

Hawaiian *Drosophila* as an Evolutionary Model Clade: Days of Future Past

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The Hawaiian *Drosophila* have been a model system for evolutionary, ecological, and ethological studies since the inception of the Hawaiian *Drosophila* Project in the 1960s. Here we review the past and present research on this incredible lineage and provide a prospectus for future directions on genomics and microbial interactions. While the number of publications on this group has waxed and waned over the years, we assert that recent systematic, biogeographic, and ecological studies have reinvigorated Hawaiian *Drosophila* as an evolutionary model system. The characteristics that distinguish good model clades from good model organisms (e.g., *Drosophila melanogaster*) are somewhat different so we first define what constitutes a good evolutionary model. We argue that the Hawaiian *Drosophila* possess many desired aspects of a good evolutionary model, describe how this group of geographically isolated flies have been used in the past, and propose some exciting avenues for future evolutionary research on this diverse, dynamic clade of *Drosophila*.

generation time to make experiments temporally feasible. Over the past 100 years, *Drosophila* researchers have developed a suite of tools and techniques that have optimized *D. melanogaster* as a model system. Massive numbers of developmental mutants in the 1980s,^[1] earning a Nobel Prize and catapulting *Drosophila* into the premiere model system for understanding animal development. Subsequently, transformation systems have been developed that allow *Drosophila* biologists to easily move DNA in and out of the organism to target specific genes and proteins and carefully dissect genetic pathways. Finally, the small and relatively simple genome of *D. melanogaster* has lent itself to modern genomic studies,^[2,3] driving the discovery of new genes and gene families that were not discoverable by classical genetic approaches.

In contrast, model systems for evolutionary studies have very different requirements.

While short generation times and the ability to produce many offspring are important in a comparative context, the true strength of an evolutionary model is diversity at the population and species level. While Hawaiian *Drosophila* do not have as short a generation time or produce as many offspring as *D. melanogaster*, the clade is spectacularly diverse, and about 25% of that species diversity in the entire family was generated from a single ancestral Hawaiian species. This makes the Hawaiian *Drosophila* appealing to evolutionary and comparative biologists. Furthermore, recent advances in genomic and computational technology means that whole genome studies can now be effectively conducted on non-laboratory organisms. Here we discuss the Hawaiian *Drosophila* as a model study system for modern next generation evolutionary studies.

1. Introduction

The problem with the future is that it keeps turning into the present

– Bill Watterson

Most biological model systems focus on single species to understand a range of biological processes. For example, at the turn of the 20th century Thomas Hunt Morgan selected *Drosophila melanogaster* as a model organism to study transmission genetics. Initially known as *D. ampelophila* before its name was cemented in biological history as *D. melanogaster*, Morgan and his associates developed this tiny fruit fly into the workhorse of modern genetics. Some of the most important characteristics for a genetic model are the ability to easily breed in the lab, highly fecund females that can rapidly produce a large number of offspring, and a short

2. The Past: Hawaiian *Drosophila* as a Model Clade

The endemic flora and fauna of the Hawaiian Archipelago are inherently interesting because of their extreme isolation, the founder events required to establish a species in the islands, and the unique geological context in which they have evolved. This provides ample biogeographic, temporal, and phylogenetic hypotheses that can be generated and tested using comparative genomic data. Several Hawaiian plant and animal radiations are quite large, often comprising hundreds of species, each of which has adapted to and diversified within the Hawaiian Islands. The

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Hawaiian *Drosophila*, encompassing an estimated 1000 species and having diverged 25 million years ago, are the largest and oldest extant lineage in the Hawaiian Islands. Other examples include plants, such as *Bidens* (flare orange drop) and *Argyroxiphium* (silverswords) and arthropods like *Laupala* crickets (rusty day-singing crickets) and *Theridion* (happy face spiders).

2.1. The Hawaiian Archipelago and Its Unique Geological History

The Hawaiian Islands, situated over 3600 km from the nearest landmass, are the most isolated island system on the planet. This isolation has prompted researchers to refer to the archipelago as an “evolutionary laboratory.”^[4] Island endemics have generally evolved in isolation, free of contact not only with closely related organisms, but also the predators, competitors, and parasites typically encountered on continents or continental islands. Colonization and establishment on a remote archipelago usually involves some type of founder event, where a reduced effective population size and possible genetic inbreeding can have a strong impact on genome content and organization. While not all organisms undergo founder events, this is a good null hypothesis for the majority of endemic Hawaiian lineages.

Finally, the geology and climatic conditions within the archipelago play a large role in the evolution of endemic lineages. The Hawaiian Islands are a so-called hot spot archipelago. A more-or-less stationary hot spot in the ocean floor slowly seeps lava that, over time, forms an island. Concurrently, the Pacific plate is moving to the northwest at the rate of a few centimeters per year. Eventually, islands migrate off the hotspot and undergo subsidence, a process where volcanic activity stops, the island begins to sink under its own weight, and erosion carves deep valleys and steep ridgelines. This “conveyor-belt” like process generates an almost linear array of temporally well-defined and pristine habitats for founding organisms over a period of millions of years. The Hawaiian Archipelago stretches across the Pacific in both space and time, from the Big Island of Hawaii that began forming about 500 000 years ago, to Kure Atoll, an island that formed about 30 million years ago and is currently sitting about 3500 km to the northwest.^[5] Moisture-laden tradewinds, which blow across the Pacific and strike the northeast surface of each island, create an inversion layer at about 4000 ft. in elevation and can produce massive amounts of rainfall. For example, Mt Waialeale on the island of Kauai is the wettest place on the planet, with an annual rainfall of nearly 12 m. The unique genetic, ecological, geological, temporal, and climatic context of Hawaiian lineages provide interesting null hypotheses for comparative evolutionary studies and are themselves desirable characteristics of a good model system.

2.2. Early Taxonomic and Phylogenetic Work

The Hawaiian *Drosophila* Project (Box 1) started in 1963^[6] and, over the past 45 years, has involved hundreds of researchers and students. This project was, from the outset, driven by a

commitment to developing a strong taxonomic framework and firm phylogenetic hypotheses. Elmo Hardy^[7] produced a revision of the Hawaiian *Drosophila*, describing hundreds of new species in a single paper. The Hawaiian flies are quite diverse, and this diversity is stunning given that there are 559 named species, and that hundreds more await description. When one considers that the total number of species in the genus *Drosophila* is about 4000 worldwide, this means that one quarter of the total number of species in the genus *Drosophila* are endemic to Hawaii an island about the size of the US state of Connecticut.

Two major phylogenetic studies, one proposing relationships among all Hawaiian Drosophilidae^[8] and another focused on the large, diverse picture wing species group^[9] provided a context to

Box 1. The genesis of the Hawaiian *Drosophila* Project

The first mention of the Hawaiian *Drosophila* in the literature occurred in the late 1800's^[75] as a short note on the entomology of the Hawaiian Islands. While the first formal taxonomic descriptions of drosophilids in Hawaii appeared in 1900 to 1902.^[76–79] it was not until the mid-20th century that research heated up on the group. Much of the accolades for the initiation of the project must go to Elmo Hardy who arrived at the University of Hawaii in 1948, he began collaborating with E. C. Zimmerman to systematize the endemic Hawaiian Diptera. This ambitious task eventually resulted in 17 volumes published in the Insects of Hawaii series and an impressive series of additional publications.^[80] Hardy began to make collections of hundreds of new drosophilid species from the islands in the archipelago. The initial hard work of collection and archiving the drosophilids in Hawaii was due to a decade (1950–1959) of extremely difficult work on Hardy's part. Hardy realized the great potential this group harbored not only for systematics but for evolutionary biology and with collaborators at the University of Texas and other institutions^[6] he and Wilson Stone started the Hawaiian *Drosophila* Project with a joint NSF-NIH funding in 1963. The goal of the project was and still is “to understand all aspects of the basic biology of the endemic Hawaiian Drosophilidae.”^[81] The first major step in getting the project off the ground was accomplished by Hardy's herculean revision of the Drosophilidae in Hawaii, where he treated all 400 known species at the time. This task required that he describe 350 new species. The Hawaiian *Drosophila* Project directed by Hardy and Stone with their initial crew of principal investigators – Marshall Wheeler, Frances Clayton, Lynn Throckmorton, William Heed, Hampton Carson, Harrison Stalker and Herman Spieth – made the Hawaiian Drosophilidae one of the most powerful evolutionary model systems and the best documented example of adaptive radiation in nature.

examine ecology and behavioral evolution within this group. Throckmorton's study^[8] suggests there are two distinct drosophilid lineages endemic to the islands, the Hawaiian *Drosophila* and the genus *Scaptomyza*. Hawaiian *Drosophila* and *Scaptomyza* are one another's closest relatives and descended from a single common ancestor roughly 25 million years ago (**Figure 1**). This relationship is somewhat troubling with respect to the higher taxonomy of Drosophilidae, as it renders the genus *Drosophila* paraphyletic. Additional taxonomic work, focusing on a comprehensive revision of the genus *Drosophila* will need to be undertaken to resolve this issue.

Carson used his more focused phylogeny to develop and test hypotheses about founder effect speciation.^[10,11] These

ideas were extended by Ken Kaneshiro in his research into mating asymmetry. Ecological studies by Bill Heed^[12] and Steve Montgomery^[13] built upon the taxonomic and phylogenetic work to examine evolutionary trends across the group. The project, while still viable peaked in the 1980's, and has waxed and waned over the most recent years (**Figure 2**). After an initial burst of activity in the 1960s and early 1970s, retirements meant fewer researchers were working in Hawaii. There was an uptick in activity when DNA sequence data started to be applied to understand the relationships of these flies in the 1980s,^[14–17] but activity on the flies in this group has settled at about half of the productivity that the group enjoyed at its peak.

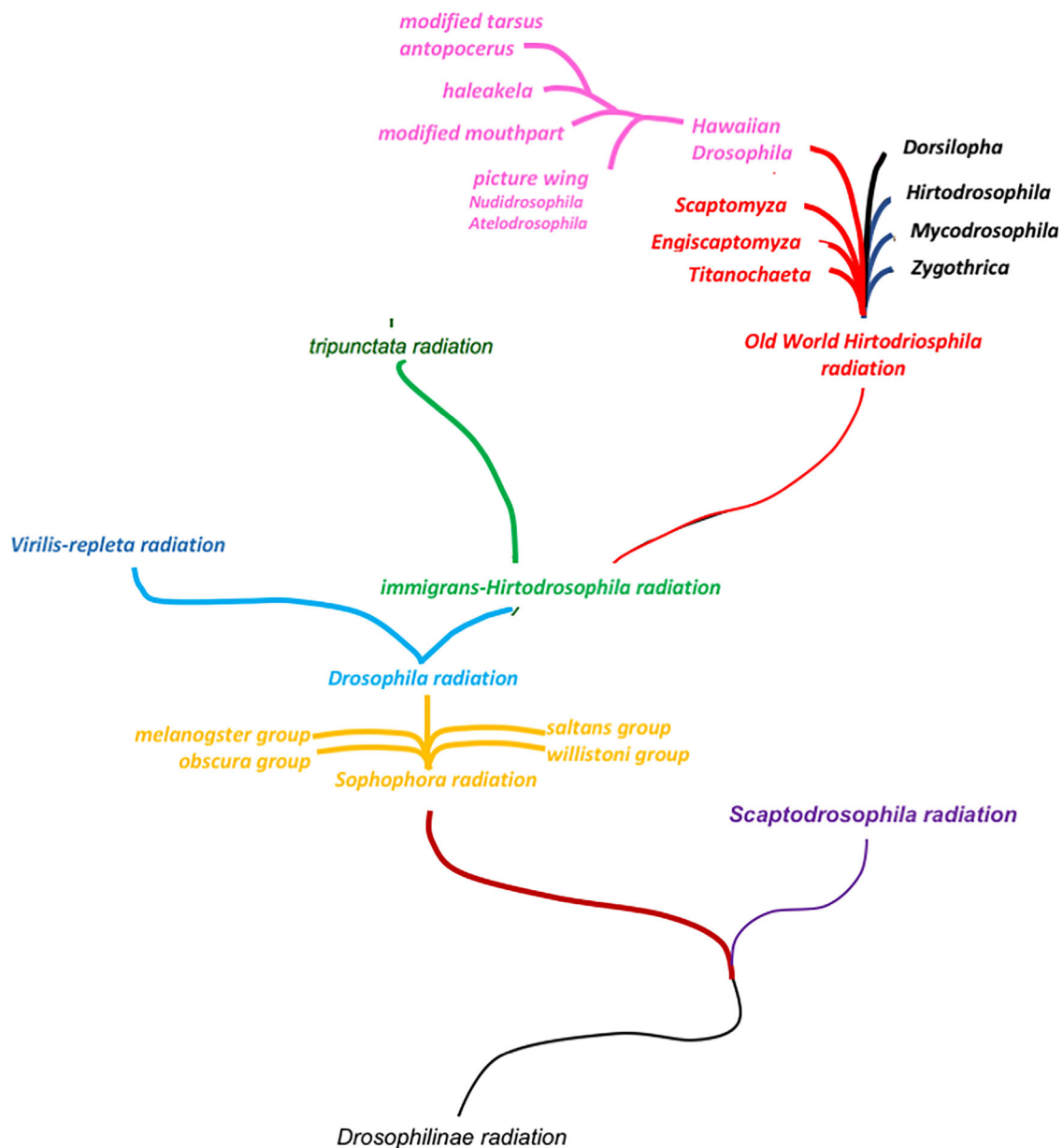


Figure 1. Phylogenetic representation of the subfamily Drosophilinae after Throckmorton.^[8] We have altered Throckmorton's original drawing to condense species groups in groups not relevant to the Hawaiian *Drosophila* and to include the different species groups in the Hawaiian *Drosophila*.

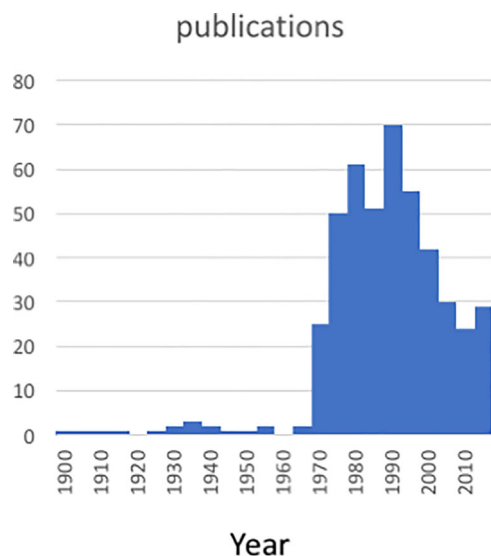


Figure 2. Graph showing the distribution of publications over the past 90 years on the Hawaiian *Drosophila*. The data for the graph were obtained by searching over 5 year periods starting with 1880 and ending with 2017. File 1, Supporting Information contains the references by year.

3. The Present: Current Hawaiian *Drosophila* Research

The groundwork laid by the Hawaiian *Drosophila* Project has made endemic *Drosophila* in Hawaii an important model system with which to address the major evolutionary questions in modern biology. We divide current research on this lineage into three major categories, with an eye toward future technical and intellectual developments across the field. These include the (a) characterization of natural variation within populations (b) role of natural selection on species formation, and (c) the use of phylogenetic systematic hypotheses in comparative analyses to understand the tempo and mode of diversification in natural populations. Gasch et al.^[18] proposed that “genetic variation represents a critical frontier for molecular studies today” and only through characterization of this variation can we hope to understand how evolution utilizes natural variation. This is clearly related to the second major topic, perhaps the holy grail of evolutionary biology, understanding the process of species formation. Butlin et al.^[19] outlined thirteen major questions in speciation research that we reiterate here and apply to the Hawaiian *Drosophila* model. Several model systems have been constructed to approach these questions at the level of species differentiation particularly in animal models and this literature has recently been reviewed by Payseur and Rieseberg^[20] and Nosil et al.^[21] The final category concerns systematics and phylogenetics. A hypothesis of phylogenetic relationships within a group is essential for reconstructing ancestral character states, examining biogeographic patterns, and understanding shifts in diversification rate.

3.1. The Hawaiian *Drosophila* Are Incredibly Diverse Anatomically and Behaviorally

The Hawaiian *Drosophila* also inhabit incredibly broad ecological niches on the islands and rely on broad ranges of host plants

for survival.^[22–25] This general statement about variation holds for comparisons between islands, as well as differences between volcanoes comprising the same island. Several recent studies address variation and the search for natural selection in Hawaiian *Drosophilidae* (mostly picture wing flies). Alves et al.^[26] examined cuticular hydrocarbons (CHCs) in 27 species of Hawaiian *Drosophila* and report significant inter and intra species variation for these cuticular molecules. Genome size^[27] and genome organization as well as transposable element effects on the evolution of the Hawaiian *Drosophila*^[28] have also been a recent focus of researchers on Hawaiian *Drosophila*. In addition, the homology of chromosomal segments has been established between *melanogaster* and several Hawaiian species.^[29] There are also fine scale differences between Hawaiian *Drosophila* species with respect to embryonic enhancer elements important in early development. Brittain et al.^[30] examined these embryonic enhancers in three Hawaiian *Drosophilidae* – *Scaptomyza anomala*, *D. grimshawi*, and *D. mimica*. These three species of flies are from very diverse Hawaiian taxa. The results of the study indicate that microsatellite repeats (MSRs) pepper the embryonic enhancers of Hawaiian taxa more frequently than in non-Hawaiian taxa, suggesting that the MSRs have been enriched in embryonic enhancer regions and perhaps serve the function of spacing enhancers. As we mentioned above natural selection has been a major focus of studies characterizing variation. Using a genus *Drosophila* wide (31 species with two from the Hawaiian *Drosophilidae*) screening approach, Cicconardi et al.^[31] examined positive diversifying selection in over 2000 ortholog groups in these species. One of the Hawaiian *Drosophilidae*, *D. grimshawi*, showed strong putative diversifying selection related to several gene ontology categories including morphological and neuronal terms.

3.2. Speciation Is a Predominant and Ubiquitous Process in the Hawaiian *Drosophila*

The uniting thread in any kind of speciation study is the capacity to catch the process getting ready for the act, “in the act” or to study the direct aftermath of speciation in great detail by focusing on hybridization and hybrid zones. Hitting the target is difficult and was best described by Rick Harrison’s warning in 1991^[32] to discern between the process of speciation and the product of speciation (**Box 2**). Flies in the genus *Drosophila* have been used extensively to study speciation, including in the *melanogaster* species complex between *D. melanogaster*, *D. simulans*, and their relatives,^[33] within the cactophilic *Drosophila repleta* species group^[34] and in the Caribbean *cardini* species group.^[35] The Hawaiian *Drosophila* have also been used as a model for the study of speciation, mainly because the rate of speciation in the Hawaiian *Drosophilidae* is extreme compared to other continental drosophilid groups. Early studies using crosses in the lab established that sexual selection had a role in the rapid proliferation of species in the genus *Drosophila* in Hawaii. Gene complexes were also pointed to as arbiters of anatomical and behavioral change in these studies at the species boundaries. While the pace of work in the lab that focused on species boundaries slowed a bit between the 1990’s and 2010, a more recent spike of these kinds of studies both using crosses

Box 2. Classical speciation studies on Hawaiian *Drosophila*

The classical work on speciation in the Hawaiian Drosophilidae was focused on a sympatric species pair that inhabit the Big Island of Hawaii – *D. silvestris* and *D. heteroneura* (Figure 5). *Drosophila silvestris* and *D. heteroneura* are differentiated morphologically with the males in the latter species having stalk like eyes that have been shown to be involved in the mating process.^[10,83,86,87] Templeton^[88] and Boake et al.^[89–94] and Kaneshiro et al.^[95,96] showed that the width of the head of *D. heteroneura* is correlated with male mating success and that the system is a classic example of sexual selection. Further work cemented the concept of sexual selection as an important evolutionary component of this species pair.^[97]

Another closely related complex of species that have been studied in the past is *Drosophila grimshawi* and its relatives, *D. craddockae* and *D. pullipes*. Initially, there were only two species in this group, *D. grimshawi* from Kauai, Oahu, and Maui Nui, and *D. pullipes* endemic to the youngest island, Hawaii (Figure 5). Kaneshiro and Kambyellis^[98] using a combination of ecological, morphological, and behavioral characters, split *D. grimshawi* into two separate species, *D. craddockae*, known from the older islands of Kauai and Oahu, and *D. grimshawi* from the Maui Nui complex of islands. This was supported by earlier work by Ohta^[99] who used lab crosses of *D. grimshawi* from different islands (Kauai, Oahu, Molokai, Maui) in the archipelago to test hypotheses about the genetics of coadaptation and mating boundaries and the role of auditory signals in the intra- and interspecific communication.^[100,101]

and genomics has happened. Price et al.^[36–38] have studied the genetics of the hybrid instability and species boundaries in the *silvestris-heteroneura* species pair. Several new Hawaiian *Drosophila* genomes have been generated with studying the *silvestris-heteroneura* boundary as a focus. By contrasting nearly complete genome sequences of this sympatric species pair with a third allopatric species (*D. planitibia*) Kang et al.^[37,38] were able to examine genomic changes at the species boundary at a fine scale. Their comparisons indicate that positive selection occur more readily in sympatry than in allopatry. More importantly, functional analysis of the genes under strong positive selection are in sensory detection and mating categories. Olfactory receptors seem to be impacted by positive selection suggesting a major role for these functional categories in the adaptive radiation of flies in this group. With the recent demonstration that auditory signals regulate aggressive behaviors in *D. melanogaster*^[39] work on the role of intra- and interspecific auditory signals could become an important component of studying the species boundary in the Hawaiian *Drosophila*.^[40]

3.3. Classic Taxonomic and Systematic Paradigm

The strong beginning on the taxonomy of the group developed by Elmo Hardy (Box 1) has been the major foundational force in the treatment of the flies in the group. About 350 species of Hawaiian *Drosophila* were described by Hardy early on and since then another ~200 have been formally described and named. There are still approximately 400 species remaining to be described and named, for a total of about 1000 species. Throckmorton^[8] examined the phylogenetic relationships both within the Hawaiian Drosophilidae and with respect to where this large lineage was placed in the family Drosophilidae. Carson^[9] took a more targeted approach, using polytene chromosomes to infer relationships within the 100 or so species of the picture wing species group. The picture wing species group is characterized by very distinctive markings on the wings, hence it's informal epithet. The flies in this group are also quite large in comparison to continental *Drosophila*, as well as to many of the other Hawaiian species. DNA sequencing was first used to examine relationships within the Hawaiian Drosophilidae in the 1990s, using one or a few target genes. Figure 3 shows the arrangement of the four groups, and depicts the picture wing group as the sister group of the four lineages of Hawaiian *Drosophila*. Some of the more interesting aspects of each group are given in the Figure 3. The recent revisionary work in the group is also summarized in Box 3. Detailed phylogenies of the group and their placement in the family Drosophilidae allow for testing of various evolutionary hypotheses related to developmental biology, behavior, ecology and genomics as we point out below.

4. The Future: The Next 50 Years of Hawaiian *Drosophila* Research

Certainly, research on Hawaiian *Drosophila* will continue. For example, the taxonomic work alone will require some attention as the collection, archiving and description of 400 taxa is no small task. But how can the system be used in the future to advance our understanding of biology? Approaching this question requires that we discuss what the characteristics of a good model system for evolutionary biology might be. In many ways asking this question is the same as asking what the current big questions in evolutionary biology might be and in addition, anticipating what they will be. The characteristics of a good model system for evolutionary studies then depends on the questions that are hot at the time. We discuss three areas of biological research where the Hawaiian *Drosophila* will be a strong model system in the future – the comparative method (including speciation and evo-devo), genomics and ecology.

4.1. The Hawaiian *Drosophila* Will Continue to Be Important in Speciation Studies, Phylogenetics, Evo-Devo Studies, and the Comparative Method

While some revisionary taxonomic work remains to be done on groups within the Hawaiian *Drosophila*, the it is clear that this taxon as a whole is monophyletic and have arisen from a single

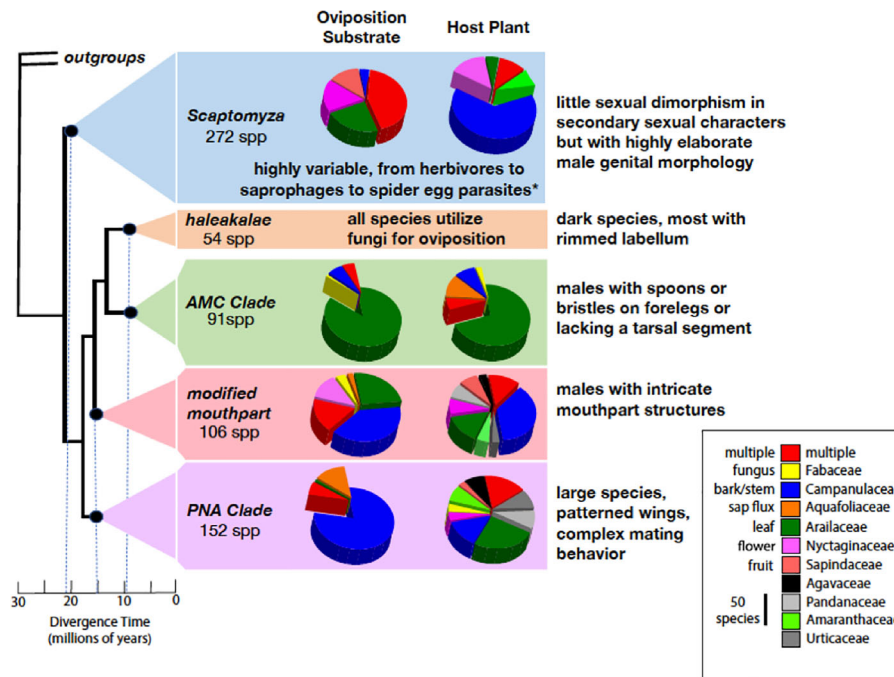


Figure 3. Phylogenetic tree showing times of divergence for the four major clades of Hawaiian *Drosophila*. The clades themselves are scaled to size for the number of species in the group, and the horizontal line indicates 50 species. To the right are some of the more conspicuous characteristics of the group. The tree is rooted with the closest continental outgroups (the *virilis-repeta* and *melanica-robusta* radiations). * Oviposition substrates and host plants are highly variable in *Scaptomyza*. Data shown are from *Elmomyza*, the largest Hawaiian lineage.

common ancestor.^[41,42] The monophyly of Hawaiian *Drosophila* makes it a powerful model for comparative biology. The large number of species in the group and sister species pairs makes the group relevant to future speciation studies. **Table 1** should

Box 3. Recent revisionary work in the Hawaiian *Drosophila*

Revision of the various species groups in the Hawaiian *Drosophila* has proceeded steadily over the past two decades.^[102–110] Multi-gene data sets have been used to infer the relationships of Hawaiian *Drosophila* groups. The taxonomic revisionary work and phylogenetic systematics of the Hawaiian *Drosophila* are fairly straightforward (Figure 3), with four major groups of species – the AMC clade (with flies in the modified tarsus group, the ciliated tarsus group and the genus *Antopocerus*), the *haleakalae* group, the modified mouthparts group and the PNA clade (with flies in the picture wing group, and the *nudidrosophila* and *ateledrosophila* species groups). In addition, a temporal framework for the divergence of the groups has been possible using island formation time points and a molecular clock. Figure 5 shows the current understanding of relationships of the four major groups of Hawaiian *Drosophila* along with some salient information about the four major groups including host plant range and food and breeding substrate.

serve as a guide for researchers interested in speciation and the use of Hawaiian *Drosophila* in such studies. Several researchers have performed comparative studies across the phylogeny. For example, Jezovit et al.^[43] have used the *Drosophila* phylogeny to examine sexual behavior and communication across the family. Their analysis specifically examined habitat distribution, mating behavior in light and dark and chemical communication via CHCs. While interesting trends over the family are uncovered with this approach only two Hawaiian species were included in the analysis. Singh^[44] used sibling species pairs across the family to examine micro-differentiation at the species boundary. He lists only two sibling species pairs within the Hawaiian *Drosophila* as part of his review of sibling species where evolutionary inferences have been made. An expansion of the sibling pair approach to the Hawaiian *Drosophila* would allow for hundreds of comparisons, and also because of the interisland movement of the members of species pairs would allow for a temporal component to be incorporated into such analyses. Many developmental studies rely on the existence of cultures for study. More species in the group will need to be made available to the research community in order to realize the group's importance in evo-devo biology. While some of the Hawaiian species are available from the *Drosophila* Species Stock Center (<http://blogs.cornell.edu/drosophila/>), most of the Hawaiian species have been difficult to rear in the lab. Novel collection and rearing approaches are needed to enhance the group's utility.

The group also offers ample morphological variation that has prompted several authors to point to the group as a model for examining evolutionary developmental phenomena.^[45–50] Some of the more evident morphological phenomena in the group include wing pigmentation,^[50,51] extreme modifications of

Table 1. The Marie Curie SPECIATION Network top 13 questions concerning speciation and how the Hawaain *Drosophila* are relevant.

SPECIATION top 13	HD roles	Examples
1. Which barriers contribute to reproductive isolation?	A large number sibling species pairs in the HD have unique reproductive barriers based on morphology and behavior.	<i>silvestris/heteroneura</i> ; <i>grimshawi</i> ; see Carson and Kaneshiro ^[10]
2. When does drift have a significant role?	Much of the evolution of this group is through founder effects that produce small population size prone to drift.	Picture wing founder effect patterns are well known; see Carson ^[87] ; Carson and Templeton ^[11]
3. What are the relative roles of natural and sexual selection?	Several species show extreme sexual dimorphism with males having extreme phenotypes as a result of sexual selection	<i>heteroneura/silvestris</i> show extreme sexual selection as do modified mouthparts and modified tarsi groups; see Kaneshiro and Boake ^[96] ; Carson ^[82,84]
4. What is the role of reinforcement?	The patterns of speciation on islands in the archipelago produces abundant capacity for reinforcement	Picture wings; <i>grimshawii</i> ; see Ohta ^[99]
5. How important is hybridization in speciation?	While the species pair <i>silvestris/heteroneura</i> has been the focus of most hybridization research, the phenomenon undoubtedly is widespread in this group.	<i>Heteroneura-silvestris</i> and other picture wings; Carson et al. ^[85] ; Kaneshiro and Val ^[95] ; Price and Muir ^[111]
6. What are the environmental and genetic conditions that promote speciation?	The ecological nature of speciation based on host plant preference and oviposition substrate is expansive	<i>silvarentis/heedii</i> ; see Kaneshiro et al. ^[68]
7. What is the nature of speciation genes?	While no speciation genes have yet to be discovered, the genetic basis of several complex phenotypes involved in speciation are available	head shape genetics of <i>heteroneura</i> ; see Templeton ^[88] ; Val ^[86]
8. What is the role of changes in gene expression and in genomic processes?	Several studies have approached this problem by examining gene expression across species boundaries.	embryonic enhancer evolution in picture wings; hybrid impact on gene expression; see Brittain et al. ^[30] ; Brill et al. ^[36]
9. What is the role of plasticity?	While not addressed in much detail the potential for studying plasticity should be abundant.	Dietary impact on courting; see Droney ^[112] ; Jones and Widemo ^[113]
10. What are the genomic patterns of reproductive isolation?	Work on this topic has just begun with the genome sequencing of multiple closely related HD species.	<i>silvestris/heteroneura/planitibia/grimshawii</i> ; see Clark et al. ^[3] ; Kang et al. ^[37,38]
11. How are biodiversity patterns related to speciation	The biodiversity of the Hawaiian Islands has been the subject of many meta-studies and this should make possible putting the HD into that context.	Overall comparison of arthropod diversity to picture wings; see Shaw and Gillespie ^[114] ; Roderick and Gillespie ^[115]
12. What causes variation in speciation rate and duration?	This group is highly amenable to a focus on rates whether speciation or other due to the unique formation of the islands	Overall Hawaiian Island comparisons are available; see Lim and Marshall ^[116]
13. What is the impact of anthropogenic change?	The habitats of the island system are rapidly diminishing and several studies on anthropogenic change and species survival have been completed.	Impact of invasive species on HD survival; see Krushelnycky et al. ^[102]

HD, Hawaiian *Drosophila*; all species names are genus *Drosophila*.

forelegs and mouthparts (**Figure 4**) and body size (the flies in the group vary from a paltry 2 mm to a robust 8–10 mm; compare this to the average size of the lab model *D. melanogaster* at about 3 mm). Biogeographic studies have been approached in the past in this group^[41,49] that took advantage of the unique formation of the islands on which these flies reside. Presently and in the future the ease with which phylogenies can now be produced in combination with the island arc system dynamics should result in a major focus on biogeographic analysis for this group of flies.

4.2. Genomics Will Greatly Enhance Hawaiian *Drosophila* Research

The small genome size of most drosophilids also makes obtaining genome level information for a large number of species or individuals tractable. Hawaiian *Drosophila* have genomes that are similar enough in size and sequence divergence to the model organism *D. melanogaster* and other

species in the *Drosophila* 12 genome project to benefit from the vast information collected on this species model. In fact, one Hawaiian species *D. grimshawi* was part of the *Drosophila* 12 genomes project. While the Hawaiian *Drosophila* are unique enough to allow focus on different biological scenarios that could be interesting in evolutionary, there are currently, fewer than ten Hawaiian species have had genomes sequenced. We anticipate an expansion of the number of genomes sequenced for the group in the next few years. Indeed, it is conceivable that many of the over 500 described species will have full genomes sequenced in the near future.

The power of this comparative approach at the genome level is illustrated by Kursel and Malik,^[52] who examined the family wide distribution of centromeric histones as a result of gene duplication. Studies such as this are impossible to do without broad sampling within a taxonomic group. At the population level genome analysis has been carried out on several model organisms – *Arabidopsis*,^[53] *Drosophila*,^[54,55] *Mus*,^[56] *Caenorhabditis*,^[57] and *Saccharomyces*.^[58,59] Similar work at the population

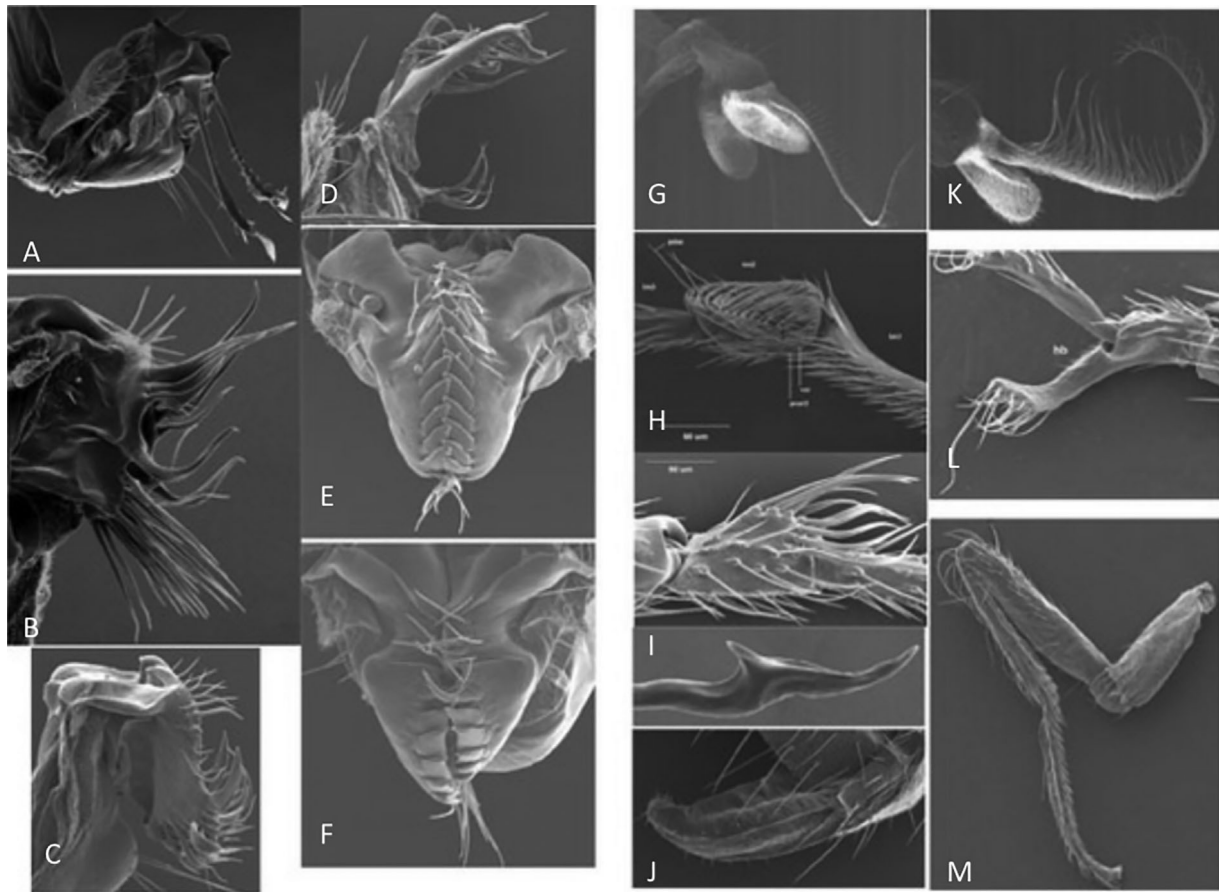


Figure 4. Electron micrographs showing sexually dimorphic morphological variation within the Hawaiian *Drosophila*. A) *Drosophila adventitia* (mouthparts, male), (B) *Drosophila hystricosa* (mouthparts, male), (C) *Drosophila xenophaga* (mouthparts, male), (D) *Drosophila aethostoma* (mouthparts, male), (E) *Drosophila prominens* (mouthparts, male), (F) *Drosophila freycinetiae* (mouthparts, male), (G) *Drosophila adunca* (antennae, male), (H) *Drosophila waddingtoni* (foreleg, male), (I) *Drosophila quasiexpansa* (foreleg, male), (J) *Drosophila hirtitibia* (aedeagus), (K) *Drosophila hirtitibia* (ovipositor), (L) *Drosophila tanythrix* (antennae, male), (M) *Drosophila proceriseta* (foreleg, male), (N) *Drosophila hirtitibia* (foreleg, male). Figures modified after the work of O'Grady et al.^[25,74]

level on humans is voluminous.^[60] These studies all focus on population level phenomena and characterization of variation in large scale model organism systems. As Gasch et al.^[18] state “genetic variation represents a critical frontier for molecular studies today” and such characterization is an important step in understanding how evolution utilizes natural variation. Whole genomes for large numbers of individuals in model organism studies also allow for whole genome scans of natural selection using a variety approaches under statistical models.^[61] The Hawaiian *Drosophila* have not been a major focus of population level studies, except perhaps for the *grimshawi* populations and the *heteroneura/silvestris* species pair described above. However, this situation could change in the near future with the availability of large scale sequencing methods for populations, allowing for genome wide scans for natural selection on a novel and important evolutionary scale. Because the biology and ecology of the flies in this group are varied and more importantly tractable (see below), correlation of statistically detectable genomic change with the ecology is possible. Such correlations will allow for better interpretations of the statistical and biological significance of genome change at the sequence level.

4.3. Modern Techniques Open Doors to the Study of the Ecology of the Group

Some prominent model systems at the population level have shed light on the workings of natural selection in the determination of ecological niches include Hoekstra's rodent model systems^[62,63] and the stickleback fish^[64,65] evolutionary model. These model systems have looked closely at adaptive evolution in behavioral traits and coat color evolution of rodents and in morphological traits of sticklebacks.^[66] The intellectual focus of the work in these model systems is on the relationship of genetic change to phenotypic change and they rely on ample phenotypic and environmental variation in the nonmodel species subjects for their utility.

Early work on the ecological niches for species in the Hawaiian *Drosophila* demonstrated a broad range of habitats and host plants for these flies.^[12,13,67] One study examined the ecology of oviposition of a sibling species pair – *D. heedi* and *D. silvarentis*^[68] and demonstrated microhabitat differentiation of their egg laying sites. Expansion of host plant descriptions for the group was accomplished^[22] and a relatively complete view of



Figure 5. Two examples of subjects of speciation studies in the Hawaiian *Drosophila*. On the top left, *D. grimshawi* is shown. This is the only picture wing species that is found on multiple islands and hence offers a unique opportunity to study the impact of biogeography on speciation and genetic divergence. On the bottom the sibling species pair of *D. heteroneura* (left) and *D. silvestris* (right) are shown. *D. melanogaster* female is shown to scale for comparison in the upper right panel.

this ecological parameter for the majority of named species now exists. Having this ecological context allows for broader tests of evolutionary hypotheses about adaptation and coevolution of the flies and their host plants. While genome scans for natural selection are often times useful and interesting, being able to test hypotheses about natural selection requires a more direct approach that involves taking non-model organisms with a focus on phenotypic and environmental/ecological variation.

The habitat/host plant context in which the Hawaiian *Drosophila* exists opens a wide range of hypotheses that can be tested about natural selection in this system. The most recent ecological work on the flies in this group is perhaps the most exciting. With the development of rapid methods to detect microbial communities in ecological samples, has come a more complete examination of the ecological context of groups of organisms. Such studies have focused in ecological settings like rivers or lakes, but there are now a large number of studies

that examine insect guts as ecological niches for microbial biodiversity.^[69–72] The Hawaiian *Drosophila* have also been examined for microbial interactions.^[24,73] While these studies are still preliminary, the results are intriguing and suggestive of a role of interaction between plants, microbes and insects and a potential driver of diversification in this group. This would no doubt be a strong potential avenue of research that would continue the multidisciplinary tradition of the Hawaiian *Drosophila* Project.

5. Conclusion

The authors of this review are, of course, partial to this group of organisms. They are our favorite organisms and most of our careers have been dedicated to understanding the biology of the group and advancing it as an evolutionary model for

future researchers. As the quote at the beginning of this paper indicates, though it is often times difficult to anticipate the future because anticipating the future is moot when it becomes the present. Our experience with this group of flies is that there is an interesting and fruitful question at every turn of a corner when studying them. We are confident that the next generation tools for genome analysis, and many of the high throughput biological methods developed in the last decade will be used to expand this model system even more than it has in the past.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no competing interests and are solely responsible for the content of the paper.

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