2001). In addition, the many choices that must be

made about inclusion/exclusion of trees and about the relative weighting of the trees and the nodes within them could make the exercise highly subjective. The first issue related to the reuse of data can be addressed by selective inclusion of those trees such that the reuse of data is minimized. We have therefore excluded some trees that added substantially less to the taxon sampling than did other studies. For example, we omitted several trees in the paper by Kopp (2006), as the same data, with a larger taxon sampling, was already used in an earlier study (Kopp & True 2002). This decision resulted in the exclusion of some species. The second issue proved more serious for our analysis. We used 117 trees (Table 2), covering about 623 species. Of those, 185 species were only included in a single tree, 141 in two trees, and 75 in three trees. Species included in a single tree can be placed on any node of the supertree between the sister taxa of the source tree (Fig. 2). Similarly, a species included in two trees can be placed at each node between the two source tree positions.

Trees based on units that were composed of two or more species (e.g., Remsen & O'Grady 2002) were excluded because they effectively represent higher taxonomic units that cannot be matched with a single species. All included trees were coded using Mesquite (Maddison & Maddison 2004) with standardized species names and relevant subgroup indications. Weighting factors were assigned according to the number of genes on which each tree was based. An exception was made when the average number of genes per species was considerably lower than the total number of genes used; in that case the average number was used. The tree based on the 12-genome analysis (Clark et al. 2007) was based on a very large number of genes, and the weighting factor for this tree was set to five. A higher weighting factor would not have affected the outcome, as the 12-genome tree was equivalent to the supertree topology. Trees based on morphology, chromosomes, and other nonmolecular sources were assigned a weighting factor of one. All outgroup species not belonging to the family Drosophilidae were marked as 'outgroup', as various distant but unrelated species have been used in various studies.

We generated the MRP-data using Mesquite. A nexus-file with the included trees and the data matrix is available in the supplementary material. The data analysis was performed with PAUP* (Swofford 2002). We generated starting trees either

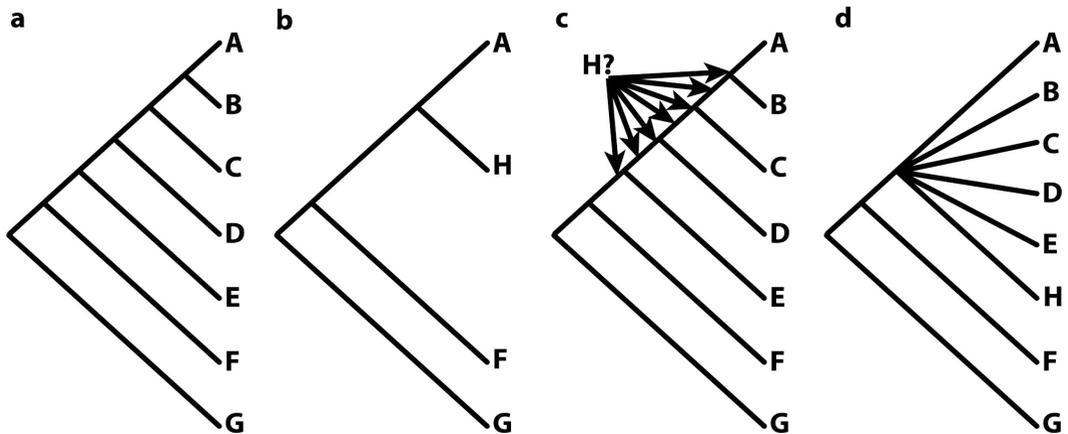


Fig. 2. The effect of a species included in a single tree. a and b: Source trees. c: The potential locations of species H. d: The consensus tree.

by sequentially adding species to the tree on the basis of closest match or by using starting tree in which species were grouped hierarchically by taxonomic classification (Bächli 1999-2008). The data were analysed with and without weighing of the data. All analyses resulted in more than 20,000 trees.

Phylogeny

The supertree analysis of the weighted data resulted in a well-resolved tree. Of the 623 species, 297 that were present in at least three trees were used for the analysis. One species, *D. sternopleuralis* (*histrion* species group) had a disproportionate effect on the resolution within the *immigrans-tripunctata* radiation and was excluded from the analysis. An overview of the whole strict consensus tree is presented in Fig. 3. Details of the tree are presented in Fig. 4 (subgenus *Sophophora*), Fig. 5 (*immigrans-tripunctata* radiation, subgenus *Dorsilopha* as well as *Zaprionus*, *Liodrosophila*, and *Samoia*), Fig. 6 ('Hawaiian *Drosophila*', *Scaptomyza*, *Hirtodrosophila*, and *polychaeta* group), and Fig. 7 (*virilis-repleta* radiation and subgenus *Siphodora*).

The discussion below works its way from the base of the tree upward. Each section addresses the current status of the clade and discusses the results of the supertree analysis in that context.

Family Drosophilidae. – The division of the family Drosophilidae (3750 species; Bächli 1999-

2008) into two subfamilies (Fig. 1), Steganinae and Drosophilinae (Hendel 1917), has been followed by many authors (Duda 1924; Throckmorton 1962, 1965, 1975; Okada 1989; Grimaldi 1990; Sidorenko 2002), but no single morphological character distinguishes the two (see Ashburner et al. 2005 for discussion). The only molecular study incorporating several genera of the Steganinae (Remsen & O'Grady 2002) suggests, on the basis of a sample of four genera of steganines and 18 of drosophilines, that both Steganinae and Drosophilinae are monophyletic.

Subfamily Drosophilinae. – The last monograph covering the whole subfamily Drosophilinae was by Grimaldi (1990). Unfortunately, most molecular analyses include only those genera closely related to *Drosophila* (all belonging to the infra-tribe Drosophilini), so we restrict our coverage to these. This decision is reflected in the genera included in the supertree analysis for which sufficient material was available. The literature (discussed below under the various subgenera) suggests that many genera are located within the subgenus *Drosophila* (including *Hirtodrosophila*, *Mycodrosophila*, *Zaprionus*, *Samoia*, *Liodrosophila*, *Scaptomyza*, and *Dichaetophora*) or within the subgenus *Sophophora* (including *Lordiphosa*). This suggestion was confirmed in the supertree analysis for the included genera. We refer to the genus *Drosophila* and its included genera as *Drosophila sensu lato*.

The genera *Scaptodrosophila* and *Chymomyza*

are generally placed basal to the genus *Drosophila* s.l. (Okada 1963; Throckmorton 1975; Grimaldi 1990; DeSalle 1992a; Kwiatowski et al. 1994, 1997; Remsen & DeSalle 1998; Kwiatowski & Ayala 1999; Tatarenkov et al. 1999; Hu & Toda 2001; Da Lage et al. 2007; Katoh et al. 2007), as was reflected in the basal placement in the supertree analysis (Fig. 4). These two genera have an extra intron in the superoxide dismutase (Sod) gene, just as does the species *Ceratitis capitata*, which is sometimes used as an outgroup for *Drosophila*. *Drosophila* and *Zaprionus* lack this intron (Kwiatowski et al. 1994). The genus *Scaptodrosophila* is characterized by three katepisternal setae almost equal in length and a pair of enlarged prescutellar setae (acrostichal hairs), a trait common to many species of the subfamily Steganinae (see Ashburner et al. 2005).

Our results accord with those of Tarrío et al. (2001) who suggested, on the basis of a study of almost 5000 bp of sequence spread over five nuclear genes, that *Scaptodrosophila* diverged before *Chymomyza*. They noted the wide variation in nucleotide composition among the major groups

and implemented an analysis that accounted for this variation and resulted in a well-supported topology. This topology is also supported by other studies using morphological (Okada 1963; Hu & Toda 2001) and molecular data (DeSalle 1992a; Kwiatowski et al. 1994, 1997). Many other studies were unable to resolve this node but were not in conflict with this topology (Throckmorton 1975; Grimaldi 1990; Remsen & DeSalle 1998; Kwiatowski & Ayala 1999; Tatarenkov et al. 1999; Katoh et al. 2007) or did not reach a firm conclusion in favour of either topology (Da Lage et al. 2007). The support for an alternative topology favoured by Remsen & O'Grady (2002) was low and likely to be an artefact of long-branch attraction.

Genera Lordiphosa and Dichaetophora. – The *tenuicauda* species group of the genus *Lordiphosa* was recently revised as the genus *Dichaetophora* (Hu & Toda 2002) after molecular (Katoh et al. 2000) and morphological (Hu & Toda 2001) analyses showed that *Lordiphosa* s.s. is closely related to the subgenus *Sophophora*, whereas the species in the *tenuicauda* group are more closely

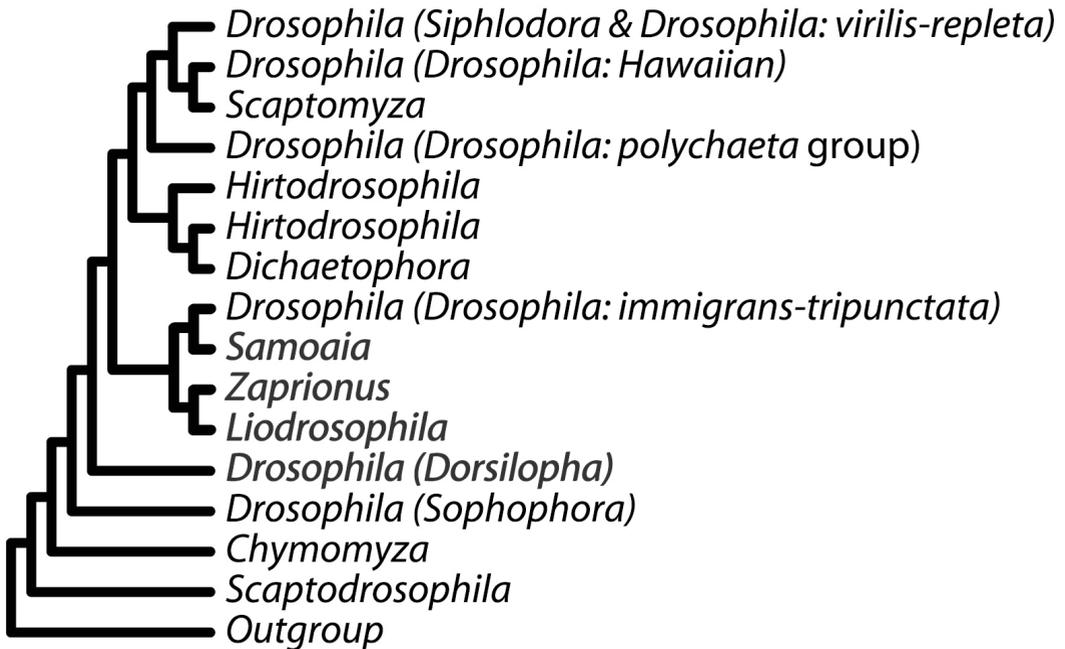


Fig. 3. Supertree phylogeny: overview of genera, subgenera in the genus *Drosophila*, and major clades within the subgenus *Drosophila*.

related to *Hirtodrosophila*. The placement of the *Dichaetophora* close to *Hirtodrosophila* was confirmed in our supertree analysis (Fig. 6). Older studies (Throckmorton 1975; Laštovka & Máca 1978; Hackman 1982; Grimaldi 1990; Pélandakis & Solignac 1993) reached contradictory conclusions because they were by chance limited to species of one of the two genera. *Lordiphosa* s.s. is the sister clade of the *willistoni-saltans* clade (Katoh et al. 2000, Y. Hu & M. Toda, pers. comm.), making the subgenus *Sophophora* paraphyletic.

Genus Drosophila. – Bächli (1999-2008) currently recognizes eight subgenera (*Drosophila* 721 species, *Sophophora* 331, *Chusqueophila* 1, *Dorsilopha* 3, *Dudaica* 2, *Phloridosa* 8, *Psilodorha* 2, and *Siphlodora* 2), whereas he follows Grimaldi (1990) in placing the Hawaiian *Drosophila* in its own genus (*Idiomya* 380, discussed below) and not in the subgenus *Drosophila* (cf. O'Grady 2002). Four of the subgenera as well as *Idiomya* are included in our supertree analysis (Fig. 3). The subgenus *Sophophora* is the first branch within the genus *Drosophila* s.l., in agreement with the results of many studies (Beverley & Wilson 1984; DeSalle 1992b; Wojtas et al. 1992; Pélandakis & Solignac 1993; Thomas & Hunt 1993; Kwiatowski et al. 1994, 1997; Russo et al. 1995; Tamura et al. 1996; Remsen & DeSalle 1998; Kwiatowski & Ayala 1999; Tataronkov et al. 1999; Tarrío et al. 2001; Remsen & O'Grady 2002; Robe et al. 2005; Da Lage et al. 2007; Katoh et al. 2007).

Several studies placed the *willistoni-saltans* clade, in at least some of their analyses, as the first branch next to a clade containing all other *Drosophila* (Pélandakis & Solignac 1993; Kwiatowski et al. 1994, 1997; Katoh et al. 2000; Tarrío et al. 2001), albeit with low statistical confidence or with lower support than the best-supported topologies within the same study (Tarrío et al. 2001). This placement is effectively explained by the large difference in nucleotide composition and codon usage in the *willistoni-saltans* clade (Anderson et al. 1993; Rodriguez-Trelles et al. 1999b; Tarrío et al. 2000, 2001; Powell et al. 2003; Tamura et al. 2004; Clark et al. 2007; Heger & Ponting 2007), and studies effectively addressing this issue confirm the monophyly of the subgenus *Sophophora*, as did our supertree analysis.

In the supertree analysis, the subgenus *Dorsilopha* is positioned between the subgenus *Sopho-*

phora and the remainder of the genus *Drosophila* s.l. (Fig. 4). The many studies (Pélandakis & Solignac 1993; Kwiatowski et al. 1994, 1997; Kwiatowski & Ayala 1999; Tataronkov et al. 1999; Hu & Toda 2001; Tarrío et al. 2001; Remsen & O'Grady 2002; Perlman et al. 2003; Robe et al. 2005; Katoh et al. 2007) that included this subgenus placed it at various positions. The subgenus *Siphlodora* is placed in the *virilis-repleta* clade in the supertree analysis (cf. Remsen & O'Grady 2002; van der Linde et al. accepted).

Most authors have concluded that the genus *Drosophila* is paraphyletic, whether they used morphological (Throckmorton 1962, 1965, 1975; Grimaldi 1990; Thomas & Hunt 1993; Hu & Toda 2001) or molecular data (Beverley & Wilson 1984; DeSalle 1992a, b; Pélandakis & Solignac 1993; Thomas & Hunt 1993; Kwiatowski et al. 1994, 1997; Kambysellis et al. 1995; Russo et al. 1995; Tamura et al. 1996; Remsen & DeSalle 1998; Tataronkov et al. 1999; Davis et al. 2000; Gailey et al. 2000; Katoh et al. 2000; Tarrío et al. 2001; Tataronkov et al. 2001; Remsen & O'Grady 2002; Da Lage et al. 2007; Katoh et al. 2007; Magnacca & O'Grady 2008). The placement of the genera included in the subgenus *Drosophila* is discussed in detail under that subgenus. The placement of the genus *Lordiphosa* within the subgenus *Sophophora* is discussed above but was not included in our supertree analysis because all members of the genus *Lordiphosa* were only represented in one or two trees.

Subgenus Sophophora. – The subgenus *Sophophora* is generally subdivided into an 'Old World' clade, containing the *melanogaster* (184 species) and *obscura* (42) species groups, and a Neotropical clade containing the *willistoni* (23) and *saltans* (21) species groups (Pitnick et al. 1999; Tataronkov et al. 1999; Bächli 1999-2008; O'Grady & Kidwell 2002; Remsen & O'Grady 2002; Da Lage et al. 2007). The supertree analysis confirmed this basal split in the subgenus (Fig. 4). Some species of the 'Old World' *obscura* clade (the *pseudoobscura* and *affinis* subgroups) have subsequently invaded the New World. Four additional species groups have been recognized (Bächli 1999-2008; Ashburner et al. 2005): *dentissima* (17 species, Africa), *dispar* (3, Australia and New Guinea), *populi* (2, North America and northern Europe), and *fima* (23, Africa, sister clade of the *ananassae* species subgroup; Pélandakis &

Solignac 1993). Recently, Da Lage & coworkers (2007) proposed to elevate the *ananassae* and *montium* subgroups to the level of species groups.

melanogaster species group. – The *melanogaster* group is subdivided into 12 species subgroups: *ananassae* (24 species), *montium* (90), *melanogaster* (9) and the ‘oriental subgroup’ cluster, *suzukii* (18), *takahashii* (14), *ficuspshila* (6), *elegans* (5), *rhopaloo* (5), and *eugracilis* (1). The remaining three (*denticulata*, 4; *flavohirta*, 1; *longissima*, 2) have not been placed in a phylogenetic context, although some evidence indicates that *longissima* is close to the *montium* species subgroup (Okada & Carson 1983a; Toda 1991), whereas *flavohirta* is within the oriental subgroup cluster (Da Lage et al. 2007). Toda (1991) gives detailed diagnoses of most species groups except *montium*, *denticulata*, and *flavohirta*. A major problem with this group is the radiation among the oriental subgroups, so explosive that even multigene studies (e.g., Kopp 2006) cannot resolve all nodes convincingly.

Our supertree analysis (Fig. 4) resolves most nodes, although several unresolved nodes remain, reflecting the situation in the literature. The first subgroup to branch off is the *ananassae* subgroup, and the second is the *montium* subgroup (cf. Inomata et al. 1997; Goto & Kimura 2001; O’Grady & Kidwell 2002; Kastanis et al. 2003; Lewis et al. 2005; Kopp 2006; Prud’homme et al. 2006; Da Lage et al. 2007). Two studies were not in conflict with the supertree results as they resolved the nodes as a polytomy (Clark et al. 1998; Kopp & True 2002). Several studies were in conflict with the supertree analysis, as they either reversed the order of the *ananassae* and *montium* subgroups, albeit with low bootstrap support (Yang et al. 2004), or placed them together as the sister clade of all remaining subgroups, also with low bootstrap support (Schwaroch 2002).

The *suzukii* and *takahashii* subgroups are sister clades based on the supertree analysis (Fig. 4), a result predicted by most studies (Pélandakis & Solignac 1993; Inomata et al. 1997; Harr et al. 2000; Goto & Kimura 2001; Kopp & True 2002; Schwaroch 2002; Kastanis et al. 2003; Ko et al. 2003; Yang et al. 2004; Lewis et al. 2005; Prud’homme et al. 2006; Da Lage et al. 2007), even though most studies are based on limited taxon sampling. The *suzukii* subgroup is polyphyletic (see below), and individual species are

placed reliably at various positions in the topology. *D. lucipennis* is the sister clade of the *elegans* subgroup (cf. Kopp & True 2002; Schwaroch 2002; Lewis et al. 2005; Prud’homme et al. 2006; Da Lage et al. 2007), whereas *D. mimetica* is within the *takahashii* subgroup (cf. Lewis et al. 2005; Da Lage et al. 2007). The *rhopaloo* subgroup is the sister group of the *elegans* subgroup combined with *D. lucipennis* (Kopp & True 2002; Schwaroch 2002; Yang et al. 2004; Kopp 2006; Prud’homme et al. 2006). The *takahashii-suzukii* clade together with the *melanogaster* and *eugracilis* subgroups form a single clade (cf. Pélandakis & Solignac 1993; Goto et al. 2000; Schwaroch 2002; Akashi et al. 2006; Kopp 2006; Prud’homme et al. 2006). The *melanogaster-eugracilis-takahashii-suzukii* clade forms together with the *elegans-rhopaloo* clade and the *ficuspshila* subgroup a polytomy in the supertree analysis, reflecting the contradicting results in the various studies (Kopp & True 2002; Schwaroch 2002; Kastanis et al. 2003; Yang et al. 2004; Lewis et al. 2005; Kopp 2006; Prud’homme et al. 2006; Da Lage et al. 2007).

ananassae species subgroup. – The *ananassae* subgroup includes three recognized complexes: *ananassae* (10 species; Bock 1971; Bock & Wheeler 1972), *biplectinata* (4; Bock 1971; Bock & Wheeler 1972; Kopp & Barmina 2005), and *ercepeae* (4; Lemeunier et al. 1997); the remaining 6 species are unplaced. The *ercepeae* complex together with *D. varians* is placed basal in our supertree (cf. Schwaroch 2002; Prud’homme et al. 2006; Da Lage et al. 2007), whereas the *biplectinata* and *ananassae* complexes are sister clades (cf. Schwaroch 2002; Yang et al. 2004; Prud’homme et al. 2006; Da Lage et al. 2007).

montium species subgroup. – Several complexes have been recognized traditionally, but only the *auraria* (Schwaroch 2002; Zhang et al. 2003a; Yang et al. 2004; Lewis et al. 2005; Prud’homme et al. 2006; Da Lage et al. 2007) and *kikkawai* complexes (Schwaroch 2002; Zhang et al. 2003a; Yang et al. 2004; Prud’homme et al. 2006; Da Lage et al. 2007) are recovered in our supertree analysis (Fig. 4). The *auraria* complex is placed basal to most other species within the subgroup (Schwaroch 2002; Zhang et al. 2003a; Yang et al. 2004; Prud’homme et al. 2006; Da Lage et al. 2007). The remaining traditionally recognized complexes are not recovered in our analysis or in

most molecular studies, nor are the various studies consistent with one another.

takahashii species subgroup. – The *takahashii* subgroup is monophyletic in our supertree analysis (Fig. 4), in agreement with the many published studies (Nigro & Grapputo 1993; Harr et al. 2000; Goto & Kimura 2001; Kopp & True 2002; Schawaroch 2002; Kastanis et al. 2003; Yang et al. 2004; Prud'homme et al. 2006; Da Lage et al. 2007). An exception in the literature is the study by Lewis et al. (2005), who suggested that the *takahashii* and the *suzukii* subgroups are essentially mixed. The topology in the supertree is well resolved, contrary to the various studies that come to different conclusions about the topology, although they agree with placing *D. pseudotakahashii* basal to all other species.

suzukii species subgroup. – The *suzukii* subgroup is polyphyletic (Fig. 4); *D. lucipennis* is the sister taxon of the *elegans* subgroup (cf. Kopp & True 2002; Schawaroch 2002; Lewis et al. 2005; Kopp 2006; Prud'homme et al. 2006; Da Lage et al. 2007), *D. mimetica* is within the *takahashii* subgroup (cf. Lewis et al. 2005; Da Lage et al. 2007). The heterogeneity of the subgroup has been recognized previously on the basis of morphological characteristics (Toda 1991).

melanogaster species subgroup. – The *melanogaster* species subgroup is monophyletic (Fig. 4), in accordance with all published phylogenies (Caccone et al. 1988; Kopp & True 2002; Schawaroch 2002; Ko et al. 2003; Yang et al. 2004; Lewis et al. 2005; Prud'homme et al. 2006; Da Lage et al. 2007). Pollard et al. (2006) used data for more than 9000 genes collected by the whole-genome project (Clark et al. 2007) to reconstruct the phylogeny between *D. melanogaster*, *D. erecta* and *D. yakuba*. The results show the majority of the genes support the grouping of *D. erecta* and *D. yakuba* as sister species, as does our supertree analysis (Kopp & True 2002; Ko et al. 2003; Parsch 2003; Lewis et al. 2005; Prud'homme et al. 2006), but also considerable incongruence in nucleotide and amino acid substitutions, insertions and deletions, and gene trees. This result explains the contradicting results obtained in other studies (see Pollard et al. 2006 for details). See also Ashburner et al. (2005: chapter 33) for an extensive discussion of this subgroup.

obscura species group. – The *obscura* species group is split into six different subgroups: *affinis* (10 species), *microlabis* (4), *obscura* (12), *pseudoobscura* (8), *subobscura* (3), and *sinobscura* (3) (Barrio et al. 1994; Gao et al. 2003). Traditionally, the subgroups are clustered according to their Old World (*microlabis*, *obscura*, *subobscura*, *sinobscura*) and New World (*affinis* and *pseudoobscura*) distributions (Lakovaara & Saura 1982; Barrio & Ayala 1997; Haring et al. 1998; see O'Grady 1999, for a summary), and this arrangement has been confirmed in our supertree analysis (Fig. 4). The *affinis*, *pseudoobscura*, and *subobscura* subgroups were monophyletic and well resolved, but the *microlabis* subgroup was positioned within the *obscura* subgroup in an unresolved polytomy.

willistoni species group. – The *willistoni* species group is split into three species subgroups: *alagittans* (5 species), *bocainensis* (12), and *willistoni* (6) (Gleason & Powell 1997; Gleason et al. 1998; Tarrío et al. 2000; O'Grady & Kidwell 2002). The monophyletic status of this group is still under discussion (Pélandakis et al. 1991; Pélandakis & Solignac 1993; Silva & Kidwell 2000; O'Grady & Kidwell 2002; Da Lage et al. 2007), but the *willistoni* and *saltans* species groups are readily distinguishable on the basis of morphological characteristics (Throckmorton 1975) as well as the deletion of an intron of the *Adh* gene specific to the *willistoni* group (Anderson et al. 1993; Tarrío et al. 2000). The group was monophyletic in the supertree analysis (Fig. 4). The *bocainensis* subgroup was paraphyletic with *D. nebulosa* placed basal to the *willistoni* subgroup.

saltans species group. – Recent studies of the *saltans* species group (O'Grady et al. 1998; Silva & Kidwell 2000) have confirmed its traditional division into five subgroups: *cordata* (2 species), *elliptica* (4), *parasaltans* (2), *saltans* (7), and *sturtevantii* (6) (de Magalhães & Bjornberg 1957; de Magalhães 1962; Throckmorton & de Magalhães 1962). Otherwise, no consensus exists on the phylogenetic relationship of the subgroups (O'Grady et al. 1998; Rodriguez-Trelles et al. 1999a, b; Silva & Kidwell 2000) or the *saltans* subgroup itself (de Campos Bicudo 1973a, b; O'Grady et al. 1998; Nascimento & de Campos Bicudo 2002). The group was monophyletic in the supertree analysis (Fig. 5), although most subgroups were only represented by a single species.

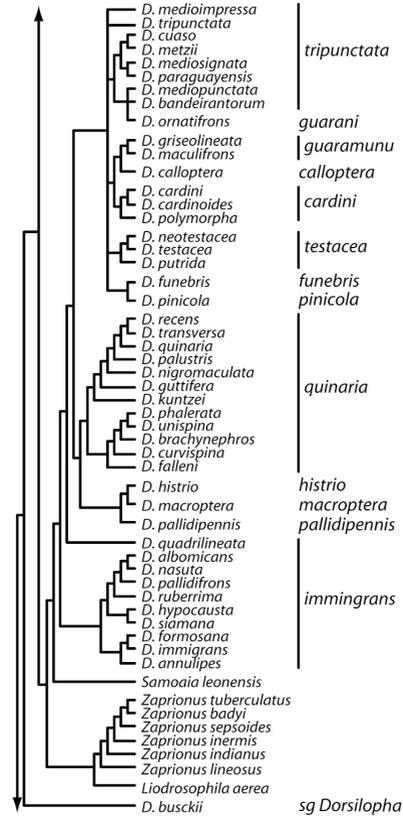
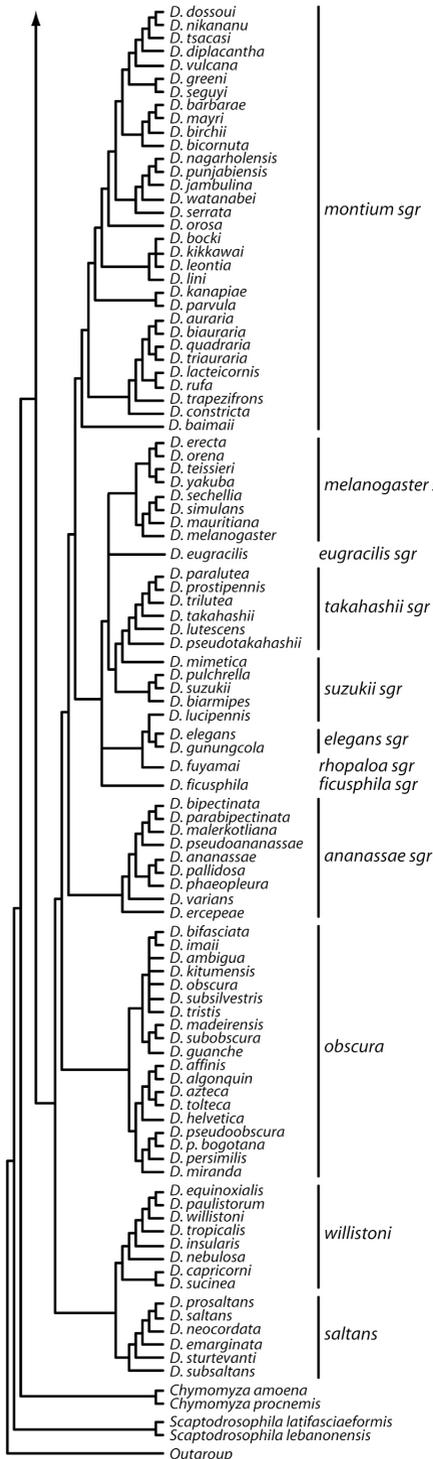


Fig. 5. Supertree phylogeny: *immigrans*-*tripunctata* radiation; subgenus *Dorsilopha*; genera *Zaprionus*, *Samoaia*, and *Liodrosophila*.

Subgenus Drosophila. – Our supertree analysis confirmed the general picture from the literature that the traditional subgenus *Drosophila* is paraphyletic (overview: Fig. 3; details: Figs 5–7). The genera *Hirtodrosophila*, *Zaprionus*, *Samoaia*, *Liodrosophila*, *Dichaetophora*, and *Scaptomyza*, as well as the ‘Hawaiian *Drosophila*’ or *Idiomyza* and the subgenus *Siphlodora*, are positioned within the subgenus *Drosophila* in the supertree analysis. The paraphyletic nature of the subgenus was first suggested by Throckmorton (1975), who included 15 genera and at least two subgenera in

Fig. 4. Supertree phylogeny: basal genera *Chymomyza* and *Scaptodrosophila* and subgenus *Sophophora*. Sgr = species subgroup; all subgroups belong to the *melanogaster* species group.

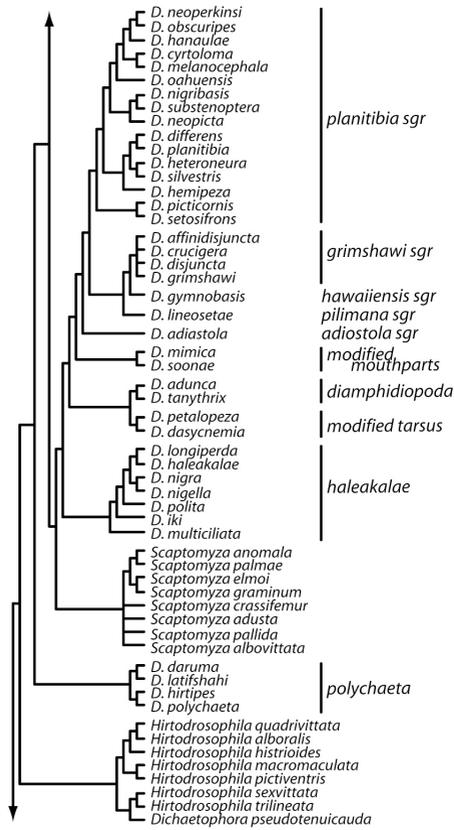


Fig. 6. Supertree phylogeny: Hawaiian *Drosophila* clade or *Idiomyia*, *polychaeta* species group, genera *Hirtodrosophila* and *Scaptomyza*.

this subgenus. Molecular research confirms that *Hirtodrosophila* (Beverley & Wilson 1984; Kwiatowski et al. 1994, 1997; Tamura et al. 1996; Remsen & DeSalle 1998; Kwiatowski & Ayala 1999; Tatarenkov et al. 1999; Katoh et al. 2000; Perlman et al. 2003; Robe et al. 2005; Da Lage et al. 2007; Katoh et al. 2007), *Mycodrosophila* (Katoh et al. 2000; Da Lage et al. 2007), *Zaprionus* (Pélandakis & Solignac 1993; Thomas & Hunt 1993; Kwiatowski et al. 1994, 1997; Russo et al. 1995; Tamura et al. 1996; Remsen & DeSalle 1998; Kwiatowski & Ayala 1999; Tatarenkov et al. 1999; Davis et al. 2000; Katoh et al. 2000; Remsen & O’Grady 2002; Robe et al. 2005; Da Lage et al. 2007; Katoh et al. 2007), *Samoaia* (Pélandakis & Solignac 1993; Tatarenkov et al. 1999; Davis et al. 2000; Robe et al. 2005),

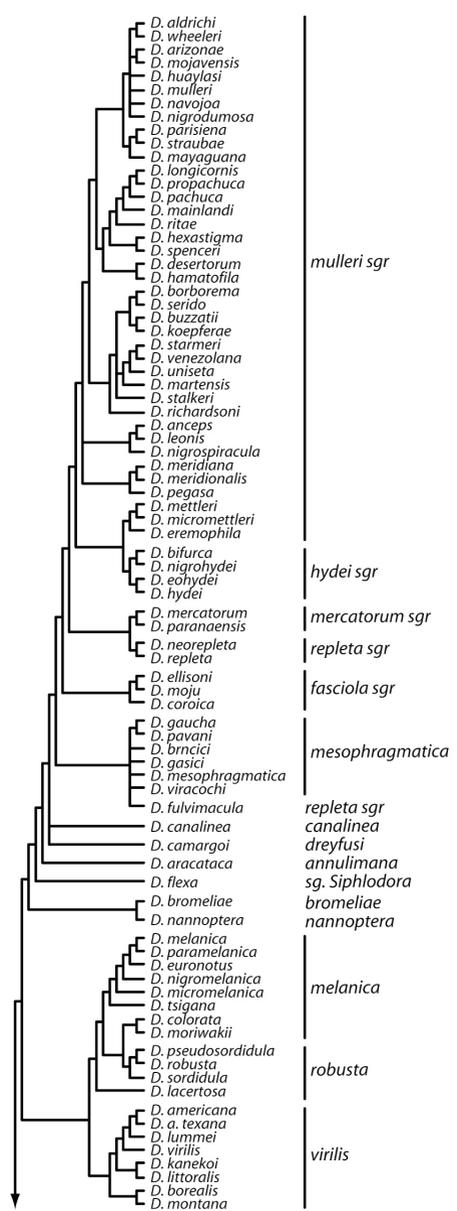


Fig. 7. Supertree phylogeny: *virilis-repleta* radiation and subgenus *Siphlodora*. Sgr = species subgroup; all subgroups belong to the *repleta* species group.

Liodrosophila (DeSalle 1992b; Tamura et al. 1996; Tatarenkov et al. 1999; Davis et al. 2000; Tatarenkov et al. 2001; Robe et al. 2005; Da Lage et al. 2007), *Dichaetophora* (Katoh et al. 2000; Hu

& Toda 2001), *Scaptomyza* (DeSalle 1992b; Pélandakis & Solignac 1993; Thomas & Hunt 1993; Kambysellis et al. 1995; Russo et al. 1995; Tamura et al. 1996; Remsen & DeSalle 1998; Kwiatowski & Ayala 1999; Tatarenkov et al. 1999; Davis et al. 2000; Gailey et al. 2000; Katoh et al. 2000; Remsen & O'Grady 2002; Da Lage et al. 2007; Katoh et al. 2007), the 'Hawaiian *Drosophila*' or *Idiomya* (Beverley & Wilson 1984; DeSalle 1992a; Thomas & Hunt 1993; Kambysellis et al. 1995; Russo et al. 1995; Tamura et al. 1996; Remsen & DeSalle 1998; Tatarenkov et al. 1999; Davis et al. 2000; Gailey et al. 2000; Katoh et al. 2000; Tatarenkov et al. 2001; Remsen & O'Grady 2002; Da Lage et al. 2007; Katoh et al. 2007), and the subgenera *Siphlodora* (Remsen & O'Grady 2002) are included in the same clade. This list is probably not complete, because other closely related genera, such as *Zygothrica*, *Phorticella*, and *Paramycodrosophila* (Grimaldi 1990; Remsen & O'Grady 2002), have not yet or rarely been included in molecular analyses.

Malogolowkin (1953) and later Throckmorton (1975) recognized two main clades within the subgenus *Drosophila*, the *virilis-repleta* radiation and the *immigrans-tripunctata* radiation. The definitions of these radiations have changed, and nowadays only species groups of the genus *Drosophila* are included; genera such as *Dettopsomyia* and *Hirtodrosophila* are no longer included (Yotoko et al. 2003; Robe et al. 2005; Markow & O'Grady 2006; Da Lage et al. 2007). This basic split has been confirmed in our supertree analysis (Fig. 3), in agreement with many studies (Grimaldi 1990; Pélandakis & Solignac 1993; Tamura et al. 1996; Remsen & DeSalle 1998; Kwiatowski & Ayala 1999; Pitnick et al. 1999; Tatarenkov et al. 1999, 2001; Davis et al. 2000; Gailey et al. 2000; Katoh et al. 2000, 2007; Remsen & O'Grady 2002; Carrasco et al. 2003; Yotoko et al. 2003; Robe et al. 2005). The Amyrel study of Da Lage et al. (2007) is an exception, as they place the *virilis-repleta* radiation within the *immigrans-tripunctata* radiation. The genera *Hirtodrosophila*, *Mycodrosophila*, and *Paramycodrosophila* are closely related (Grimaldi 1990; Katoh et al. 2000; Remsen & O'Grady 2002; Da Lage et al. 2007).

An overview of the relationships within the subgenus *Drosophila* based on the supertree analysis is presented in Fig. 3. Two major clades can be recognized in the subgenus *Drosophila*. The first clade consists of the *virilis-repleta* radiation, the

'Hawaiian *Drosophila*' or *Idiomya*, and the subgenus *Siphlodora*, as well as the genera *Hirtodrosophila*, *Scaptomyza*, and *Dichaetophora*. The second clade consists of the *immigrans-tripunctata* radiation as well as the genera *Zaprionus*, *Liodrosophila*, and *Samoia*.

The Hawaiian drosophilids consist of the genus *Scaptomyza* and the 'Hawaiian *Drosophila*' or *Idiomya* (cf. Throckmorton 1966, 1975; DeSalle 1992a; Thomas & Hunt 1993; Kambysellis et al. 1995; Russo et al. 1995; Tamura et al. 1996; Remsen & DeSalle 1998; Kwiatowski & Ayala 1999; Tatarenkov et al. 1999, 2001; Davis 2000; Davis et al. 2000; Remsen & O'Grady 2002; Da Lage et al. 2007; Katoh et al. 2007). Grimaldi (1990) placed them in different clades, but a more exhaustive reanalysis of his data did not support that conclusion (Remsen & O'Grady 2002). In turn, the Hawaiian drosophilids are the sister clade of the *virilis-repleta* radiation (cf. Kambysellis et al. 1995; Russo et al. 1995; Tamura et al. 1996; Remsen & DeSalle 1998; Kwiatowski & Ayala 1999; Tatarenkov et al. 1999, 2001; Gailey et al. 2000; Tarrío et al. 2001; Tatarenkov & Ayala 2001; Remsen & O'Grady 2002; Da Lage et al. 2007; Katoh et al. 2007). The supertree analysis places the *polychaeta* species group outside the *virilis-repleta* radiation basal to the combined clade of the Hawaiian drosophilids and the *virilis-repleta* radiation (Fig. 3 and Fig. 6). In the literature, studies differ in the placement of the *polychaeta* group, placing it variously basal to either the genus *Drosophila*, subgenus *Drosophila*, the Hawaiian drosophilids combined with the *virilis-repleta* radiation, the *virilis-repleta* radiation, or the *repleta* clade or within the *immigrans-tripunctata* radiation (Throckmorton 1975; Pélandakis & Solignac 1993; Tamura et al. 1996; Katoh et al. 2000; Tatarenkov & Ayala 2001; Remsen & O'Grady 2002; Carrasco et al. 2003; Wang et al. 2006; Da Lage et al. 2007), although it is generally considered to be part of the *virilis-repleta* radiation. The genera *Hirtodrosophila* and *Dichaetophora* form a single clade in the supertree analysis (cf. Katoh et al. 2007; van der Linde et al. accepted, but contra Hu & Toda 2001), whereas the results suggest that the genus *Hirtodrosophila* is paraphyletic. These results could very well reflect the limited knowledge of this genus, as it is often represented as a single species in an analysis. The *Hirtodrosophila-Dichaetophora* clade is placed basal to the *polychaeta* species group in the supertree analysis.

The second clade consists of two subclades. The first contains the *immigrans-tripunctata* radiation together with the genus *Samoia* (Pélandakis & Solignac 1993; Gailey et al. 2000; Tatarenkov et al. 2001). The second consists of the genera *Zapri- onus* (Pélandakis & Solignac 1993; Gailey et al. 2000; Tatarenkov et al. 2001; Robe et al. 2005) and *Liodrosophila* (Gailey et al. 2000; Tatarenkov et al. 2001; Robe et al. 2005).

Studies differ considerably in their placements of genera and subgenera within the subgenus *Drosophila*, and several conflicting trees have been published. A major problem is the large variation in nucleotide content, especially at the third codon position (Moriyama & Hartl 1993; Tarrío et al. 2001). The *immigrans-tripunctata* and *virilis-repleta* radiations, as well as the Hawaiian *Drosophila*, form generally well-defined monophyletic clades. Two species groups – *polychaeta* and *tumiditarsus* – are generally included in the *virilis-repleta* radiation, but several studies cast doubt on that assignment (*polychaeta*: see above; *tumiditarsus*: Tan et al. 1949; Throckmorton 1962, 1982; Tatarenkov & Ayala 2001; Da Lage et al. 2007; Yassin 2007). Furthermore, several small groups within the subgenus *Drosophila* (Bächli 1999–2008) have not been assigned to either of the two main lineages of the subgenus because of the lack of molecular data (*antioquia*, 3 species; *aureata*, 1; *nigrosparsa*, 4; *onychophora*, 16; *picta*, 1; *simulivora*, 6; and *xanthopallescens*, 4).

The immigrans-tripunctata clade. – The *immigrans-tripunctata* clade encompasses 15 species groups: *bizonata* (7 species), *calloptera* (8), *cardini* (15), *funebri* (7), *guarani* (17), *histrion* (16), *immigrans* (101), *macroptera* (5), *pallidipennis* (1), *pinicola* (3), *quinaria* (34, including *D. guttifera*), *rubrifrons* (9), *sticta* (1), *testacea* (4), and *tripunctata* (79) (Bächli 1999–2008). The *immigrans* group itself is subdivided into five groups: *curviceps* (10 species), *hypocausta* (9), *immigrans* (34), *nasuta* (12), and *quadrilineata* (22). The *guarani* group (King 1947) consists of two species subgroups, *guarani* and *guaramunu* (Bächli 1999–2008; Remsen & O’Grady 2002) that are more often treated as separate species groups (Kastritsis 1969; Clayton & Wheeler 1975; Throckmorton 1975; Yotoko et al. 2003; Robe et al. 2005). Note that *D. guarani* and *D. guaramunu* have been synonymized with *D. ornatifrons* and *D. maculifrons*, respectively (Vilela & Bächli 1990).

Twelve species groups are represented in the supertree analysis (Fig. 5). The *immigrans* species group is basal to all other groups in our analysis (cf. Pélandakis & Solignac 1993; Remsen & O’Grady 2002; Carrasco et al. 2003; Perlman et al. 2003; Yotoko et al. 2003; Robe et al. 2005; Da Lage et al. 2007). The next clade consists of three small clades, the *histrion*, *macroptera*, and *pallidipennis* species groups. The third clade is the *quinaria* species group; the remaining species groups are clustered in a large polytomy. This radiation is the least resolved of all clades in the supertree analysis, a reflection of the limited number of studies covering a substantial part of this clade as well as the large variety of topologies found in various studies (Pélandakis & Solignac 1993; Remsen & O’Grady 2002; Carrasco et al. 2003; Yotoko et al. 2003; Robe et al. 2005; Da Lage et al. 2007). Furthermore, the species-group delineations do not necessarily provide an adequate basis for further analyses of this radiation, especially because the *tripunctata* group as currently defined is polyphyletic (Frota-Pessoa 1954; Throckmorton 1975; Carrasco et al. 2003; Yotoko et al. 2003; Robe et al. 2005; Da Lage et al. 2007).

All but two studies (Da Lage et al. 2007; Katoh et al. 2007) concluded that the *immigrans-tripunctata* radiation is monophyletic. The study by Da Lage et al. (2007) positioned the *virilis-repleta* radiation between the *immigrans* species group and the remainder of the radiation, whereas that by Katoh et al. (2007) casts doubt on the placement of *quadrilineata* species subgroup within the *immigrans* species group or even the *immigrans-tripunctata* clade, although older studies suggest that placement (Wakahama et al. 1983; Kumar & Gupta 1987).

cardini species group. – The *cardini* group is split into two subgroups: *cardini* and *dunni* (Heed 1962). Hollocher (1996) investigated the phylogenetic relations within this group on the basis of morphological characteristics (male genitalia, Heed 1962; cytology, Heed & Krishnamurthy 1959; Heed & Russell 1971), biogeographical data, and mitochondrial DNA sequences. See Wilder & Hollocher (2003) for a more detailed study of the *dunni* subgroup. The *dunni* subgroup was not included in the supertree analysis, but the *cardini* subgroup was monophyletic (Fig. 5).

quinaria species group including D. guttifera. – Eggs of the *quinaria* and *guttifera* species groups

differ from those of other subgenus *Drosophila* species in having three rather than four egg filaments. This group is monophyletic in the supertree analysis (Fig. 5), and *D. guttifera* is positioned firmly within this species group. The most extensive phylogeny of the *quinaria* species group was published by Perlman *et al.* (2003), and most other studies agree with it either fully (Spicer & Jaenike 1996; Yotoko *et al.* 2003) or to a large degree (Carrasco *et al.* 2003; Da Lage *et al.* 2007). All studies that include *D. guttifera* place it within the *quinaria* group, contrary to its assignment to its own species group by Sturtevant (1942).

tripunctata species group. – Frota-Pessoa (1954) subdivided the *tripunctata* group into four clusters (numbered I through IV). Much work remains to be done in this group as Yotoko *et al.* (2003), Carrasco *et al.* (2003), and Robe *et al.* (2005) found little support for these groups, but found ample evidence for the paraphyletic nature of this group (Frota-Pessoa 1954; Throckmorton 1975; Carrasco *et al.* 2003; Yotoko *et al.* 2003; Robe *et al.* 2005; Da Lage *et al.* 2007).

The virilis-repleta clade. – Most authors (Tamura *et al.* 1996; Pitnick *et al.* 1999; Katoh *et al.* 2000; Carrasco *et al.* 2003; Robe *et al.* 2005; Wang *et al.* 2006) agree that the *virilis-repleta* clade consists of two main lineages, but Da Lage *et al.* (2007) place the *melanica* and *bromeliae* clades at different places in the topology. Some studies report minor deviation from this basal split, generally with poor bootstrap support (Pélandakis & Solignac 1993; Tatarenkov & Ayala 2001). The *virilis* lineage consists of the *virilis* (12 species), *robusta* (16), *melanica* (13), *angor* (5), and *quadrisetata* (12) species groups (Watabe & Peng 1991; Wang *et al.* 2006). The *robusta* group is polyphyletic, but the three subgroups – *lacertosa* (7), *okadai* (3), *robusta* (4) – form well-defined monophyletic clusters (Wang *et al.* 2006). The *repleta* clade includes the *repleta* (100 species), *mesophragmatica* (13), *bromeliae* (5), *dreyfusi* (9), *annulimana* (16), *flavopilosa* (17), and *canalinae* (11) species groups (Pitnick *et al.* 1999; Tatarenkov & Ayala 2001; Carrasco *et al.* 2003; Robe *et al.* 2005; Wang *et al.* 2006; Da Lage *et al.* 2007). Remsen & O'Grady (2002) placed the subgenus *Siphlodora* in this clade, a position confirmed by van der Linde *et al.* (accepted). The *repleta*, *mesophragmatica*, *dreyfusi*, and *canalinae* groups form a

well-supported clade (Throckmorton 1975; Pélandakis & Solignac 1993; Durando *et al.* 2000; Tatarenkov & Ayala 2001; Remsen & O'Grady 2002; Robe *et al.* 2005; Da Lage *et al.* 2007). The *nannopectera* (4) species group is generally placed within the *repleta* lineage (Pitnick *et al.* 1999; Tatarenkov & Ayala 2001; Carrasco *et al.* 2003; Wang *et al.* 2006), although Robe *et al.* (2005) place it basal to the whole clade. The *bromeliae* group is placed basal to the *repleta s.l.* clade (Pélandakis & Solignac 1993; Tatarenkov & Ayala 2001), whereas Da Lage *et al.* (2007) placed it with the *polychaeta* group. The placement of the *annulimana* group differs in different studies, but its placement basal to the previous groups is well supported (Tatarenkov & Ayala 2001; Robe *et al.* 2005; Da Lage *et al.* 2007). The *annulimana* and *flavopilosa* groups are sister clades according to Robe *et al.* (2005). The position of the remaining groups – *carbonaria* (1), *carsoni* (1), *coffeata* (4), *peruviana* (1) – is unclear as they have not been included in any molecular study. The inclusion of the *tumiditarsus* group (1) in the clade is doubtful (Tan *et al.* 1949; Throckmorton 1962, 1982; Tatarenkov & Ayala 2001; Da Lage *et al.* 2007; Yassin 2007).

The supertree analysis reflects the above-described topology (Fig. 7). In the *virilis* lineage, the *melanica* and *robusta* species groups formed a single clade, but neither group was monophyletic. The *virilis* group was the sister clade of the *robusta-melanica* clade. The *repleta* group was monophyletic except for *D. fulvimacula*, which was placed in the *mesophragmatica* species group. The *repleta*, *mesophragmatica*, *dreyfusi*, and *canalinae* groups formed a single clade. The *bromeliae* and *nannopectera* species groups were sister clades and placed basal to the subgenus *Siphlodora*. The *annulimana* group was placed between the subgenus *Siphlodora* and the *repleta-mesophragmatica-dreyfusi-canalinae* clade.

mesophragmatica species group. – In the supertree analysis, the *mesophragmatica* species group was resolved as a polytomy, including *D. fulvimacula* of the *repleta* subgroup (Fig. 7), contrary to the literature that resolves it as a monophyletic group (Carrasco *et al.* 2003; Robe *et al.* 2005). The placement of *repleta* subgroup's species is the average position of five trees, but only one places the species basal to the *mesophragmatica* group (Durando *et al.* 2000: ND2). The branching with-

in the group differs slightly in different studies and according to the genes used and also differs slightly from that in an earlier study using hybridization, genetic, and morphological characteristics (Brncic & Santibanez 1957).

melanica species group. – Eggs of several *melanica*-group species have two filaments rather than the usual four of the subgenus *Drosophila* (Stalker 1964). *D. moriwakii* and *D. colorata* are placed basally (Narayanan 1973; Flores et al. 2008), although Wang et al. (2006) suggested that *D. moriwakii* might be placed basal to the *robusta* species subgroup. Wang et al. (2006) recognized an Old World and a New World clade, and the relationships within the New World clade have been studied previously in detail by Stalker (1966, 1972), who used a wide range of morphological and physiological characteristics, as well as banding patterns of salivary gland chromosomes; by Levitan (1982), who used chromosomal, especially karyotype, information; and by Pitnick et al. (1999), who used sequences of cytochrome oxidase II. In the supertree analysis, *D. moriwakii* and *D. colorata* formed a separate clade basal to the *robusta* species subgroup, making both species groups non-monophyletic (Fig. 7). This result suggests that additional studies are needed to resolve the relationships between the various subclades.

repleta species group. – Six subgroups have been recognized within the *repleta* species group: *inca* (3 species), *mulleri* (50), *hydei* (7), *mercatorum* (4), *repleta* (9), and *fasciola* (21) (Bächli 1999–2008). Molecular evidence suggests that the *mulleri* clade is paraphyletic or polyphyletic (Durando et al. 2000; Katoh et al. 2000) and includes the *repleta* and *mercatorum* subgroups (Durando et al. 2000). Durando et al. (2000) suggested that Wasserman originally defined the *mulleri* subgroup as a ‘waste paper basket’ to include several complexes and clusters that he could not place elsewhere. Durando et al. (2000) and Katoh et al. (2000) used different genes for their analyses but produced identical trees for the *repleta* clade. The supertree analysis confirms the parafyly of the *mulleri* subgroup, but contrary to Durando et al. (2000), it includes the *hydei* subgroup as the sister clade of the *eremophila* complex (Fig. 7). The *fasciola* subgroup is placed basal to the remaining subgroups, and the *repleta* and *mercatorum* subgroups are sister clades.

hydei species subgroup. – The *hydei* subgroup is defined by the extensive coiling in both the testes and the ventral receptacles (Wharton 1944; Wasserman 1982). Most molecular studies support monophyly of the subgroup (Durando et al. 2000; Carrasco et al. 2003; Yotoko et al. 2003; Morán & Fontdevila 2005), but that of Robe et al. (2005, including three species) does not. The subgroup is traditionally split into the *hydei* (3 species) and *bifurca* (4) complexes; the first differs from the second in having specialized spermathecae and a chromosomal inversion (Wasserman 1962, 1982, 1992). This split is consistent with the mitochondrial DNA studies (Spicer & Pitnick 1996; Carrasco et al. 2003; Yotoko et al. 2003), although all of these included few species. The study by Moran and Fontdevila (2005), using nuclear DNA (*Xdh*), indicated that both complexes are paraphyletic and provided a more detailed discussion.

mulleri species subgroup. – The *mulleri* subgroup is subdivided into several complexes, *mulleri*, *buzzatii*, *eremophila*, *anceps*, and *meridiana* (Durando et al. 2000), but sources differ in the assignment of species (Bächli 1999–2008). As discussed under the *virilis-repleta* radiation, this subgroup is not monophyletic (Durando et al. 2000; O’Grady et al. 2001a). The phylogeny of the largest recognized cluster in the *buzzatii* complex, the *buzzatii* cluster (7 species), has been investigated in detail separately (*Xdh*: Rodriguez-Trelles et al. 2000a; COI mtDNA: Manfrin et al. 2001; wing morphology: Morães et al. 2004). The various complexes are recovered in the supertree analysis (Fig. 7). The *mulleri* and *buzzatii* complexes are sister clades, whereas the *eremophila* complex is placed basal in the subgroup, as the sister clade of the *hydei* subgroup.

Zaprionus genus group. – The sister genera *Phorticella* (11 species) and *Zaprionus* (56) are easily recognized by the white striping on the head and mesonotum (Chassagnard 1988). The genus *Zaprionus* has two subgenera, *Zaprionus* (44 species; even number of stripes) and *Anaprionus* (12 species; odd number of stripes) (Chassagnard 1988; Pélandakis & Solignac 1993). The subgenus *Zaprionus* is divided in two species groups, *inermis* (14 species) and *armatus* (30); the latter is subdivided into three species subgroups (*armatus*, 14 species; *tuberculatus*, 3; and *vittiger*, 13) (Chassagnard 1988; Chassagnard & Tsacas 1993;

Da Lage et al. 2007). The genus *Phorticella* has two subgenera, *Phorticella* (7 species) and *Xenophorticella* (4) (Okada & Carson 1983b). The genus *Zaprionus* and its two subgenera are monophyletic in the supertree analysis, but the species groups were not (Fig. 5).

Genus *Scaptomyza*. – O’Grady et al. (2003a) provide a tree of the subgenera based on both molecular (Bonacum 2001) and morphological data and include the subgenus *Engiscaptomyza*, which was originally included as a separate subgenus in *Drosophila* before Grimaldi (1990) proposed removing it from *Drosophila* (p. 120). He placed the subgenus implicitly in *Scaptomyza* because it was closely related to *Scaptomyza* for the time being as he argued that it ‘should eventually be elevated to generic status’ (p.123). Many studies have confirmed the proximity of *Engiscaptomyza* to *Scaptomyza* (Throckmorton 1966; DeSalle & Grimaldi 1991; Thomas & Hunt 1991, 1993; DeSalle 1992a; Russo et al. 1995; Kambysellis & Craddock 1997; Remsen & DeSalle 1998; Kwiatowski & Ayala 1999; Katoh et al. 2000; Bonacum 2001; Remsen & O’Grady 2002). The monophyly of the genus and the inclusion of *Engiscaptomyza* were confirmed in the supertree analysis (Fig. 6).

‘*Hawaiian Drosophila*’. – Authors differ in the taxonomic status of the ‘*Hawaiian Drosophila*’; a minority (e.g., Powell & DeSalle 1995; Powell 1997; Bächli 1999-2008; Starmer et al. 2003; Ashburner et al. 2005) follow Grimaldi (1990) and place them in either the genus *Idiomyia* or as the subgenus *Idiomyia* within *Drosophila*. O’Grady (2002) has proposed that *Idiomyia* be synonymized with the subgenus *Drosophila*, as it is placed within the subgenus *Drosophila*, but does not apply the same reasoning for the genus *Scaptomyza*, the sister clade of the ‘*Hawaiian Drosophila*’ (O’Grady et al. 2003a). Molecular studies show that this group is monophyletic and firmly placed within the subgenus *Drosophila* (Throckmorton 1966; Beverley & Wilson 1984; DeSalle 1992a; Thomas & Hunt 1993; Kambysellis et al. 1995; Russo et al. 1995; Remsen & DeSalle 1998; Tataronov et al. 1999, 2001; Davis 2000; Davis et al. 2000; Gailey et al. 2000; Remsen & O’Grady 2002; Da Lage et al. 2007; Katoh et al. 2007). The group includes 7 species groups: *antopocerus* (15 species), *haleakalae* (54),

‘modified mouthparts’ (47), ‘modified tarsus’ (48), ‘picture wing’ (143), *rustica* (3), *nudidrosophila* (28), and *ateledrosophila* (3) (species counts for the last two: Magnacca & O’Grady 2008). The *planitibia* subgroup is sometimes considered a species group (Bonacum et al. 2005; Markow & O’Grady 2006). All studies place the *haleakalae* group basal to all other groups (Kambysellis et al. 1995; Baker & DeSalle 1997; Bonacum 2001). The ‘modified mouthparts’ and ‘picture wing’ groups are sister clades according to most authors (Thomas & Hunt 1991, 1993; Baker & DeSalle 1997; Bonacum 2001), whereas Bonacum (2001) places the *nudidrosophila* within the ‘picture wing’ clade. The *antopocerus* group is the sister clade of the ‘modified mouthparts’-‘picture wing’ clade (Baker & DeSalle 1997). The ‘modified tarsus’ group is the sister group of the *antopocerus* group (Baker & DeSalle 1997; Bonacum 2001). The single study in disagreement obtained weak bootstrap support for the alternative (DeSalle 1992a). The supertree analysis confirms the general pattern as described (Fig. 6).

Several studies detailing specific groups within the ‘*Hawaiian Drosophila*’ have been published for the ‘picture wing’ species group (Kambysellis et al. 1995; Kaneshiro et al. 1995), *planitibia* subgroup (Bonacum et al. 2005), *rustica* group (O’Grady et al. 2001b), *mimica* subgroup (O’Grady et al. 2003b), and *haleakalae* group (Hardy et al. 2001; O’Grady & Zilversmit 2004).

Discussion

Here, we present the results of our examination of the current phylogenetic status of the genus *Drosophila* and the related genera. The resulting phylogenetic tree is the first detailed summary of the relevant phylogenetic studies since the last family-wide cladistic analysis by Grimaldi (1990), which was based on morphological data. Most studies we review analysed DNA sequences (Table 1), although a few studies still employed more traditional morphology-based cladistic (Hu & Toda 2001; Sidorenko 2002) or chromosome-inversion analyses (Durando et al. 2000; O’Grady et al. 2001a; Diniz & Sene 2004).

Our review and construction of a supertree from the large volume of work covering the genus *Drosophila* and related genera shows that our insight into the phylogenetic history of this group has greatly improved over the last 17 years. Later

work has sometimes confirmed the phylogeny proposed by earlier researchers (e.g., Throckmorton 1975; Grimaldi 1990) and sometimes resulted in new insights. This improved understanding of the phylogenetic history will facilitate comparative studies covering a wide range of species but might also direct researchers toward studying clades for which molecular studies are lacking or poorly executed, such as the genera *Zaprionus*, *Hirtodrosophila*, and *Scaptodrosophila*. Furthermore, various groups have been identified as polyphyletic or paraphyletic by molecular studies, most notably the genus *Drosophila* itself (see below).

In some cases, we were unable to find evidence that supported one topology over others. We treat those as polytomies in our trees. Some of these polytomies probably reflect rapid branching, such as the *D. simulans*, *D. mauritiana*, and *D. sechellia* complex, which probably reflects nearly simultaneous speciation (Kliman et al. 2000). Such polytomies may never be resolved. For many others, suitable data are simply lacking. The most noteworthy polytomies are the placement of the *melanogaster* species subgroup and the various oriental species subgroups and the various polytomies within the *immigrans-tripunctata* radiation.

Supertrees. – Supertrees can be used to build a phylogenetic hypothesis based on averaging of the phylogenetic signal present in published phylogenies, the source trees. Our analysis of the complete data resulted in many aspects in a generally well-resolved tree, even though more than 20,000 equally parsimonious trees were found. The large number was a result of a limited number of small polytomies, which, when combined, result in the large number of unique trees, but supertrees are only as good as the resource trees used for the analysis. The number of unique trees available for specific clades varied dramatically; a greater number of trees generally resulted in better resolved clades. In general, the supertree was fairly well resolved and provides a solid hypothesis for further studies.

The taxonomic status of the genus *Drosophila*. – The genus *Drosophila* Fallén 1823, is paraphyletic, and various genera and subgenera are even located within the subgenus *Drosophila*, making it also paraphyletic (see subgenus *Drosophila* for discussion). Several authors have suggested that

this situation should be addressed by a change in nomenclature (Kwiatowski et al. 1997; Tatarenkov et al. 1999; Hu & Toda 2001; Remsen & O'Grady 2002; Robe et al. 2005; Markow & O'Grady 2006; Da Lage et al. 2007; Magnacca & O'Grady 2008). On the basis of our review, a revision of the genus seems warranted, and the issue will be addressed in a separate article (van der Linde et al. 2007, accepted).

Challenges for the future. – Of primary importance is the placement of the genera *Hirtodrosophila*, *Zaprionus*, *Liodrosophila*, *Samoia*, and related genera relative to each other. Another issue is the poorly resolved topology in the *tripunctata* clade. Current studies make clear that the *tripunctata* group is paraphyletic. Many species and probably multiple genes will have to be sequenced before the topology within this group can be considered resolved. In addition, the current group designation must be altered to reflect current phylogenetic insights. The *repleta* species group presents a similar problem; it might include several other species groups.

Finally, many species and genera have never been included in any molecular study. Many of these species are difficult to culture or have been collected only rarely for studies. On the basis of more traditional classifications (e.g., Grimaldi 1990), several other genera might be positioned within the current limits of *Drosophila*, e.g. *Zygothrica*, *Phorticella*, and *Paramycodrosophila*. We also expect inclusion of more species to result in the discovery of more paraphyletic taxa and subsequent taxonomic changes. Finally, if the genus *Drosophila* and subgenus *Drosophila* are split as we suggest the lower-level taxonomic assignments will have to be revisited and adjusted accordingly. We expect that many of these uncertainties will be resolved in the next 10 years, allowing this well-known family to become a model for comparative biology as it is already for genetics.

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