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**SUBSTRATE USE WITHIN THE
DROSOPHILA MELANOGASTER SPECIES SUBGROUP**

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INTRODUCTION

Introduction to Fly Olfaction

Survival and reproduction of living organisms, such as *Drosophila*, requires effective sensory perception with which to navigate the environment. Clear sensory perception is vital to the survival of living organisms such as *Drosophila*, commonly known as vinegar flies. More specifically, olfaction, or the sense of smell, is beneficial for *Drosophila* survival and fitness. The detection of odors allows it to identify potential food, danger, or mates without having to come in direct contact with the source. Innate and adaptive mechanisms allow these organisms to recognize each odor and its implications or lack thereof. Flies are able to integrate both mechanosensory and chemosensory information in order to advise feeding and oviposition behaviors. Using their olfactory system involves not only fitness for themselves, but also ensures the fitness of the next generation.

The olfactory system is integral in the *Drosophila*'s ability to navigate the environment. Thus, the system is composed of a highly organized circuit, arranged in a way that allows for phenotypic plasticity, depending on the species (Figure 1). The *Drosophila* circuit has been mostly studied in *Drosophila melanogaster*. On the antenna of the fly, there are sensory hairs, otherwise referred to as sensilla, that house groups of up to four olfactory sensory neurons (OSNs). OSNs generally express one type of olfactory receptor (OR). From the antenna, OSNs project to a region of the brain known as the antennal lobe. OSNs of the same type, that is, expressing the same OR, innervate a discrete region of the antennal lobe, known as a glomerulus. From the antennal lobe, projection neurons innervate two higher-order brain centers: the mushroom body, which is associated with learned behaviors, and the lateral horn, which is involved with innate behaviors. In the mushroom body, projection neurons will synapse on Kenyon cells, another type of neuron found specifically in the mushroom body (Couto et al., 2005; Vassar et al., 1994; Vosshall et al., 2000; Grabe & Sachse, 2018; Su et al., 2009).

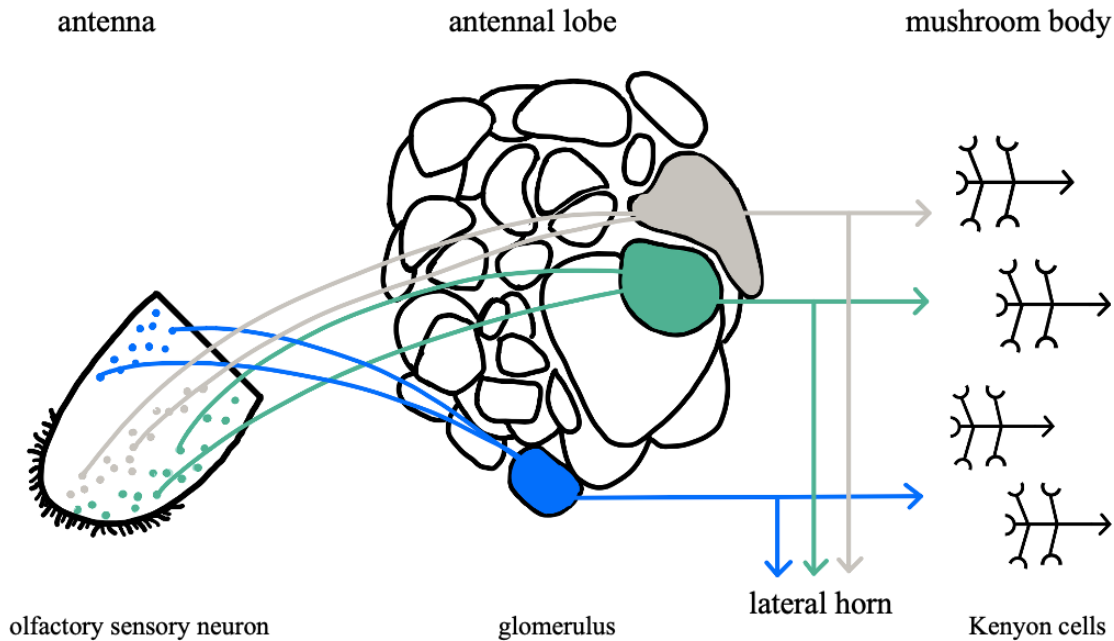


Figure 1. The *Drosophila* olfactory circuit.

OSNs in the antenna generally express one type of OR, and these OSNs project to a specific region of the antennal lobe called a glomerulus. From the antennal lobe, projection neurons innervate either the mushroom body (learned behaviors) or the lateral horn (innate behaviors). Projection neurons innervating the mushroom body synapse on Kenyon cells, another type of neuron found within the mushroom body.

Flies detect volatiles with the ORs expressed by OSNs. These ORs are each tuned to a certain subset of volatiles, activating particular glomeruli or glomerular subsets (Buck & Axel, 1991). This olfactory information is then combined with other sensory cues, memories of past experiences, and details regarding the animal's spatial and temporal state. All of this information is integrated to instruct attraction or aversion behaviors (Su et al., 2009).

Introduction to Oviposition

Successful oviposition, or the process of ejecting an egg from the oviduct to the outside environment, is a significant aspect of an organism's fitness (Ubuka & Bentley, 2011). Egg laying animals, such as *Drosophila*, can guarantee offspring survival by locating and ovipositing on a suitable substrate, since most insect larvae are quite limited in their mobility (Dweck et al., 2013). A given substrate usually acts as the *Drosophila* habitat and food, so habitat choice directly affects both the nutrition and fitness of her offspring (Shettleworth, 2001; Dweck et al., 2013).

There is a strong selection pressure for accurate oviposition site selection in *Drosophila* species, not only for the progeny's nutrition but also due to parasitization and predation of the insect larvae once it pupates (Rifell, 2013). There is evolution of site selection from how *Drosophila* behaviors vary based on their environments (Kimura, 1980). While *Drosophila* display innate behaviors that are programmed through their genes, these innate behaviors can also vary based on their internal state and external signals, such as environmental or social cues (Aranha and Vasconcelos, 2018). For instance, a female is more likely to lay her eggs on a site where another female has already laid her eggs, using the other female's oviposition behavior as a social cue to guide her own. Thus, females display significant adaptations in their oviposition behavior, which

have the potential to change with space and time, such as delaying egg laying due to lack of appropriate substrate (Wertheim et al., 2003).

Furthermore, *Drosophila* reproductive ecology is characterized by a wide diversity of resources that are used differently by each species. *Drosophila* utilize different substrates based on their microbial and chemical properties, presenting a significant variety of obstacles for each species. Additionally, *Drosophila* must be able to utilize the substrates that are available in its habitat during any given time of the year (Markow & O'Grady, 2008). Therefore, armed with behavioral plasticity and faced with environmental variation, natural selection favors females who have high success in finding suitable substrates for their offspring (Mayhew, 1997; Thompson, 1988; Jaenike, 1978; Mery & Kawecki, 2004; Stastny et al., 2006; Yang et al., 2008).

Natural selection acts specifically on the cues that allow *Drosophila* to discriminate between various substrates, a skill referred to as chemosensory perception (Whiteman & Pierce, 2008). Flies can identify chemosensory cues through senses such as vision, olfaction, and gustation (Markow & O'Grady, 2008; Zhu et al., 2014). Using these senses, flies can select for ethanol content, temperature, fermentation volatiles, microbial composition, and taste (McKenzie & Parsons, 1972; Dillon et al., 2009; Reed, 1938; Stökl et al., 2010; Stensmyr et al., 2012; Riffell, 2013). Microbial composition is especially important because larvae feed on the microbe community of the plant tissue instead of on the plant itself (Bernays & Chapman, 1994). Additionally, texture and the density of other existing larvae on the substrate can play a role in the oviposition decision. A high density of other larvae on the substrate will cause the female to avoid ovipositing in a site because their offspring will face competition with the other existing larvae. Olfaction also plays a vital role in this skill, as the activation of certain glomeruli will determine attraction or aversion to a substrate (Kobayakawa et al., 2007; Semmelhack & Wang, 2009; Suh et al., 2007).

Furthermore, gene loss or gain allows an even greater ability to recognize (and specialize) on particular substrates. Since insects can use the chemical structure of given odorants to determine whether they can undergo feeding and oviposition on the substrate, host specialization is likely the target of selection in *Drosophila* species (Whiteman & Pierce, 2008). Females are either generalist or specialist in terms of substrate choice. A specialist female is very strict about the conditions in which she will oviposit and can recognize and select against non-host substrates. The current hypothesis is that changes in both olfactory and gustatory receptors underlie the evolution of host specialization in specialists or a lack thereof in generalists (McBride, 2007; McBride & Arguello, 2007). Oviposition site selection can therefore involve behavioral, phenotypic, and genetic changes in *Drosophila* species, causing the diversification of adaptations that results in speciation (Dworkin & Jones, 2009; Futuyma & Moreno, 1988). As such, oviposition represents an ideal entry point to understand how neuronal circuits adapt to the chemosensory ecology.

Introduction to the *Drosophila melanogaster* species subgroup

Alfred Henry Sturtevant believed that understanding the differences between intimately related species provides the most extensive understanding of the speciation process (Provine, 1991). As the field of speciation continues to expand, ecologists have discovered that increases in biodiversity of coexisting species are accompanied by variation in ecological niches and adaptation of the preferences of each species to the available resources in these diverse habitats as a result of competition avoidance (Hutchinson, 1978). The *Drosophila melanogaster* species subgroup is one such group of coexisting species that have evolved to adapt to different niches in order to increase their fitness.

The *Drosophila melanogaster* group originated in and is distributed over three adjacent biogeographic regions: Afrotropical, Australasian, and East Palearctic (Figure 2), with each region having its respective endemics (Lachaise et al., 1988). This group is composed of 141 different species, which are further categorized into 12 subgroups, such as the *suzukii*, *ananassae*, and *melanogaster* subgroups (Seetharam & Stuart, 2013).

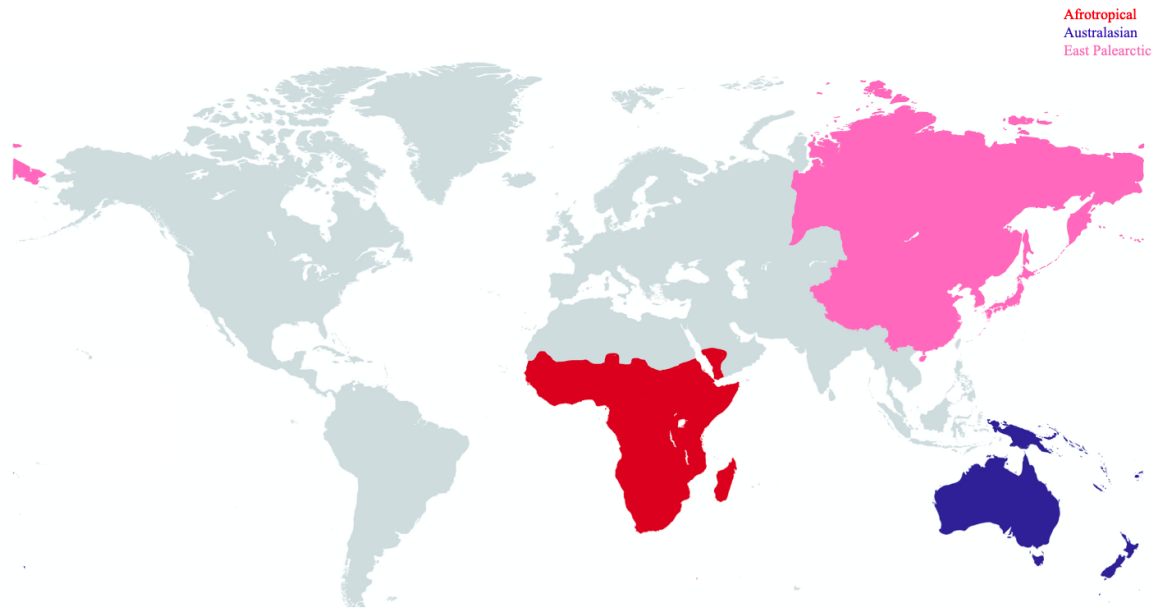


Figure 2. Distribution of the *melanogaster* group.

The *Drosophila melanogaster* group originated in and is distributed over three adjacent biogeographic regions: Afrotropical, Australasian, and East Palearctic. The *melanogaster* subgroup is endemic to the Afrotropical region.

The *D. melanogaster* species subgroup is endemic to the Afrotropical region. The ancestor of the *melanogaster* subgroup originated in Southeast Asia, much like the ancestors for the other species in the *melanogaster* group (Lachaise et al., 1988). When the ancestor of the *melanogaster* subgroup colonized Africa during the late Miocene, the olfactory system played a significant role in how this ancestor was able to adapt to the available substrates and environmental conditions specific to the Afrotropical region (Ometto et al., 2013). Furthermore, the consensus view is that the diversification of the *melanogaster* species subgroup took place due to allopatric speciation. Since all of these species can be found within a very specific region, this allopatric speciation could have occurred anywhere within this region, either in mainland Africa or in the islands found in the Indian Ocean (Lachaise & Silvain, 2004). From the incidence of geographic barriers, the species within the subgroup diverged.

The *melanogaster* species subgroup is comprised of nine species: *Drosophila melanogaster*, *Drosophila sechellia*, *Drosophila simulans*, *Drosophila mauritiana*, *Drosophila teissieri*, *Drosophila yakuba*, *Drosophila santomea*, *Drosophila orena*, and *Drosophila erecta*. These species have varying relationships with one another (Figure 3) but all descended from a common ancestor (Lachaise et al., 1988).

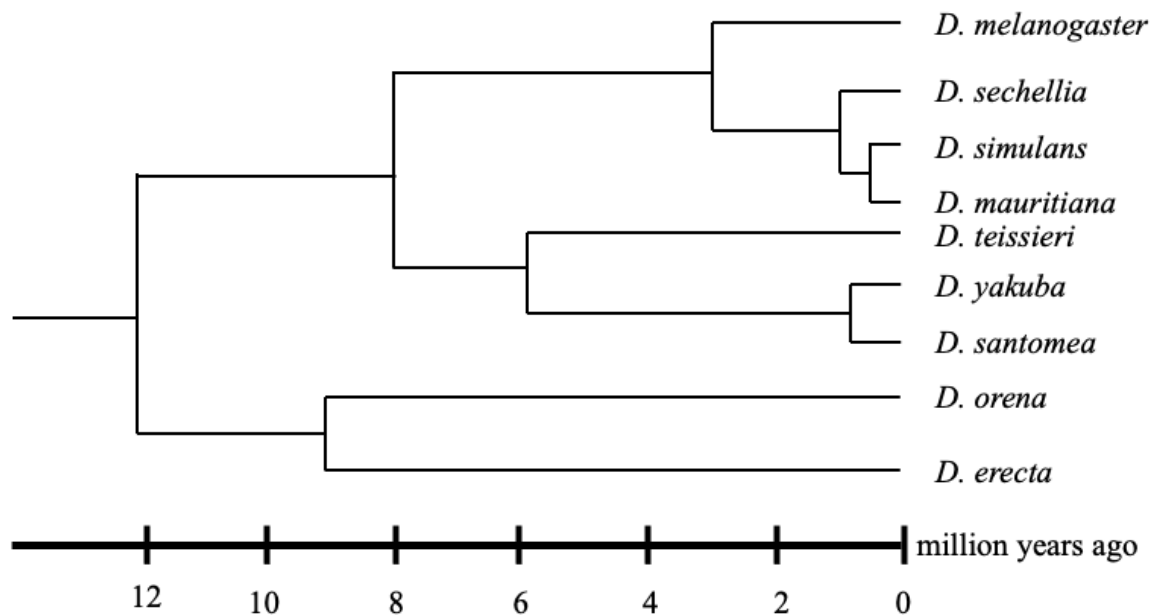


Figure 3. Consensus phylogeny of the *melanogaster* subgroup.

These are approximate values. Initially, three lineages (*D. melanogaster-simulans-mauritiana-sechellia*, *D. erecta-orena*, and *D. teissieri-yakuba*,) emerged from a common root and diverged into the nine species that we know of today (Lachaise et al., 1988). *D. sechellia* diverged from *D. melanogaster* about 3 million years ago and from *D. simulans* and *D. mauritiana* 0.1-0.24 million years ago. *D. yakuba* and *D. santomea* diverged about 0.4 million years ago. (adapted from David et al., 2007).

Over the last 10 to 15 million years, the *melanogaster* subgroup has evolved to utilize a great diversity of ecological niches (Lachaise et al. 1988; Tamura et al. 2004; David et al. 2007). The majority of *Drosophila* are saprophagous species, feeding and developing on decaying organic matter, generally utilizing material such as sweet fruits and vegetables (R’Kha et al., 1991). However, the various substrates that are suitable for feeding and oviposition differ widely from one species to the next. For example, some species are considered specialists, while others are considered generalists (Table 1).

Generalist Species	Specialist Species	To Be Determined
<i>D. melanogaster</i> <i>D. simulans</i> <i>D. mauritiana</i> <i>D. yakuba</i> <i>D. teissieri</i> *	<i>D. sechellia</i> <i>D. erecta</i> ^	<i>D. santomea</i> <i>D. orena</i>

*potentially a specialist in certain environments

^seasonal specialist

Table 1. Current classifications of species in the *melanogaster* subgroup.

D. melanogaster, *D. simulans*, *D. mauritiana*, *D. yakuba*, and *D. teissieri* are generalist species, having the ability to use a wide variety of substrates for feeding and oviposition. *D. sechellia* is a specialist species with one host plant for feeding and oviposition. *D. erecta* is a seasonal specialist, feeding and breeding on one host plant during the fruiting season, but is a generalist during the rest of the year. *D. santomea* and *D. orena* have yet to be fully classified as generalist and specialist species.

Overall, because of its impressive ability to adapt to a variety of niches, the *melanogaster* subgroup has an appreciable number of species found in central equatorial Africa (Lachaise et al., 1988). While most of these species remain on or around the African mainland (Figure 4a), two species, *D. melanogaster* and *D. simulans*, have evolved massive distribution ranges all over the world (Figure 4b; Meigen, 1830; Sturtevant, 1920).

In the following literature review, we will characterize each of these species with both field and laboratory experimental data. The following results section will discuss the geographic distribution and substrate use of each of the nine species in the *melanogaster* subgroup, starting from the generalist species and ending with the specialist species.

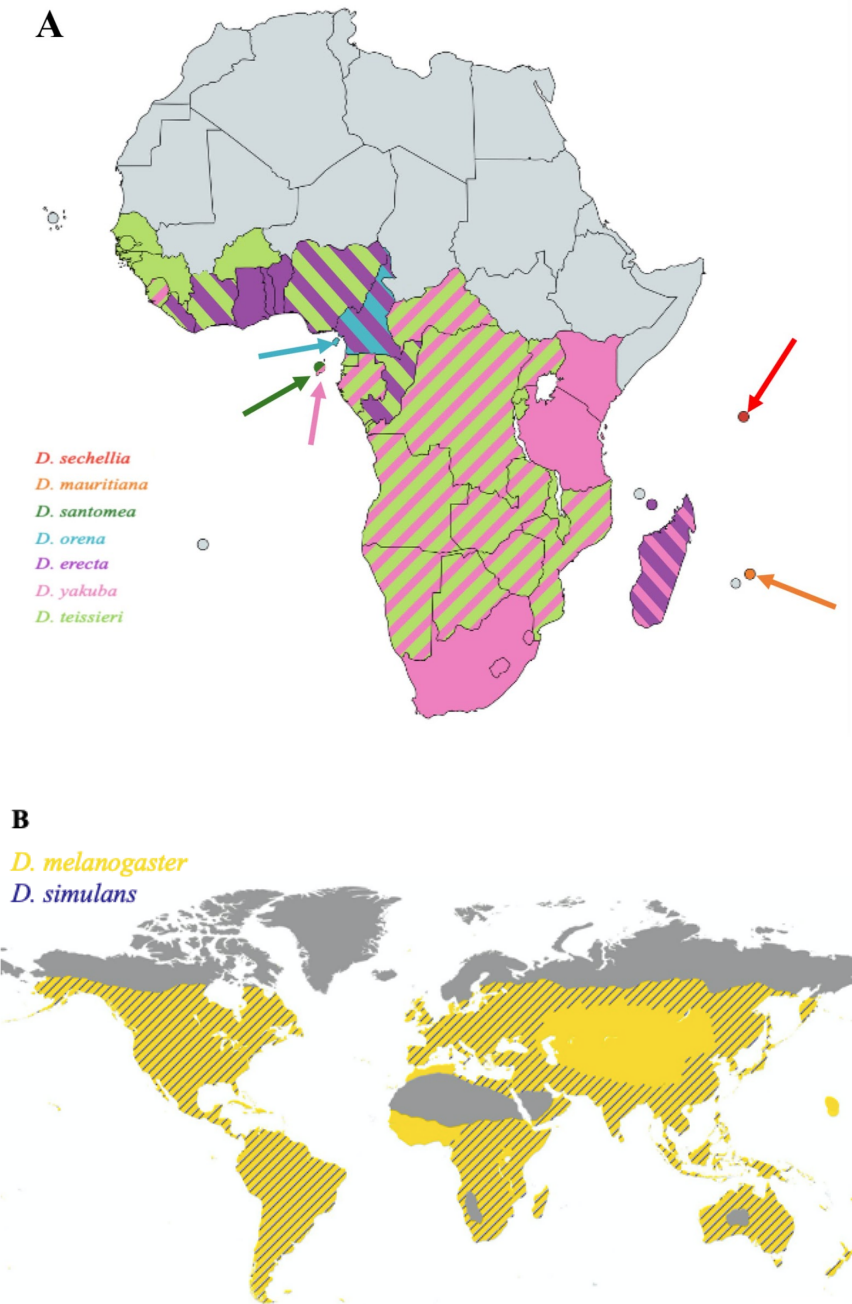


Figure 4. Distribution of species in the *melanogaster* subgroup.

(a) distribution of *melanogaster* subgroup species on the African mainland, except for *D. melanogaster* and *D. simulans*, whose distributions are shown in Figure 4b. All nine species of the *melanogaster* subgroup are either found on the African mainland or in the islands surrounding it. Arrows corresponding to each species point to islands where they are found. We see that the *melanogaster* subgroup has successfully dispersed throughout the Afrotropical region. (b) distribution of *D. melanogaster* and *D. simulans*. *D. melanogaster* and *D. simulans* are cosmopolitan species, distributed worldwide in addition to being found in the Afrotropical region. These species are not found at high latitudes or in extreme deserts. *D. simulans* distribution is adapted from David et al. 2007.

RESULTS

***Drosophila melanogaster* and *Drosophila simulans* as worldwide generalists**

D. melanogaster is found all over the world (Figure 4b; Meigen, 1830). Abundant in mainland continents and oceanic islands alike, this species can be identified in all places where climatic conditions, such as temperature and humidity, promote their survival (David et al., 2004).

The most significant catalyst in the worldwide spread of this species is human transportation. Because of its association with humans and their constructions, *D. melanogaster* has been able to travel to and colonize almost every continent with the exception of Antarctica. This advantage is further exponentiated by *D. melanogaster*'s ability to make substantial populations under various climates that may or may not be the same as their native climate (David et al., 2004). Therefore, this species can be considered a domestic species that is exceptionally differentiated into numerous populations (David & Capy, 1988). In fact, its commensalistic relationship with humans is what separates it from its closest relatives within the *melanogaster* subgroup (David et al., 2004; McKenzie & Parsons, 1972). However, while the association with humans plays a significant role in *D. melanogaster* distribution, this species still demonstrates adaptations to environments that are not related to human activity (David et al., 2007).

While the M-type commensal *D. melanogaster* is found worldwide, this species has an Afrotropical origin, like the other members of the *melanogaster* subgroup (Tsacas & Lachaise, 1974; Lachaise et al., 1988). Adaptation to this native area is demonstrated in the high degree of polymorphism in *D. melanogaster* Afrotropical populations compared to anywhere else in the world, where populations do not show such significant adaptations (David et al., 2007). Through genome sequencing, studies suggest that the origin of *D. melanogaster* was somewhere in Zambia or Zimbabwe. The genomes of the strains found in sub-Saharan Africa differ from those of cosmopolitan strains. The diversity of genomes in cosmopolitan strains are accordant with geographical expansion from the ancestral sub-Saharan Africa range (Pool et al., 2012). Between 10-15,000 years ago, the *D. melanogaster* migration out of Africa caused the evolution of two different lineages: the Zimbabwe line, remaining in the ancestral range of Southern Africa, and the M-type, which became widely distributed throughout the world (Baudry et al., 2004; Li & Stephan, 2006; Thornton & Andolfatto, 2006; Wu et al., 1995; Hollocher et al., 1997). Evidently, the exact location of origin is yet to be confirmed, but scientists do know that it was somewhere around the African mainland (Dweck et al., 2013).

D. melanogaster is a generalist species that tends to utilize all types of fruit. More specifically, this species uses sweet, fermenting fruit for feeding and oviposition (David et al., 2004; Dweck et al., 2013). During oviposition on fruits that have just begun to decompose, *D. melanogaster* females will deposit eggs into the stem cavity, while also laying additional eggs into the rotting abscess (Miller et al., 2011).

In general, there is a vast amount of potential substrates for this species, but *D. melanogaster* has exhibited a preference for fruits with citrus characteristics bearing the compound valencene (Dweck et al., 2013; Sharon-Asa et al., 2003). Additionally, *D. melanogaster* presents a high level of adaptation to ethanol, making it one of the most ethanol resistant species of *Drosophila*. This expands the number of potential hosts for this species even more, as *D. melanogaster* can exploit substrates with ethanol concentrations that are lethal to most others (Merçot et al., 1994). In the laboratory, researchers have identified other preferences of this species. For instance, some *D. melanogaster* strains show somewhat of a mysterious preference for *Morinda citrifolia* (Amlou et al., 1998). *M. citrifolia* is the preferred substrate of *D. sechellia*,

which will be discussed later in this paper, and is toxic to most other Drosophilids, due to the high concentrations of hexanoic and octanoic acid (Farine et al., 1996). Some laboratory strains of *D. melanogaster* collected from the Seychelles, include females that prefer to oviposit on this substrate, even if all of their embryos are killed by *M. citrifolia* toxins. The preference of Seychelles *D. melanogaster* females for *M. citrifolia* is most likely made possible by their high alcohol tolerance, but besides this, not much is known about this preference, especially since it seems to lower individual fitness (Amlou et al., 1998).

The ancestral line of *D. melanogaster*, known as the Zimbabwe line, shows some characteristics that diverge from other current populations of this species. Found in Africa, these ancestral *D. melanogaster* populations are forest dwelling, wild populations, which is significantly different from the common *D. melanogaster* found throughout the world as a human commensal. The Zimbabwe line *D. melanogaster* have a close association with and preference for *Sclerocarya birrea*, otherwise known as the marula fruit (Supplemental Figure 1a). Marula appears to be exclusively used by ancestral *D. melanogaster*. However, close examination reveals that *D. melanogaster* only tends to utilize marula during the fruiting season, suggesting that these populations are seasonal specialists for this fruit (Mansourian et al., 2018). Therefore, this association is classified as host partiality, but not complete host specialization.

During the off-season for marula fruit, forest dwelling Zimbabwe line *D. melanogaster* are opportunists, using alternative breeding substrates (Mansourian et al., 2018). For instance, some field experiments show evidence that wild *D. melanogaster* can utilize figs as an alternate host, highlighting their ability to use a wide variety of substrates and paying tribute to their generalist characteristics (Lachaise, 1974).

The association of ancestral *D. melanogaster* populations with marula may offer insights into the origin and evolution of *D. melanogaster*. First, this relationship may suggest that marula fruit may be the primary host for ancestral *D. melanogaster* populations. During its evolution into the contemporary species, *D. melanogaster* may have had a close association with specific fruits that have characteristics of citrus, one of them being marula fruit. Since citrus fruits are part of a genus that comes from Asia, ancestral *D. melanogaster* may have developed a relationship with marula fruit after its migration to the Afrotropical region, where species of the Asian genus citrus were no longer available. Furthermore, the preference of modern-day *D. melanogaster* for citrus fruits is likely explained by the ancestral association with marula fruit, which shows citrus characteristics (Mansourian et al., 2018).

The other worldwide generalist within the *melanogaster* subgroup is *D. simulans* (Supplemental Figure 1b). *D. simulans* is classified as a worldwide generalist (Figure 4b; Sturtevant, 1920). *D. simulans* has a considerable environmental variability and is often grouped with *D. melanogaster* because both of these species are seen all over the world and are sympatric in most areas (R'Kha et al., 1991; Capy & Gibert, 2004).

Because of its shared Afrotropical origin with the rest of the *melanogaster* subgroup, *D. simulans* is native to the eastern side of the world and is considered an invader to the western side (Lachaise et al., 1988). The *D. simulans* species as a whole is composed of three groups that have separate geographical distributions. The first of these groups is a Malagasy race native to Madagascar which has continued to persist in this area. The second group is an Indo-Pacific race which has a widespread distribution from the Seychelles Islands to New Caledonia and Hawaii. Lastly, there is a cosmopolitan race known for its extraordinary ability to colonize new habitats (Baba Aïssa & Solignac, 1984; Solignac & Monnerot, 1986). This is the *D. simulans* group that has a large overlap with *D. melanogaster*.

D. simulans is plentiful during the springtime, emerging from locations that lack sweet fruits (David et al., 2007). Among the available rotting, non-sweet fruits, this generalist species is

presented with a great diversity of resources (R’Kha et al., 1991). Adult *D. simulans* can be collected in open areas, such as orchards during the fruiting season, where their larvae are found in rotting, non-sweet fruits (David et al., 2007). *D. simulans* is active and still able to use rotting, non-sweet fruits during the remaining seasons of the year (David et al., 2007). Moreover, *D. simulans* is abundant in North Africa, Southern Spain, and Uruguay within habitats with mild temperatures and a diversity of vegetation (David et al., 2007). It especially succeeds in areas with high humidity, as is common for most of the members of the *melanogaster* subgroup (David et al., 2007). Lastly, *D. simulans* is able to proliferate well in the Mediterranean and subtropical areas (David et al., 2007). Compared to *D. melanogaster*, *D. simulans* has larger population sizes in temperate and semi tropical areas (David et al., 2004). The widespread distribution of *D. simulans* in seemingly random areas suggests that we have yet to realize the entirety of its expansive ecological niche (David et al., 2007).

As with most species in the *melanogaster* subgroup, *D. simulans* generalists show avoidance of octanoic and hexanoic acid, which is toxic to the fly and its offspring (Prieto-Godino et al., 2017; R’Kha et al., 1991). Thus, in the laboratory, we see that they have no preference for ripe *M. citrifolia*, and females show avoidance behavior when presented with this substrate for oviposition (Mansourian et al., 2018). However, when *M. citrifolia* is rotten, and therefore elicits a different array of nontoxic odorants, *D. simulans* can utilize this fruit as a substrate (David et al., 1989). This differential preference in substrate may play a part in how this species lives in sympatry with *D. sechellia* (Cariou et al., 1990).

Compared to *D. simulans*, *D. melanogaster* is found to be more plentiful in areas with lower temperatures and can be collected earlier in the year (Gravot, 2000; McKenzie & Parsons, 1974; Nielsen & Hoffmann, 1985). Additionally, because of its association with human beings, *D. melanogaster* is potentially able to take refuge in buildings or cellars during the winter time, enabling a higher chance of survival during these colder months, as *D. melanogaster* has also been observed to be more tolerant to fluctuations in temperature than *D. simulans* (Capy & Gibert, 2004). While both species are cosmopolitan generalists, *D. melanogaster* occupies a more extensive niche than *D. simulans*, highlighting *D. melanogaster*’s world colonization even more (David et al., 2007). Unlike *D. melanogaster*, *D. simulans* is very unlikely to enter a building and less often exists alongside human activities (Rouault & David, 1982; David & Tsacas, 1983). Additionally, even though *D. simulans* is successful in several climates, such as tropical and temperate climates, is not found in all of the places that offer suitable climates. For instance, *D. simulans* is not found in most of West Africa, most of continental Asia, and some islands in the French Caribbean, such as Martinique and Guadeloupe. It is also infrequently found in the Ivory Coast (David et al., 2007).

While it is generally considered that *D. simulans* and *D. melanogaster* have substantial overlap, both in geographical distribution and oviposition substrate choice, there are some behavioral differences that distinguish each species. For example, *D. simulans* has been characterized to be more sensitive to stresses, such as temperature extremes and high alcohol and CO₂ concentrations, than *D. melanogaster*, as *D. simulans* is less geographically differentiated than *D. melanogaster* (David et al., 2004; David & Bocquet, 1975; Hoffmann & Watson, 1993; Boulétreau et al., 1984). This could have a large impact on which habitats they actually choose to occupy versus which habitats are suitable for them. In addition, *D. simulans* cannot use artificial fermenting resources rich in alcohol (McKenzie & Parsons, 1974). This observation is explained by its low tolerance to alcohol and has been demonstrated in its inability to use grape residues from wineries (McKenzie & Parsons, 1974; Parsons, 1983). Its inability to use these substrates is made more clear when considering their avoidance behavior towards human constructions (Rouault &

David, 1982). Lastly, *D. simulans* do not have a preference for marula fruit as does wild African *D. melanogaster* (Mansourian et al., 2018).

***Drosophila mauritiana* as a generalist species**

D. mauritiana is an abundant species endemic to Mauritius, an island in the Indian Ocean (Figure 4a; Supplemental Figure 1c; David & Tsacas, 1975). This volcanic island is a relatively young island compared to others that surround it in this region, with historical records suggesting it has been around since at least 900 AD (Stanford Mauritian Archaeology, 2021). Mauritius provides a broad ecological niche for *D. mauritiana* (Lachaise et al., 1988). For instance, *D. mauritiana* is prominent at both sea level and high altitude areas such as montane forests. Additionally, *D. mauritiana* appears to have a wide distribution in both domestic and natural habitats. Within this island, it seems as though the population density of *D. mauritiana* fluctuates depending on the structure of the habitat or the altitude wherein it exists (David et al., 1989). However, *D. mauritiana* seems to be unaffected by the presence of buildings, even entering them, and appears even more domestic than *D. simulans*, its cosmopolitan sister species that also exists on the island (Lachaise et al., 1988).

As an exception to the endemism of *D. mauritiana* on Mauritius, *D. mauritiana* was also identified on the island of Rodrigues, another small volcanic island located east of Mauritius. Experts hypothesize that this population of *D. mauritiana* was recently brought to Rodrigues Island by man in 1985. As a result, *D. mauritiana* is considered endemic to Mauritius but also exists on the Rodrigues Islands (David et al., 1989).

The island of Mauritius, where *D. mauritiana* resides, offers an abundance of resources that this species can utilize. It is no surprise, then, that *D. mauritiana* can be classified as broad-niched and opportunistic and is considered a domestic species that coincides with humans. As a comparison to other insect species on the Mauritius that breed on fruit, *D. mauritiana* is one of the most widely distributed species on the island, not to mention one of the most plentiful. *D. mauritiana* is able to breed in a variety of sweet and fermenting resources. This species is remarkable in its ability to utilize unfamiliar resources, highlighting its capacity for colonization and opportunistic tendencies. We consider that, relative to later ones, early populations of *D. mauritiana* on the Mauritius were small and utilized only a small number of native fruits. However, in less than five centuries, the colonization of the island by humans and its demographic expansion allowed *D. mauritiana* to become a broad, widespread generalist (David et al., 1989).

The generalist ability of *D. mauritiana* is comparable to that of its cosmopolitan sibling, *D. simulans*; the breeding preference of these two species appears to be strongly diverged from that of their other sibling, *D. sechellia*, an extreme specialist on *Morinda citrifolia* (Lachaise & Tsacas, 1983; Tsacas & Baechli, 1981). However, scientists have been surprised with the finding that, much like *D. sechellia*, *D. mauritiana* has a preference for ovipositing on *M. citrifolia* when investigated with a two choice assay (Amlou et al., 1998). This preference also exists in the field on Mauritius island (Moreteau et al., 1994). This is unexpected because *D. mauritiana* embryos are unable to survive the toxicity of *M. citrifolia* (Amlou et al., 1998). However, we can resolve this apparent contradiction with the finding that *D. mauritiana* breeds in fully decayed *M. citrifolia*, while *D. sechellia* breeds on fresh *M. citrifolia* (Lachaise et al., 1988). Ripe *M. citrifolia* consists of hexanoic and octanoic acids in high concentrations, which are poisonous to most flies except for *D. sechellia* (Rhka et al., 1991; Jones, 1998; Amlou et al., 1997). The reason that *D. mauritiana* does not show a strong preference for ripe *M. citrifolia* is because of its toxicity. In nature, not many *D. mauritiana* individuals appear to utilize *M. citrifolia*, showing that it is certainly not the most significant breeding site of the species (David et al., 1989). Since *D. mauritiana* and *D.*

sechellia diverged less than one million years ago (Figure 3), the evidence of *D. mauritiana*'s preference towards *M. citrifolia* is potentially a phenotype from its common ancestor .

***Drosophila yakuba* as a generalist species**

D. yakuba is found in the ancestral range from eastern Guinea in the northwest region of Africa to Zimbabwe which is in the southeast region of Africa (Figure 4a; McEvey et al., 1988). It has an eastern overlap with *D. teissieri*, and prefers warm and dry conditions (Cooper et al., 2018). *D. yakuba* is however more widely distributed than *D. teissieri*, found widely across sub-Saharan tropical Africa (Lachaise et al., 2000). In fact, *D. yakuba* is the most abundant of the *Drosophila* species endemic to Africa and has an elevational range that extends from sea level to 3000 meters (Lachaise et al., 1988; David et al., 2007). On the African mainland, this species is highly domestic (David et al., 2007).

In addition, *D. yakuba* is found on the islands surrounding the African mainland, such as Madagascar, Zanzibar, and all of the islands in the Gulf of Guinea (Llopart et al., 2005). One of these islands is São Tomé, where *D. yakuba* lives below the elevation of 1450 meters and has an overlap zone with *D. santomea* between 1150 and 1450 meters, where the two species form hybrids (Figure 5; Lachaise et al., 2000). Furthermore, within the island of São Tomé, *D. yakuba* is found within areas occupied by humans (Cooper et al., 2018).

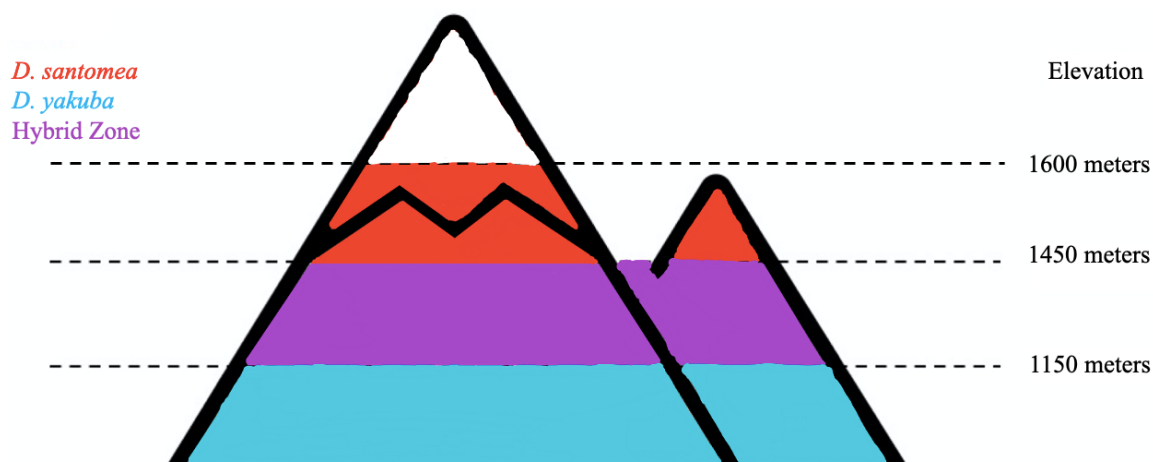


Figure 5. Elevation ranges of *D. santomea* and *D. yakuba* on São Tomé.

D. yakuba is found at elevations below 1450 meters. *D. santomea* is found at elevations between 1150 and 1600 meters. *D. yakuba* and *D. santomea* have an overlap zone between 1150 and 1450 meters, where they are able to form hybrids.

Within its range, *D. yakuba* lives chiefly in open areas, where it has adapted to live at altitudes ranging from 200-2,020 kilometers, but most commonly at the lower altitudes (Cooper et al., 2018). These open areas can include habitats in semi-arid areas, savannas, grasslands, woodlands, secondary forests, and semi-domestic habitats, such as plantations for coffee and cacao. More specifically, secondary forests make an appropriate location for *D. yakuba* because of the mosaic of habitats that secondary forests provide. These forests provide great biodiversity because they are rainforests whose vegetation has been altered, either naturally or unnaturally by human activity. This species is also found around the edges of the rainforests but is not found within them (Llopart et al., 2005).

D. yakuba has been observed to breed on the rotten fruits produced by over 28 plant species, both on the African mainland and the islands in the oceans surrounding it (Lachaise et al.,

1988). It is also a human commensal (David et al., 2007). *D. yakuba* can make use of double the amount of host plants for breeding sites than *D. melanogaster* can (Lachaise et al., 1988). *D. yakuba* has a remarkable ability to use several different hosts, providing a competitive advantage that undoubtedly increases its fitness.

In both field and laboratory experiments, a number of specific substrates have been shown to be attractive to *D. yakuba*. *D. yakuba* shows attraction to *Pandanus* syncarps (Supplemental Figure 1d; Lachaise & Tsacas, 1974). However, it was observed that this species utilizes *Pandanus* fruit only as a feeding resource and not for oviposition (Lachaise & Tsacas, 1974). Additionally, field experiments were able to identify large populations of *D. yakuba* on the syncarps of *Ficus mucuso* (Moraceae) in lowland environments above sea level (Lachaise et al., 1988; Llopart et al., 2005). It was also observed that *D. yakuba* shows preference for cacao fruits of *Theobroma cacao* (Malvaceae) and the fleshy fruits of *Landolphia hirsuta* (Apocynaceae; Lachaise, 1974). In the laboratory, experiments have shown that *D. yakuba* can oviposit effectively on jackfruit of *Artocarpus heterophyllus* and figs of *Ficus thonningii* (Llopart et al., 2005).

In addition to being able to utilize several host plants on the mainland, *D. yakuba* has a great ability to adapt to insular environments. Prior researchers hypothesized that this ability is related to its adaptation to open areas, including semi-arid habitats (Cariou et al., 2001). A special case of *D. yakuba* adaptation is found in populations residing on the island of Mayotte, located in the Indian Ocean. This population has been shown to be strictly adapted to utilization of *M. citrifolia*. This specialization is surprising considering the fact that *D. yakuba* is a generalist species (Burla, 1954). However, flies in this population do have significant olfactory attraction to *M. citrifolia*, and much like *D. sechellia*, this strain has remarkable tolerance to the toxicity of *M. citrifolia* volatiles. Seeing that neither mainland *D. yakuba* populations nor other *Drosophila* species can effectively utilize this plant, we can see that this is an extreme case of adaptation. To support this, it has been shown that the adaptation of Mayotte *D. yakuba* to *M. citrifolia* coincides with partial reproductive isolation. This species of *Drosophila* is found only within two small regions at the Bay of Soulou, where there is a great abundance of *M. citrifolia*. *D. yakuba* colonized this island about 29,000 years ago, during a period where *M. citrifolia* was an easily accessible, year-round resource. Therefore, *D. yakuba* adapted to *M. citrifolia* due to its abundance on Mayotte. However, compared to *D. sechellia*, experimentally less *D. yakuba* flies from Mayotte survive on *M. citrifolia*, perhaps showing us that while some populations are adapted to this substrate, *M. citrifolia* is not as suitable a substrate for *D. yakuba* as it is for *D. sechellia*. Surprisingly, a small percentage of mainland *D. yakuba* showed greater attraction to *M. citrifolia* compared to the rest of the species in the *Drosophila melanogaster* subgroup, perhaps as a result of some Mayotte strains being transported back to the mainland (Yassin et al., 2016).

***Drosophila teissieri* as a generalist species with potential to become a specialist**

D. teissieri is widely distributed throughout mainland Africa (Figure 4a; McEvey et al., 1988). Endemic to the Afrotropical region, this species has a similar distribution to *D. yakuba* (Lachaise et al., 1988). However, the distribution of *D. teissieri* has a more western distribution than that of *D. yakuba*, which has a more eastern distribution and prefers warmer and drier conditions than *D. teissieri* (Lachaise et al., 1988; Cooper et al., 2018). Additionally, these two species coexist on the island of Bioko, where they are geographically separated by means of elevation (Cooper et al., 2018). Within Bioko, *D. teissieri* is less abundant than *D. yakuba*, and it has a more restricted geographic distribution (Lachaise et al., 1988).

D. teissieri is a chiefly forest dwelling species, presumably because it was able to successfully exploit the vegetation within the forests of the savanna. Most of the observations of

D. teissieri's association with plants do not allow us to clearly define whether it is a generalist or a specialist species. Some observations suggest that *D. teissieri* only utilizes plant types that are used by either *D. yakuba* or *D. melanogaster* (Lachaise et al., 1988). Since the behavior of both of these species allow them to be adequately classified as generalists, we can hypothesize that *D. teissieri* is also a generalist. However, as mentioned previously, *D. teissieri* and *D. yakuba* have adapted to different locations and elevations. *D. teissieri* is therefore likely able to exploit different plant substrates that may be out of *D. yakuba*'s range.

For instance, it has been proposed that *D. teissieri* has a close association with plants in the genus *Parinari* (Supplemental Table 1; Supplemental Figure 1e; Comeault et al., 2017). This genus coexists with *D. teissieri* in forest areas across sub-Saharan Africa (Kew Science, 2021). This close association could suggest some degree of specialization on *Parinari* in certain areas. More specifically, it has been put forward that *D. teissieri* is a seasonal specialist on the *Parinari* fruit and utilizes alternative substrates during the remainder of the year (Rio et al., 1983; Comeault et al., 2017; David et al., 2007). *D. teissieri*'s specialization on *Parinari* could explain the restriction of *D. teissieri*'s range to forest habitats (Lachaise et al., 1988). We might conclude that perhaps *D. teissieri* can act as a generalist in certain areas where it coexists with *D. yakuba* and *D. melanogaster* but acts as a specialist in areas where it is isolated from these other species.

Drosophila sechellia* as a specialist on *Morinda citrifolia

D. sechellia is exclusively found in the Seychelles archipelago (Figure 4a, Lachaise et al., 1988). The Seychelles islands are located in the Indian Ocean, on the eastern side of the coast of Africa. The islands found within this archipelago are made of granite, which suggests their limited ability to support a wide variety of vegetation (Stodart, 1984). Since it is exclusively found on these islands, *D. sechellia* is considered an endemic species (Lachaise et al., 1988).

Among the relatively limited variety of flora found in the Seychelles, *M. citrifolia* has a significant presence year round (Burns et al., 2020). Commonly known as noni fruit, *M. citrifolia* is found in the areas around the Indian and South Pacific Oceans and is a reliable resource for a variety of organisms in the area (David et al., 1989).

D. sechellia is a specialist that utilizes ripe *M. citrifolia* as a host plant for both feeding and oviposition (Supplemental Table 1; Supplemental Figure 1f; Lachaise & Tsacas, 1983; Tsacas & Baechli, 1981). This relationship developed gradually after *D. sechellia* diverged from the generalist species *D. melanogaster* and *D. simulans* (Figure 3). After its divergence from *D. melanogaster* 3 million years ago and from *D. simulans* 0.1 to 0.24 million years ago, *D. sechellia* has become an extreme specialist that has evolved to use this novel resource, presumably as a way to combat interspecific competition with other *Drosophila* species in the area (Lachaise & Silvain, 2004; Garrigan, 2012; Schrider et al., 2018).

The major driving force of *D. sechellia*'s specialization on ripe *M. citrifolia* was its development of a tolerance to the fruit (R'Kha et al., 1991; Legal et al., 1992). Ripe *M. citrifolia* consists of hexanoic and octanoic acid in high concentrations. These compounds are toxic to most flies, including the cosmopolitan generalist *D. melanogaster* (Farine et al., 1996). Several physiological adaptations are responsible for this behavior, including but not limited to loss of olfactory and gustatory avoidance behaviors through gene interactions and differential gene expression (Matsuo et al., 2007; Whiteman & Pierce, 2008). Changes in circuitry also underlie this behavior (Supplemental Table 1). Specific *D. sechellia* OSNs are more highly tuned to hexanoates, such as methyl hexanoate and ethyl hexanoate. Finer tuning to hexanoates occurs by increasing the number of ab3 OSNs and a greater expression of OR22a. Since methyl hexanoate and ethyl hexanoate are the ligands of OR22a, an increase in these receptors allows

D. sechellia to have an increased sensitivity to *M. citrifolia* volatiles. Additionally, the volume of the DM2 glomerulus in *D. sechellia* is approximately 3 times larger than the DM2 glomerulus in *D. melanogaster*. The increase in glomerular size is associated with an increased number of OSNs expressing OR22a and allowing for an increased sensitivity to the volatile (Dekker et al., 2006). Overall, the significant changes in *D. sechellia* olfactory circuitry highlight its extreme specialization to *M. citrifolia*.

In addition to its unique ability to utilize this resource, *D. sechellia* shows clear behavioral responses to *M. citrifolia* (Rhka et al., 1991; Jones, 1998; Amlou et al., 1997). For instance, *M. citrifolia* can stimulate oogenesis in females, plays a part in mate interactions, and is preferred in oviposition (R'Kha et al., 1991). Meanwhile, the eggs of other *Drosophila* species are unable to hatch properly on *M. citrifolia* substrate (Amlou et al., 1998).

D. sechellia can survive on typical *Drosophila* medium under laboratory conditions if that food is supplemented with juice from *M. citrifolia* or at the very least, levodopa, the compound that is vital for *D. sechellia*'s survival on this substrate. However, they are not able to thrive on plain medium, and their reproductive success has been found to decrease significantly when provided with this substrate.

As mentioned above, the preference for ripe *M. citrifolia* provides an important advantage in interspecies competition (R'Kha et al., 1991). Relative to other species, *D. sechellia* has a smaller number of ovarioles and produces larger eggs, which results in low fecundity, the number of offspring an organism produces over its lifetime (Markow et al., 2009; Hodin & Riddiford, 2000; R'Kha et al., 1997). These limitations highlight the need for *D. sechellia* to quickly recognize and effectively utilize its host plant while it is still ripe. Because of this, adult *D. sechellia* have evolved to be able to detect and navigate over long distances to various *M. citrifolia* trees, even if they are widely dispersed (R'Kha et al., 1991). When *M. citrifolia* fruit is rotten, it is no longer toxic to other species and thus creates competition for *D. sechellia*. This is seen in areas where *D. sechellia* lives in competition with species such as *D. simulans* and *Drosophila malerkotiliana*, a member of the *Drosophila ananassae* subgroup (Lachaise & Silvain, 2004; Louis & David, 1986; R'Kha et al., 1991). Ergo, the specialization on ripe *M. citrifolia* fruit gives *D. sechellia* an opportunity to colonize this resource earlier and make up for its reproductive limitations (Amlou et al., 1998).

The specialization on *M. citrifolia* is influential in *D. sechellia* fitness, not only because it allows early colonization but also because of the hormones found in the fruit that increase the fitness of *D. sechellia* with respect to its eggs. Within *D. sechellia*, the molecular makeup of the dopamine regulatory protein, Catsup, has a variety of polymorphisms that result in infertility. These differences in molecular structure cause arrests in oogenesis, the production of eggs. When the female *D. sechellia* utilizes *M. citrifolia*, it is able to obtain L-DOPA which is a precursor of dopamine. The presence of L-DOPA allows *D. sechellia* to overcome ineffective dopamine metabolism, therefore allowing oogenesis to continue and even resulting in larger eggs. Overall, the effects of L-DOPA compensate for the reproductive obstacles that *D. sechellia* faces. These advantages allow us to conclude that L-DOPA is beneficial for *D. sechellia* fitness, which is why the species has evolved specialization for *M. citrifolia* (Lavista-Llanos et al., 2014).

Current knowledge of *Drosophila santomea* substrate use

D. santomea is another endemic species exclusively found in São Tomé (Figure 4a; Lachaise et al., 2000). This volcanic island is found in the Gulf of Guinea off the coast of Gabon. Across the island, *D. santomea* is found only in montane rain and mist forests, specifically in the elevational range of 1153-1600 meters (Coyne et al., 2002; Llopart et al., 2005). The area occupied

by *D. santomea* is also characterized by overlapping ranges and hybrid zones with *D. yakuba*, where *D. santomea* lives at elevations above 1150 meters and *D. yakuba* at elevations below 1450 meters (Figure 5, Coyne et al., 2002).

D. santomea is thought to be linked to *Ficus chlamydocarpa*, a fig tree endemic to the Cameroon Volcanic Line (Supplemental Table 1; Supplemental Figure 1g; Cariou et al., 2001). More specifically, the *F. chlamydocarpa Fernandesiana* subspecies is found in the 1200 to 1750 meter elevational range of *D. santomea* (Llopart et al., 2005). To date, *D. santomea* has been observed only to emerge from the fallen syconia of this fig (Cariou et al., 2001). While the elevational range of *D. santomea* presents a variety of feeding and breeding substrates appropriate for *Drosophila*, including *Artocarpus heterophyllus* (jackfruit), *Craterispermum montanum* plants, and other *Ficus* species, this species appears not to utilize these resources in the field (Excell 1944; White, 1986). *D. santomea* breeds predominantly on fig patches, and while *D. santomea* is currently considered a generalist species, there appears to be substantial evidence that in nature this species breeds solely on *F. chlamydocarpa*, since this species has not been found to emerge from any other substrates in nature. Flies observed breeding on *F. chlamydocarpa* in nature support the specialization hypothesis, whereas *D. santomea* raised in the laboratory can adequately utilize standard cornmeal medium (Cariou et al., 2001). Further work must be done in order to determine whether *D. santomea* is a specialist or a generalist species and to determine why *D. santomea* avoids the other substrates available on São Tomé.

Current knowledge of *Drosophila orena* substrate use

D. orena is a mainland endemic species found in western equatorial Africa (Figure 4a). It shares this region with about 40 other Drosophilid species, but its exact habitat is unresolved (Lachaise et al., 1988). For several decades, most knowledge of this species came from the collection of *D. orena* on Mount Lefo in western Cameroon in 1975 (Linz et al., 2013). In 2017, Comeault et al. obtained a population of *D. orena* on the island of Bioko, West Africa. However, the overall lack of field data from only two collections makes *D. orena* the least known species of the *melanogaster* subgroup (Comeault et al., 2017).

While its exact habitat is unknown, the two collections of *D. orena* provide clues about oviposition and feeding preferences. After the 2017 Bioko collection of *D. orena*, researchers compared the proportion of *D. orena* flies relative to other *melanogaster* subgroup species at various elevations and showed that *D. orena* is the chief species found at high elevations (Comeault et al., 2017). At high elevations, this species is found in the submontane forest, which is rich with myrtaceous and epiphytic plants (Tsacas & David, 1978; Tsacas et al., 1981). Both the Cameroon and Bioko populations of *D. orena* are likely seasonal specialists, utilizing the waterberry from *Syzygium staudtii*, a plant in the family Myrtaceae (Supplemental Table 1; Supplemental Figure 1h; Lachaise et al., 1988). This hypothesis was put forth during the initial collection in Cameroon after observing *D. orena* near waterberry plants, and later experiments with the Bioko population supported this, with *D. orena* showing a preference for *S. staudtii* over other possible hosts in the area (Comeault et al., 2017). However, we have limited knowledge about this species, so it can potentially utilize other substrates, and perhaps different substrates in different seasons.

Investigation of the specialization of *D. orena* on waterberry reveals an association with seven olfactory receptors expressing odorant binding proteins: *Obp99a*, *Obp99c*, *Obp99d*, *Obp57d*, *Obp57e*, *Obp93a*, and *Obp83a*. In its olfactory system, *D. orena* ORs show an enhanced ability to undergo positive selection on odorant binding proteins and chemosensory proteins for the waterberry preference. This preference appears to be advantageous for *D. orena*'s fitness as its behavioral bias towards waterberry also encourages a female's inclination to lay eggs (Comeault

et al., 2017). This encompasses our current knowledge of *D. orena*'s geographical distribution and substrate use. Future work is necessary to discover if *D. orena*'s waterberry preference is a result of habitat adaptation and whether this adaptation is a strict specialization .

Seasonal Host Specialization of *Drosophila erecta*

D. erecta is a mainland species found in the forests of west-central Africa (Figure 4a, Lachaise & Tsacas, 1974). *D. erecta* is abundant in areas such as the Ivory Coast, Nigeria, Cameroon, and the Republic of Congo (Lachaise & Tsacas, 1974). It can be found in the islands surrounding the mainland, including Madagascar (Lachaise et al., 1988). Additionally, *D. erecta* has been identified at Bafut, N'Guemba on Mt. Lefo in the Cameroon Volcanic line, at an altitude of 2100 meters, a rare case considering the usual distribution of *D. erecta* at low altitudes (Lachaise et al., 1988). Within its distribution, *D. erecta* is found in the gallery forests, a type of forest that grows along a watercourse. In areas devoid of much tree vegetation, gallery forests increase the biodiversity of the ecosystem by providing a moist, water-rich environment in which species needing moisture, such as *D. erecta*, can exist.

In the gallery forests of west-central Africa, the genus *Pandanus*, otherwise known as screwpine, also exists widely (Lachaise & Tsacas, 1974). There are 20 to 24 species of *Pandanus* found in continental Africa (Stone, 1973). Ripe, mature *Pandanus* syncarps have been observed to be a suitable substrate for *D. erecta* (Supplemental Table 1; Supplemental Figure 1i). Populations of *D. erecta* have been shown to thrive on at least three *Pandanus* species, and *D. erecta* can hypothetically utilize most *Pandanus* trees that produce large, fleshy syncarps (Couturier et al., 1986; Lachaise & Tsacas 1983; Lachaise et al., 1988). Additionally, these monocots are able to thrive best in lowland swamp areas (Cariou et al., 2001). The abundance of *Pandanus* in lowland moist areas provides insight into why *D. erecta* may have evolved to be swamp and streamside dwellers (Lachaise et al., 1988).

The relationship between *D. erecta* and its host *Pandanus* is not as straightforward as it initially seems. *Pandanus* fruits are available for as short as two months, lasting several months at most (Rio et al., 1983; David et al., 2007). This implies that the specialization of *D. erecta* on *Pandanus* is limited to the annual fruiting season, making the species a seasonal specialist (Rio et al., 1983). Thus, a generation of *D. erecta* that is alive during the fruiting season is solely dependent on *Pandanus* for feeding and oviposition; they will show absolute exclusion of other host plants, even if they exist with greater availability than *Pandanus*. Furthermore, generations that coincide with mature *Pandanus* fruit have large population sizes, perhaps due to the fact that they can congregate in lowland swamp areas with *Pandanus* trees, accelerating the process of reproduction (Lachaise et al., 1988).

D. erecta populations that coincide with the *Pandanus* fruiting season are more responsive to the syncarp's volatiles than are other generalist species of the *melanogaster* subgroup (Lachaise et al., 1988). Specifically, the *Pandanus* volatile 3-methyl-2-butenyl acetate (3M2BA) elicits strong responses among ab3 OSNs housed in the ab3a sensilla in *D. erecta* when compared to *D. melanogaster* (Supplemental Table 1). These OSNs activate three glomeruli, DM2, DM5, and DM6, with DM2 being the most strongly activated during functional calcium imaging experiments. In *D. erecta*, the DM2 glomerulus has a larger relative volume than the homologous *D. melanogaster* glomerulus. The same is true for DM4 and VM5d, which are also activated by 3M2BA in *D. erecta* (Linz et al., 2013). These findings parallel the increased size of the *D. sechellia* DM2 glomerulus activated by *M. citrifolia* volatiles (Dekker et al., 2006). While 3M2BA is sufficient to initiate oviposition behaviors in *D. erecta*, it is likely that a mixture of *Pandanus*

volatiles leads gravid females to *Pandanus* fruits and prompts them to lay their eggs on the substrate (Linz et al., 2013).

In contrast to the more specialized generations, *D. erecta* that live outside of the *Pandanus* fruiting season are shown to be more opportunistic and generalistic. These populations must rely on alternate substrates as food sources and breeding sites (Lachaise et al., 1988). Figs have been proposed as a preferred substrate for these populations, although they have not been strictly defined as such (Rio et al., 1983). Because they must seek out alternatives, these populations of *D. erecta* are more widely distributed. Lower population densities and challenges to locating suitable substrates lead to slower mating and lower rates of survival among off-season generations, which may explain the differences in population size between specialized and non-specialized *D. erecta* (Lachaise et al., 1988).

DISCUSSION

Conclusion

Throughout this literature review, we have examined the importance of olfaction in oviposition decisions that are vital for the fitness of animal species. We also focused on the current knowledge of the history, relationships, geographic distributions, and substrate preferences of each of the nine species in the *Drosophila melanogaster* subgroup. From our findings, we revealed the plasticity of ecological adaptations such that adaptations can only provide a selective advantage if the conditions are favorable; the organism must find ways to survive when the conditions are not.

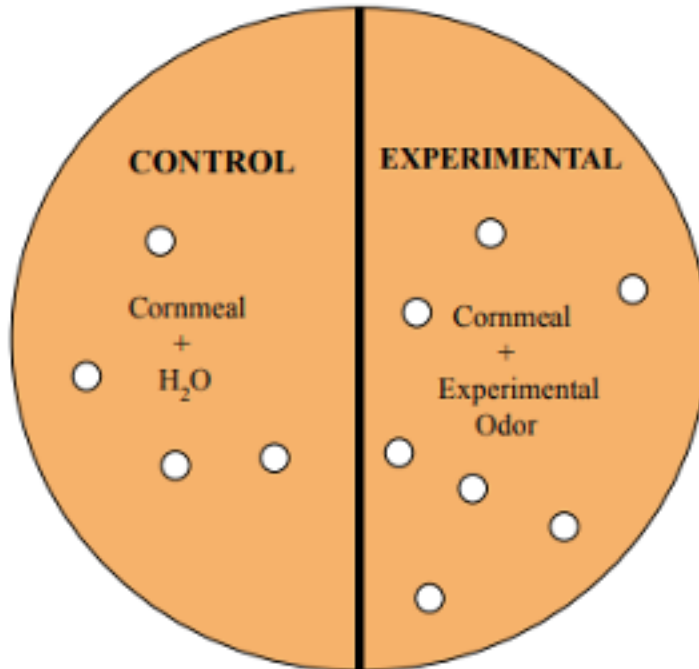
We concluded that *D. melanogaster* and *D. simulans* are worldwide generalist species, *D. mauritania* is a geographically restricted generalist species found only on Mauritius, and *D. yakuba* is a geographically restricted generalist species found on the African mainland and the islands surrounding it. We also discussed *D. teissieri* and its capacity to act as a seasonal specialist on *Parinari* fruit. We found that *D. sechellia*, found in the Seychelles archipelago, is an extreme specialist on *M. citrifolia*. We outlined the current knowledge about the substrate preferences of *D. santomea* and *D. orena*, highlighting their potential to be classified as specialist species. Lastly, we found that *D. erecta* is a seasonal specialist on *Pandanus* syncarps during the fruiting season and that it acts as a generalist for the remainder of the year. Overall, generalist or specialist behavior towards particular substrates allow us to make inferences about the ecological niche of each species and gives insight into how they were able to so efficiently adapt within their respective distributions.

Future Directions

From the data discussed in this literature review, we observe the different substrate preferences of these species in nature. Moving forward, we intend to quantify these preferences in the laboratory using oviposition assays (Figure 6). As previously discussed, we found that while *D. yakuba* is classified as a generalist species, certain populations have shown preference towards fallen syconia of *Ficus* plants. Furthermore, populations of *D. yakuba* on the island of Mayotte have shown a strict attraction to *Morinda citrifolia*. Observations such as these prompt us to investigate whether this species should appropriately be labeled as generalist. With oviposition assays, we can begin to explore the seemingly contradictory classification of *D. yakuba*, examine the classification of *D. santomea* and *D. orena*, and provide further support for the classifications of the remaining *melanogaster* subgroup species.

An ovipositional index will allow us to compare the ovipositional preference of mated females for a control substrate versus a specific odor. During a robust oviposition assay (as is outlined in Figure 6a), mated females will lay eggs for 24 hours, after which the results will be quantified using the ovipositional index calculation (Figure 6b).

To examine the behaviors of both the generalist and more specialist strains of *D. yakuba*, we will compare the oviposition tendencies of both strains using the described assay. We will then determine whether these strains exhibit the same preferences and, if not, which conditions prompt them to showcase different behaviors.

A**B**

$$\text{Ovipositional Index} = \frac{\text{number of eggs on experimental food} - \text{number of eggs on control food}}{\text{total number of eggs}}$$

Figure 6. The two choice oviposition assay

(a) Schematic of the two choice oviposition assay. One side is the control, with cornmeal and H₂O. The other side is the experimental, with cornmeal supplemented experimental odor. If the experimental odor is a preferred substrate, we expect more eggs (white circles) to be laid in the experimental food relative to the control. **(b)** Ovipositional index calculation. A higher ovipositional index implies a higher oviposition preference for the experimental food than the control food.

While laboratory experiments will help us identify the explanations for field evidence, we must be aware that laboratory conditions present a potential confounding variable in our data. The results section of this paper has provided concrete evidence about the significant role of ecology in the niches and substrate preferences of these flies. However, the term ecology is more broadly defined as “the study of the relationships between living organisms, including humans, and their physical environment” (The Ecological Society of America, 2021). Therefore, if we want to obtain accurate data from behavioral experiments in the laboratory, we must also be able to replicate the organism’s natural habitat. This will include factors such as: how we take care of each species in the lab, how interactions with handlers will impact their behavior, and how an organism reared in the laboratory might differ from ones found in the field. In the context of *Drosophila*, laboratory-reared flies exist in very different conditions than they do in the wild, so a better understanding of the natural history of these species will allow us to better replicate those conditions in a laboratory

environment. These steps ought to improve the reliability of behavioral data, especially with respect to species comparative studies.

First, we must be aware of how well we are taking care of each species in the laboratory. For instance, in the Caron laboratory, we observe that our strain of *D. yakuba* does not thrive on standard cornmeal food as well as other *Drosophila* species we maintain. With a greater understanding of each species' ecology, we can adjust dietary, temperature, humidity, space, and lighting conditions to better match each species' needs. By providing optimal conditions within the laboratory, we might be able to better imitate the natural habitats of each species, and in turn, obtain data that is more representative of their natural tendencies.

Next, we can determine how interactions with a human handler can affect the behavior of laboratory animals. Social interactions often differ based on conditions, and we have seen that these different social interactions can prompt changes in behavior of the organisms that we are studying. For instance, mice will behave differently depending on how they are picked up by the handler. Mice that are picked up by the tail, a stressful interaction for the mice, are less likely to perform conclusive behaviors during laboratory experiments, whereas mice that are handled in less aversive ways are able to provide more tangible data for behavior experiments (Gouveia & Hurst, 2017). While this impact on behavior has been studied in mice, there has been less consideration of this effect in *Drosophila* work. Specifically, in laboratory conditions, humans provide a novel social interaction for *Drosophila*. Save for human commensal species, such as *D. melanogaster*, we can assume that the natural ecology of these *Drosophila* do not involve regular interactions with human beings. We can then observe the manner in which we handle the *Drosophila* species in the laboratory and ask: what kind of influence does this have on the experiments that we are performing? If our interactions with the *Drosophila* species are affecting the data that we collect, we can make changes in our protocols in order to more accurately mimic natural conditions and improve our data collection as a whole.

Lastly, we can examine the benefit of collecting natural populations for our behavior experiments. A recent study investigating a population of *D. melanogaster* reared in the dark for 60 years showed that trade-offs in sensory modalities might affect which sense an organism invests more resources over long periods of time. These so-called "dark flies," while able to survive in both dark and light environments, exhibited a reduced investment in olfaction as indicated by significantly smaller olfactory structures. In turn, this reduced investment in olfaction presented a higher potential energy cost for dark fly fitness when placed back into light saturated environments (Ozer & Carle, 2020). This study highlights the importance of considering how long term laboratory environments have affected not only the behavior of *Drosophila* species, but also their morphology and genetic information. If we are to improve the accuracy of our behavior experiments, it would potentially be beneficial to collect and use natural populations of *Drosophila*.

Overall, the field of ecology-based *Drosophila* species studies has the potential to rapidly advance if findings can be grounded in the natural environments of our laboratory subjects. Therefore, it is vital that we take into account observations about the respective niches of *Drosophila* species and use them to more accurately perform our experiments. This literature review, along with other ecology based publications, demonstrates the movement towards an increased emphasis on creating more natural but still controlled conditions within laboratories, ultimately allowing us to refine our understanding of the behavior of *Drosophila* species.

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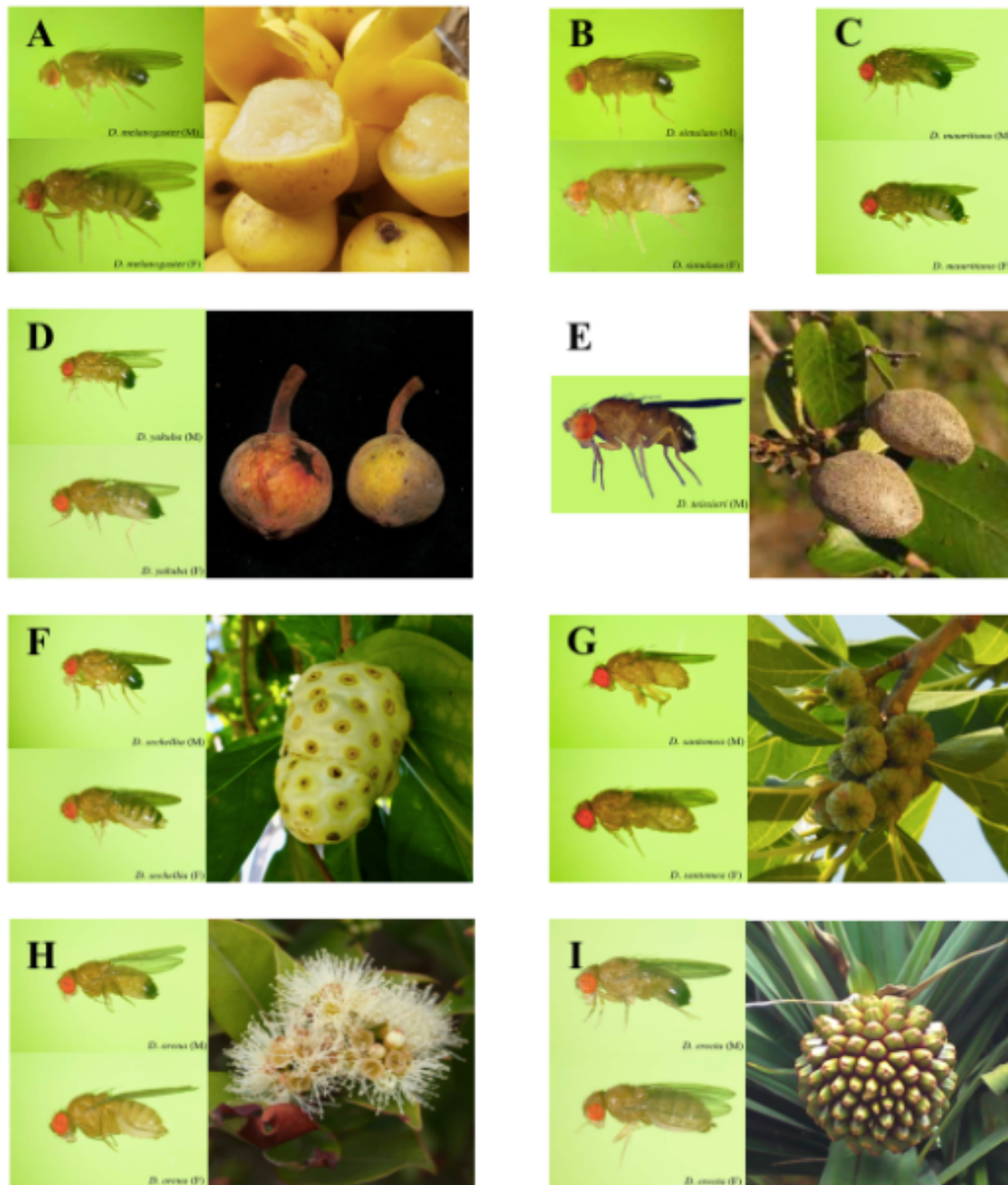
SUPPLEMENTAL MATERIALS

Species	Host Fruit	Volatiles	Odorant Receptor Responsible	Glomerulus Activated
<i>D. sechellia</i>	<i>Morinda citrifolia</i>	Hexanoic and octanoic acid	OR22a	DM2
<i>D. erecta</i> (seasonal)	<i>Pandanus</i>	3M2BA	OR22a, OR59c, OR85a, OR67a	DM2, DM4, DM5, DM6, VM5d
<i>D. teissieri</i> *	<i>Parinari</i>	Ethyl butyrate	OR42a OR43b	VM7 VM2
<i>D. santomea</i> *	<i>Ficus chlamydocarpa</i>	furfural*	OR10a OR67a	DL1 DM6
<i>D. orena</i> *	<i>Syzygium staudtii</i>	alpha-pinene*	OR98a	VM5v

*Hypothesis

Supplemental Table 1. Circuitry underlying host plant specialization of specialist species.

This table outlines specialist species (and hypothesized specialist species), host fruits, volatiles (and hypothesized volatiles) that accompany host fruits, odorant receptors responsible for the specialization, and the glomeruli activated by the OSNs that express these odorant receptors. *D. sechellia* is a specialist on *M. citrifolia*, containing hexanoic and octanoic acid volatiles, its specialization is mediated by OR22a, and OSNs activate the DM2 glomerulus. *D. erecta* is a specialist on *Pandanus*, containing 3-methyl-2-butenyl acetate (3M2BA), mediated by OR22a, and glomeruli activated are DM2, DM4, DM5, DM6, and VM5d. *D. teissieri* is hypothesized to be a seasonal specialist on *Parinari*, of which ethyl butyrate is a primary volatile (Shoko et al., 2014). It can therefore be hypothesized that OR42a and OR43b, and the associated VM7 and VM2 may play a role in the association between *D. teissieri* and *Parinari*. *D. santomea* is hypothesized to be a specialist on *Ficus chlamydocarpa*. *D. orena* is hypothesized to be a seasonal specialist on *Syzygium staudtii*. *Ficus chlamydocarpa* and *Syzygium staudtii* primary volatile have yet to be identified, but based on primary volatiles from other *Ficus* and *Syzygium* species (Li et al., 2012; Quijano-Célis et al., 2013), we can begin to hypothesize possible important volatiles and their associated receptors and glomeruli.



Supplemental Figure 1. Species of the *melanogaster* subgroup and their associated substrates.

(a) Male and female *D. melanogaster* and marula fruit, the seasonally preferred substrate for the ancestral line. Non-ancestral *D. melanogaster* is a generalist. (b) Male and female *D. simulans*. (c) Male and female *D. mauritiana*. (d) Male and female *D. yakuba* and *Ficus mucoso*, the substrate for which lowland populations show a preference but not a strict association. (e) Male *D. teisseiri* and *Parinari campestris*, the preferred substrate during the fruiting season. Outside of the fruiting season, *D. teissieri* is a generalist. (f) Male and female *D. sechellia* and its host plant *Morinda citrifolia*. (g) Male and female *D. santomea* and *Ficus*, a potentially preferred substrate. (h) Male and female *D. orena* and *Syzygium* waterberry, a potentially preferred substrate. (i) Male and female *D. erecta* and *Pandanus*, the seasonally preferred substrate. Outside of the *Pandanus* fruiting season, *D. erecta* is a generalist.

Photo credit:

All fly images taken from Ehime-Fly (https://kyotofly.kit.jp/cgi-bin/ehime/photo_data_list.cgi) with the exception of *D. teissieri*, which was adapted from the Drosophila Stock Center Mexico (<http://bancodemoscas.fcencias.unam.mx/Drosophilidae.htm>). Fruit images were taken from the following:

- (a) Marula fruit (<https://www.demandafrica.com/food/from-marula-to-amarula/>)
- (d) *Ficus mucoso* (<https://congotrees.rbge.org.uk/species/details/ficus-mucoso>)
- (e) *Parinari campestris* (<http://tropical.theferns.info/viewtropical.php?id=Parinari+campestris>)
- (f) *Morinda citrifolia* (<https://ourtropicalsoil.com/2019/01/22/noni/>)
- (g) *Ficus* (<https://www.inaturalist.org/taxa/50999-Ficus>)
- (h) *Syzygium* (http://www.africanplants.senckenberg.de/root/index.php?page_id=78&id=2276#)
- (i) *Pandanus* (<https://commons.wikimedia.org/wiki/File:Pandanusutilisfruit.JPG>)