Rapid morphological, ecological and behavioral evolution in *Drosophila*:

Comparisons between the cactophilic *repleta* species group and the

endemic Hawaiian Drosophila.

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Introduction

Flies in the genus *Drosophila* have served as model systems in genetics, ecology, and evolutionary biology for over 100 years. In addition to their ease of culture and numerous attractive genetic attributes, species in this group also feature a wealth of naturally occurring diversity in morphological, ecological and behavioral characters. These three character systems evolve in concert, sometimes rapidly, as the flies interact with and adapt to their environment. For example, historical changes in the environment, such as geological processes (e.g., island formation, continental movement), long-term climatic patterns (e.g., sea level rise, temperature and rainfall shifts) and host plant availability (e.g., cladogenesis and extinction in plant lineages), have been instrumental in laying the basis for the genera, radiations and species groups currently seen in the Drosophilidae. These long-standing processes can have a direct impact on ecological associations and, in turn, morphological and behavioral characters associated with feeding and breeding ecology.

More recent environmental phenomena mediated by man's impact on climate and species distributions, are also having an impact on the evolution of this genus. An example of this type of perturbation is seen in Hawaiian *Drosophila* where predatory species (ants and wasps) are introduced and native species are extirpated from part of their ranges and driven higher upslope where invasive species have yet to gain a foothold (Foote and Carson, 1986). Similar range reductions are predicted for alpine taxa with increasing temperatures. As

temperatures increase, less heat tolerant species will be pushed out of the lower parts of their traditional ranges to higher elevations. This effect will be greatest on those *Drosophila* species that are adapted to montane environments or have seasonal shifts in species distributions that are mediated by temperature. However, desert adapted species may be unable to undergo altitudinal or latitudinal shifts, because they specialize on cacti, whose slow growth may prevent the host distribution from shifting before the flies become extinct. Those species able to undergo rapid evolution with respect to climatic or host variables will be the ones to survive.

Sexual selection on both morphological and behavioral characters can likewise play a role in driving rapid change in morphology, behavior and ecological associations. Environmental factors are known to influence the chemical and morphological underpinnings of sexual selection in *Drosophila*, and thus reproductive behaviors and the characters that execute them are also of interest in responding to environmental changes.

Ecology

Likewise, the ecology of this group is quite diverse. When considering ecological associations in Drosophilidae, it is useful to divide preference into two discrete categories: (1) adult feeding, oviposition site preference and larval feeding substrates and (2) mating site preference. While these are identical for the majority of drosophilid species, a number of taxa, such as the Hawaiian *Drosophila*, have evolved separate preferences for mating.

Throckmorton (1975) was the first to summarize ecological adaptations across the family Drosophilidae and place them in an evolutionary context. He considered most ecological adaptation in the family as a reflection of "opportunism and versatility centering around the saprophagous leafmold habit" (Throckmorton, 1975). He proposed that basal drosophilids were able to exploit a number of different types of substrate, although they may have preferred one (Throckmorton, 1975). Subsequent elaboration and specialization generated broad "feeding and oviposition guilds," such as sap feeders, fungivores, frugivores, flower breeders, or those taxa that utilize some other decomposing vegetative structure (*e.g.,* leaves, bark). Recent phylogenetic results (Figure 1) broadly support Throckmorton's hypotheses and show support for clades of present-day drosophilid species adapted to various guilds of rotting plant material (Markow & O'Grady, 2005; Markow & O'Grady, 2008).

Species can be classified as either generalists or specialists depending on the breadth of feeding and oviposition substrate types they use and the phylogenetic relationships of the taxa they specialize upon. For example, a **broad generalist** would be able to capitalize on multiple resources spread across feeding guilds. *Drosophila melanogaster* is an example of a broad generalist and can be reared from flowers, fungi, fruits and a number of other rotting plant materials. Interestingly, broad generalist species have evolved multiple times on the phylogeny and can often be seen nested within clades of more specialized taxa. More **substrate generalists** are restricted to a given feeding guild (*e.g.,* fruit) but can utilize this resource across a wide array of

unrelated plant species. Many members of the *tripunctata* radiation are generalist frugivores and can be found on many different rainforest fruits. **Substrate specialists** are a class of taxa that have adapted to a single substrate type from a single clade of host plant. Most members of the *repleta* species group fit in this category because they have evolved to necrotic parts of a single host plant family, Cactaceae. Likewise, most Hawaiian *Drosophila* are also classified as substrate specialists because individual species use one substrate type from a single clade (e.g., leaves of Araliaceae in the case of *Drosophila waddingtoni*). **True specialists** are the most narrowly defined category and can use only a single type of plant resource from a single species of host plant. *Drosophila sechellia* is a classic example, larvae develop only rotting fruits of *Morinda citrifolia* (reference). A small number of specialist taxa have shifted away from rotting plant material to become **parasitic** on some animal species (Ashburner, 1981; Throckmorton, 1975).   Broad generalists, substrate generalists and substrate specialists can all be considered **polyphagous** species, while true specialists and parasitic taxa are **monophagous**.

Morphology

Morphology is quite variable across the genus, with the various species groups and radiations adapting to different ecological niches and microhabitats. In addition, there is also a high degree of sexual dimorphism, with males of many taxa possesses species-specific secondary sexual characteristics used in courtship and/or mating. A classic example of such a character is the sex comb

of *Drosophila melanogaster*, a row of stout, peg-like bristles on the foreleg of males. Sex combs are quite variable within the broader *melanogaster* species group and have been studied extensively because of their evolutionary significance and relatively simple genetic architecture (Tanaka et al. 2009). While sex combs are restricted to the *melanogaster* and *obscura* species groups, male-specific foreleg modifications are widespread in the family and can be found in most species groups and genera (*e.g.,* (Stark & O'Grady, 2009), suggesting that evolution of these characters is rapid and critical to mating success in many species.

Primary sexual characters, particularly the male genitalia, also evolve rapidly (Masly et al. 2011; Richmond et al 2011). These traits are critical to species recognition and success during courtship and intromission. While male characters have been studied extensively (Ashburner et al. 2005; Coyne 1983; Hsu 1949, Vilela 1983), female characters, such as spermathecae (Pitnick et al 1999), are also subject to rapid evolutionary change.   Other characteristics of the female terminalia also evolve rapidly, but are linked more to ecology than sexual behavior. The ovipositor of many Hawaiian *Drosophila* species has diversified rapidly and has been correlated with oviposition substrate (Craddock and Kambysellis 1997; Hardy et al. 2001).

Behavior

Behavioral evolution in *Drosophila* entails two broad classes of change: modifications to actual behaviors (e.g., the evolution of male guarding in some species of the *repleta* group and lek behavior in Hawaiian *Drosophila*) and changes in the structures employed in the various behaviors. Some of the structures involved in this latter category may be either external, like wing patterns used in courtship display, or internal, such as in the nervous system or reproductive organs.

Rapid Evolutionary Change

Two of the largest and most interesting groups of *Drosophila* are the cactophilic *Drosophila repleta* species group, a clade of over 100 species endemic to the New World, and the Hawaiian *Drosophila*, a lineage consisting of over 600 species that have diversified rapidly in the Hawaiian Islands. As these lineages have been well-studied, we will focus this chapter on the rapid evolution of behavior and related morphology in the species within them.

Hawaiian *Drosophila* Radiation

Phylogenetic relationships

The Drosophilidae endemic to the Hawaiian Archipelago are comprised of two large sister lineages, Hawaiian *Drosophila* and the genus *Scaptomyza* (Russo et al. 1995; Remsen & DeSalle, 1998; Remsen & O'Grady 2002; O'Grady &

DeSalle 2008). Hawaiian *Drosophila* contains over 400 described species

(O'Grady, Magnacca, & Lapoint, 2008) placed in eight major lineages including

the *picture wing*, *modified mouthparts*, *antopocerus*, *modified tarsus*,

nudidrosophila, *ateledrosophila*, *rustica*, and *haleakalae* species groups.

Relationships among the various lineages of Hawaiian *Drosophila* (Figure) have

recently been summarized by (O'Grady, et al., 2011). The sister lineage of the

Hawaiian *Drosophila* is the genus *Scaptomyza*, a clade of about 300 species,

over 50% of which are endemic to the Hawaiian Islands. The remainder of taxa

in the genus *Scaptomyza* are found throughout the world, but particularly on

islands. O'Grady and DeSalle (2008) examined phylogenetic relationships

across the family Drosophilidae to demonstrate (1) a single common ancestor of

Hawaiian *Drosophila* and all *Scaptomyza* on Hawaii roughly 25 million years ago,

(2) the origin of *Scaptomyza* in Hawaii, and (3) the subsequent escape from and

colonization of the remainder of the world by members of the genus *Scaptomyza*.

Molecular clock analyses place the divergence between Hawaiian *Drosophila*

and the genus *Scaptomyza* at ~20 million years, a number that fits well with a

Scaptomyza species in Dominican amber. However, the movement of this genus

out of Hawaii seems to have taken place independently within several subgenera

and more likely is on the scale of the past 5-10 million years.

Sexual adaptations to morphology and behavior

The majority of species in Hawaiian *Drosophila* possess secondary sexual

modifications in male wings, forelegs, mouthparts and other head structures.

Mating behavior in this group is among the most complex in all Diptera. Males of most species lek, guarding specific territories away from the feeding and oviposition substrate. Male-male aggression is common and a single male will often need to spar with multiple males over the course of a single day. This ranges from 10-15 minute contests occurring 3-4 times an hour in *D. percnosoma* to 30 second interactions taking place 4-5 times in a five minute period in *D. imparisetae* (Shelley 1987, 1989; O'Grady unpublished). The genus *Scaptomyza* provides an interesting contrast to Hawaiian *Drosophila* because they have a markedly lower degree of sexual dimorphism. While males of most Hawaiian *Drosophila* species possess extreme secondary sexual dimorphism, differences between males and females in most *Scaptomyza* species are rare and restricted to the male genital apparatus. Corresponding sexual behaviors are likewise simpler, with courtship in *Scaptomyza* being of much shorter duration and less dependent upon male display. It is possible that these two sister clades represent distinct avenues of sexual selection, one favoring "showy" displays using energetically expensive secondary sexual characters with correspondingly complex behaviors and another employing the rapid elaboration of primary sexual characters (male genitalia) while maintaining relatively simple behaviors.

Ecological adaptations to morphology and behavior

O'Grady et al. (2011) examined the evolution of substrate and host plant species preference across the Hawaiian *Drosophila*. This work builds on

previous studies by Heed (1968, 1972), Carson (1971), Kambysellis et al. (1995), and Magnacca et al. (2008) by placing oviposition and larval feeding preference in the context of a broadly sampled phylogeny of Hawaiian *Drosophila*. This study suggested that host plant family was a much more plastic character than substrate type. This gives insight into how oviposition preference and larval feeding might evolve within this group. Based on these results, a species that has adapted to leaves of *Cheirodendron* (Arialaceae) could more readily shift to leaves of another plant, such as *Pisonia* (Nyctaginaceae), than it is change substrate types within *Cheirodendron* and oviposit on stems. While additional work is needed to fully understand this phenomenon, this constraint might be explained by differences in physical or chemical properties of leaves vs. stems or by microbial communities adapted to different substrate types across plant groups.

Cactophilic *Drosophila* Radiation in the New World

Phylogenetic relationships

While the cactophilic lifestyle has arisen more than once in the Western Hemisphere, the largest radiation of cactus-breeding *Drosophila* belong to the *repleta* species group which contains over 100 species. The separation between the *repleta* and the *virilis* group is estimated to have occurred approximately 10 mMYA. Five subgroups are recognized within the *repleta* group: *hydei, mercatorum, fasciola, repleta,* and *mulleri.*

At least 60 of the *repleta* group species, particulary members of the *mulleri* supbgroup, are endemic to Mexico, primarily residing in arid or semi arid areas. Additional species are endemic to South America, where radiations of different cactus species, both *opuntia* and columnar provide niches for them. Members of the *fasciola* subgroup appear restricted to the wetter areas of the West Indies, Central and South America. *Drosophila hydei* and *D. mercatorum,* while utilizing cactus in rural areas, also are cosmopolitan, using other decaying fruit and vegetable materials associated with humans. Because cacti can be characterized chemically and their necroses can be tracked at both spatial and temporal scales, the system is an ideal one for studies of the interaction of genes and ecology in evolution.

Endemic to the Western Hemisphere, the Cactaceae family contains about 1,800 species. The major divisions of the family Cactaceae used by *Drosophila* are presented in Figure 2. The Pachecereae, Cactae, and Opuntiodiae. Clearly, then, there are more cactus species than cactophilic *Drosophila* species, reflecting the fact that while some *Drosophila* species tend to be associated with only one species of cactus, many are cactus generalists and can utilize multiple cactus hosts. In some cases, multiple host use is restricted to hosts of the same genus, but in other cases, flies are able to utilize cacti from three different cactus lineages. The majority of cactophilic *Drosophila* utilize species of *Opuntia*, which is not surprising, given that these represent the majority of cactus species available and are basal to the other cacti.

Phylogenetic analyses also indicate that opuntia-breeding is the ancestral state for cactophilic *Drosophila*.

Most well-studied of the cactophilic *Drosophila* are *D. mojavensis* and *D. arizonae* in North America. William Heed and his students and colleagues (Fellows and Heed 1972; Heed1982; Ruiz and Heed 1988) identified the host specificities of each, and his subsequent group members characterized the yeast communities associated with each cactus species (Starmer et al 1982). In addition, the whole genome of one cactophilic fly species, *D. mojavensis* (Figure 3,*)* was published as part of the *Drosophila* Genomes Consortium effort (2007), and the genome of *D. buzzatii* currently is being sequenced by Alfredo Ruiz and his colleagues in Barcelona.

The South American *D. buzzatii* is another important focal taxon for studies of ecology, evolution and behavior (Manfrin and Sene 2006). An assortment of related species, *D. koepferi, D. anonieta, D. gouviae, D. b* and *D. serido*, with different evolutionary and reproductive relationships provide a parallel system to the cactophilic Drosophilids in North America.

Rapid evolution of ecological adaptations

Cactophilic *Drosophila* are able to utilize the moist, nutritious habitats afforded by cactus tissue only after it becomes necrotic, a condition that occurs following an injury to the plant and subsequent invasion and decomposition by bacteria and yeasts. Adult flies feed and mate at or near these necrotic cacti and

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females deposit their eggs in the necrotic tissue, which then serves as the food for the developing larvae.

In order to exploit the cactus niche, however, the flies face several challenges.   With respect to abiotic variables (Gibbs et al 2003), many cactophilic species exhibit rapid adaptation to high temperature (Stratman and Markow 1998) and low humidity (Matzkin et al 2007, 2009) that typifies many cactus habitats. While these resistances are physiological in nature, often related to cuticular hydrocarbon composition (Toolson et al 1990; Markow and Toolson 1999; Etges and Jackson 2001), flies also disperse at night when the termperatures are lower and humidities higher (Markow and Castrezana 2000).

The cacti themselves, with their various toxic compounds, present additional, biotic challenges to the flies. Partial detoxification of the plant tissue is accomplished by the microbial communities, unique to each cactus species, which are responsible for the decay process. Although the flies feed on these microbes, they are still confronted with the need to process many cactus compounds. By-products of microbial decomposition and the chemical profiles of unaltered plant tissue constitute the specific environment that each *Drosophila* species must deal with in its own particular host cactus. Oligoarrays based upon the *D. mojavensis* genome have revealed candidate genes for changes in host use and population genetic analyses of these genes, especially alcohol dehydrogenase and glutathione S-transferase D-1, show that they are evolving rapidly among subspecies using different hosts (Matzkin et al 2006; Matzkin 2005; 2008).

Finding the appropriate cactus host necroses is assumed to occur via olfactory cues from the volatiles specific to that cactus. Flies behaviorally have been reported to move toward material from their native host in a laboratory setting (Newby and Etges 1998). More recently, however, population genetic differentiation in olfactory receptor genes among different host-specific subspecies of *D. mojavensis* suggest the possibility of connecting particular receptors with particular hosts and their cues (Matzkin and Shoemacher 2011).

Rapid evolution of behavioral traits

In contrast to the frequently spectacular mating behaviors exhibited by Hawaiian *Drosophila*, visual cues appear to play little of any role in mating behavior. Among the cactophilic *repleta* group flies, a moving fly of any sex or species will trigger interest from a male, especially in mating chambers or vials in the laboratory, but this appears to be the primary role of vision in the system. Courtship consists of males closely following females, constantly licking the tip of her abdomen. At the same time, males are producing species specific auditory cues or "songs" with the their wings. Unlike the auditory courtship component of *melanogaster* group flies, the females sing as well as the males, resulting in a dueting between members of the courting pairs. Female flies of all repleta species examined so far will indicate their acceptance of a courting male by a characteristic wing spreading behavior without which the males will not attempt to mount. Because the male songs of close relatives are distinctive (Etges et al 2006), they obviously have evolved quite rapidly which could explain the sexual

isolation reported among different subspecies of *D. mojavensis* (Krebs and Markow 1989; Zouros and d'Entremont 1980).

Courtship and mating in at least some species of cactophilic *Drosophila* take place on the cactus, but away from the necrotic sites where feeding is taking place. For example, in *D. nigrospiracula,* males space themselves on healthy tissues in ways that suggest they are defending small territories (Markow 1988). Females land near a male and they approach each other with three different outcomes: (1) females depart rather quickly; (2) courtship takes place and the female departs; and (3) copulation takes place (Markow 1988). In *D. mojavensis* males, in addition to locating themselves on healthy cactus arms, also can be found in groups on healthy tissue near the necrosis. Females arrive and there is a flurry of courtship activity some of which results in copulation. Rapid evolution in male genitalia within species and between closely related species has been reported for both South American (Soto et al 2007) and North American (Richmond et al 2011) *repleta* group species.

Oviposition specificity also has been extensively studied in cactophilic *Drosophila* (Fanara et al 1999; Fanara and Hasson 2001; Barker and Starmer (1999). While *D. buzzatii* prefers to oviposit in it's native hosts compared to other cacti, its sister species, *D. koepferi*, is more of a generalist in its preferences (Soto et al 2011). Males of both species have greater success in mating when reared in their own host (Hurtado et al 2011), so in the case of *D. buzzatii,* the oviposition preference supports an evolutionary association between maternal preference and offspring performance.

Conclusions

Adaptive radiation vs. adaptive infiltration

Adaptive radiations are characterized by high ecological and phenotypic diversity in a rapidly evolving lineage.   The Hawaiian *Drosophila*, with their impressive ecological breadth, high diversity in male secondary sexual characters, and over 600 described species evolving in the past 25 million years, are a classic example of adaptive radiation in nature (Kaneshiro 1997; O'Grady et al. 2008). The *repleta* group also constitutes an impressive radiation of over 100 species evolving in the past ~30 million years, with physiological adaptations (e.g., desiccation tolerance) and diverse reproductive strategies, but lacking the ecological breadth and phenotypic diversity seen in the Hawaiian taxa. Interestingly, most species in this group are adapted to a single plant family, Cactaceae, although this family is very diverse and presents significant biological challenges to any species attempting to exploit it.

The differences in the degree to which these two groups have diversified, in terms of species numbers, the degree of ecological breadth and the magnitude of morphological, behavioral, and physiological adaptation suggests two separate modes of radiation acting in Hawaiian *Drosophila* and the *repleta* group. One where the species spatially segregate into a number of different host plant family and substrate types, each with it's own ecological requirements, and another where the species diversify on a single plant lineage but maintain separation from close relatives via a variety of differences in reproductive morphology (male

genitalia and female reproductive tracts) and/or behavior (specific lek sites away from a common feeding area, male guarding in some species, courtship song characteristics and pheromone profiles).

Heed and Mangan (1986) used the term "adaptive infiltration" when discussing the ecology of Sonoran desert *Drosophila*, three of which were in the *repleta* group. They applied the term narrowly and discussed only four endemic species in this region, *D. pachea, D. nigrospiracula, D. mettleri* and *D. mojavensis*, all of which have adapted to the harsh climate and cactophilic lifestyle. However, one can apply this term more broadly to the entire *repleta* group, the majority of which are cactophilic and occupy harsh, arid environments. The specific differences between taxa which have adaptively radiated compared to those that have adaptively infiltrated can be reflected in the degree of ecological breadth, physiological tolerance, rapid morphological innovation (e.g., primary and secondary sexual characters), and behavioral diversity.

Rapid evolution of behavioral and physical traits can occur under either scenario, adaptive radiation or adaptive infiltration. In the case of the Hawaiian radiation, the most obvious physical traits to have evolved are the morphological ones employed in behavior and behavioral displays. Among the repleta species, the most obvious physical traits are chemical ones, especially those used in aggregation and mating. In both cases, however, behavioral and physical traits are linked, although it is unclear whether behavioral changes precede physical changes and promote their evolution or *vice versa.*

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Figure Legends

Figure 1. Overview of phylogenetic relationships and substrate type usage in the genus *Drosophila*.

Figure 2. Phylogenetic relationships among the major lineages in the repleta species group and Hawaiian Drosophila, with proportions of species using specific substrate types and host plant lineages.
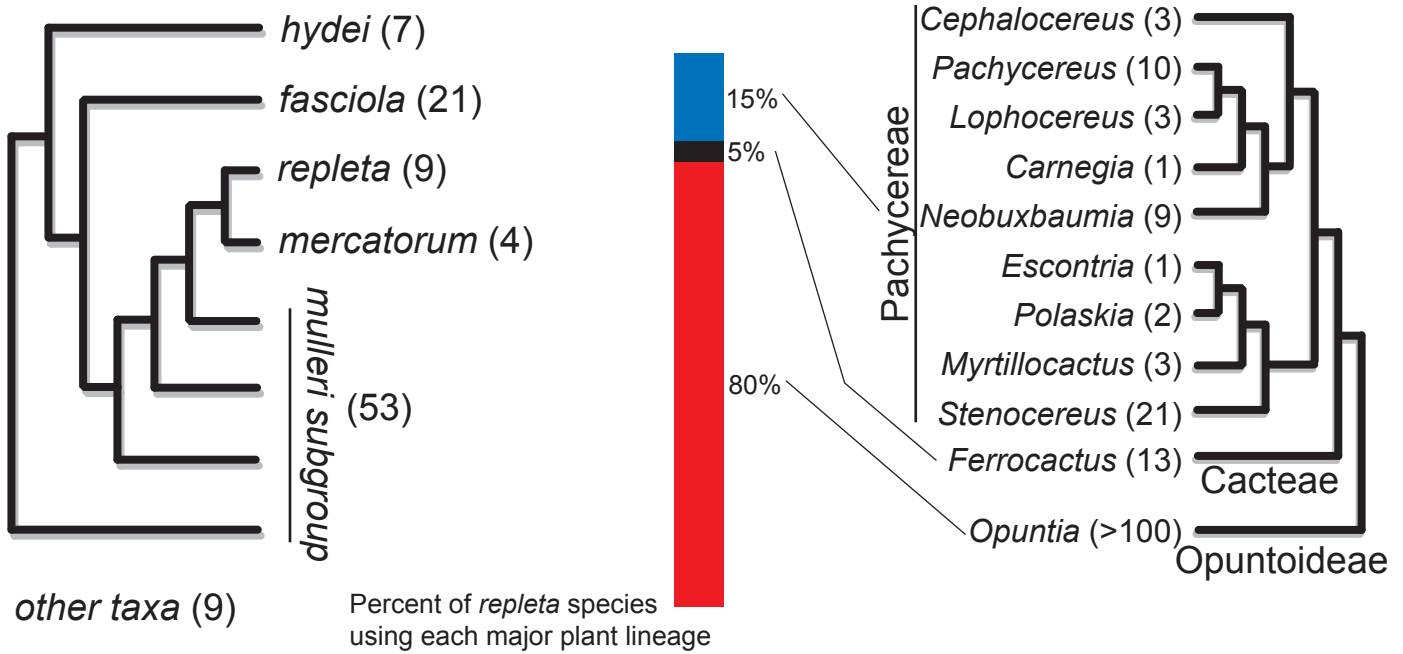
Figure 1
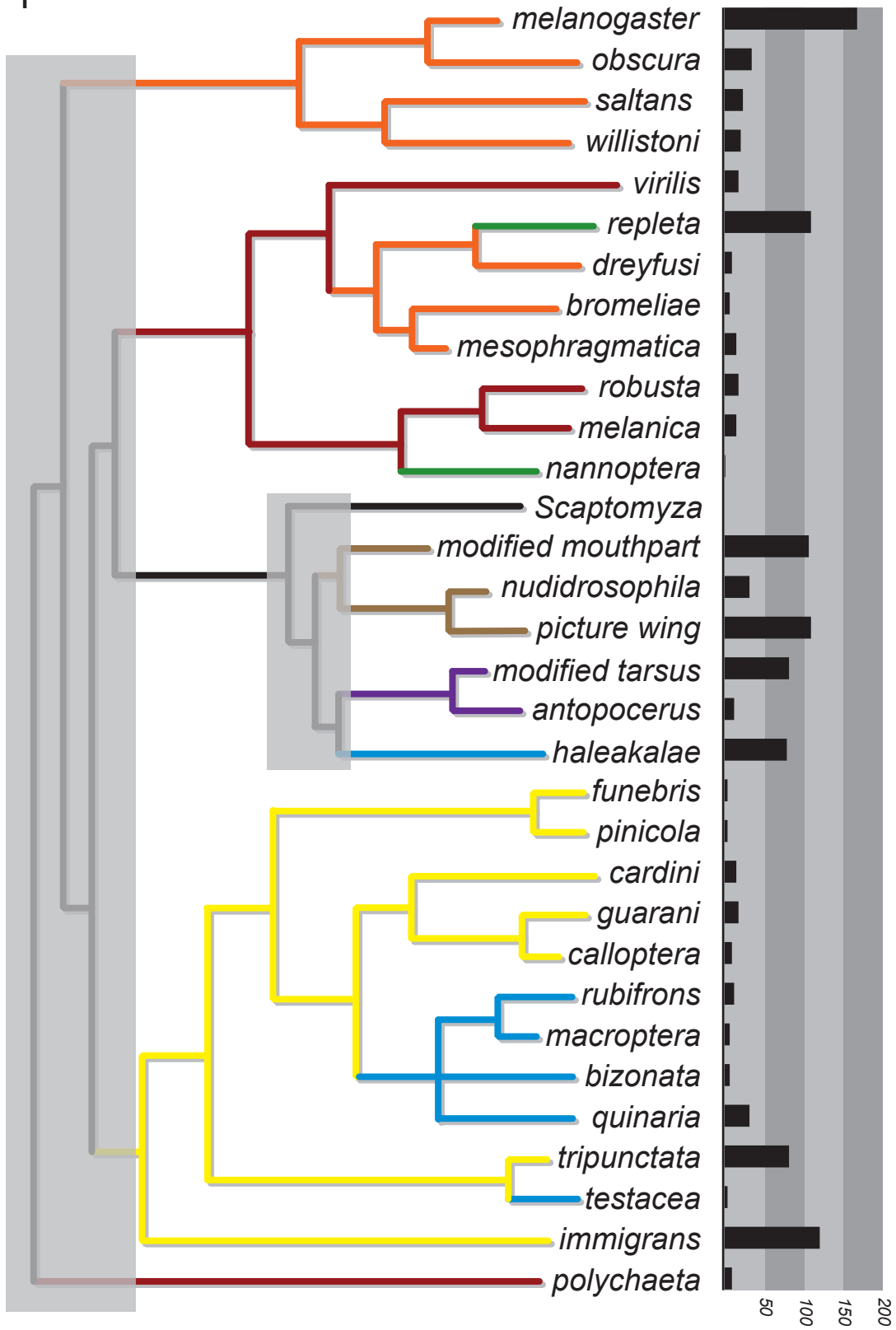
cactus · leaves · sap, slime flux · bark · fruits, flowers · fungus · fruits, flowers, fungus · equivocal reconstruction

Figure 2

	spp.	Ecological Breadth	Morphological Diversity	Mating Behavior			
				visual	auditory	tactile	other
repleta	>100	low (1 family)	primary sexual characters	No	Yes	Yes	Lek (some) Male guarding (some)
Hawaiian Drosophila	>600	high (34 families)	secondary sexual characters	Yes	Yes	Yes	Lek (most)