

Phylogeny of the Genus *Drosophila*

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ABSTRACT Understanding phylogenetic relationships among taxa is key to designing and implementing comparative analyses. The genus *Drosophila*, which contains over 1600 species, is one of the most important model systems in the biological sciences. For over a century, one species in this group, *Drosophila melanogaster*, has been key to studies of animal development and genetics, genome organization and evolution, and human disease. As whole-genome sequencing becomes more cost-effective, there is increasing interest in other members of this morphologically, ecologically, and behaviorally diverse genus. Phylogenetic relationships within *Drosophila* are complicated, and the goal of this paper is to provide a review of the recent taxonomic changes and phylogenetic relationships in this genus to aid in further comparative studies.

KEYWORDS *Drosophila*; taxonomy; phylogenetics; nomenclature; evolutionary history; Hawaiian *Drosophila*; *Scaptomyza*; *virilis-repleta* radiation; *immigrans-tripunctata* radiation; FlyBook

TABLE OF CONTENTS

Abstract	1
Introduction	2
A History of Phylogenetic Studies in <i>Drosophila</i>	2
<i>Sturtevant</i>	2
<i>The Texas group: Patterson, Stone, and colleagues</i>	3
<i>Margaret Kidwell</i>	3
<i>American Museum of Natural History Group</i>	4
<i>Ayala and colleagues</i>	4
<i>Toyohi Okada and the Asian school of drosophilists</i>	4
<i>Georges Teissier, Leonidas Tsacas, and the French drosophilists</i>	5
<i>Corowaldo Pavan, Danko Brncic, and the South American drosophilists</i>	5
Taxonomy and Phylogeny in <i>Drosophila</i>	5
<i>Drosophila</i> taxonomy	6
Phylogenetics	8
<i>Drosophila</i> : A Genus Divided	8
Phylogenetic Relationships Within the Family Drosophilidae	9

Continued

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CONTENTS, *continued*

<i>Steganinae and early-branching Drosophilinae</i>	12
<i>Relationships within major lineages of Drosophila</i>	12
<i>The subgenus Sophophora</i>	12
<i>The subgenus Drosophila</i>	12
<i>Hawaiian Drosophilidae</i>	14
<i>The subgenus Siphodora</i>	15
An Emerging Picture of Phylogenetic Relationships in Drosophilidae	15
Future Research	17
<i>Taxonomy</i>	18
<i>Outgroups</i>	19
<i>Morphological characteristics</i>	19
<i>Whole-genome phylogenetics</i>	19
<i>Conclusions</i>	21

THE family Drosophilidae is one of the premiere model systems in genetics, developmental biology, and genomics (Gompel and Carroll 2003; Gompel *et al.* 2005; Prud'homme and Gompel 2010, 2011; Kuntz and Eisen 2014; Wangler *et al.* 2015; Ugur *et al.* 2016). Many of these studies rely heavily on a knowledge of the phylogenetic relationships in this family. While there have been several phylogenetic treatments over the years (Throckmorton 1975; Grimaldi 1990; DeSalle 1992; Kwiatowski and Ayala 1999; Tatarenkov and Ayala 2001; Remsen and O'Grady 2002; O'Grady *et al.* 2008b, 2011; O'Grady and DeSalle 2008; van der Linde *et al.* 2010), these have suffered from either poor taxon sampling, poor support, or both. In fact, the monophyly and placement of a number of important groups remains either poorly supported or contentious in spite of extensive molecular and morphological evidence. Open questions remaining to be resolved include the monophyly and placement of the major lineages in the genus *Drosophila*, particularly the placement of the subgenus *Sophophora*, and the placement and monophyly of the Hawaiian species of Drosophilidae.

The goal of this review is to provide a summary of *Drosophila* phylogeny, including the origin of this field and its development in the past 100 years, by summarizing the major phylogenetic studies throughout the last decade. The first section provides a historical account of the researchers working on taxonomic and phylogenetic studies. The second section summarizes and discusses the recent hypotheses of relationships between and within the major lineages (*e.g.*, including multiple species groups, subgenera, and/or “radiations”) of the Drosophilidae. This includes a discussion of the ramifications of the paraphyly of *Drosophila*. Finally, we discuss the prospects for future taxonomic, phylogenetic, and comparative genomic work in *Drosophila* as a result of the expansion of genome sequencing.

A History of Phylogenetic Studies in *Drosophila*

Unfortunately, it is not possible to provide a comprehensive list of the many excellent studies done at the species group level,

but these can be found elsewhere (*e.g.*, Powell 1997; Markow and O'Grady 2006). Although we have made an attempt to divide this section into “schools” or groups of researchers, this is largely artificial. The history of *Drosophila* phylogenetic research is reticulate, with researchers influencing, and being influenced by, multiple groups. For example, O'Grady's research has been influenced by the genetics community through his major professor Margaret Kidwell, the Texas group via his friend and mentor Bill Heed, and by his post-doctoral advisor Rob DeSalle's own phylogenetic and population genetic experience. DeSalle, in turn, has been influenced by his work as an undergraduate with Lynn Throckmorton, a member of the Texas group, and during his graduate and post-doctoral research with Alan Templeton, Dan Hartl, and Alan Wilson.

Sturtevant

While Sturtevant was one of the most influential geneticists of the 20th Century, he was also interested in a broad range of biological questions, including the genetics of species formation and insect taxonomy (Sturtevant 1920; Sturtevant and Wheeler 1953). Sturtevant was one of the first drosophilid taxonomists to fully catalog the family (Sturtevant 1921). He recorded a total of 398 species placed in 40 genera. Of this total, 242 species were placed in the genus *Drosophila*. Sturtevant was also one of the first *Drosophila* biologists to think broadly about the evolution of this genus. He treated the North American taxa (Sturtevant 1916, 1921), introduced the concept of species groups to *Drosophila* (Sturtevant 1939), and subdivided the genus *Drosophila* into subgenera (Sturtevant 1939, 1942). Since Sturtevant's initial catalog almost 100 years ago, both the family Drosophilidae and the genus *Drosophila* have grown significantly, and each is now ~10 times larger than they were in Sturtevant's day.

Sturtevant thought more clearly about characters than most of his contemporaries, both in terms of their use as tools

for species identification and in how they might be used to define groups of related species. He also tried to be as objective as possible about the process of phylogenetic inference. In a short note to the *Proceedings of the National Academy of Sciences* (Sturtevant 1939), Sturtevant outlined his “attempt to derive a classification by a method as free of personal equation as can be made.” And later, in a larger revision of the genus *Drosophila* (Sturtevant 1942), he carefully outlines the character and character systems used, and provides matrices of binary and continuous characters. He even had clear concepts of synapomorphy and autapomorphy, and their relative usefulness in inferring relationships. Clearly, Sturtevant’s access to the Dipteran literature brought him in contact with Willi Hennig’s thoughts on phylogenetic systematics long before the publication of “Phylogenetic Systematics” in German (Hennig 1950) or English (Hennig 1966). It is intriguing to consider the possible cross-fertilization of ideas between these two great scientists, and provocative to think that many of Hennig’s ideas may have been inspired by Sturtevant.

The Texas group: Patterson, Stone, and colleagues

J. T. Patterson and Wilson Stone established a fly laboratory at the University of Texas (UT), Austin in the early 1900s (Painter 1965; Wagner and Crow 2001). This group, like Morgan’s fly laboratory at Columbia, initially focused on the genetics of *Drosophila melanogaster*. However, over time, Patterson and his colleagues became more interested in correlating morphological differences with polytene chromosome banding patterns to examine differences among species. The *Drosophila* community created by Patterson included many prominent *Drosophila* workers, Nobel prize winner Herman J. Muller among them (Crow and Abrahamson 1997). This group became a focal point of ecological and evolutionary studies on *Drosophila* from the 1940s through the 1960s. Several prominent *Drosophila* researchers were either trained by or collaborated with the Texas group during these years, including William Heed, Hampton Carson, Herman Spieth, Marshal Wheeler, Lynn Throckmorton, Marvin Wasserman, Harrison Stalker, Frances Clayton, Michael Kambysellis, D. Elmo Hardy, Jong Sik Yoon, and many others. Important contributions of this group include the Hawaiian *Drosophila* Project, the *Drosophila*-cactus model system of the *repleta* species group, and the National *Drosophila* Species Stock Center. It is clear that the atmosphere created by Patterson was conducive to many aspects of *Drosophila* biology, not just ecology and evolution. Other members of the Texas group also went on to study population genetics (James F. Crow, Charles Langley, and John Gillespie), developmental biology (Thom Kauffman and Burke Judd), and the history of genetics (James F. Crow).

Marshall Wheeler, a student of Patterson, produced a number of catalogs over the years (Wheeler 1959, 1981). His last (Wheeler 1986) listed a total of 2822 species in 62 genera in the family Drosophilidae. Over the course of his career, Wheeler described > 250 drosophilid species

and, more importantly, created identification keys for most of the known genera and species. One of his most significant contributions was a revision of the *melanogaster* species group, where he treated the ~100 species known at the time, describing ~30 new species in this group (Bock and Wheeler 1972). Wheeler also was the person responsible for starting and maintaining the National *Drosophila* Species Stock Center at UT Austin from the 1940s until his retirement in the mid-1970s. This resource, still available to the *Drosophila* community today, is due largely to the work of Wheeler and the other members of the UT Austin group.

Throckmorton, also a member of the *Drosophila* group at UT Austin, published several seminal papers on the phylogenetic relationships and biogeography of Drosophilidae (Throckmorton 1962, 1966, 1975, 1982). Together, these papers are some of the most influential works on phylogeny in *Drosophila* and have served as the starting point for many comparative studies. While no explicit method was ever discussed for how these relationships were constructed and some of the work predated the publication of Hennig’s (1966) book on “Phylogenetic Systematics” in English, it is clear that whatever “algorithm” Throckmorton used was based on some repeatable notion of shared character states, or synapomorphy. This may have been informed by Throckmorton’s interactions with Hennig and/or Sturtevant. Throckmorton published a significant paper in *Systematic Biology* (Throckmorton 1968) that discussed the concordance and discordance of taxonomic characters in the genus *Drosophila*. This paper demonstrates that Throckmorton was thinking deeply about how to score and assess characters, in the Hennigian sense, as least as early as 2 years after Hennig’s book. This is an impressive theoretical contribution that presaged the molecular vs. morphological character debate that consumed much of the literature in the 1980s and early 1990s (DeSalle and Grimaldi 1991, 1992, 1993).

Margaret Kidwell

While Margaret Kidwell was not a systematist *per se*, she did have a large influence on the field of *Drosophila* phylogenetics through her work on the evolution of transposable elements in the genus *Drosophila*. She produced several papers comparing species and transposon phylogenies (O’Grady *et al.* 1998; Silva and Kidwell 2000, 2004; O’Grady and Kidwell 2002), and was instrumental in some of the early discussions between gene tree and species trees. Kidwell trained several graduate students (O’Grady and da Silva), postdoctoral fellows (Clark, Wojciechowski, and Dickerman) and visiting scholars (Loreto, Robe, Valente, and Watada), who went on to produce taxonomic revisions and/or phylogenetic hypotheses at the species group, generic, and family levels. Her training was strongly influenced by several *Drosophila* biologists during her time at Brown University, including her husband James Kidwell, her mentor and dissertation advisor Masatoshi Nei, and her colleague Francisco Ayala.

American Museum of Natural History Group

Rob DeSalle began working on phylogenetic relationships in *Drosophila* in the late 1970s while he was an undergraduate. He generated some of the first molecular phylogenetic hypotheses for generic level relationships in the family (DeSalle 1992) and among the *picture wing* species group of Hawaiian *Drosophila* as a graduate student. He also trained a number of researchers, (Remsen, Bonacum, Stark, Durando, Almeida, Oliveira, Baker, Yassin, and O'Grady), many of whom have continued to contribute to *Drosophila* systematics.

Patrick O'Grady, a student of Margaret Kidwell's, produced an early multigene phylogeny of relationships in the family Drosophilidae (O'Grady 1998). This work was expanded in collaboration with James Remsen (Remsen and O'Grady 2002) to include > 40 species, representing 16 genera and 27 species groups of Drosophilidae. Subsequent work has expanded taxon sampling to > 300 species from over half of the 64 recognized genera in the Drosophilidae. O'Grady and his collaborators, including James Bonacum, Richard Lapoint, Karl Magnacca, and Noah Whiteman, have also focused on the Hawaiian Drosophilidae, producing several extensive phylogenies of hundreds of species in this lineage (Lapoint *et al.* 2011, 2013, 2014; O'Grady *et al.* 2011).

James Remsen and Richard Baker, both graduate students with Rob DeSalle, published several important phylogenetic studies on relationships within the Drosophilidae, two focusing on Hawaiian *Drosophila* (Baker and DeSalle 1997; Remsen and DeSalle 1998) and another examining the phylogeny of major lineages within the Drosophilidae (Remsen and O'Grady 2002; see above). Another DeSalle student, James Bonacum, made important contributions to our understanding of the phylogenetic relationships within the endemic Hawaiian Drosophilidae (Bonacum 2001; O'Grady *et al.* 2011).

Grimaldi (1990) produced the most recent, and comprehensive, taxonomic and phylogenetic treatment of the family Drosophilidae, examining 180 morphological characters from ~208 species. This was the first study to take an explicit algorithmic approach using morphological characters, rather than gestalt, to revise the Drosophilidae based on phylogenetic relationships, not gestalt. While some of Grimaldi's analytical methods have been questioned (Remsen and O'Grady 2002) and some of his hypothesized relationships conflict with DNA analyses (DeSalle and Grimaldi 1991, 1992), this is a landmark study and many of the relationships proposed are well supported by molecular evidence.

Ayala and colleagues

Francisco Ayala, a former Dominican priest, was the first student of Theodosius Dobzhansky. He has published widely in evolutionary biology and has contributed extensively to our understanding of relationships within the *obscura* species group, particularly among populations of *D. pseudoobscura*. Starting in the late 1990s, Ayala and his students began using DNA sequences from several loci to infer broad-scale

phylogenetic relationships in Drosophilidae. Kwiatowski and Ayala (1999) examined relationships among *Drosophila* and several closely allied genera. Later, Tatarenkov and Ayala (2001) focused on inferring phylogeny within the *virilis-repleta* radiation. Ayala's other academic descendants have also had a large impact on *Drosophila* evolutionary biology. Several of these (*e.g.*, Fontdevilla, Ruiz, and Ranz) have contributed to our understanding of *Drosophila* phylogeny and taxonomy, particularly within the cactophilic *D. repleta* species group.

Toyohi Okada and the Asian school of drosophilists

Toyohi Okada was one of the earliest Asian *Drosophila* systematists. He worked on a number of questions in drosophilid systematics and taxonomy, starting in the 1950s and continuing until the early 1990s. His taxonomic work was impressive and included descriptions of hundreds of new drosophilid species from the Asian region. Okada, much like Throckmorton, also thought deeply about morphological characters and their role in inferring phylogenetic relationships. Two of his systematic papers, one on the Drosophilidae of Japan (Okada 1956) and another on the early life stages of Drosophilidae (Okada 1968), are significant contributions to our understanding of morphological evolution in this family. Over the course of his career, Okada, produced several character matrices and phylogenies that were clearly inspired by the field of numerical taxonomy.

A number of modern researchers, including Masanori Toda, Toru Katoh, Hide-aki Watabe, Masaoshi Watada, and Koichiro Tamura, have made important contributions to our understanding of phylogenetic relationships in the family Drosophilidae. Toda has succeeded Okada as the leading *Drosophila* taxonomist. He has described hundreds of new *Drosophila* species over the course of his career and produced treatments of many of the genera of Drosophilidae. He has also trained or collaborated with an impressive number of students, all of whom continue to make significant contributions to *Drosophila* phylogenetic systematics. Koichiro Tamura began publishing on *Drosophila* molecular evolution in the early 1990s and produced his first *Drosophila* phylogeny in 1995 (Tamura *et al.* 1995). Since that time, he has produced a number of important phylogenetic papers on higher-level relationships within drosophilid flies (*e.g.*, Katoh *et al.* 2002), as well as a number of molecular clock studies placing *Drosophila* evolution within an explicit temporal context (Tamura *et al.* 2004).

Toru Katoh began publishing on phylogenetic relationships within Drosophilidae in the late 1990s, mainly focused on the phylogeny of *Lordiphosa*, a lineage initially considered a subgenus of *Drosophila*, and its relationships to the subgenus *Sophophora* (Katoh *et al.* 2000; Gao *et al.* 2011). *Lordiphosa* had not been included in previous studies because it was difficult to culture, and was restricted to the tropical and subtropical regions of Asia. Katoh's work demonstrated that both subgenera were paraphyletic with respect to one another and that a large number of *Lordiphosa* species actually

belonged within the subgenus *Sophophora* (Katoh *et al.* 2000, 2002; Gao *et al.* 2011). His subsequent work has focused on the *immigrans* species group (Katoh *et al.* 2007), the genus *Scaptomyza* (Katoh *et al.* 2017), and biogeography in the genus *Drosophila* (Izumitani *et al.* 2016).

A number of Chinese *Drosophila* systematists, such as Hong-wei Chen and J. J. Gao, have been trained by Toda or Katoh and are becoming active in the field. They and their students have contributed much to our understanding of relationships among the early diverging lineages in the Steganiinae (*e.g.*, Chen *et al.* 2004; Zhao *et al.* 2009, 2013; Li *et al.* 2014).

Georges Teissier, Leonidas Tsacas, and the French drosophilists

A number of French workers, many based at the Gif-sur-Yvette laboratory of the Centre National de la Recherche Scientifique, have contributed significantly to our understanding of relationships within the genus *Drosophila*. This laboratory was founded in 1951 by the Georges Teissier, a *Drosophila* population geneticist and crustacean biologist. He was succeeded by his student Charles Bocquet in 1965. Leonidas Tsacas joined the lab in the mid-1960s and worked on the Afrotropical fauna until his retirement in the 1990s. Over his career, he described > 200 species, including *D. teissieri* (Tsacas 1971). He also trained and collaborated with an entire generation of French *Drosophila* workers, and was integral to the development of many of the resources available to the *Drosophila* community, particularly those studying the *melanogaster* species group. Marie-Thérèse Chassagnard was a collaborator, contributing many beautiful illustrations to Tsacas' species descriptions. Two of Tsacas' students, Daniel Lachaise and Françoise Lemeunier, began working on *Drosophila* ecology and phylogeny in the 1970s (*e.g.*, Tsacas *et al.* 1981). Lachaise focused on the African fauna and discovered *D. santomea* (Lachaise *et al.* 2000). Lemeunier, in collaboration with Michael Ashburner at Cambridge, proposed the first phylogeny of the *melanogaster* subgroup based on polytene chromosome banding patterns (Ashburner and Lemeunier 1976).

Tsacas collaborated extensively with Jean David, first when David was at Lyon and later when he moved to Gif in 1979 to serve as Director of the laboratory. Australian researcher Shane McEvey joined the laboratory in the late 1980s, and began to work on the fauna of Madagascar and French Polynesia. During the 1980s, two of Bocquet's students, Michel Solignac and Marie-Louise Cariou, converted to working on *Drosophila* molecular phylogenetics. Solignac, along with his student Michel Pélandakis, addressed phylogenetic relationships in the Drosophilidae using 28S rDNA sequences (Pélandakis *et al.* 1991; Pélandakis and Solignac 1993). These two studies were the first to sample broadly at the species level, greatly expanding on the taxon sampling of previous work (DeSalle 1992). Their first study, a phylogeny of the subgenus *Sophophora* (Pélandakis *et al.* 1991), is an important companion to Bock and Wheeler's (1972) revision

of the *D. melanogaster* species group, proposing phylogenetic placements for many of the species treated in the earlier work, as well as the first outline of relationships among species groups within the subgenus *Sophophora*. Their latter paper (Pélandakis and Solignac 1993) included > 70 species representing multiple genera in Drosophilidae, and species groups within the genus and subgenus *Drosophila*.

Cariou succeeded David as a director of the Gif laboratory in 1992. The majority of David's phylogenetic work over the past decade has been in collaboration with younger colleagues, including Amir Yassin and Jean-Luc Da Lage. Da Lage, a student of Cariou's, generated several phylogenetic papers, including one examining relationships across Drosophilidae using the Amylase locus (Da Lage *et al.* 2007). Amir Yassin, one of Jean David's students, has worked on understanding phylogenetic relationships both at the genus level, and within the genus *Zaprionus* (Yassin 2007; Yassin and David 2010). Perhaps his most significant contribution to date is a revision of the family Drosophilidae, where he attempted to resolve some of the issues with paraphyly in the genus *Drosophila* (Yassin *et al.* 2010; Yassin 2013).

Corowaldo Pavan, Danko Brncic, and the South American drosophilists

The Brazilian school of drosophilists was initiated by Theodosius Dobzhansky (Pavan and da Cunha 2003). He had several early collaborations with Crodowaldo Pavan (Dobzhansky and Pavan 1943a,b, 1950). These led to further work by da Cunha and others (Pavan and da Cunha 1947). Dobzhansky also worked with other members of the South American *Drosophila* community, often via the Rockefeller Institution (Pavan and da Cunha 2003; de Magalhães and Vilela 2014). Danko Brncic at the University of Chile worked on chromosome inversions and taxonomy in Chilean *Drosophila* (Brncic 1955; Brncic and Dobzhansky 1957). The UT Austin fly group also collaborated widely in South America, working with Hermoine Bicudo, Luis Magalhaes, and Danko Brncic, among others.

Recent workers continue to add to our understanding of taxonomic and phylogenetic relationships in Drosophilidae. Violeta Rafael and her students Andrea Acurio and Doris Vela have made important contributions to the fauna of Ecuador, as well as to our broader understanding of evolution in several species groups (Rafael and Vela 2000; Acurio and Rafael 2009). Interestingly, several of the present-day Brazilian workers were trained in molecular techniques while investigating the evolution of transposable elements in Margaret Kidwell's laboratory. These include Vera Valente and Elgion Loreto. Other influential Brazilian researchers include Carlos Vilela, Francisca do Val, and Claudia Russo (Val *et al.* 1981; Vilela and Baechli 1990; Russo *et al.* 1995, 2013).

Taxonomy and Phylogeny in *Drosophila*

The related fields of taxonomy, nomenclature, and phylogeny overlap in the minds of many *Drosophila* evolutionary

biologists. However, each is quite unique and has its own specific focus. Taxonomy is the field dedicated to the description, identification, naming, and classification of species. α taxonomy provides a framework for identifying and describing new species. Type specimens are designated and deposited in museum collections to serve as a standard by which to compare other putative members of a given species. Nomenclature, specifically biological nomenclature, is a sub-discipline of taxonomy that provides a series of rules to guide the naming of species. *Drosophila* species names are governed by The International Code of Zoological Nomenclature (ICZN; International Commission for Zoological Nomenclature 1999). Taxonomists also produce lists of known species and create a classificatory hierarchy above the level of species, placing taxa into genera, families, orders, and other higher groups. Phylogenetics, the study of evolutionary relationships among organisms, relies on character-based data and repeatable, explicit analytical analyses. While phylogenetic analyses can be used to classify species, this discipline is distinct from taxonomy because of its explicit evolutionary focus and reliance on an objective, repeatable, and analytical framework in which to generate hypotheses of evolutionary relationships.

Kim van der Linde and colleagues published several important papers in *Drosophila* phylogenetics in the late 2000s, two of which aimed to split the subgenus *Drosophila* into multiple genera (van der Linde and Houle 2008; van der Linde *et al.* 2010), and another which attempted to reassign the type of the genus from *D. funebris* to the genetic model species *D. melanogaster* (van der Linde *et al.* 2007). Neither attempt was successful, but this work did stimulate discussion within the community that may, in time, lead to a comprehensive revision of the family (O'Grady and Markow 2009; O'Grady 2010; van der Linde and Yassin 2010).

The issues involved with the abortive reorganization of *Drosophila* are complex, but important to touch upon in detail. The type of a genus, subgenus, or other higher-level taxonomic group is determined by priority, or which species in that genus was described first. *D. funebris* is the type of the genus *Drosophila*. This species was described by Fabricius (1787) as *Musca funebris* and then later transferred to the genus *Drosophila* when it was erected by Fallén (1823). It is the type of the genus *Drosophila* because it was the first species described in this genus. *D. melanogaster* was described over 40 years later by Meigen (1830). When Sturtevant (1939) divided *Drosophila* into subgenera, *D. funebris* and *D. melanogaster* were designated as the types of the subgenera *Drosophila* and *Sophophora*, respectively. When van der Linde and colleagues (van der Linde and Houle 2008; van der Linde *et al.* 2010) proposed dividing *Drosophila* into multiple genera, the newly proposed genus names needed to track the earliest described types in each of the subgenera. Therefore, the genus name *Drosophila* would apply to *D. funebris* and its relatives, and the genus name *Sophophora* would apply to *D. melanogaster* and its relatives. To avoid this potentially confusing name change, where *D. melanogaster* would

become *Sophophora melanogaster*, van der Linde proposed redesignating the type from *D. funebris* to *D. melanogaster*, thus preserving the binomen *D. melanogaster*. Such nomenclatural changes are rare, but not unheard of, provided there is a strong justification for the move. The critical factor with the proposed van der Linde reorganization was that, while the rules of nomenclature and taxonomy were followed, none of the proposed genera were statistically supported by phylogenetic analyses, meaning that there were arbitrary entities not supported by any data.

***Drosophila* taxonomy**

The most recently published print catalog of the family Drosophilidae lists 3962 described species (3950 extant and 12 extinct), placed in > 70 genera (Brake and Bächli 2008). While the placement and monophyly of some genera is controversial (see below), it is generally accepted that all genera are placed into two subfamilies, Steganinae and Drosophilinae. Brake and Bächli (2008) listed 28 genera and 697 species in the subfamily Steganinae. The subfamily Drosophilinae is considerably larger, with 43 genera and 3265 species. The online database TaxoDros (<http://www.taxodros.uzh.ch/>) now lists nearly 4500 species of Drosophilidae. The current numbers from the TaxoDros Database (Bächli 2015, accessed March 2017) are 29 genera and 963 species of Steganinae, and 48 genera and 3497 species of Drosophilinae (Table 1).

The genus *Drosophila* is placed within the subfamily Drosophilinae and, as traditionally described, includes ~2000 species, or roughly 50% of the species in the family (Table 2). This unbalanced situation is partly the result of the actual evolutionary process, where members of the genus *Drosophila* have adapted to and radiated in a variety of niches, and subsequently diversified rapidly (Markow and O'Grady 2005, 2006; Markow *et al.* 2008). It is also due to shortcomings of human perception and how species are placed into genera. For example, the genus *Drosophila* has been used as a “catch all” for most of the last 150 years, meaning that all taxa not easily placed within a more distinctive genus were placed as *incertae sedis* in *Drosophila*.

With such a large number of species placed within the genus *Drosophila*, > 80% of which are in a single subgenus (*Drosophila*), drosophilid taxonomists have introduced a number of informal taxonomic ranks to help organize the diversity within this large clade. Arguably the two most useful are the radiation, introduced by Throckmorton (1962), and the species group, first employed by Sturtevant (1942). Both ranks reflect the complex evolutionary history of this family. Radiations reflect the polyphyletic nature of both the genus and subgenus *Drosophila*, and are composed of a number of genera, subgenera, and species groups. Species groups are closely related assemblages of species erected based on a series of shared morphological traits (*e.g.*, sex combs) and other characteristics. For example, polytene chromosome banding patterns can generally be aligned (*i.e.*, a homology statement can be made with confidence)

Table 1 Diversity of genera and subgenera placed in Drosophilidae

Genus	Subgenus	Notes
Steganinae (963)		
<i>Acletoxenus</i> (4)	N/A	
<i>Allopygaea</i> (3)	N/A	
<i>Amiota</i> (135)	<i>Amiota</i> (134) Unplaced (1)	
<i>Apenthecia</i> (16)	<i>Apenthecia</i> (10) <i>Parapenthecia</i> (6)	
<i>Apsiphortica</i> (6)	N/A	
<i>Cacoxenus</i> (40)	<i>Cacoxenus</i> (1) <i>Gitonides</i> (24) <i>Nankangomyia</i> (6) <i>Paracacoxenus</i> (5) <i>Phragmitoxenus</i> (1) Unplaced (3)	
<i>Crincosia</i> (3)	N/A	
<i>Electrophortica</i> (1)	N/A	
<i>Eostegana</i> (13)	N/A	
<i>Erima</i> (1)	N/A	
<i>Gitona</i> (16)	N/A	
<i>Hyalistata</i> (6)	<i>Hyalistata</i> (6)	
<i>Leucophenga</i> (242)	<i>Leucophenga</i> (225)	
<i>Luzonimyia</i> (8)	N/A	
<i>Mayagueza</i> (1)	N/A	
<i>Neorhinoleucophenga</i> (2)	N/A	
<i>Paraleucophenga</i> (12)	N/A	
<i>Paraphortica</i> (1)	N/A	
<i>Pararhinoleucophenga</i> (11)	N/A	
<i>Parastegana</i> (6)	<i>Allstegana</i> (4) <i>Parastegana</i> (1) Unplaced (1)	
<i>Phortica</i> (131)	<i>Alloparadisa</i> (2) <i>Allophortica</i> (5) <i>Ashima</i> (21) <i>Phortica</i> (99) <i>Shangrila</i> (2) <i>Sinophthalmus</i> (2)	
<i>Pseudiasata</i> (6)	<i>Pseudiasata</i> (6)	
<i>Pseudocacoxenus</i> (1)	N/A	
<i>Pseudostegana</i> (39)	N/A	
<i>Pyrgometopa</i> (1)	N/A	
<i>Rhinoleucophenga</i> (30)	N/A	
<i>Soederdomia</i> (1)	N/A	
<i>Stegana</i> (226)	<i>Ceratostylus</i> (1) <i>Orthostegana</i> (7) <i>Oxyphortica</i> (28) <i>Stegana</i> (26) <i>Steganina</i> (160) Unplaced (4)	
<i>Trachyleucophenga</i> (1)	N/A	
Drosophilidae (3497)	N/A	
<i>Arengomyia</i> (3)	N/A	
<i>Baeodrosophila</i> (5)	N/A	
<i>Bialba</i> (1)	N/A	
<i>Calodrosophila</i> (1)	N/A	
<i>Celidosoma</i> (1)	N/A	
<i>Chymomyza</i> (57)	N/A	
<i>Cladochaeta</i> (137)	N/A	
<i>Colocasiomyia</i> (30)	N/A	
<i>Dettopsomyia</i> (14)	N/A	
<i>Diathoneura</i> (39)	<i>Calatila</i> (1) <i>Diathoneura</i> (38)	
<i>Dicladochaeta</i> (1)	N/A	

(continued)

Table 1, continued

Genus	Subgenus	Notes
<i>Dichaetophora</i> (62)	N/A	
<i>Drosophila</i> (1665)	<i>Chusqueophila</i> (1) <i>Dorsilopha</i> (4) <i>Drosophila</i> (875) <i>Dudiaca</i> (2) Hawaiian <i>Drosophila</i> (427) <i>Phloridosia</i> (8) <i>Psilodorha</i> (2) <i>Siphlodora</i> (2) <i>Sophophora</i> (344)	Table 2 Table 2 Table 2
<i>Hirtodrosophila</i> (160)	N/A	
<i>Hypselothyrea</i> (35)	<i>Hypselothyrea</i> (20) <i>Deplanothyrea</i> (11) <i>Baechlia</i> (4)	Table 2
<i>Impatiophila</i> (42)	N/A	
<i>Jeannelopsis</i> (3)	N/A	
<i>Laccodrosophila</i> (5)	N/A	
<i>Liodrosophila</i> (65)	N/A	
<i>Lissocephala</i> (35)	N/A	
<i>Lordiphosa</i> (63)	N/A	
<i>Marquesia</i> (2)	N/A	
<i>Microdrosophila</i> (78)	<i>Microdrosophila</i> (51) <i>Oxystyloptera</i> (27)	
<i>Mulgravea</i> (14)	N/A	
<i>Mycodrosophila</i> (130)	<i>Mycodrosophila</i> (75) <i>Promycodrosophila</i> (14) Unplaced (41)	
<i>Neotanygastrella</i> (17)	N/A	
<i>Palmomyia</i> (1)	N/A	
<i>Palmophila</i> (3)	N/A	
<i>Paraliodrosophila</i> (5)	N/A	
<i>Paramycodrosophila</i> (16)	N/A	
<i>Phorticella</i> (11)	<i>Phorticella</i> (7) <i>Xenophorticella</i> (4)	
<i>Poliocephala</i> (1)	N/A	
<i>Protochymomyza</i> (1)	N/A	
<i>Samoaia</i> (8)	N/A	
<i>Scaptodrosophila</i> (284)	N/A	
<i>Scaptomyza</i> (269)	<i>Alloscaptomyza</i> (8) <i>Boninoscaptomyza</i> (1) <i>Bunostoma</i> (13) <i>Dentiscaptomyza</i> (5) <i>Elmomyza</i> (86) <i>Engiscaptomyza</i> (7) <i>Euscaptomyza</i> (3) <i>Exalloscaptomyza</i> (6) <i>Grimshawomyia</i> (2) <i>Hemiscaptomyza</i> (15) <i>Lauxanomyza</i> (1) <i>Macrosaptomyza</i> (2) <i>Mesoscaptomyza</i> (18) <i>Metascaptomyza</i> (3) <i>Parascaptomyza</i> (25) <i>Rosenwaldia</i> (9) <i>Scaptomyza</i> (37) <i>Tantalia</i> (6) <i>Titanochaeta</i> (11) <i>Trogloscaptomyza</i> (1) Unplaced (10)	
<i>Sphaerogastrella</i> (10)	N/A	
<i>Styloptera</i> (10)	N/A	
<i>Tamborella</i> (3)	N/A	

(continued)

Table 1, continued

Genus	Subgenus	Notes
<i>Zaprionus</i> (64)	<i>Anaprionus</i> (14) <i>Zaprionus</i> (50)	
<i>Zapriothrica</i> (5)	N/A	
<i>Zaropunis</i> (1)	N/A	
<i>Zygothrica</i> (131)	N/A	
Unplaced Genera	N/A	
<i>Apacrochaeta</i> (1)	N/A	
<i>Balara</i> (1)	N/A	
<i>Collessia</i> (5)	N/A	
<i>Miomymia</i> (1)	N/A	
<i>Sphyrnoceps</i> (1)	N/A	

N/A, not applicable.

within a species group, but only with great difficulty between species groups (Wasserman 1982). Furthermore, it is common for some members of the same species group to produce viable offspring in hybrid crosses (Bock 1984). Therefore, while both the radiation and species group ranks are useful for organizational purposes, species groups better reflect biological processes such as chromosome evolution and partial reproductive isolation occurring during diversification, and radiations reflect historical patterns at deeper evolutionary divergences.

Phylogenetics

Phylogenetic studies can be divided into two major synthetic approaches: those based on primary genetic or morphological data, and meta-analyses that summarize two or more previously published phylogenies. Primary data analyses utilize a variety of methodologies, including character-based (maximum parsimony, maximum likelihood, and Bayesian analysis) and distance-based (neighbor joining) methods. Some meta-, or summary, analyses are analytical, taking either a supermatrix approach and analyzing data using the same methods for primary analyses (van der Linde *et al.* 2010; O'Grady *et al.* 2011; Russo *et al.* 2013), or a supertree approach (O'Grady *et al.* 2008b; van der Linde and Houle 2008) that uses a number of computational methods to combine tree topologies. Other meta-analyses simply “graft” different phylogenetic studies onto a backbone tree. This latter approach is similar to the taxonomy trees that are used to organize data in GenBank. While it is beyond the scope of this paper to provide a review of all of these methods, a recent review has discussed the pros and cons of each (O'Grady *et al.* 2008b).

Two points that are useful to discuss are statistical support and the independence of data. For example, a poorly analyzed data matrix that has little statistical support for most relationships cannot be in conflict with a data set where relationships are fully resolved and highly supported. In such cases, the poorly resolved and weakly supported study should not be taken as having the same value as one with a high degree of support. Unfortunately, because of the focus on topology over support, many supertree analyses consider

well-supported and poorly supported analyses to be of equivalent value. Review of the primary data that underlie these analyses is essential to making an informed decision about the relative weight that each should possess and of the overall value of the resultant supertree.

Now that multigene and genomic-scale data matrices are more easily generated, the independence of data becomes an important issue. This is true of both primary data studies that may utilize some of the same genes, as well as large meta-analyses summarizing phylogenies generated using some (or most) of the same loci (Figure 1). It is necessary to make a distinction between primary and meta-analyses, and to clearly discuss the primary data that goes into meta-analyses, as the latter are not truly independent inferences of phylogeny. Again, an understanding of the underlying data is of critical importance when assessing the value of a given study. Regardless of the approach taken, it is important to be aware of the limitations of each when utilizing published phylogenies for subsequent ecological and evolutionary analyses.

Drosophila: A Genus Divided

Several studies (*e.g.*, Throckmorton 1975; DeSalle and Grimaldi 1991; Katoh *et al.* 2002; Remsen and Grady 2002; O'Grady *et al.* 2011) have suggested that the genus *Drosophila* is paraphyletic with respect to various genera, including *Scaptomyza*, *Zaprionus*, *Lordiphosa*, and *Samoia*. Although a global picture of phylogeny in this group has yet to emerge, it is likely that *Drosophila* is not monophyletic, and that some taxonomic revision will be necessary to resolve the conflicts between traditional taxonomy and molecular phylogenetics (Yassin 2013).

A proposal was put forward to the ICZN to address the fact that many phylogenetic studies did not support the monophyly of *Drosophila* at the level of genus or subgenus. Kim van der Linde and colleagues (van der Linde *et al.* 2007) suggested that *Drosophila* be split into a series of smaller genera, each roughly corresponding to some of Throckmorton's radiations. They also proposed redesignating the type of the genus from *D. funebris*, traditionally in the subgenus *Drosophila*, to *D. melanogaster*, a member of the subgenus *Sophophora*. This would prevent the necessity of changing the genus name of *D. melanogaster*, an important genetic model system.

Several researchers argued against this proposal on a variety of grounds (O'Grady *et al.* 2008a). O'Grady and colleagues (O'Grady *et al.* 2008a; O'Grady and Markow 2009) pointed out that, while there is strong statistical support for the nonmonophyly of *Drosophila*, the placement, exact content, and monophyly of some groups remains unclear based on the current data. Furthermore, none of the proposed new genera possess strong morphological synapomorphies, meaning that diagnosis of these groups without molecular characters is not possible. The ICZN rejected the proposal to transfer the type of the genus (International Commission

Table 2 Species group-level diversity in *Sophophora*, *Drosophila*, and Hawaiian *Drosophila*

Subgenus	Species group	Species
<i>Drosophila</i> (875)	<i>angor</i>	5
	<i>annulimana</i>	18
	<i>antioquia</i>	3
	<i>asiri</i>	4
	<i>atalaia</i>	2
	<i>aureata</i>	1
	<i>bizonata</i>	7
	<i>bromeliae</i>	14
	<i>calloptera</i>	8
	<i>canalinae</i>	14
	<i>caponei</i>	1
	<i>carbonaria</i>	1
	<i>cardini</i>	16
	<i>carsoni</i>	1
	<i>coffeata</i>	4
	<i>dreyfusi</i>	9
	<i>flavopilosa</i>	16
	<i>funebri</i>	7
	<i>guarani</i>	16
	<i>guttifera</i>	1
	<i>histrion</i>	16
	<i>immigrans</i>	106
	<i>macroptera</i>	5
	<i>melanica</i>	14
	<i>mesophragmatica</i>	17
	<i>moerlia</i>	5
	<i>nannoptera</i>	4
	<i>nigrosarsa</i>	4
	<i>onychophora</i>	22
	<i>pallidipennis</i>	1
	<i>peruensis</i>	6
	<i>picta</i>	1
	<i>pinicola</i>	3
	<i>polychaeta</i>	8
	<i>quadrisetata</i>	15
	<i>quinaria</i>	34
	<i>repleta</i>	106
	<i>robusta</i>	16
	<i>rubifrons</i>	10
	<i>simulivora</i>	6
	<i>sticta</i>	1
	<i>testacea</i>	4
<i>tripunctata</i>	83	
<i>tumiditarsus</i>	1	
<i>virilis</i>	14	
<i>xanthopallescens</i>	4	
Unplaced	222	
<i>Sophophora</i> (344)	<i>dentissima</i>	18
	<i>dispar</i>	2
	<i>fima</i>	23
	<i>melanogaster</i>	193
	<i>obscura</i>	44
	<i>populi</i>	2
	<i>saltans</i>	21
	<i>setifemur</i>	2
	<i>willistoni</i>	24
	Unplaced	15
Hawaiian <i>Drosophila</i> (427)	<i>antopocerus</i>	15
	<i>ateledrosophila</i>	3
	<i>haleakalae</i>	55
	<i>modified mouthpart</i>	106

(continued)

Table 2, continued

Subgenus	Species group	Species
	<i>modified tarsus</i>	76
	<i>nudidrosophila</i>	28
	<i>picture wing</i>	130
	<i>rustica</i>	3
	Unplaced	11

for Zoological Nomenclature 2010). A thorough taxonomic treatment, with examination of type material and a full circumscription of the newly proposed genera, will be required before further action can be taken to revise the genus *Drosophila* and the genera related to it.

Phylogenetic Relationships Within the Family Drosophilidae

The first modern phylogenetic tree of the family Drosophilidae was proposed by Throckmorton (1962) using a number of internal morphological characters. He proposed a series of radiations, each containing a number of genera, subgenera, and species groups (Figure 2). These radiations did not stress monophyly as an organizing principle and, as a result, many lineages were paraphyletic with respect to other genera, subgenera, and/or species groups. For example, Throckmorton considered the subgenus *Sophophora* (genus *Drosophila*) to be “basal” within the subfamily Drosophilinae (Figure 2). This radiation gave rise not only to the *melanogaster* and *obscura* species groups, but also the genera *Chymomyza* and *Drosophila*. Likewise, many of the major lineages within the subgenus *Drosophila*, such as the *immigrans*–*tripunctata* radiation, also included a suite of related genera like *Zygothrica*, *Hirtodrosophila*, and *Mycodrosophila* (Figure 2). Throckmorton updated and reviewed this work in 1975 and 1982, the former of which is one of the more widely cited papers on *Drosophila* phylogeny (Throckmorton 1975, 1982). A number of studies, mostly based on molecular characters, have been conducted in the 50 years since Throckmorton’s review paper on *Drosophila* phylogenetics. Many of these have been reviewed elsewhere (Markow and O’Grady 2006).

Here, we review the major phylogenetic studies that have been conducted on the genus *Drosophila* since the ICZN decision in 2010. This is not a comprehensive list. Several excellent studies (e.g., Mota *et al.* 2008; Robe *et al.* 2010a–c, 2013) focusing on individual species groups are not reviewed here. Instead, we focus on those studies that examine relationships among multiple species groups. These include Yassin’s (2013) revision of the family, Russo *et al.*’s (2013) supermatrix analysis of ~9000 bp of nuclear DNA, O’Grady and colleagues’ studies of Hawaiian *Drosophila* and *Scaptomyza* (O’Grady *et al.* 2011; Lapoint *et al.* 2013), Gao *et al.*’s (2011) treatment of *Lordiphosa* and *Sophophora*, Katoh *et al.*’s (2017) phylogeny of *Scaptomyza*, and Robe and colleagues’ excellent studies of Neotropical Drosophilidae (Robe *et al.* 2010b,c).

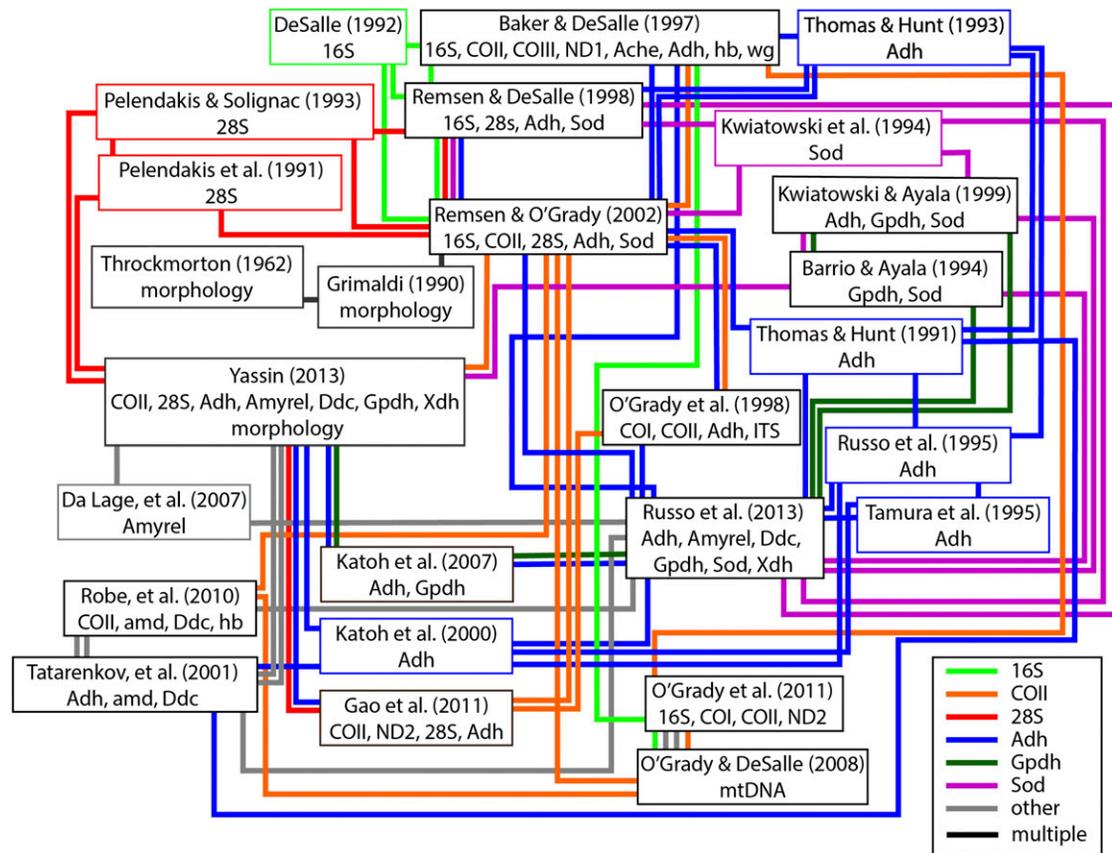


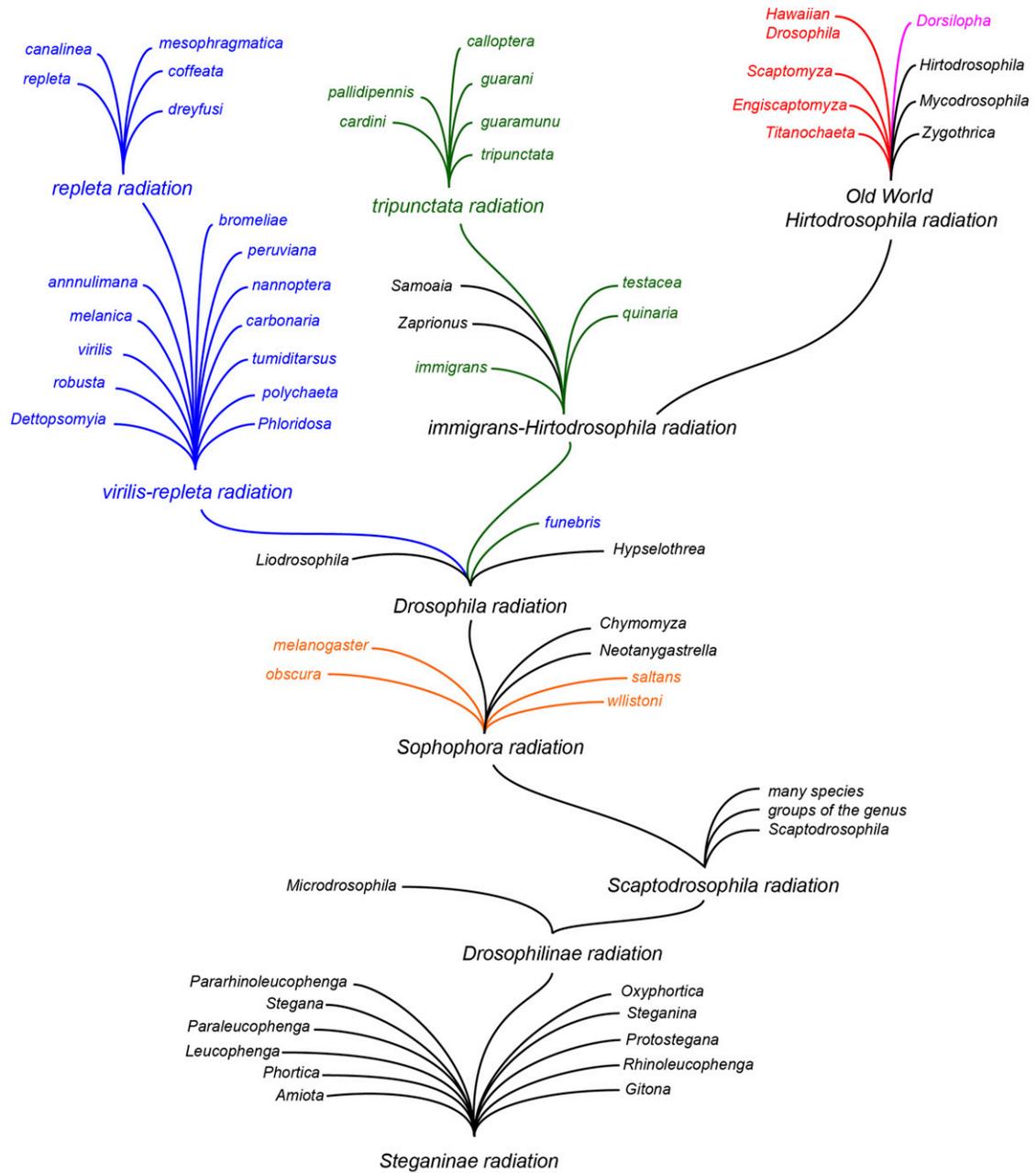
Figure 1 Summary of character overlap between major phylogenetic studies of Drosophilidae. Rectangles surrounding reference are color-coded based on whether they used a single gene (light green, 16S; orange, COII; red, 28S; blue, AdH; dark green, Gpdh; purple, Sod; gray, other; and multiple loci, black). Lines linking studies indicate which genes are shared between the various analyses. Many smaller studies were omitted for clarity.

Yassin (2013) included 30 genera in Steganinae and 43 in Drosophilinae in his recent revision of the Drosophilidae (Figure 3A). He performed a molecular analysis on a subset of genera and species in the family, and then grafted additional taxa that had been examined for a suite of 37 morphological characters. The resulting phylogeny was then used to generate a revised classification of the family Drosophilidae and the genus *Drosophila*. Yassin (2013) proposed a compromise to the “lumping vs. splitting” conundrum prompted by the ICZN decision. He suggested keeping the overall structure at the level of genus and making changes to the subgenera placed in *Drosophila*. This would avoid dividing the genus into multiple genera, as suggested by van der Linde and colleagues (van der Linde *et al.* 2007, 2010; van der Linde and Houle 2008). It would also maintain the binomials of several important model organisms and eliminate the ~500 secondary homonymies created by synonymizing many genera into a single, extremely large genus *Drosophila*, a solution not explicitly suggested but possible nonetheless (O’Grady and Markow 2009; van der Linde and Yassin 2010). This is very similar to the proposals suggested by O’Grady and Markow (2009), in that it stresses phylogenetic relationships, rather than adherence to monophyletic genera. It differed in that Yassin (2013) set aside Throckmorton’s (1975) informal

radiations in favor of revisions of the subgeneric taxonomy within the genus *Drosophila* (Figure 3).

Yassin’s (2013) treatment resulted in several significant shifts in our understanding of relationships within this family. Two drosophilid genera, *Cladochaeta* and *Diathoneura*, were transferred to Ephydriidae, the sister family of Drosophilidae. His study also included several genera, particularly within the subfamily Steganinae, that had never been sampled in previous molecular studies. Explicit hypotheses of relationships among these taxa are an important contribution to our understanding of evolution within the Drosophilidae.

There were also significant changes within the genus *Drosophila*. While several drosophilid genera (*Apacrochaeta*, *Sphyrnoceps*, and *Palmophila*) were synonymized with the genus *Drosophila*, other taxa, such as the Hawaiian *Drosophila*, were removed and placed in the genus *Idiomyia* following Grimaldi’s (1990) suggestion. Finally, several previously recognized subgenera (*Phloridosia*, *Pslidorha*, and *Chusqueophila*) were transferred to the subgenus *Drosophila*. Yassin’s (2013) revised genus *Drosophila* contains five subgenera: *Sophophora*, *Dorsilopha*, *Drosophila*, *Siphlodora*, and *Dudiaca*. Yassin (2013) was explicit in which species groups were included in each of these newly constituted subgenera, a large step forward from previous studies.



Throckmorton (1975)	Yassin (2013)
■ Hawaiian <i>Drosophila</i>	<i>Idiomyia</i> , as genus
■ <i>virilis-repleta rad.</i>	<i>Drosophila</i> (<i>Siphlodora</i>)
■ <i>immigrans-tripunctata rad.</i>	<i>Drosophila</i> (<i>Drosophila</i>)
■ <i>Drosophila</i> (<i>Sophophora</i>)	<i>Drosophila</i> (<i>Sophophora</i>)
■ <i>Drosophila</i> (<i>Dorsilopha</i>)	<i>Drosophila</i> (<i>Dorsilopha</i>)

Figure 2 Throckmorton's phylogeny of the family Drosophilidae. The radiations proposed by Throckmorton (1975) are redrawn and compared with Yassin's (2013) classification scheme. Major lineages are color-coded as follows: *virilis-repleta* radiation (blue), *immigrans-Hirtodrosophila* radiation (green), Hawaiian Drosophilidae (red), *Dorsilopha* (pink), and *Sophophora* (orange).

Steganinae and early-branching Drosophilinae

Yassin (2013) found that the subfamily Steganinae was monophyletic and sister to the Drosophilinae (Figure 3A, I). Russo *et al.* (2013), in contrast, suggests that two steganine genera, *Leucophenga* and *Phortica*, are sister to *Chymomyza*, and that a paraphyletic *Scaptodrosophila* is sister to all other drosophilids (Figure 3B, I–II). This result has not been seen in any previous study and may be the result of using an extremely distant outgroup (see below).

There are a number of genera that form a clade (II) that is the sister group of clades III–IX, the most recent common ancestor of extant members of the genus *Drosophila* (Yassin 2013; Figure 3A). This group is not well known but contains two genera, *Chymomyza* and *Scaptodrosophila*, that have been sampled extensively in molecular studies [reviewed in Markow and O’Grady (2006)]. There are also a number of less-well-known genera, such as *Neotanygastrella* and *Lissocephala*, that Throckmorton (1975) considered to be basal to the genus *Drosophila*. This clade is not exactly seen in the Russo *et al.* (2013) study due to the inclusion of several members of the subfamily Steganinae (Figure 3, I) embedded within it. Interestingly, the genus *Scaptodrosophila*, once considered a subgenus of *Drosophila*, is not monophyletic in either study (Russo *et al.* 2013; Yassin 2013). This is a large lineage of ~300 described species (Bächli 2015), most of which are endemic to Australia or other regions in the Australo-oceanian Region (Bock and Parsons 1978; Evenhuis 1989).

Relationships within major lineages of Drosophila

Markow and O’Grady (2006) reviewed the hypotheses of phylogenetic relationships in Drosophilidae, focusing specifically on relationships among drosophilid genera and species-level relationships within various species groups. The recent revision of the family (Yassin 2013) focused heavily on relationships within the genus *Drosophila*, which will be the focus of this section of the review. The “crown” Drosophilidae, which contain all the members currently placed in *Drosophila* along with a variety of other genera, can be divided into seven major lineages (Figure 3, III–IX). Lineage III, which includes the subgenus *Sophophora* and the genus *Lordiphosa*, is sister to lineages IV–IX (Russo *et al.* 2013; Yassin 2013). Lineage IV, which contains the subgenus *Dorsilopha* and the genus *Styloptera*, is sister to lineages V–IX in both studies (Russo *et al.* 2013; Yassin 2013). Lineages V and VI are a heterogeneous group of genera. In Yassin’s study, lineage V is sister to lineages VI–IX. Lineage VI is a paraphyletic grade (VIa and VIb) that is sister to lineage VII. The affiliations of taxa in lineages V and VI are slightly different in Russo *et al.*’s (2013) phylogeny, possibly because of the reduced taxon sampling in their study or Yassin’s (2013) use of the morphological grafting technique. Lineage VII includes Yassin’s (2013) revised subgenus *Drosophila*, a group that is also supported in other studies (Russo *et al.* 2013). Finally, lineage VIII, which contains the Hawaiian Drosophilidae, is supported as the sister taxon to lineage IX, the subgenus

Siphlodora (Figure 3), in several studies (Russo *et al.* 2013; Yassin 2013).

The subgenus *Sophophora*: The subgenus *Sophophora* (Figure 3, lineage III) currently contains 344 described species placed in nine species groups (Table 1 and Table 2; Kopp and True 2002; Schawaroch 2002; Kopp 2006). This subgenus also contains the important genetic model species, *D. melanogaster*, as well as *D. pseudoobscura*, a species that has been used extensively in evolutionary biology. Traditionally, the *obscura* and *melanogaster* groups were considered sister taxa (Figure 6A). The *obscura*–*melanogaster* clade was the sister group of the lineages formed by the Neotropical *saltans* and *willistoni* species groups (Figure 6A).

Recently, the monophyly of the subgenus *Sophophora* has been called into question. Several studies (Katoh *et al.* 2000; Gao *et al.* 2011) that have sampled extensively within the genus *Lordiphosa* suggest that *Sophophora* is paraphyletic with respect to *Lordiphosa*. These studies recover the same sister group relationship between the *saltans* and *willistoni* groups (Figure 6B). Interestingly, several species groups within the genus *Lordiphosa* (*Lordiphosa miki*, *L. fenestratum*, *L. denticeps*, and *L. nigricolor*) are the sister lineage to the Neotropical *saltans* and *willistoni* taxa (Figure 6B), to the exclusion of the *melanogaster*–*obscura* species. The *melanogaster*–*obscura* clade is sister to the combined *Lordiphosa*–*saltans*–*willistoni* clade (Figure 6B). The paraphyly of *Sophophora* has also been recovered in several larger analyses examining relationships across Drosophilidae (Russo *et al.* 2013; Yassin 2013).

The subgenus *Drosophila*: The subgenus *Drosophila* (Figure 3, lineage VII), which contains the type of the genus, *D. funebris*, was also supported as monophyletic in Yassin’s (2013) study. This group corresponds loosely to the large *immigrans*–*tripunctata* radiation (Throckmorton 1975), a group of species that have not been extensively studied. Throckmorton (1975) proposed that this lineage gave rise to two further radiations, the *tripunctata* radiation in the New World and the *immigrans* radiation in the Old World (Figure 2). The *immigrans* radiation contains the *immigrans*, *testacea*, and *quinaria* species groups, as well as the genera *Zaprionus* and *Samoaia*. The *tripunctata* radiation is made up of the large *tripunctata* group and several closely related species groups, including *calloptera*, *cardini*, *rubifrons*, and *macroptera*.

Membership within the redefined subgenus *Drosophila* (Yassin 2013) corresponds well with previous studies (Remsen and O’Grady 2002; Perlman *et al.* 2003; Russo *et al.* 2013), although some species group-level relationships are not perfectly concordant between studies (Figure 3). Relationships within the *tripunctata* and *immigrans* species groups, two of the larger lineages in the subgenus *Drosophila* (Markow and O’Grady 2006), were problematic. For example, the *tripunctata* species group (Figure 3B and Figure 5, A and B) is not monophyletic in some studies (*e.g.*, Robe *et al.*

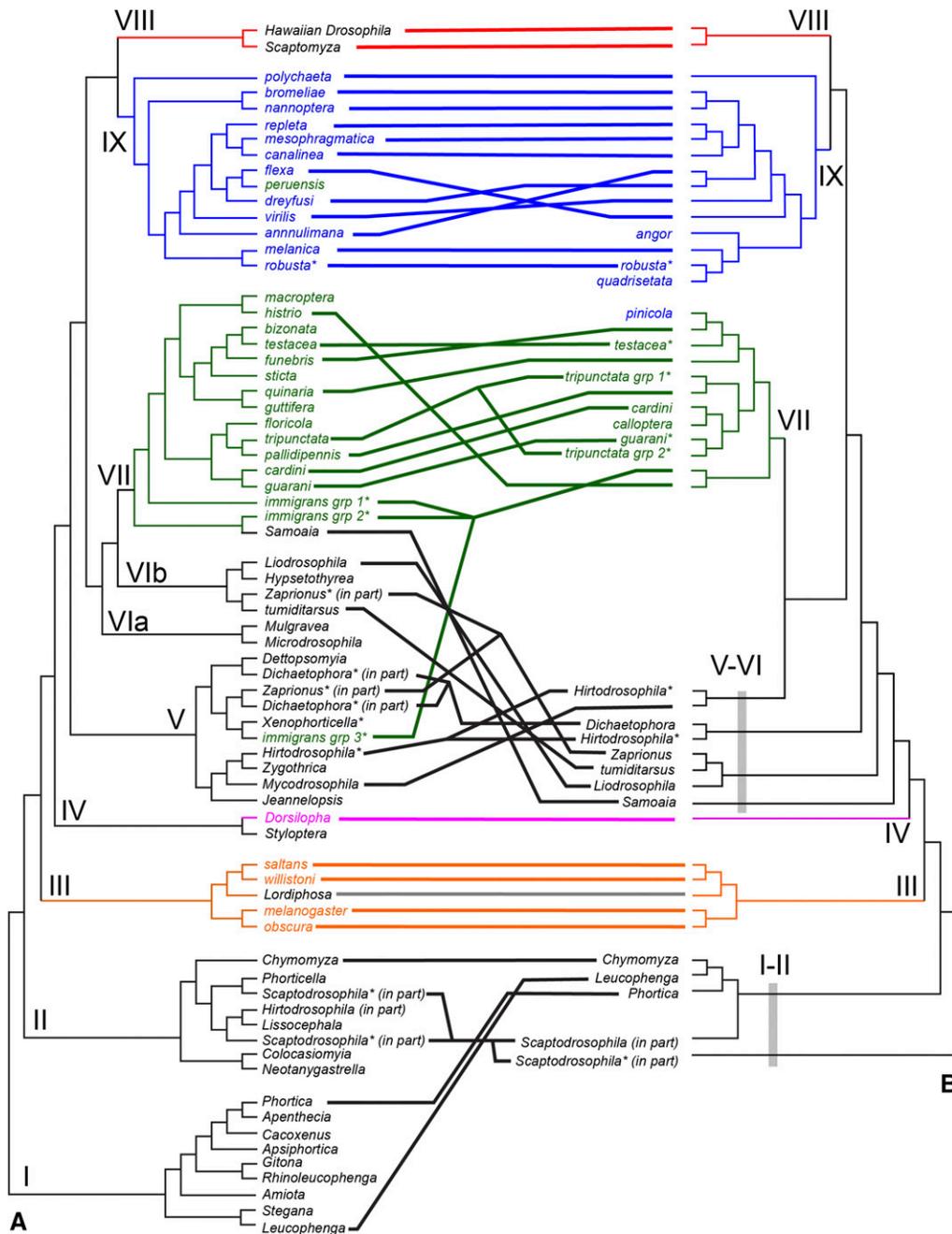


Figure 3 Comparison of (A) Yassin (2013) and (B) Russo *et al.* (2013) phylogenies, showing species group sampling and points of agreement and conflict. Major lineages are color-coded as follows: *virilis*–*repleta* radiation (IX, blue), *immigrans*–*Hirtodrosophila* radiation (VII, green), Hawaiian Drosophilidae (VIII, red), *Dorsilopha* (IV, pink), and *Sophophora* (III, orange).

2010b,c; Russo *et al.* 2013). The Robe *et al.* (2010c) study, in particular, sampled extensively in the *tripunctata* group, including members of each of the major subgroups defined by various workers. They found that some *tripunctata* lineages were closely related to the *guaramunu* species group, while others were more closely to the *pallidipennis* species group (Robe *et al.* 2010b,c; Figure 5, A and B). These affiliations are reflected in the more recent studies (Russo *et al.* 2013; Yassin 2013), although Yassin (2013) did not sample extensively within this group.

The placement of the *immigrans* species group was also different between the studies. Robe and colleagues (Robe *et al.* 2010b,c) found that the *immigrans* group was monophyletic

and sister to the remainder of the subgenus *Drosophila* (Figure 5, A and B). Russo *et al.* (2013) showed that the *histrio* and *immigrans* groups were sister taxa and that this clade was, in turn, sister to the remainder of the subgenus *Drosophila* (Figure 3B). However, sampling of *immigrans* group species within the Robe and Russo studies (Robe *et al.* 2010b,c; Russo *et al.* 2013) was not as extensive as they were in Yassin’s analysis (Yassin 2013). Yassin’s (2013) phylogeny suggested that the *immigrans* group, a large heterogeneous assemblage of species found primarily in the Australasian and Oceanian regions (Evenhuis and Okada 2012), was polyphyletic and formed three main lineages. Two of these were in the subgenus *Drosophila*, the other was in a large clade of genera in

lineage V (Figure 3A). One lineage was sister to the genus *Samoaia*, and this lineage was sister to the remainder of species in *Drosophila* (Figure 3A). Another lineage was sister to all *Drosophila*, exclusive of the *immigrans*–*Samoaia* clade (Figure 3A). Yassin (2013) sampled multiple lineages of the *immigrans* group and suggested that this lineage might not be monophyletic (Figure 3A). A third *immigrans* lineage was nested within a clade that included the genera *Zaprionus* and *Xenophorticella* (Figure 3A).

Yassin (2013) showed that the genus *Samoaia*, a clade of large-bodied and spectacularly pigmented species endemic to the Samoan Archipelago, was nested within the subgenus *Drosophila*, close to some members of the *immigrans* species group (Figure 3A). Russo *et al.* (2013), in contrast, reconstructed *Samoaia* as sister to most other lineages in the broader genus *Drosophila*, including the subgenera *Siphodora* and *Drosophila*, the Hawaiian Drosophilidae, and several other drosophilid genera (Figure 3B). The placement by Robe *et al.* (2010b) was more similar to that of Russo *et al.*'s (2013), although they did not sample as extensively from drosophilid genera (Figure 5A).

Hawaiian Drosophilidae: While Yassin (2013) and Russo *et al.* (2013) did not sample extensively within the Hawaiian Drosophilidae (Figure 3, lineage VIII), there have been several recent studies on both the Hawaiian *Drosophila* (O'Grady *et al.* 2011) and the genus *Scaptomyza* (Lapoint *et al.* 2013; Katoh *et al.* 2017). The endemic Hawaiian Drosophilidae consist of two major lineages: Hawaiian *Drosophila* (*Idiomya* of some authors) and the genus *Scaptomyza* [reviewed in O'Grady (2002) and Markow and O'Grady (2006)]. The Hawaiian Drosophilidae contain nearly 700 described and at least 200 undescribed species (Kaneshiro 1997). There are currently eight species groups recognized in the Hawaiian *Drosophila* (*picture wing*, *modified mouthparts*, *modified tarsus*, *antopocerus*, *ateledrosophila*, *nudidrosophila*, *haleakalae*, and *rustica*) and 21 subgenera known from *Scaptomyza*, 10 of which (*Elmomyza*, *Rosenwaldia*, *Alloscaptomyza*, *Tantalia*, *Exalloscaptomyza*, *Engiscaptomyza*, *Celidosoma*, *Titanochaeta*, *Bunostoma*, and *Grimshawomyia*) have endemic Hawaiian taxa. The remainder of *Scaptomyza* species are outside of Hawaii, and can be found on many island chains and most continental land masses (O'Grady *et al.* 2010).

O'Grady *et al.* (2011) examined the phylogenetic relationships within the endemic Hawaiian *Drosophila* lineage. This was the most extensively sampled study to date and examined representatives of seven of the eight described species groups. They found that the *antopocerus*, *modified tarsus*, and *ciliated tarsus* groups formed a large monophyletic lineage that they referred to as the AMC clade (Figure 4, Figure 5, and Figure 6). While most groups and subgroups within this clade were monophyletic, the *bristle tarsus* subgroup and *ciliated tarsus* species group were not. Subsequent work (Lapoint *et al.* 2014) showed that these two lineages actually formed a paraphyletic grade of at least three distinct clades. Additional taxonomic work within the AMC clade will be

needed to resolve this issue. The AMC clade was the sister taxon of the mycophagous *haleakalae* species group (Figure 4). The *modified mouthpart* species group was sister to the AMC–*haleakalae* clade (Figure 4). A large clade containing the *picture wing*, *ateledrosophila*, and *nudidrosophila* species groups (PNA clade) was sister to the remaining Hawaiian *Drosophila* (Figure 4). Magnacca and Price (2015) examined the phylogeny of the *picture wing* and *nudidrosophila* species group with expanded sampling, and found that many of the species groups and subgroups traditionally placed within the *picture wing* clade were monophyletic.

Lapoint *et al.* (2013) examined the phylogeny of the genus *Scaptomyza* using an expanded data set from the O'Grady and DeSalle (2008) study. *Scaptomyza* was strongly supported as monophyletic and sister to the endemic Hawaiian *Drosophila* (Figure 4A). There were two main clades of Hawaiian *Scaptomyza*: one containing the subgenera *Exalloscaptomyza* and *Bunostoma* (clade H1, Figure 4A) and another composed of the remainder of the endemic Hawaiian lineages (clade H2, Figure 4A). Interestingly, several non-Hawaiian *Scaptomyza* subgenera, such as *Parascaptomyza*, *Scaptomyza*, and *Hemiscaptomyza*, were sister to the larger Hawaiian clade (Figure 4A). The *Bunostoma*–*Exalloscaptomyza* clade was sister to all the other Hawaiian and non-Hawaiian *Scaptomyza* (Figure 4A).

Lapoint *et al.* (2013) acknowledged that support was weak along the backbone of the phylogeny (see * in Figure 4A) and outlined two possible scenarios to explain the distribution of this group. One scenario was a single colonization of Hawaii by the ancestor of the Hawaiian *Drosophila* and *Scaptomyza*, followed by at least one, and possibly as many as three, escapes from Hawaii to give rise to the mainland *Scaptomyza* subgenera, and at least two more independent escapes in two small, pan-Pacific subgenera *Rosenwaldia* and *Bunostoma* (gray ovals, Figure 4A). They also proposed a second scenario, where a single colonization by the ancestor of the Hawaiian Drosophilidae was followed by a migration back to the mainland after the divergence of the Hawaiian *Drosophila* and clade H2, and a final, secondary colonization of Hawaii by clade H1 (Figure 4A). While they discounted this second scenario as being unlikely due to the difficulty of colonizing Hawaii multiple times, Lapoint *et al.* (2013) were also clear that the existing data would support either hypothesis, and cited several studies where multiple colonizations had been inferred in snails (Rundell *et al.* 2004) and spiders (Arnedo *et al.* 2007). Subsequent work on Hawaiian Dolichopodidae (Goodman *et al.* 2014, 2016) have also demonstrated multiple colonizations of the Hawaiian Archipelago.

Katoh *et al.* (2017) have recently examined the phylogenetic relationships within the genus *Scaptomyza*, expanding on earlier work (O'Grady and DeSalle 2008; Lapoint *et al.* 2013). This study increased the sampling of within *Scaptomyza* to 14 of the 21 known subgenera, including the first molecular sequence of *Boninoscaptomyza*, and an expanded number of species in the subgenera *Scaptomyza* and *Parascaptomyza*. Like most previous studies, this analysis

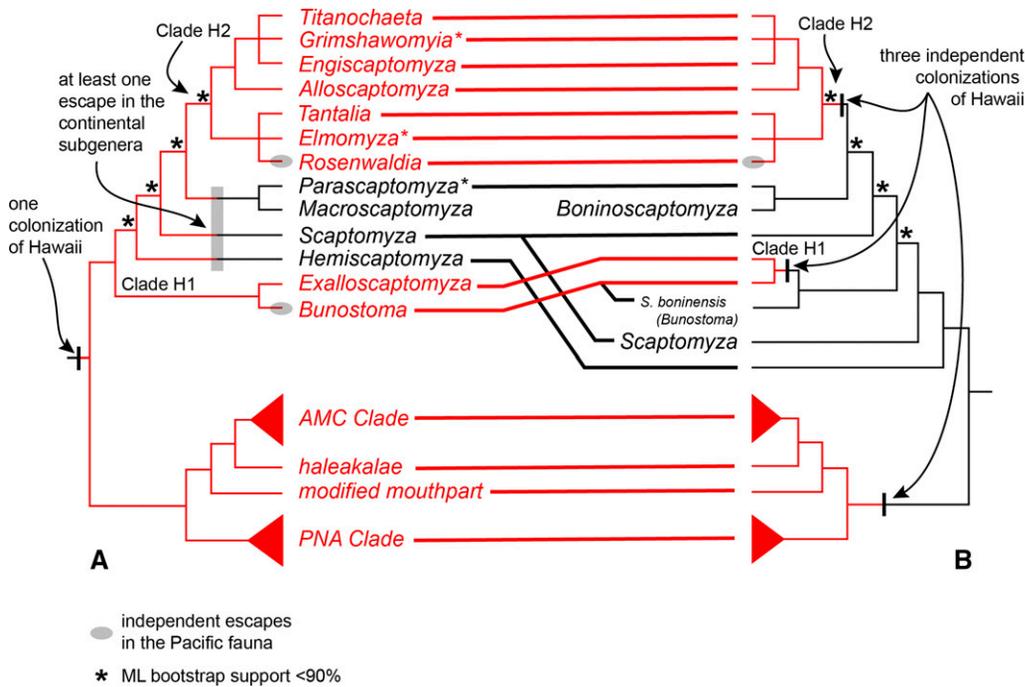


Figure 4 Phylogenetic relationships within the Hawaiian Drosophilidae based on (A) Lapoint *et al.* (2013) and (B) Katoh *et al.* (2017). Possible scenarios of colonization and escape are indicated on each phylogeny. Gray ovals represent independent escapes in the Pacific fauna and gray boxes show possible escapes that generated continental taxa. Asterisks indicate poorly supported nodes on the phylogeny. Hawaiian endemic species are in red, non-Hawaiian groups are in black. ML (maximum likelihood).

supported the sister group relationship of Hawaiian *Drosophila* and *Scaptomyza* (Figure 4B). In contrast to the topology of the Lapoint *et al.* (2013) study, the Katoh *et al.* (2017) analysis supported two non-Hawaiian subgenera as sister to the remainder *Scaptomyza* (Figure 4B).

They proposed that the Hawaiian Islands have been colonized at least three times by Drosophilidae: once in the Hawaiian *Drosophila* lineage, once in clade H1, and again in clade H2 (Figure 4B). While support for these individual clades is strong, as is the support for the monophyly of *Scaptomyza* as a whole, there is weak support at many internal nodes for the relationships between the various Hawaiian and non-Hawaiian subgenera (see * in Figure 4B). Furthermore, while the study by Katoh *et al.* (2017) is an improvement over the sampling at the species (63–72 species) and subgeneric (13–14 subgenera) levels, there is a clear impact of sampling on the support and relationships within the backbone of this phylogeny. Clearly, additional sampling will be required before the question of whether the Hawaiian Drosophilidae are the results of a single or multiple colonization events can be confidently resolved.

The subgenus *Siphlodora*: The subgenus *Siphlodora* (Figure 3, lineage IX) was originally described as including only a few species with sinuate posterior cross veins (Sturtevant 1916). These are now included in the *flexa* species group. The redefined subgenus *Siphlodora* (*sensu* Yassin) now contains several large clades, including the *repleta*, *virilis*, and *robusta* species groups. This group roughly corresponds to the *virilis–repleta* radiation, a lineage proposed by Throckmorton (1975) for a number of species groups that were allied either through some ecological habit (*e.g.*, a sap-feeding or cactophilic lifestyle) or evolutionary affinity

based on morphological characters. The *virilis–repleta* radiation was divided into two main groups, the monophyletic *repleta* radiation and a number of unresolved species groups basal and external to the *repleta* radiation (Throckmorton 1975). The *repleta* radiation includes the *repleta*, *canalineae*, *coffeata*, *mesophragmatica*, and *dreyfusi* species groups. The remainder of the *virilis–repleta* radiation include species in the *virilis*, *carsoni*, *melanica*, *robusta*, *annulimana*, *bromeliae*, *peruviana*, *nannoptera*, *tumiditarsus*, *carbonaria*, and *polychaeta* groups, members of the genus *Dettopsomyia* and the subgenus *Phloridosa* (Throckmorton 1975).

Membership within the newly redefined subgenus *Siphlodora* (Yassin 2013) corresponds quite well with previously generated work (Durando *et al.* 2000; Tatarenkov and Ayala 2001; Remsen and O’Grady 2002; Robe *et al.* 2010b,c; Oliveira *et al.* 2012; Russo *et al.* 2013), although some relationships are not preserved across studies (Figure 3, lineage IX). However, the placement of *D. peruviana*, a member of the *peruensis* species group, was problematic. While the phylogeny clearly shows this taxon is nested in the subgenus *Siphlodora* (Figure 3A), Yassin’s (2013) taxonomic treatment placed this in the subgenus *Drosophila*. Clearly, additional taxonomic and phylogenetic research needs to be done on the less-well-studied lineages, such as the *peruensis* species group, within the genus *Drosophila*.

An Emerging Picture of Phylogenetic Relationships in Drosophilidae

A number of recent studies are revolutionizing our understanding of phylogenetic relationships across the family Drosophilidae (Russo *et al.* 2013; Yassin 2013), as well as within the major lineages of this important family (Robe *et al.*

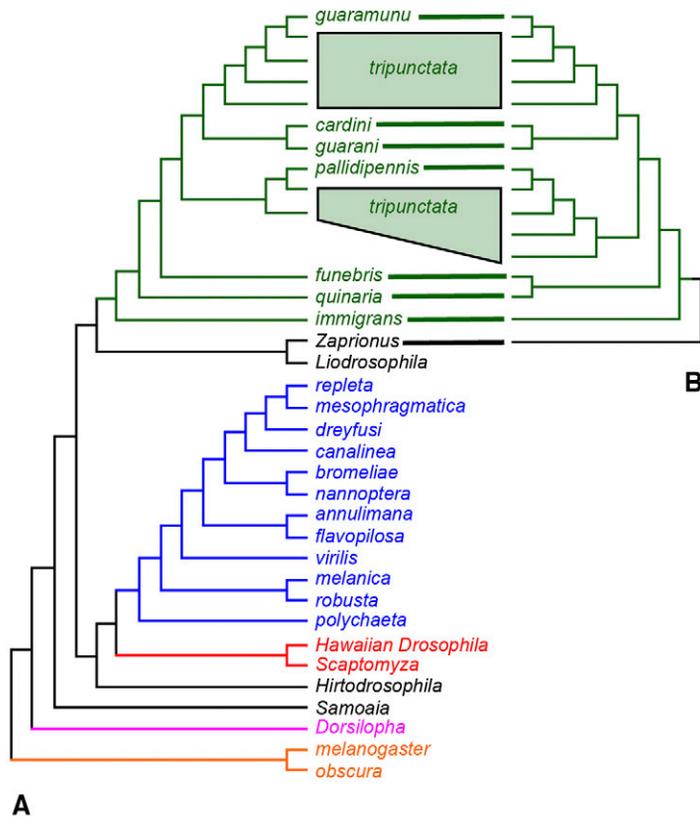


Figure 5 Comparison of Robe's phylogenetic studies in the genus *Drosophila*: (A) Robe *et al.* (2010b) and (B) Robe *et al.* (2010c). Major lineages are color-coded as follows: *virilis-repleta* radiation (IX, blue), *immigrans-Hirtodrosophila* radiation (VII, green), Hawaiian Drosophilidae (VIII, red), *Dorsilopa* (IV, pink), and *Sophophora* (III, orange).

Throckmorton (1975)	Yassin (2013)
■ <i>Hawaiian Drosophila</i>	<i>Idiomyia</i> , as genus
■ <i>virilis-repleta rad.</i>	<i>Drosophila (Siphlodora)</i>
■ <i>immigrans-tripunctata rad.</i>	<i>Drosophila (Drosophila)</i>
■ <i>Drosophila (Sophophora)</i>	<i>Drosophila (Sophophora)</i>
■ <i>Drosophila (Dorsilopa)</i>	<i>Drosophila (Dorsilopa)</i>

2010b,c; Gao *et al.* 2011; O'Grady *et al.* 2011; Lapoint *et al.* 2013; Katoh *et al.* 2017). There are several common threads in all of these studies. First, they are expanding taxon sampling within *Drosophila* and related groups impressively. More genera sampled across the family and more species groups sampled within each subgenus means that more rigorous tests of monophyly are possible, and a more comprehensive view of diversification is possible. Another similarity is that the numbers of characters, both molecular and morphological, are increasing. More characters potentially translate into increased support at controversial nodes within the phylogeny or the resolution of novel relationships among taxa. We predict that these trends will continue, converging on whole-genome analysis with nearly complete taxon sampling.

It is clear that the two subfamilies within the family Drosophilidae, Steganinae and Drosophilinae, are monophyletic and sister to one another (Yassin 2013). While Russo

et al. (2013) suggested that they are both paraphyletic (Figure 3B, I–II), this result is unique to their study and may be the result of long branches at the base of the phylogeny obscuring relationships (Felsenstein 1978). Within Steganinae, sampling is still quite poor. The most extensive study to date (Yassin 2013) sampled only 9 of the 31 genera within this clade and found this group to be monophyletic (Figure 3A, I). Additional sampling within Steganinae is needed before any firm conclusions can be made about relationships among included lineages.

The subfamily Drosophilinae accounts for the bulk of species-level and generic diversity in the family. A clade of genera (Figure 3A, II) is the sister taxon of *Drosophila* and related groups (Figure 3A, III–IX). While taxon sampling within this clade as a whole is poor, it is clear that the genus *Scaptodrosophila* is not monophyletic. This is a large, widespread genus with ~300 described species. *Scaptodrosophila* will require thorough revisionary work and will, most likely,

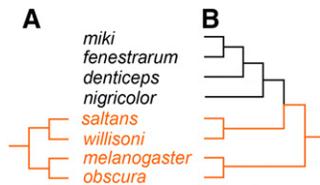


Figure 6 Phylogenetic relationships within the subgenus *Sophophora*, contrasting the (A) traditional view (O’Grady and Kidwell 2002) with the (B) recent inclusion of the genus *Lordiphosa* within this subgenus (Katoh *et al.* 2000; Gao *et al.* 2011). Traditional *Sophophora* species groups are shown in orange.

be divided into multiple genera. Other genera in this clade include *Chymomyza*, some members of *Hirtodrosophila*, and several smaller genera (Table 1). The genus *Hirtodrosophila*, with ~160 known species, is a large, heterogeneous group with many undescribed species in the Old and New World tropics. Revisionary work on this group will also be necessary to resolve the evident paraphyly in this genus (Figure 3A, II and V).

The subgenus *Sophophora* (Figure 3A, III) is the sister group to lineages IV–IX. *Sophophora* contains ~350 described species and several studies (Katoh *et al.* 2000; Gao *et al.* 2011) have shown that it is paraphyletic with respect to some members of the genus *Lordiphosa* (Figure 3A, III). This group has been revised to include several species groups of the latter (Table 2). The Neotropical *saltans* and *willisoni* species groups are sister to the *Lordiphosa* species groups. The *melanogaster* and *obscura* species groups are sister to the clade formed by the *saltans*, *willisoni*, and *Lordiphosa* species (Figure 3A, III).

Lineages IV–VI mainly contain a number of non-*Drosophila* genera. Lineage IV is a small clade containing the subgenus *Dorsilopha* and the genus *Styloptera* (Figure 3A). It is the sister group of lineages V–IX. Lineage V is a large group of ~15 genera. It contains several diverse mycophagous genera, such as *Hirtodrosophila*, *Zygothrica*, and *Mycodrosophila*. *Hirtodrosophila*, as mentioned above, is not monophyletic and will require revision of both the Old and New World taxa. It is likely that there are as many as 100 species remaining to be described in this genus. Other groups, like *Zygothrica*, are poorly known in the Neotropics and may also contain many undescribed species (Grimaldi 1987). Lineage VI, broken into two separate clades, A and B, are sister to the remainder of species in the family Drosophilidae. These lineages include both well-known genera, such as *Zaprionus* (Yassin and David 2010), and lesser-studied groups like *Microdrosophila* and *Mulgravea*. It is likely that additional genera allied with these lineages and expanded sampling, particularly in the Asian tropics, will be required before these relationships can be elucidated. These lineages are supported as four separate lineages in Yassin’s (2013) study, although additional character and taxon sampling may alter the sister group relationships in this part of the phylogeny [see Russo *et al.* (2013)].

Lineage VII corresponds to the subgenus *Drosophila* (*sensu* Yassin 2013). This group is paraphyletic with respect to the genus *Samoaia*, a distinctive clade of eight species that is endemic to the Samoan Archipelago. It is possible that the members of this endemic genus, while morphologically distinct, are actually just highly modified members of the genus *Drosophila*. The *immigrans* species group, one of the largest groups in the subgenus *Drosophila*, is not monophyletic. Revisionary work, along with expanded sampling and phylogenetic analyses, will be needed to resolve this issue. The other members of this subgenus include the *floricola*, *tripunctata*, *cardini*, *pallidipennis* and *guarani* species groups, a clade of flower and fruit breeding taxa from the New World. A second clade containing *D. funebris*, the type of the genus, includes mycophagous taxa in the *quinaria*, *testacea*, *bizonata*, *macroptera*, and *guttifera* species groups.

The Hawaiian Drosophilidae, including the genus *Scaptomyza* and members of the Hawaiian *Drosophila* (*Idiomya* of some authors), is sister to the subgenus *Siphlodora* (*sensu* Yassin 2013). Relationships within the major lineages of Hawaiian *Drosophila* and the subgenera of *Scaptomyza* are summarized in Figure 4 (O’Grady *et al.* 2011; Lapoint *et al.* 2013, 2014; Katoh *et al.* 2017). While sampling in the Hawaiian *Drosophila* is comprehensive at the level of species groups and major lineages, species-level sampling could be expanded. The recent conflict in the colonization history of the Hawaiian Drosophilidae (Lapoint *et al.* 2013; Katoh *et al.* 2017) indicates that increased sampling within the genus *Scaptomyza*, particularly among the non-Hawaiian subgenera, will be required to create a robust hypothesis of relationships within this impressive lineage.

The subgenus *Siphlodora* includes a number of well-known species groups, including the *repleta*, *virilis*, *melanica*, and *robusta* groups (Caletka and McAllister 2004; Etges and Levitan 2004; Flores *et al.* 2008; O’Grady *et al.* 2008a; Oliveira *et al.* 2012; Markow 2015; Sanchez-Flores *et al.* 2016). This is an ecologically diverse group that utilizes sap fluxes, fungi, cacti, and fruits as oviposition and larval substrates. Members of this lineage have independently evolved the use of cacti as a host substrate at least twice, once in the *repleta*–*mesophragmatica* clade and again in the *nannoptera* species group (Figure 3A). The *peruensis* species group was placed in the subgenus *Drosophila* by Yassin (2013), yet seems to belong in this subgenus, where it is closely related to the *dreyfusi* and *flexa* species groups. Additional sampling within this lineage from the Neotropics and Asian tropics should be done to expand the coverage of species groups in this subgenus (Table 2).

Future Research

While much progress has been made to date, there are several issues that remain unresolved, including creating a stable taxonomic structure, ingroup and outgroup sampling, and the integration of large-scale molecular and morphological data matrices. These initiatives will require extensive field

Table 3 Drosophilidae whole-genome sequences available as of February 2017

Species	Subgenus	Group	Reference
<i>D. busckii</i>	<i>Dorsilopha</i>		Vicoso and Bachtrog (2015)
<i>D. albomicans</i>	<i>Drosophila</i>	<i>immigrans</i>	Zhou <i>et al.</i> (2012)
<i>D. arizonae</i>	<i>Drosophila</i>	<i>repleta</i>	Sanchez-Flores <i>et al.</i> (2016)
<i>D. mojavensis</i>	<i>Drosophila</i>	<i>repleta</i>	<i>Drosophila</i> 12 Genomes Consortium <i>et al.</i> (2007)
<i>D. navojoa</i>	<i>Drosophila</i>	<i>repleta</i>	Sanchez-Flores <i>et al.</i> (2016)
<i>D. neotestacea</i>	<i>Drosophila</i>	<i>testacea</i>	Hamilton <i>et al.</i> (2014)
<i>D. americana</i>	<i>Drosophila</i>	<i>virilis</i>	Fonseca <i>et al.</i> (2013)
<i>D. virilis</i>	<i>Drosophila</i>	<i>virilis</i>	<i>Drosophila</i> 12 Genomes Consortium <i>et al.</i> (2007)
<i>D. grimshawi</i>	<i>Drosophila</i>	Hawaiian <i>Drosophila</i>	<i>Drosophila</i> 12 Genomes Consortium <i>et al.</i> (2007)
<i>D. heteroneura</i>	<i>Drosophila</i>	Hawaiian <i>Drosophila</i>	Kang <i>et al.</i> (2016)
<i>D. planitibia</i>	<i>Drosophila</i>	Hawaiian <i>Drosophila</i>	Kang <i>et al.</i> (2016)
<i>D. silvestris</i>	<i>Drosophila</i>	Hawaiian <i>Drosophila</i>	Kang <i>et al.</i> (2016)
<i>D. ananassae</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> 12 Genomes Consortium <i>et al.</i> (2007)
<i>D. biarmipes</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> modENCODE ^a
<i>D. bipectinata</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> modENCODE ^a
<i>D. elegans</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> modENCODE ^a
<i>D. erecta</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> 12 Genomes Consortium <i>et al.</i> (2007)
<i>D. eugracilis</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> modENCODE ^a
<i>D. ficusphila</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> modENCODE ^a
<i>D. kikkawai</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> modENCODE ^a
<i>D. melanogaster</i>	<i>Sophophora</i>	<i>melanogaster</i>	Adams <i>et al.</i> (2000)
<i>D. rhopaloea</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> modENCODE ^a
<i>D. santomea</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila santomea</i> ^b
<i>D. sechellia</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> 12 Genomes Consortium <i>et al.</i> (2007)
<i>D. simulans</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> 12 Genomes Consortium <i>et al.</i> (2007)
<i>D. suzukii</i>	<i>Sophophora</i>	<i>melanogaster</i>	Chiu <i>et al.</i> (2013)
<i>D. takahasiii</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> modENCODE ^a
<i>D. yakuba</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>Drosophila</i> 12 Genomes Consortium <i>et al.</i> (2007)
<i>D. miranda</i>	<i>Sophophora</i>	<i>obscura</i>	Zhou <i>et al.</i> (2012)
<i>D. persimilis</i>	<i>Sophophora</i>	<i>obscura</i>	<i>Drosophila</i> 12 Genomes Consortium <i>et al.</i> (2007)
<i>D. pseudoobscura</i>	<i>Sophophora</i>	<i>obscura</i>	Richards <i>et al.</i> (2005)
<i>D. willistoni</i>	<i>Sophophora</i>	<i>willistoni</i>	<i>Drosophila</i> 12 Genomes Consortium <i>et al.</i> (2007)
<i>Scaptodrosophila lebanonensis</i>			Vicoso and Bachtrog (2015)

^a modENCODE (<https://www.hgsc.bcm.edu/arthropods/drosophila-modencode-project>).

^b *D. santomea* (http://genomics.princeton.edu/AndolfattoLab/Dsantomea_genome.html).

sampling, molecular laboratory work, and bioinformatic analysis. This is a daunting task and will require training of the next generation (or generations) of *Drosophila* taxonomists to complete this work. Clearly, this is more than a single laboratory—or even a small, coordinated group of laboratories—can accomplish alone. A number of other taxonomic-focused working groups (e.g., The Legume Phylogeny Working Group 2013) have been very successful, and additional coordination of efforts within the *Drosophila* Phylogenetics Community will be necessary in order for the field to rapidly progress.

Taxonomy

A number of surveys, primarily in the tropical regions of the New and Old Worlds, and in the Hawaiian Islands, have projected the eventual number of Drosophilidae species (Kaneshiro 1997; Grimaldi and Nguyen 1999; Grimaldi *et al.* 2000). A conservative estimate, based on these rough surveys, is that only ~75% of drosophilid species are currently known to science. Given these estimates, the species-level diversity in this family may eventually reach ~5200 species, meaning that over 1000 species await discovery, description, and placement in the *Drosophila* phylogeny. While

the recent revisionary and phylogenetic work (e.g., Russo *et al.* 2013; Yassin 2013) is a strong step toward a comprehensive understanding of evolution in this group, it also highlights several areas that need additional work. Obviously, expanded sampling to the non-*Drosophila* genera within the family will be necessary, not only to understand biogeographic, ecological, and evolutionary patterns across the family, but also to place the disparate lineages that comprise the genus *Drosophila*. Within the genus *Drosophila*, several lineages require additional work. For example, the large *immigrans* and *tripunctata* species groups, each of which were inferred as polyphyletic in some of the studies discussed above, require additional taxon sampling and taxonomic revision. Furthermore, several of the smaller, less studied lineages, such as the *peruensis* species group, will require additional study and incorporation into the existing taxonomic framework.

Related to α taxonomy and the discovery of new species is the classification of these taxa into a hierarchy that is stable both taxonomically and nomenclaturally. Yassin's (2013) focus on defining monophyletic subgenera within *Drosophila* that can be used to organize species diversity is an important step. This increases stability in the sense that names do not

Table 4 Uses of the *Drosophila* 12 Genomes data set

Field	Question	References
Function/regulation	Discovery of functional elements	Stark <i>et al.</i> (2007b)
	Prediction of regulator targets	Kheradpour <i>et al.</i> (2007)
	Whole-genome presence/absence	Rosenfeld <i>et al.</i> (2008)
	Genome-wide enhancer activity maps	Arnold <i>et al.</i> (2014)
	Genome organization and function via BEAF-32	Yang <i>et al.</i> (2012)
Gene families	Hox post-transcriptional regulation	Patraquim <i>et al.</i> (2011)
	Gene family evolution and natural selection	Hahn <i>et al.</i> (2007)
	Cellular immune response	Salazar-Jaramillo <i>et al.</i> (2014)
	Evolution of glutathione transferases	Saisawang and Ketterman (2014)
	Insulin/TOR signal transduction pathway	Alvarez-Ponce <i>et al.</i> (2009)
Genome evolution	Odorant-binding protein family	Vieira <i>et al.</i> (2007)
	Genome size and satellite DNA content	Bosco <i>et al.</i> (2007)
	Inferred chromosomal rearrangements	Bhutkar <i>et al.</i> (2007)
	Principles of genome evolution	Ranz <i>et al.</i> (2007)
	Protein-coding gene catalog	Lin <i>et al.</i> (2007)
Natural selection	Comparative gene identification	Lin <i>et al.</i> (2008)
	Rate analyses of orthologs and paralogs	Heger and Ponting (2007a)
Evolution and evolutionary rates	False positives in the estimates of positive selection	Markova-Raina and Petrov (2011)
	Incomplete lineage sorting and natural selection	Pollard <i>et al.</i> (2006), Rosenfeld <i>et al.</i> (2012)
	Evolutionary patterns of amino acid substitutions	Heger and Ponting (2007b), Yampolsky and Bouzinier (2010)
	Gene family evolution and natural selection	Hahn <i>et al.</i> (2007)
	hAT transposable elements	de Freitas Ortiz and Loreto (2009), de Freitas Ortiz <i>et al.</i> (2010)
Genome structure and transposable elements	LTR retrotransposons roo and rooA	de la Chau and Wagner (2009)
	Species-specific DINE-1 transposable elements	Yang and Barbash (2008)
	MicroRNAs in <i>Drosophila</i>	Stark <i>et al.</i> (2007a)
	Amino acid repeats	Huntley and Clark (2007)

need to change and types do not need to be redesignated. However, uniform conventions should be employed. For example, the subgenera *Sophophora* and *Drosophila* are all paraphyletic with respect to *Lordiphosa* and *Samoia*, respectively. They retain their status as members of the genus *Drosophila*, as does the subgenus *Dorsilopha*. The Hawaiian *Drosophila*, in contrast, are placed into an entirely different genus, *Idiomyia*, in spite of the fact that they are nested deep into what is considered “*Drosophila*.” It would have been more stable in terms of preserving links to the historical literature and consistent to simply consider them members of *Drosophila*, subgenus *Idiomyia*.

Outgroups

Outgroup selection is essential, not only to critically test ingroup monophyly, but also in determining relationships among early-diverging lineages. Grimaldi’s (1990) classic study did an excellent job of summarizing the acalyprate families that are closely related to Drosophilidae and would serve as acceptable outgroups. Ephydriidae is the most closely related and widely available outgroup, although Diastatidae and Camillidae would also be excellent choices. Unfortunately, several studies (Kwiatowski and Ayala 1999; Tatarenkov and Ayala 2001; Russo *et al.* 2013) employ outgroups from divergent acalyprates (*e.g.*, Tephritidae) or even Nematocera (*Culex*, *Aedes*, and *Anopheles*). While there are whole genomes available for some of these taxa, meaning that power is increased in terms of character sampling, it also means that these are effectively random outgroups (Watrous and Wheeler

1981; Rosenfeld *et al.* 2012) and that the outgroup may adhere to the longest branch of the ingroup (Felsenstein 1978). This may lead to problems in phylogeny inferences, especially if there are undersampled regions of the tree, like in the Steganinae and basal Drosophilinae. Future studies should pay careful attention to outgroup choice.

Morphological characteristics

The two most comprehensive studies to date (Russo *et al.* 2013; Yassin 2013) are largely congruent with one another, although there are some minor points of disagreement (Figure 3). The Yassin (2013) study is more taxonomically comprehensive, and should be used as a guide for future sampling and hypothesis testing (Figure 3A). His morphological grafting approach expanded sampling to taxa without DNA samples and this is an important step forward. However, this approach is also poorly understood computationally and may not provide a rigorous, or globally optimized, hypothesis of relationships. Furthermore, it does not allow for the same statistical testing that large numbers of DNA sequence data can. Clearly, additional work needs to be done in this area, and future advances may make it possible to expand sampling to museum specimens, making comprehensive taxon sampling a reality, even for rare species.

Whole-genome phylogenetics

Genome sequencing of *Drosophila* species has proceeded in two “dimensions.” The first dimension has been whole-genome

sequencing of representative species across the genus *Drosophila*. The earliest *Drosophila* genome to be sequenced was, of course, the model species, *D. melanogaster* (Adams *et al.* 2000). The sequencing of this genome was followed several years later by the sequencing of *D. pseudoobscura* (Richards *et al.* 2005) and, shortly afterward, 12 selected species within the genus (*Drosophila* 12 Genomes Consortium *et al.* 2007). To date, over 30 *Drosophila* genomes have been sequenced, with more being generated every year (Table 3). These initial *Drosophila* genomes have had an impressive impact on the field (Table 4) and continue to be used for a variety of research questions. Since the publication of the 12 genomes data set, 12 new genomes of species of *Drosophila* have been sequenced. These whole-genome studies can be divided into five major categories: gene function and regulation, gene family evolution, genome evolution, natural selection and evolutionary rates, and structural and transposable elements (Table 4).

Multiple whole-genome sequences from closely related taxa greatly enhance the capacity for the discovery of functional elements and regulatory sequences (Stark *et al.* 2007b), especially enhancer sequences (Arnold *et al.* 2014). Likewise, large gene families are difficult to analyze, and orthology is difficult to assign, without comprehensive genomes. The dynamics of gene family evolution (Hahn *et al.* 2007) and the targeted analysis of specific gene families, such as odorant receptors (McBride 2007; Vieira *et al.* 2007), glutathione transferases (Saisawang and Ketterman 2014), and the insulin/TOR pathway genes (Alvarez-Ponce *et al.* 2009), have been facilitated by the increased number of genomes available for analysis.

Whole-genome sequences also make it possible to examine genome-level phenomena in an evolutionary context (Ranz *et al.* 2007). For example, gene gain and loss, large-scale genome rearrangements, and the evolution of transposable elements are all elucidated through the use of whole-genome sequences. *Drosophila* species have served as a model system for understanding chromosomal rearrangements. Polytene chromosomes from *Drosophila* salivary glands have long been a powerful tool in the hands of *Drosophila* biologists. Whole-genome sequences allow for mapping of polytene chromosome banding patterns and breakpoints, uniting the physical, genetic, and genomic maps of the genome (Bhutkar *et al.* 2007; Schaeffer *et al.* 2008). Furthermore, the evolutionary dynamics of genome size can be examined at high resolution with whole genomes (Bosco *et al.* 2007). The known *Drosophila* genomes show an impressive variation in size, ranging from 130 Mbp in *D. mojavensis* to 362 Mbp in *D. virilis*, a nearly threefold difference. Genome composition is likewise highly variable and correlated with genome size (Bosco *et al.* 2007). *D. mojavensis*, for example, has the smallest percentage of satellite DNA (2%), while *D. virilis* has the largest (44%). Transposable and other repetitive elements, each of which can be major determinants of genome size, have also been studied using the whole-genome approach. The evolutionary dynamics of the hAT transposable element (de Freitas Ortiz and Loreto 2009; de Freitas Ortiz *et al.* 2010), LTR retrotransposons *roo* and *rooA* (de la Chaux

and Wagner 2009), and DINE-1 transposable elements (Yang and Barbash 2008) have been examined. In addition, amino acid repeats and microRNAs have been studied in genomes across the genus *Drosophila* (Huntley and Clark 2007; Stark *et al.* 2007a).

Pollard *et al.* (2006) examined the phylogenetic relationships of the *melanogaster* subgroup using the 12 genomes data set. They were particularly interested in the *D. yakuba*, *D. erecta*, and *D. melanogaster* triad of species. There are three possible topologies of the three species: [(*D. yakuba* and *D. erecta*) and *D. melanogaster*], [(*D. melanogaster* and *D. erecta*) and *D. yakuba*], and [(*D. yakuba* and *D. melanogaster*) and *D. erecta*]. The [(*D. yakuba* and *D. erecta*) and *D. melanogaster*] topology is favored based on morphology and earlier genetic studies Lachaise *et al.* (1988). Surprisingly, an analysis of ~10,000 genes found evidence for widespread lineage sorting within the genomes of these three species. For example, 50% of the genes supported the “traditional” view of relationships within these species, [(*D. yakuba* and *D. erecta*) and *D. melanogaster*]. The remaining 50% of genes examined were equally split, with 25% supporting the [(*D. melanogaster* and *D. erecta*) and *D. yakuba*] hypothesis and 25% supporting the [(*D. yakuba* and *D. melanogaster*) and *D. erecta*] scenario. These results were robust to the kind of data used (*e.g.*, nucleotide positions, amino acid positions, indels, and genes). Since only one of the topologies can reflect the history of the three species in this group, Pollard *et al.* (2006) suggested that coalescent processes were at the heart of the rampant incongruence present in the data set.

Rosenfeld *et al.* (2012) took a different approach to understanding this interesting pattern and suggest that while lineage sorting does occur, the degree to which it does occur is biased by outgroup choice with respect to the three ingroup taxa. Pollard *et al.* (2006) used *D. ananassae*, a member of the *D. melanogaster* species group, as an outgroup. This taxon is actually quite distant from the ancestor of *D. erecta*, *D. yakuba*, and *D. melanogaster*. Rosenfeld *et al.* (2012) showed that when outgroup taxa closer to the *melanogaster* subgroup were used, the incongruence is less evident.

Other evolutionary phenomena were also examined using the 12 genomes data, including several studies that examined the degree of natural selection in protein-coding genes across the genus *Drosophila* (*Drosophila* 12 Genomes Consortium *et al.* 2007; Hahn *et al.* 2007; Markova-Raina and Petrov 2011), which examined natural selection using the dN/dS approach in proteins in the 12 genomes data set. Perhaps the most interesting result of these studies is that, for the most part, annotated genes with known functions show large evolutionary constraint and high levels of purifying selection. Putative coding regions that are either difficult to annotate or where gene products cannot be easily classified into functional groups tend to have fewer evolutionary constraints.

The second dimension that *Drosophila* genome sequencing has explored is to generate large numbers of whole-genome sequences for both mutational lines (Wang *et al.* 2015) and geographically diverse strains in one species, *D. melanogaster*.

Although these studies are phylogenetic and are important to systematics of Drosophilidae, they also shed light on the process of speciation and provide a framework for understanding species boundaries within this group. Several studies have been accomplished analyzing the population genomics of *D. melanogaster* strains (King *et al.* 2012; Langley *et al.* 2012; Mackay *et al.* 2012; Pool *et al.* 2012; Campo *et al.* 2013; Bergman and Haddrill 2015; Grenier *et al.* 2015; Kao *et al.* 2015; Lack *et al.* 2015, 2016). Lack *et al.* (2016) combined these previously sequenced genomes with another 305 new genomes of this species to create the *Drosophila* Genome Nexus, a population genomics network that provides information on the polymorphisms within this species. The recent surge of sequencing in 2016 raised the number of *D. melanogaster* genomes available for analysis to 1121 wild-derived genomes. The Nexus was constructed so that “genomic questions can be addressed from multi-population allele frequencies and haplotypes in this model species” (Lack *et al.* 2016), and to enhance gene and function discovery. The worldwide distribution of the genomes for the Nexus is impressive and includes samples from five different continents, excluding South America.

Conclusions

Flies in the family Drosophilidae have been an important part of biology since Charles Woodworth suggested that T. H. Morgan adopt the “fly” as a study animal over a century ago. From the first visible mutants discovered in Morgan’s fly laboratory to the many whole genomes that are being generated today, these flies have made important contributions to our understandings of nearly every aspect of modern biology. Twelve drosophilists have been awarded Nobel Prizes (Thomas Hunt Morgan, Hermann Joseph Muller, Christiane Nüsslein-Volhard, Eric Wieschaus, Edward B. Lewis, Jules A. Hoffmann, George Wells Beadle, Edward Lawrie Tatum, Richard Axel, Jeffrey Hall, Michael Rosbash, and Michael Young), and the tradition of biological research on *D. melanogaster* genetic and development is long and storied.

Few groups of organisms have received as much attention as *D. melanogaster* and its close relatives in the genus *Drosophila* and family Drosophilidae. One reading the historical account above might wonder why, in spite of the progress that has been made over many years, so much remains unknown concerning the phylogenetic relationships within this important model system. However, much like *D. melanogaster* serves as a model for human genetics and development, the family Drosophilidae is emblematic of how systematics is accomplished. It serves as a wonderful example of the revisionary nature of systematics, and the way that systematists continually incorporate disparate biological information and exploit new technologies to refine classification schemes. Drosophilidae is also a powerful model for how future systematic research will be undertaken. For example, it is likely that within the next decade many drosophilid species will have fully sequenced genomes, extensive amounts of morphological and behavioral trait information, nearly complete life history data, and a detailed understanding of genetic architecture

linking genotype with phenotype. This system, and how *Drosophila* biologists work toward a complete understanding of their study organism, will help set the tenor of integrative systematic research across the tree of life.

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