7-20-2017

The Evolution of Fear Ecology: A Fruit Fly (Drosophila melanogaster) Perspective

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A Thesis Submitted to The Graduate School at the University of Missouri-St. Louis
in partial fulfillment of the requirements for the degree
Master of Science in Biology

August
2017

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Dedication

First, I would like to dedicate my thesis to my parents, who let me choose my own path and supported my interests, even if it meant having too many pets in the house. Next, I would like to dedicate this thesis to the menagerie pets I have owned over the years, without whom I may never have developed a love for animals. This thesis is especially for my first cat, Puss-in-Boots, who taught me that humans are not the only intelligent creatures on Earth, and for my Budgies, Sansuke, Drift and Sasami, who kept me sane through those long months of graduate school.

Acknowledgements

I would like to thank several members of the Dunlap Lab for their assistance with my projects: Michael Austin, Magnus Creed, Hannah Franko, Pablo Iturralde, Sukvir Kaur, Naomi Raible, Lynnsey Street, and Adam Thompson. I would like to also thank University of Missouri Research Board for providing funding for the generalized use of preference project described in Chapter 2. I would also like to thank the Whitney R. Harris World Ecology Center for their funding, which enabled me to build the apparatus described in Chapter 3.

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Abstract

Several mechanisms underlie how evolutionary lineages respond to predation pressures or predation risk. Further mechanisms link evolutionary predation responses to how animals forage, or find mates. However, gaps remain in our understanding about how predation and foraging interact in an evolutionary context.

In my first chapter, I elaborate on how predation and foraging relate in to one another in ecological, evolutionary and behavioral contexts. I start out with an overview of fear ecology. Then, I outline how trade-offs influence the evolution of morphological, chemical and behavioral responses to predation. I further elaborate on how these trade-offs influence reproduction. Finally, I go into detail on how the fruit fly, *Drosophila melanogaster*, has been used to study predation and foraging, and how it can also be used to study the gaps in our knowledge of the mechanisms behind evolutionary responses to predation in a foraging context.

In the second chapter, I delve into innate bias and how it can aid a forager when choosing between patches. Innate bias can be influenced by several factors such as spatial scale and the decoy effect. Additionally, innate bias sometimes cannot be generalized across contexts. I do this in the context of a large scale patch study with experimentally evolved lines of *Drosophila melanogaster.* These lines have been selected for an innate preference for laying eggs on either an orange or pineapple substrate.

Finally, in my third chapter, I explore how predation can influence the decisions of the same innate preference lines of flies. I do this in a study where I give the flies a choice of laying eggs on a safe patch without predators and one with a live Chinese mantid (*Tenodera sinensis*). Additionally, these patches reflected the innate preference of the line being tested. Here I looked at how the fly might take more risks to go to a preferred patch or change their patch preference in the presence of a predator.

Chapter 1

The Evolution of Fear Ecology: A Fruit Fly (*Drosophila melanogaster*) Perspective

INTRODUCTION

Predation is important in the lives of animals. Animals with the highest fitness are those that survive long enough to successfully reproduce, and not succumb to a predator (Westneat and Fox 2010). In this way, only those animals who can avoid their predators will pass on their genes to subsequent generations. This is a mechanism upon which selection will act. Furthermore, it is impossible for prey to expend all their energy remaining safe from predators (Vincent 2002). Energy spent on predatory defense takes energy away from other important tasks such as foraging or finding mates. Thus, balancing predator defense and foraging is necessary to maximize fitness.

Animals do not evolve in static environments, but act within an ecological landscape. This makes understanding their ecology very important when describing how an animal evolves. Predation and foraging are key aspects of ecology. Together they describe the most basic mechanisms of a food web. Behavior ties into this picture through the interactions of prey and predator. It is better for a prey animal to avoid being killed by a predator. Being eaten would destroy or diminish the prey's fitness. For the same reason, it is better for the prey to not leave its offspring vulnerable to predation. Additionally, the prey must also utilize its environment to forage for food and find mates. All of these factors are distributed throughout the landscape in a patchy manner. These patches are not of equal quality, and present various trade-offs. Prey are forced to evaluate their environment while considering the presence of food, predators and mates. An entire field of study focuses on these interactions. It is called "fear ecology".

More specifically, fear ecology describes how prey forages in an ecological landscape in the presence of predators.

Many study systems have been used to examine the ideas behind fear ecology (Longland and Price 1991; Ripple et al 2001). Many have been pure ecological studies: only a few studies have approached more evolutionary questions (Ruehl and Texler 2015). Fewer yet have utilized experimental evolution. There is, however, one animal that is primed for such a use. It is the fruit fly (*Drosophila melanogaster*). The fruit fly has been long used as a model organism in genetics, but it is perfect for furthering our understanding of how fear ecology can influence the evolution of an animal.

Animals must make economic choices in order to manage the balance of safety and foraging in an ecological landscape. However, this “choice” can involve behavioral decisions, or it may result from selective pressure that forces an evolutionary change within the population. Many behaviors are described by one or more genes which selection can act (Westneat and Fox 2010). This means that an animal’s individual decisions can not only affect its fitness, but can be selected upon. An animal can use these decisions to make choices about whether to remain safe at the cost of starving and having no offspring, or risk being eaten and have a chance at both (Longland and Price 1991). Many studies have focused on the trade-offs this entails, either behaviorally or morphologically (Westneat and Fox 2010).

An animal must be able to evaluate the state of predation risk. This perception can range from personal encounters with a predator to an innate

aversion to certain cues. In either case, the behavioral response is very pronounced and leads to rapid evolutionary selection in favor of the best response. A strong selection pressure such as predation usually results in very rapid evolutionary responses (reviewed in Burnham et al. 2015; Zuk et al. 2006). Commonly, twenty generations are sufficient for evolution to occur in the presence of predation pressure. Some cases are more rapid (Zuk et al. 2006).

It is necessary to understand how balancing predation and foraging can relate to a particular animal's fear ecology. Then, one can more effectively explore the trade-offs that guide ecological, behavioral and evolutionary processes. These trade-offs usually either affect an animal's behavior, morphology or chemistry, which ultimately affects the animal's reproductive fitness. I will describe these factors in terms of a wide array of animals. Afterwards, I will delve into how these aspects have been explored in the fruit fly. Finally, I will elaborate on what knowledge is missing about the fruit fly and how it has evolved to forage effectively and still avoid predators.

FEAR ECOLOGY

Fear ecology describes the study of predation risk and how it influences an animal's movement through an ecological environment. In particular interacts with foraging and an animal's use of resources. The basic tenet of fear ecology is that prey tend to avoid locations in which predators are also present. Additionally, this spatial displacement of prey can result in cascading effects. Classically, fear ecology has been studied in Yellowstone (Kauffman et al. 2010; Ripple et al.

2001). Yellowstone is unique in that the top predator, wolves, were removed for a time from the ecosystem, and then subsequently reintroduced. This has made it perfect for studying the effects of a predator, the wolf, on its primary prey, the elk. Upon the reintroduction of wolves, elk immediately switched from using their preferred lowland riparian areas, which wolves also prefer, to using upland steppes. This changed the elks' foraging habits from browsing riparian species of trees, such as willow, to upland conifers. This change in foraging by elk has markedly altered the prevalence of aspen in these areas, and has demonstrated ecological cascades reaching as far as beavers (Kauffman et al. 2010).

Fear ecology has also been explored in other habitats as well. Guinea pigs clearly demonstrate the tenets of fear ecology. When choosing between foraging patches, Guinea pigs will prefer patches closer to shelter over those in areas more accessible to predators (Cassini et al 1991). The same effect exists in desert rodents when avoiding owl predators (Longland and Price 1991). Sea turtles also base their foraging habits on the presence of sharks. They will venture further into open areas when they know there are fewer sharks about (Heithaus et al. 2007). Thus, the foraging habits of animals can sometimes be described and predicted by utilizing fear ecology.

BEHAVIORAL AND MORPHOLOGICAL TRADE-OFFS

Animals may not already have an optimal morphological adaptation that makes them more resistant to predators. Therefore, the interaction between prey and predator in a foraging context will often involve an initial behavioral response

which may result in morphological or chemical traits, or even a pairing of such traits. For example, the tendency for an animal to remain still against a certain substrate, such as bark, may cause a selection by the predator in favor of individuals with more cryptic coloration (Skelhorn 2010). Both predation and foraging have long been studied, apart or together. Consequently, depending on the trophic level of the animal, it can be seen as either the prey or the predator. For example, a flycatcher can either be seen as the predator of insects, or the prey of hawks (Thompson et al. 2011). This means that the principles that apply to one are directly linked to the other. Therefore, understanding the principles of foraging theory is paramount to understanding how trade-offs affect animals when avoiding predators while foraging.

Morphological Trade-offs

Behavioral responses can evolve hand in hand with morphological adaptations. In fact, morphological adaptations are much more thoroughly studied than behavioral ones in an evolutionary context. They range over a variety of forms including, but not limited to crypsis, Batesian mimicry and masquerade (Skelhorn 2010). Many of these characteristics are aided by a complementary behavioral response.

Some classic examples of morphological traits are found in Order Lepidoptera. The case of short term selection for crypsis in the geometrid moth, *Biston betularia,* in Europe due to industrial activity is perhaps the most well-known (Bishop 1972). *B. betularia* is ancestrally light in color, with a peppered pattern that helps it blend into lightly colored bark. However, during the industrial

revolution, large amounts of black soot coated the trees. This favored a small portion of the population of moths that were melanistic, and thus more able to blend into the darkened bark. Consequently, melanistic individuals started to dominate the population because it was more difficult for predators to find them. While the validity of this case is currently under debate, it illustrates how morphological adaptation can evolve.

One of the many examples of Batesian mimicry is the snake mimicry used by *Hemeroplanes* sp. (Hossie and Sherratt 2014). The caterpillar of this species has a posterior end that can resemble a snake. When threatened, the caterpillar will extend it downward and swish it about as though it were a snake's head. This fools potential bird predators, which are preyed upon by snakes, into perceiving the caterpillar as a predator, not prey. This ultimately scares the potential predator away, and allows the caterpillar more of an opportunity to reach maturity and reproduce.

Similarly, there are cases of masquerade, where a prey animal resembles a non-prey item. There are several examples of caterpillars that resemble twigs (reviewed in Skelhorn 2010). The resemblance is mostly from color and texture, but the prey will even hold their body out from a branch in order to enhance the effect. This makes them mistaken for an actual twig, not just visually difficult to distinguish from the twig as in crypsis.

Evolving armor is another way an animal can develop a morphological defense against predators. Armor makes the animal more robust against attack. For example, hedgehogs protect their backs with spines, and will curl into a ball

when harassed (Stankowich and Campbell 2016). This makes it difficult for a predator to reach the hedgehog due to the spines. Ideally, the predator will realize its mistake and leave the hedgehog alone. Another example of an armor-bearing prey animal is the box turtle (Iverson 1991). The box turtle possesses a thick shell into which it may retreat if attacked. It can also close the shell much like a clam. However, unlike the hedgehog, its armor is heavy, and requires a lot of energy to develop and carry around (Vincent 2002). Its central purpose is also predator defense. These factors add extra energetic costs that the lightly armored hedgehog does not have. The lighter spines the hedgehog uses may have been co-opted from another functional use. They are used as cushioning when the animal falls from branches. It remains unclear as to which of the two uses of spines, predator defense and fall cushioning came first; nonetheless, this additional use makes the spines relatively cheaper for the hedgehog to invest in due to the spines' additional benefits (Stankowich and Campbell 2016). Theoretically, the land turtle compensates for the higher cost of its shell by not moving around as much, and thus saving energy. Such energetic trade-offs are demonstrated in an array of armor forms.

The nature of the predators present can also induce a trade-off for variance in armor between similar species. For example, if there is no predator, a prey animal may lose armor over generations that has previously evolved. This is evident in the case of three-spine sticklebacks. These fish can be found in either freshwater or marine environments (Marchinko 2008). Freshwater populations of sticklebacks have reduced armor compared to those in marine populations. This

is due to two basic factors: the type of predators in each environment, and the fact that armor and predation affect body size. Armor is important in the presence of fish predators, but not insect predators. Armor is favored in marine environments primarily because there are more fish predators in marine environments and fewer insect predators. The reverse is true for freshwater environments. Having armor produces a trade-off in body size. Armored individuals are smaller. Consequently, since larger body sizes are favored for both foraging and mate acquisition, it is better for sticklebacks to have less armor in environments with fewer fish predators.

As the stickleback example illustrates, armor impacts the growth of an animal. Furthermore, growth is more generally a part of an animal's life history traits. Consequently, it is a prominent morphological factor that is influenced by predation risk (Ferrari and Chivers 2009; Marchikno 2008). In the case of Everglades snails, the presence of predators negatively impacted a snail's growth rate despite the fact that it also had access to higher quality food (Rhuel and Trexler 2015). Effects on an animal's growth can also have cascading effects on other aspects of an animal's biology, such as the three-spine stickleback's ability to forage competitively or procure a mate (Marchinko 2008). Several instances in tadpoles, water fleas and other taxa indicate that larger individuals are much more robust, and can compete better for food and mates than smaller individuals (Bennett and Murray 2015; Walsh et al. 2015). This demonstrates that the trade-off between body size and various other attributes that can be impacted by predators. This makes growth an important factor when studying predation

and foraging trade-offs.

Some cases of morphological adaptations can be developmentally plastic, allowing animals to thrive in rapidly changing environments where normal evolutionary responses are too slow, or where the loss of a trait could be detrimental later on. Tadpoles are a common focus of these kinds of studies (Relyea 2007). Tadpoles will often use scent cues to detect the presence of predators. By perceiving predator cues, they can respond plastically while undergoing development. A common response is the growth of thicker tails during development (Ferrari and Chivers 2009). Thicker tails make the tadpoles more capable of escape should a predator find them. However, this response does not always result in a perceptible trade-off (Bennett and Murray 2015). Another nice example of this can be found in *Daphnia*, the water flea. Water fleas are low trophic level aquatic arthropods. They experience varying predation risk throughout the year. Because of this, various plastic responses have evolved in water fleas with the presence of predators. Probably the most striking of these is the ability of water fleas to develop armor across generations when placed in areas of high predation (Petrusek et al. 2009). This armor is costly, so it will quickly disappear from a population over generations when water fleas receive less predation. Another plastic response by water fleas is that they will decrease their development time based on perceived predator cues (Walsh et al. 2015). This can be done both within and across generations. Of course, as in tadpoles, increased development time decreases fitness in conditions of low predation (Relyea 2007; Walsh et al. 2015).

Perhaps morphological traits are much more frequently studied because they are more readily quantifiable than behavioral traits. For example, it is easy to measure color or shell thickness, but not so easy to measure active foraging time. Behavioral traits are further complicated in an evolutionary context because they need to be segregated between learned and innate traits. Often this involves extensive genetic work that narrows down the exact genes involved in the expression of the traits that may describe a behavioral tendency (Keene and Waddell 2007; Yamamoto et al. 2008). Sometimes, the genes interact in very complex ways, and require further studies to determine how they act in a specific species (Rohner et al. 2013). This makes understanding the trade-offs an animal faces in predation and foraging contexts even more important.

Chemical Defense

Chemical defense is similar to morphology, but is not always immediately apparent to the predator. There is an array of chemical defense types that range from venom to conspecific death cues. Sometimes, as in the case of aposematism, the chemical defense is accompanied by a distinct morphological trait. In this case, bright color is used on prey animals to signal to predators that there is something nasty about them. Often this something nasty is a toxin such as in African monarchs (Huheey 1975), but it can also be an unpleasant odor such as in skunks (Lartviere and Messier 1996). Sometimes animals will evolve Müllarian mimicry for these cues even if they are not toxic themselves. An example of this is the non-toxic common acraea, which shares a similar orange and black coloration with the toxic African monarch (Huheey 1975). In these

cases, the prey has been evolved with a purely morphological trait because of the chemical defense of another species.

Another type of chemical defense is conspecific death cues. Often animals are wary of volatiles released by the members of their own species, or closely related species (Iliadi 2009; Dukas 1999). The cue can be associated with the presence of a predator, and usually results in a simple aversion reaction by prey. However, there are a large number of fishes that produce volatiles under their skin that are only released when they are injured (Chivers and Smith 1998). Typically this injury is made when a predator attacks the fish. This is interpreted as a predation cue by all nearby fish. Although it is not beneficial to the individual that was attacked, it benefits others of its own species nearby by indicating the presence of a predator.

Foraging Theory and Predators

Foraging theory describes foraging economics and trade-off models within the ecological landscape of an animal, and how it maximizes foraging activities (Brown and Kolter 2004). Furthermore, these theories are often used to describe real-time decisions, but, by extrapolating the foraging efficiency to the population level, they can be extended to an evolutionary context as well. There is a large array of classical foraging models, but two of them stand out when also considering predation risk (Brown and Kolter 2004). They are known as tenacity and vigilance.

Tenacity

Essentially, tenacity refers to an animal's ability to maintain foraging

activities despite predation risk (Brown and Kolter 2004). This may result from a variety of mechanisms ranging from morphological to behavioral tendencies. Often tenacity is driven by morphological aspects such as camouflage and armor, but behavioral tendencies may also lend themselves to it. An example of this is the snake-mimicking caterpillar mentioned earlier. Tenacity can manifest as a way to avoid detection from a predator, or a way to thwart a predator's attempts to capture and kill prey.

Prey can avoid detection morphologically, or behaviorally. Camouflage is an example of a morphological adaptation that is used this way. As in the example with the Geometrid moths, camouflage can evolve based on a predator's perception of its prey (Bishop 1972). If a predator cannot see its prey, the prey is more likely to evade detection, thus increasing its ability to remain within a good foraging patch. Similarly, there is a behavioral response that is universal for a great variety of animals: freezing (Iliadi 2009). Freezing allows for prey to avoid detection from predators even if they are present. Many predators hunt based on movement or sound, so ceasing motion diminishes these cues the prey may be giving the predator (Westneat and Fox 2010). Often the effects of camouflage can even be enhanced by freezing.

Predators may be thwarted from prey capture even if they find prey. For example, if an animal is too large, the predator may not be able to capture or consume the prey. In the tadpole example, an increase in the tail width during development is hypothesized to produce tadpoles that are too big for a predator to swallow in addition to aiding in escape (Ferrari and Chivers 2009; Relyea

2007).

Another way prey can thwart a predator is to have armor. Rather than hide be forced to hide from predators, prey may have evolved stronger physical defenses. This is evident in the armor of hedgehogs and box turtles. The spines of the hedgehog will defend it, which will allow it to remain within a good foraging patch. The hedgehog, by having the tenacity to stay at a patch of food, will gain an advantage over less tenacious predators. The hedgehog will lose some foraging opportunity as it is attacked, but because it did not leave it can still fully take advantage of the patch, especially if it is rich. By not moving, the hedgehog saves some time in finding another patch, or losing food to a competitor that may be able to come in before the hedgehog returns (Stankowich and Campbell 2016). Like the hedgehog, the turtle can use its robust shell to stake out a rich patch of food at the cost of foraging time if attacked, and be able to get to the food before its less tenacious competitors (Iverson 1991).

Vigilance

Vigilance is often used to elicit a flight response. In vigilance behavior, an animal spends time or energy to perceive predators in the environment (Brown and Kolter 2004). Essentially, it allows the animal to balance safety with other activities. In foraging this means an animal must spend time and energy keeping watch for predators instead of foraging. It is common in group-oriented animals (Westneat and Fox 2010). When multiple individuals exhibit the same time trade-off, the cost is divided among participating individuals, thus allowing each individual to spend more time foraging. If there are no predators, it is best for an

animal to spend its time and energy on foraging and other activities such as finding a mate. However, except for a few cases on islands, animals always have to balance vigilance with foraging (Westneat and Fox 2010).

Recall the foraging habits of Guinea pigs. They are much safer in sheltered areas (Cassini et al 1991). However, if food is only in open areas, an individual will be forced to forage in the riskier open areas. The Guinea pig will give up some of its foraging time to vigilance for predators, but in return it will get some food, while staying in safer areas gives it none. Similar studies on vigilance have been done on other taxa as well (Brown and Kolter 2004). One of these examples is in tadpoles. When tadpoles detect a predator cue, they may opt to remain still and less detectable to predators in exchange for foraging opportunities (Ferrari and Chivers 2009). This is an example of freezing. Decreased time foraging does negatively affect tadpole size. Because smaller tadpoles are much weaker, they are less likely to survive to adulthood and find a mate. This form of vigilance demonstrates a direct trade-off between predation and foraging in a way that can affect the animal's fitness.

TRADE-OFFS WITH REPRODUCTION

With a fundamental understanding of fear ecology, and trade-offs in foraging theory, one can delve further into reproductive trade-offs. Reproduction is ultimately the key to an animal's fitness (Westneat and Fox 2010). Trade-offs in animals can come in a variety of forms. Two of these forms are mate acquisition and care of offspring.

Mate acquisition is the next step after survival towards genes successfully being transmitted to the next generation. In terms of trade-offs, having the chance to mate is considered to have a high reward value, and in several cases it can outweigh the risk of being eaten by a predator (Westneat and Fox 2010). As a result, several behaviors that seem overly risky can result. Most notable are several courtship displays such as those seen in birds-of-paradise. However, these risk-taking behaviors are more frequently found in more subtle cases. For instance, a male Iberian rock lizard will hide from predators, and will remain hidden until he feels he is safe (Martin et al. 2003). However, when exposed to the same predation risk, and presented with an opportunity to mate, he will come out earlier. In other words, he is more willing to risk predation when he has an opportunity to increase his reproductive fitness. Similarly, *Achroia grisella,* a lekking pyralid moth, will also risk predation to acquire mates (Brunel-Pons and Greenfield 2010). Males often gather in leks to attract females. In order to win a female over his competitors, a male produces a song. However, this song can be eavesdropped on by predatory bats. Upon perceiving the echolocation pulse of a bat, males become silent in order to avoid detection. If a male is alone, he will remain silent for an extended period of time until he is sure the threat has passed. However, if he is in a lek, he will resume singing faster. This is because, in a lek, every second he remains silent is a second potentially lost to his rivals in wooing a female.

Evolutionary responses to trade-offs in mate acquisition can evolve rapidly. In the case of the Hawaiian field cricket, the response occurred in less

than twenty generations (Zuk et al. 2006). Hawaiian field crickets suffer from a lethal parasitoid fly that is comparable to a predator. The parasitoid locates its cricket prey by the song of the courting male. Because the effect of the parasitoid is so strong, males have evolved to be predominantly silent. This is not to say there are no more singing crickets, but that a wing mutation that prevents singing, which was already present within the population, was selected for by female preferences. Consequently, female crickets even prefer silent males over their singing rivals in the presence of the parasitoid, so this selection is reinforced.

Maximizing an animal's reproductive fitness should ultimately maximize the survival of its offspring. This is not to say that each individual offspring needs to survive to the next generation, but that an optimal amount does. There is a great array of tactics an animal may be utilizing to this end. These tactics range from poorly caring for numerous offspring to nurturing just a few offspring until they also reproduce (Vincent 2002). Both extremes rely to some degree on the predators present in the environment. In spider mites, females predominantly lay their eggs on leaves that lack predators (Hackland and Schausberger 2014). This ensures that the likelihood of at least one egg making it to the next generation is maximized. In another instance, Pied Flycatchers will vary their antipredator behavior near the nest based on how far they are from a nearby hawk nest (Thompson et al. 2011). Flycatchers nearer to hawk nests will resume normal activity after hiding more quickly than flycatchers that nest farther away. This varied allocation of antipredator behavior demonstrates the trade-off in vigilance

for predators between risk taking and successfully rearing offspring. Flycatcher nests closer to hawk nests experience more frequent exposure to hawks than flycatcher nests that are further away. If the flycatchers near hawk nests reacted every time the hawks were spotted they would no longer have enough time to care for their young. Therefore, it is better for the flycatchers near hawk nests to take more risks around hawks so that they can raise their own offspring.

From mate acquisition to offspring survival, trade-offs in predation and foraging affect fitness. Thus, selective pressure is placed on relevant traits which ultimately guide an animal's evolutionary trajectory. Now that I have presented an overview of a how a great variety of animals deal with balancing predation risk and foraging, I would like to focus extensively on a single species: the fruit fly, *Drosophila melanogaster*.

DROSOPHILA MELANOGASTER

The Model Organism

As a lower trophic level animal, both the larval and adult fruit fly are prey to generalist predators such as frogs, spiders, birds, ants and many others. The fruit fly eats rotting fruit as both a larva and as an adult. Female fruit flies evaluate their environment for optimal patches of fruit to eat and on which to lay eggs. Some of these patches inevitably have more predators than others (Huffaker 1958). Being aware of these predators via cues, fruit flies will readily leave areas they see as dangerous (Gibson et al. 2015). Additionally, a recent review has explored the use of fruit flies as a model organism to study the

neurology and psychology of fear (Iliadi 2009). Several labs have identified which cues and mechanisms are associated with a fear response (Gibson et al. 2015; Iliadi 2009; Yamamoto et al. 2008). Furthermore, fruit flies are also known to use various cues to determine the quality of a patch (Dunlap and Stephens 2009). Such cues include the quality of the resources or food within the patch (Ruehl and Texler 2015). In particular, females look at qualifying factors such as color, texture, taste, and sugar and yeast content where choosing to lay their eggs. Perhaps most notably, the fruit fly has been long studied in the field of genetics (Iliadi 2009). It was one of the first species to be fully sequenced, and has frequently been used to identify how specific genes influence behavior (Iliadi 2009; Keene and Waddell 2007). Because of this, its short life cycle and lab adaptability, it has been used in several experimental evolution studies (Dunlap and Stephens 2009; reviewed in Burnham et al. 2015). All of these factors together make fruit flies a perfect study organism for studying evolutionary responses within a predation and foraging context.

What Flies Fear

As fear ecology suggests, a prey animal's perception of predation risk can result in an avoidance type response. In the case of the fruit fly, this avoidance is driven by a fear response. Fear is defined as an emotion, which is a highly debated topic among scientists (Iliadi 2009). While some define emotions to be distinct "feelings", others see emotion as a physiological reaction. Despite disagreements, there is obvious support that fear is a fundamental response among animals (Iliadi 2009).

Several studies have addressed fear in fruit flies (Iliadi 2009). Earlier studies on flies were performed simply by measuring locomotor responses or exploratory behavior. Locomotor responses indicate a “flight” response, and exploratory behavior indicates a “boldness” or “risk taking” response (Sih and Giudice 2012). These are both classical behavioral study measures. Later studies have shifted to more neurological and molecular assays (Iliadi 2009).

Often, behavioral assays include simple adverse stimuli such as electric shock, heat treatments or spinning the flies in a centrifuge while exposing them to a cue they are being conditioned to avoid (Iliadi 2009). Typically, a fly may respond to a fear-inducing stimulus in one of two ways: startle-flight, or freezing (Gibson et al. 2015). Some particularly innovative studies have looked at how flies react to these stimuli in real time (Mendoza et al. 2014). After these assays, flies are often sacrificed to look at their neurological responses and genetics.

A fly’s neurology is commonly studied by counting the number of mushroom bodies in its brain (Iliadi 2009). Mushroom bodies are centers of neurological activity that relate to memory. The presence of more mushroom bodies is associated with increased learning and memory. Ultimately, a fly can invest more or less energy in learning what to avoid. Another neurological method that can be used is a single-sensillum recording (SSR) screen (Dweck et al. 2013). This method involves a live recording of synaptic responses in insects in response to olfactory cues. Essentially, it allows one to determine if a fly is neurologically receiving a cue. This method can be used to determine how sensitive a fly has evolved to be to a given olfactory cue, which may give it

insight to the presence of a predator, or if a place is optimal for laying eggs (Dweck et al. 2013). Further molecular data in flies are analyzed through DNA and RNA sequencing (Yamamoto et al. 2008). All of the genes associated with fly senses, neurology and memory have been studied in depth (Iliadi 2009; Keene and Waddell 2007; Yamamoto et al. 2008). This makes it easy to track any evolutionary changes in these genes in selected lineages, and thus any changes in fear response.

As good as it is to know how to measure a fear response, it is equally important to understand what cues flies are using to illicit such a response. There are three predation cues known to be used by flies: conspecific death smell, vibration, and shadow (Iliadi 2009; Gibson et al. 2015). The first of these cues is a bit odd. An animal should learn to fear or avoid the smell of death from its own kind. Fruit flies do this, but only in higher odor concentrations (Iliadi 2009). Although it remains unexplained, they seem to be attracted to lower odor concentrations of conspecific death smell. The second cue, vibration, can indicate a predator's approach. Vibrations induce a distinct startle response. The third cue, shadow, can indicate a predator's looming presence; however, flies are also attracted to light. This complicates understanding this cue slightly. Are flies attracted to light, or adverse to shadow? This question is cleared up by a distinct startle response exhibited by flies exposed to a passing shadow (Gibson et al. 2015). Flies obviously reacted to the passing shadow as if it were a predator.

There are no published studies on *Drosophila melanogaster* I am aware of that link predation risk to evolution; however, there is a study with fruit flies and

parasitoid wasps that involves an innate (not learned) preference for citrus (Dweck et al. 2013). The fly's citrus preference has been backed up by behavioral assays and genetic analysis (Dweck et al. 2013; reviewed in Burnham et al. 2013). However, this preference for citrus seems to be linked primarily with ovipositional preference, and not preference in other contexts. Notably, the parasitoid wasp that preys on fruit fly larvae is deadly to the larvae, but is susceptible to compounds found in citrus fruits such as oranges. However, there are no oranges in the native range in Africa that both fruit flies and the parasitoid wasp share. Instead, a native fruit, the squirrel nutmeg, shares a nearly identical chemical profile to oranges. It is believed that this is the fruit with which the preference for citrus preference evolved. This example demonstrates that the foraging and egg laying habits of fruit flies have changed over evolutionary time in the presence of parasitoid risk, which can be considered functionally equivalent to predation risk.

Past Fly Foraging and Life History Work

Several studies have been done on fruit fly foraging (reviewed in Burnham et al. 2015; Dunlap and Stephens 2009; Mery and Kaweki 2002). These studies have examined aspects of fruit fly foraging in economical and evolutionary contexts. In particular, they use experimental evolution to explore classical patch economics. Essentially, an animal will evolve an innate preference if an environment has low reliability and is highly fixed, but will evolve learning in an environment of high reliability and uncertainty (Mery and Kawecki 2004; Dunlap and Stephens 2009). Utilizing this principle, several lines of flies were selected

over many generations in environments that favored an innate preference, or learning (reviewed in Burnham et al. 2015). Additionally, flies were evolved for preferences for egg laying on orange or pineapple flavored substrate. Notably, an orange preference, which is already innate, was easily amplified, but a pineapple preference was more difficult to evolve due to the fact it goes against the fly's innate preference for orange. Additional studies have shown that evolving learning results in life history trade-offs (Mery and Kawecki 2004). Flies that evolve learning lay fewer eggs and live shorter lives than flies with innate preference. Associative learning has previously been studied in larvae. Dukas (1999) tested this in flies that were known to perform well when giving learning tasks. He tested the foraging responses of these flies to a variety of odor cues. Of particular interest is his test that showed that larvae can learn to associate the odor of conspecific death with a food substrate, and thus learn to avoid predators.

FUTURE DIRECTIONS

This brings me to what the fruit fly has yet to help us learn. As I mentioned earlier, surprisingly few *Drosophila melanogaster* studies combine the effects of predation on foraging in an evolutionary context. Further studies on that topic would be beneficial, especially for a species with a genome that is thoroughly studied. The fruit fly is a perfect study organism for this. With its genome so well understood, it makes for a good opportunity to add to our understanding of how fruit flies evolve responses to predators such that their foraging efforts become

maximized. Furthermore, because these underlying principles of predation and foraging are so fundamental in all animals, studies on fruit flies can easily be a good starting point to understanding the evolutionary responses of other animals to predation in a foraging context.

Chapter 2

Patch Use and Innate Preference in *Drosophila melanogaster*

INTRODUCTION

Innate bias can be an important factor when an animal is optimizing its choice of food, homes, mates, and when to flee for safety. Innate bias is bias that has been inherited in an animal. It can be used as a form of inherited information when the animal makes decisions. Having innate bias can decrease the chances of making the wrong choice and increase the chances of making the right choice. Innate bias is also an important concept in many fields from animal behavior to neuroscience and economics. This is mostly due to the fact that it is used as the "baseline" for measuring and understanding behavioral plasticity as mediated by learning (Stephens and Krebs 1986). It is often described as an evolutionary constraint for potential behaviors, and explained away with "just so" stories about the probable past or present adaptive context. Consequently, there is very little overarching theory explaining how biases evolved originally. There are several reasons for this. One is the disparity of the fields. For example, the people who study behavioral economics do not often collaborate with the people who study neuroscience. Another is controlling environmental variables. In an evolution study, controlling environmental variables requires things like experimental evolution. Experimental evolution is impractical or impossible for many species, so it is not always an option. Ultimately, the classic "time machine" problem in evolution is the largest of these problems. We cannot simply go back in time to observe the factors that lead to the evolution of a bias. This makes discovering the exact cause of a bias difficult or imposable to pinpoint.

Additionally, innate bias may be applicable only to one context, or it may

be more generalized over several contexts. For example, innate bias can evolve as a specific set of genes designed for one behavior (i.e. only for oviposition), or it could evolve more generally (i.e. as a sensory bias, which influences several behaviors). Regardless of its generality, innate preference may also be influenced by things such as spatial scale. In short, context matters in decision making (e.g. Stephens & Dunlap 2011).

One important factor that influences decision making is the decoy effect. This is where a novel option is presented that is of intermediate value to two extreme options. This third option, the decoy, is of intermediate quality of the other two options (e.g. Bateson & Healy 2005). It has been shown to have an effect on several species of animal, including humans, and in slime molds (e.g. Latty & Beekman 2011; Shafir et al 2002; Stephens and Krebs 1986). Ultimately, the animal should choose one of the two quality options, or the option that best suits its preference. However, the decoy, will appear to have qualities from both options even if those qualities are inferior. This theoretically draws the animal's attention away from the best option, in favor of the decoy.

Spatial scale is another influential factor when it comes to decisions. In foraging, an animal's use of patches is thought to be very important (Stephens and Krebs 1986). Because resources do not occur in the environment homogeneously, but rather is heterogeneous patches, animals need to make choices when locating and optimizing the use of resources. An animal's use of patches is most often understood through the Marginal-value Theorem. The Marginal-value Theorem describes how long an animal should stay at a patch in

order to optimize its use of the patch's resources. The resource quality and travel time between patches are the major deciding factors for this. Because travel time is important, the scale of the environment or an assay can influence an animal's patch use. Small scale arenas are frequently used in patch studies to minimize the effect of travel time on patch use.

In our lab, we have successfully evolved populations of *Drosophila melanogaster* that show innate bias towards specific substrates with respect to laying eggs using fruit cues. These flies give us the unique opportunity to pursue questions in the evolution of bias. Furthermore, extensive work on patch use across contexts has only previously been addressed in larvae in other labs (e.g. Dukas 1999; Scherer et al. 2003).

Our flies have been tested exclusively in a small scale for their oviposition preference when given the choice between choosing orange and pineapple substrate. Our flies' innate bias makes them prime subjects for approaching questions of contextual patch use, environmental scaling and the decoy effect. In this particular study, we looked not only at oviposition preference in a larger scale arena (as compared to the smaller arena they were selected in), but also at courtship preference and where males and females spend their time. Additionally, we provided the flies with a novel decoy. We hypothesized that (1) females will retain their oviposition preference in a large scale arena, (2) females decide the location of courtship and males seek out females to court, (3) flies will not retain their oviposition preference across every context, and (4) oviposition preference may be influenced by environmental factors such as scale.

METHODS

Subjects

We used flies that have been previously selected for an innate preference for oviposition on either orange or pineapple substrate. The fly lines were selected identically to the lines described in Dunlap and Stephens (2009). These selected lines were formed from a wild-caught population from Fenn Valley, Michigan. For each line, approximately 480 flies were reared from eggs between six vials on standard cornmeal fly food. Flies were then kept at 24°C in 24-hour light.

Once each generation, all the adult flies were transferred into a shoebox sized cage. After emergence, adult flies were allowed to acclimate for a few days in the cage with standard fly food. Then, in an experience phase, the flies were presented with two fruit agar substrates (patches), orange and pineapple, one of which was mixed with quinine, which gave it a bitter but non-toxic taste. This allowed them to learn about their environment. The agar was then taken away and replaced in a test phase with a new set of orange and pineapple agar plates. These did not have any quinine. The flies were allowed to lay their eggs freely on both plates; however, their eggs were only taken from one of the agar plates (either orange or pineapple depending upon selection history) for the next generation.

During the selection the quinine served as a cue to inform the female flies where not to lay their eggs; however, the quinine was alternated inter-generationally between the two substrates while the flies' eggs of a given

selected line were only ever taken from one substrate type. This simulated an environment where evolving to be able to learn the quinine cue better was ineffective because the meaning of the cue changed, but the correct egg laying substrate did not. Theoretically, flies should always evolve an innate bias, or preference, in this case where the learning is unreliable, but the environment never changes. Flies were either selected for an orange oviposition preference, or a pineapple oviposition preference. Orange preference lines were always taken off orange agar, and pineapple selected lines were always taken off pineapple agar. The control lines were only ever presented with standard fly food during the selections.

In all, there are 12 selected lines, 6 orange selected and 6 pineapple selected, used in this experiment plus 6 controls. One of each type of selected line, orange and pineapple, were paired with a control. These three lines were each assigned an identity and were always selected, tested and reared simultaneously as a triplicate. Triplicates were formed to allow for better between treatment comparisons when testing for effects.

At the time of our tests in this experiment, the lines had undergone over 160 generations of continuous selection. Each line has since undergone at least one thorough assay to test every 50 generations, and one had just been recently run prior to this experiment. As with selections, the fly lines are always assayed in triplicate in the same boxes in which they were selected. Preference assays were measured by counting the eggs laid on both the orange and pineapple substrate. The counts are then used to calculate a preference ratio: (eggs on

orange - eggs on pineapple)/ (eggs on orange + eggs on pineapple). In this ratio, a 1 indicates a strong preference for orange, and a -1 indicates a strong reference for pineapple. Our lines have exhibited expected scores in recent oviposition preference assays. In the most recent assay the scores were as follows: orange lines were 0.526108971, pineapple was 0.3029270351 and the controls were 0.4486794455.

These preference lines have been selected in a purely oviposition-based context. We have also conducted other assays on these flies to test the generality of their innate preference. For example, we have tested the larvae of these lines to see if they have the same preferences when feeding. We have also tested feeding preference in the adults, and if they spend time in the same places as where they like to lay their eggs. We have even tested males and females separately. What we have not done is look at the effects of a large scale assay arena on preference and patch use.

We collected 4 vials of eggs from each line. Like with the selections, each vial had about 80 eggs. The flies were also reared in the same conditions as during selections. However, instead of transferring the adults into a cage after emerging, approximately 100 individuals were selected (~50 male, ~50 female). We marked them with different colors by sex for easier identification. For both sexing and marking, flies were knocked out with cold. Marking was first done by tossing the sexed flies in florescent powder. The flies were then able to groom off most of the powder overnight, but left some on the top of the thorax for identification. However, there were some difficulties with some flies even

grooming off that thoracic spot, so we only marked flies this way for the first two triplicates. The remaining triplicates were hand painted with Testor's enamel modeling paints. We also randomly assigned each line within a triplicate a unique pair of colors so that any flies not removed from the previous day could be identified and removed from the dataset. After marking the flies, we housed males and females together in a glass bottle with standard fly food for at least 24 hours. Just prior to testing, we transferred the flies into a similar glass bottle without any food.

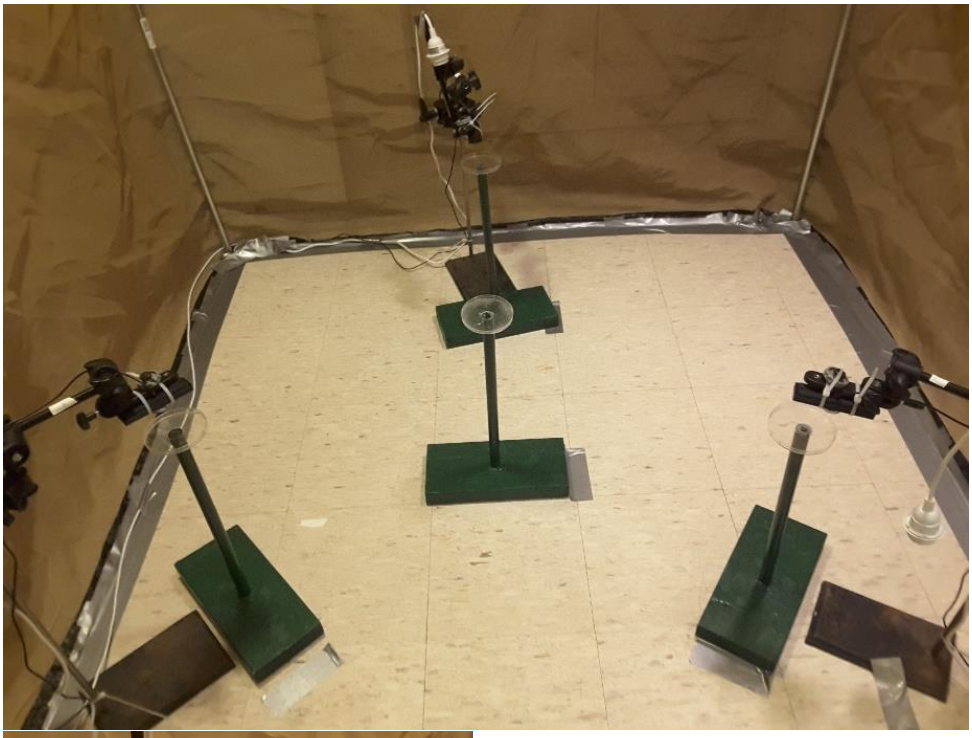
Flight Cage and Video Recording

For testing, we released the marked flies from their bottle on a central pedestal (48 cm in height) into a large 6 ft^3 flight cage. The cage was originally designed for work with aphids, so the mesh was sufficiently small to prevent flies from escaping. The mesh was formed to fit around a metal frame with an open bottom. We sealed the bottom with duct tape, so that no flies could escape out the bottom. They were given three different flavored patches: orange and pineapple plus a novel flavor, apple. The orange and pineapple patches were to mimic the flies' selection conditions, and the apple patch was introduced as a decoy. Each patch was placed on one of three pedestals of the same height as the central pedestal. Each pedestal was placed equidistant from each other (100 cm between the centers of the pedestal patches), and the same distance from the central pedestal (57 cm). Above each patch was mounted a camera and an LED light (Figure 1). All lighting other than the three patch lights were turned off prior to starting the assay.

The cameras used to observe the flies' activities on each patch were manual focus webcams (Genius WideCam F100 HD). Each camera was hooked up to the same computer, where video could be recorded in Noldus Media Recorder. Media Recorder allows for multiple videos to be recorded simultaneously. This is important to keep the time stamps the same on all of the videos, and thus make time comparisons between the three patches accurate.

Once the videos stopped recording at 6 hours, the assay was considered to be over. Within 15 minutes of this time, the patches were removed and imaged for egg locations on an EPSON scanner or with a Cannon EOS Rebel. The eggs were counted at a later time in ImageJ using the Multipoint tool. Then the flies were removed from the cage by a combination of starving them to death or catching them in traps overnight and catching them manually the next morning so that another assay within the triplicate could be run.

Videos were watched in PotPlayer, which allows for millisecond accuracy while skipping through time points in the video. This is important because fly activities are fast, and what a fly is doing at the beginning of a second can differ significantly by the end of that second. While watching the videos we took observations at 1 minute intervals for three hours. Three hours is a sufficient amount of time to observe the range of behaviors under investigation.



A

B

Figure 1. The spatial arrangement of patches. Photographs of the 6 ft ^{3} flight cage patch set up: A) The three patches are placed on pedestals equidistant from the central relese pedestal. Cameras are placed above each patch on an ajustable camera mount arm. Note that the LED bulbs have been removed here. B) A closer view of one patch.

Experimental Design and Procedure

For this experiment, each triplicate was assigned to run in a randomized order across several weeks. Each of the 6 triplicates (18 lines) was assayed once. Each of the lines within the triplicate was further randomized to run on different consecutive days within that week. Each line was given three food patches to choose from: orange, pineapple and a novel flavor, apple. The substrate for each patch was made the same way they were during the flies' selection: a mixture of agar and frozen fruit juice in a round petri dish. The assay was run for a total of 6 hours. Media Recorder allows one to terminate a recording automatically based on a timer, so the length of the videos is uniform. However, egg plates where removed as much as 15 minutes after this time. In our lab, 15 minutes is not considered to be biologically significant to the flies.

Behavioral Observations

Oviposition data taken from the patches were used to measure the flies' oviposition preference. This was so they could be compared to all other previous preference assays done with the flies within their selection boxes. Using time-stamped videos, we took several behavioral measures on each patch: male and female residency, males courting, females being courted, matings and oviposition. Residency consisted of counting the number of males and females on each patch at every time point. Males courting is a count of the males on that patch actively wooing a female. Females being courted is the number of females being wooed by one or more males. Matings refers to the number of mating pairs on one patch. Oviposition is the number of females actively laying eggs on a

patch. Other observations such as resting and eating were too difficult to observe with this camera set-up. Each of these behaviors reflects an alternative context in which a fly may exhibit a preference outside of its oviposition preference.

Statistical Analysis

Data were compiled and formatted in Microsoft Excel, and statistical values were evaluated in STATISTICA. Oviposition and fly residency data were analyzed separately in a repeated measures ANOVA. This was so that more than one factor, such as selection history, could be analyzed for either oviposition or fly residency while testing the null hypothesis of patch use. In other words, it uses "repeated measures" for the independent variable of patch type, and could analyzed the effect of all of the factors, such as lineage, sex, and time, together. A repeated measures ANOVA uses several output values to describe a model. A sum of squares (SS) value is an error measure that describes how well the data fits the model. Essentially it describes how far a factor deviates from the mean. The mean sum of squares (MS) incorporates the sample size of the factor and its degrees of freedom into the sum of squares value. This describes how the sum of squares value relates to what is expected. The degrees of freedom (df) simply describes how much the model can vary. The *F*-statistic (F) uses the mean sum of squares to fit the dataset to the null hypothesis model, and the *p*-value (*p)* describes how likely the null hypothesis is to be true based on this fit.

Oviposition and courtship data were analyzed as observed versus expected values. Because flies in different assays laid different numbers of eggs and had varying numbers of courtship events, we first calculated a ratio of the

numbers of eggs or courtship events observed based on how many we would expect on each patch by chance: (Observed-Expected)/Expected. For oviposition, observed was simply the number of eggs counted on each patch. Expected was calculated as Egg Total/Number of Patches. Egg total is the sum of all eggs laid on all three patches in the cage. Number of patches is the sum of the patches on the cage (3). The formula was the same for courtship, only with expected calculated thus: Total Number of Females Being Courted/Number of Patches. Total Number of Females Being Courted is the sum of the average number of females being courted by males across all three patches.

Residency was analyzed using a large factorial repeated measures ANOVA. A large factorial was necessary to incorporate the increased number of factors measured by the dataset. Selection history was replicated within the data by using different lines (6 total for each history). Each line was itself only assayed once.

RESULTS

Oviposition

We analyzed the egg counts of each patch in a repeated measures ANOVA with each egg measure per patch type per line as the repeated measure for a given line, and a factor of evolutionary history. We find a significant effect of patch type ($F_{2,24}$=16.35, p=0.000033) (Figure 2, Table 1).

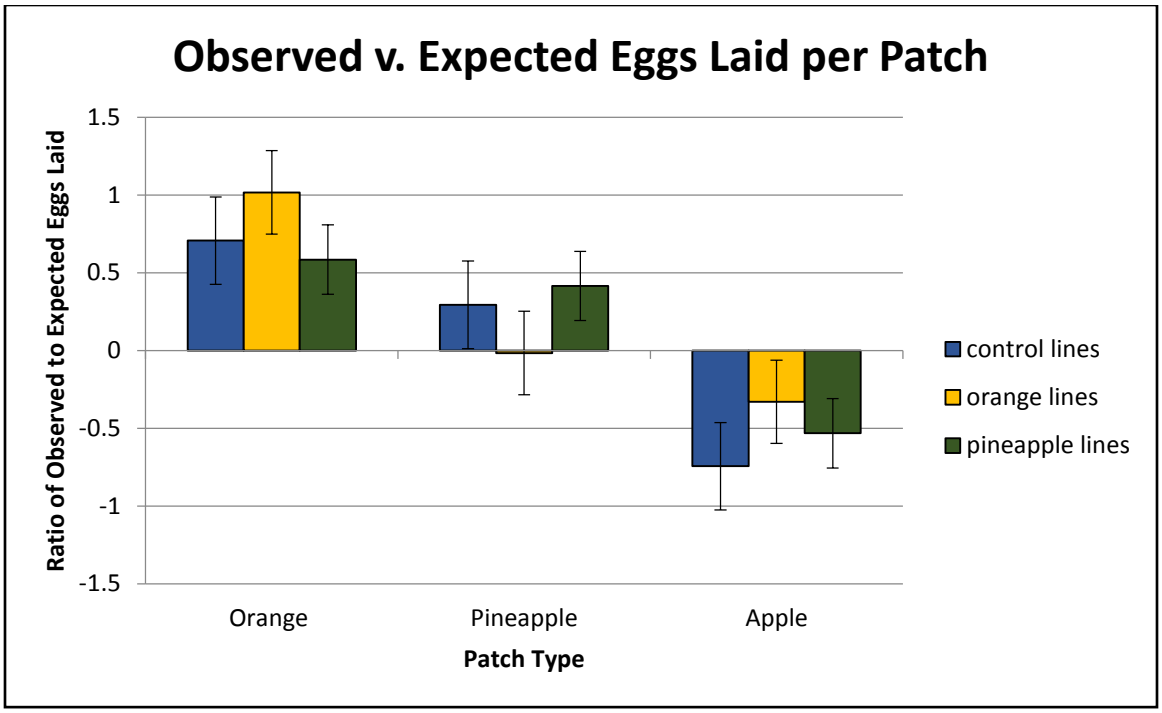


Figure 2. Oviposition per patch. The number of eggs on each patch for each line were counted to calculate a ratio of observed eggs per patch over the expected number of eggs laid by chance. Zero represents the expected value, and values above and below zero are values that are above and below expected respectively. Each cluster collects values for each patch type. Each color within a cluster represents a selection history. Sample size: N=15 (5 lines per selection history); standard errors for orange lines=0.267933608, pineapple lines=0.222643793 and control lines=0.281436365.

Table 1. Repeated Measures ANOVA for Oviposition Preference

Effect	SS	df	MS	F	*p*
Intercept	*1.07858*	*1*	*1.078582*	*17.99806*	*0.001143*
Selection History	0.14307	2	0.071534	1.19368	0.336668
Error	0.71913	12	0.059928		
Patch Type	*12.89709*	*2*	*6.448546*	*16.35845*	*0.000033*
Patch Type * Selection History	1.27777	4	0.319442	0.81035	0.530890
Error	9.46087	24	0.394203		

Sample size: N=15 (5 lines per selection history).

Courtship

The results for patch use by flies for courtship are shown in Figure 3, which shows the ratio of observed to expected number of females being courted. The ratio was calculated the same way as with the egg counts. Because one of the lines showed no courtship events, we analyzed these data using a nonparametric approach. Evolutionary history is not statistically significant for any patch type (Kruskal Wallis ANOVA, Orange: H(2)=0.0582, p=0.9713; Pineapple: H(2)=5.43, p=0.3279; Apple: H(2)=5.43, p=0.062.

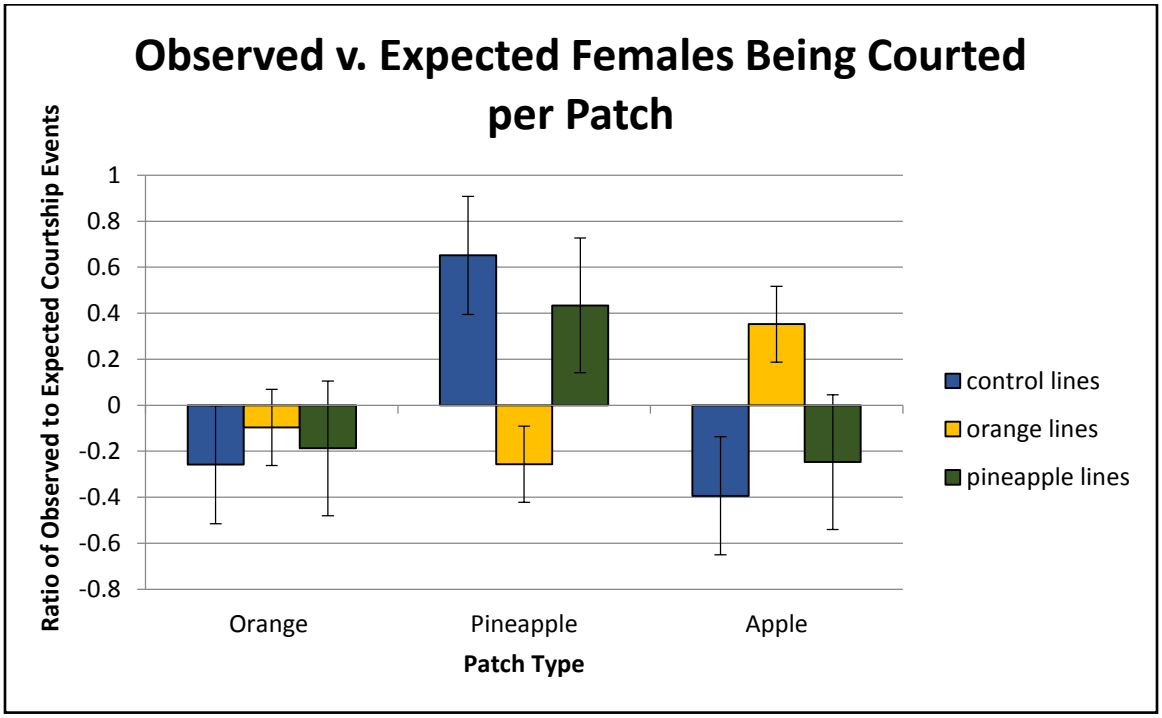


Figure 3. Fly courtship per patch. The number of courtship events on each patch for each line were counted used to calculate a ratio of observed courtship events per patch over the expected number of courtship events that occur by chance. Zero represents the expected value, and values above and below zero are values that are above and below expected respectively. Each cluster collects values for each patch type. Each color within a cluster represents a selection history. Sample size: N=17 (6 lines each for control and pineapple selection histories and 5 lines for orange selection history); standard errors for orange lines=0.164997304, pineapple lines=0.292482197 and control lines=0.256641777.

Residency

We averaged the observed numbers of males and females on each patch for each of the selected and control lines and performed a repeated measures ANOVA on square-root transformed data to account for equal variance (Figure 4, Table 2). The only significant effect is an interaction between sex and patch type ($F_{2,30}$=4.98, p=0.0133).

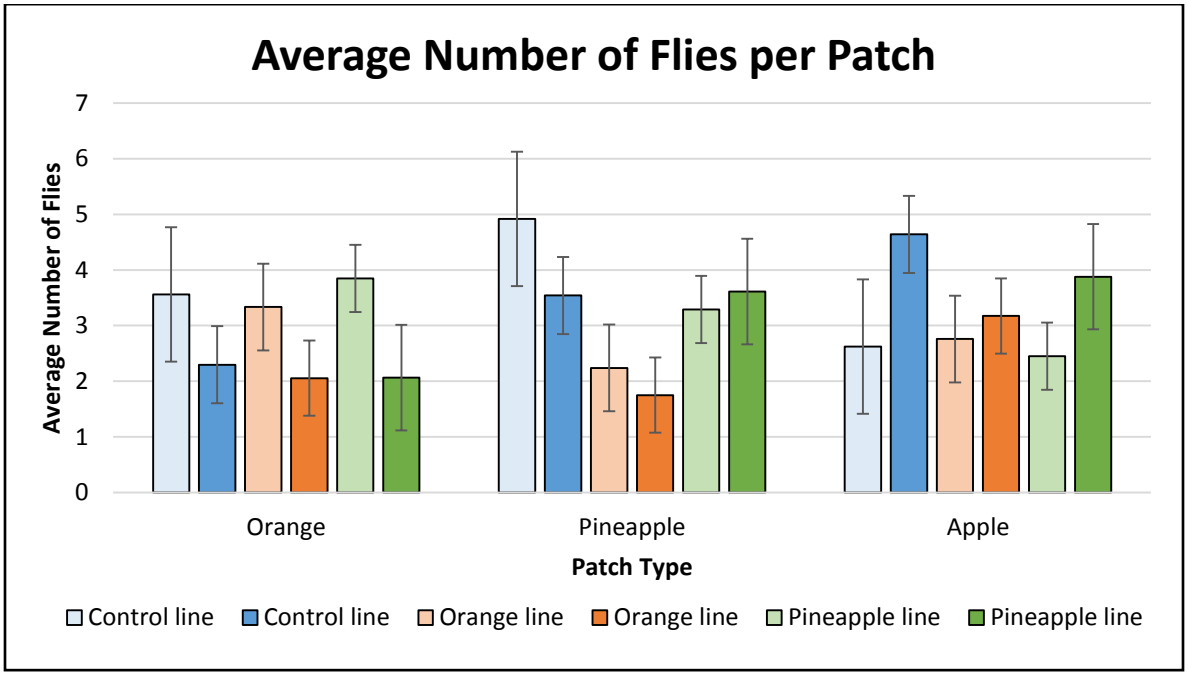


Figure 4. Male and female fly residency per patch. Each value is the average accumulation of flies over 180 minutes on each patch (x-axis) for each selection history (colored bars). Control lines are shown in blue, orange lines are orange, and pineapple lines are green. Male and female fly residency is shown here in different bars. Female bars are shown in the same color as the male bars, but as a lighter tint. Sample size: N=18 (6 lines per selection history); standard error for control line female=1.208256475, control line male=0.692605214, orange line female=0.780059716, orange line male=0.67604486, pineapple line female=0.603846032 and pineapple line male=0.949028761.

Table 2. Repeated Measures ANOVA (Square root Transformed) of Fly Residency by sex

Effect	SS	df	MS	F	*p*
Intercept	*270.6472*	*1*	*270.6472*	*146.7852*	*>0.000000*
Selection History	1.4963	2	0.7482	0.4058	0.673564
Error	27.6575	15	1.8438		
Sex	0.1287	1	0.1287	0.3488	0.563613
Sex * Selection History	0.1450	2	0.0725	0.1965	0.823707
Error	5.5361	15	0.3691		
Patch Type	1.1735	2	0.5867	1.6633	0.206514
Patch Type * Selection History	2.5308	4	0.6327	1.7936	0.156190
Error	10.5827	30	0.3528		
Sex * Patch Type	*1.8906*	*2*	*0.9453*	*4.9988*	*0.013376*
Sex * Patch Type * Selection History	0.5013	4	0.1253	0.6627	0.622765
Error	5.6731	30	0.1891		

Sample size: N=18 (6 lines per selection history).

Because the time course of patch residence may differ, we also looked at how both males and females use each available patch over time. We did this by taking the raw counts of males and females for each 1 minute interval, and averaging it over 10 minute intervals to form 10 minute time bins. First we present a snapshot of a single triplicate to show an example of individual line data (Figure 5).

The combined data for all of the lines can be found in Figure 6. We analyzed these data using a factorial repeated measures ANOVA, with factors of evolutionary history and then time block, sex, and patch type repeated for each line. Here, while we find significant effects of time ($F_{17,255}$=4871, p>0.0000), a significant interaction of sex and patch type ($F_{2,30}$=5.11, p=0.012335), and some of their further interactions (Table 3), we did not find significant effects of evolutionary history.

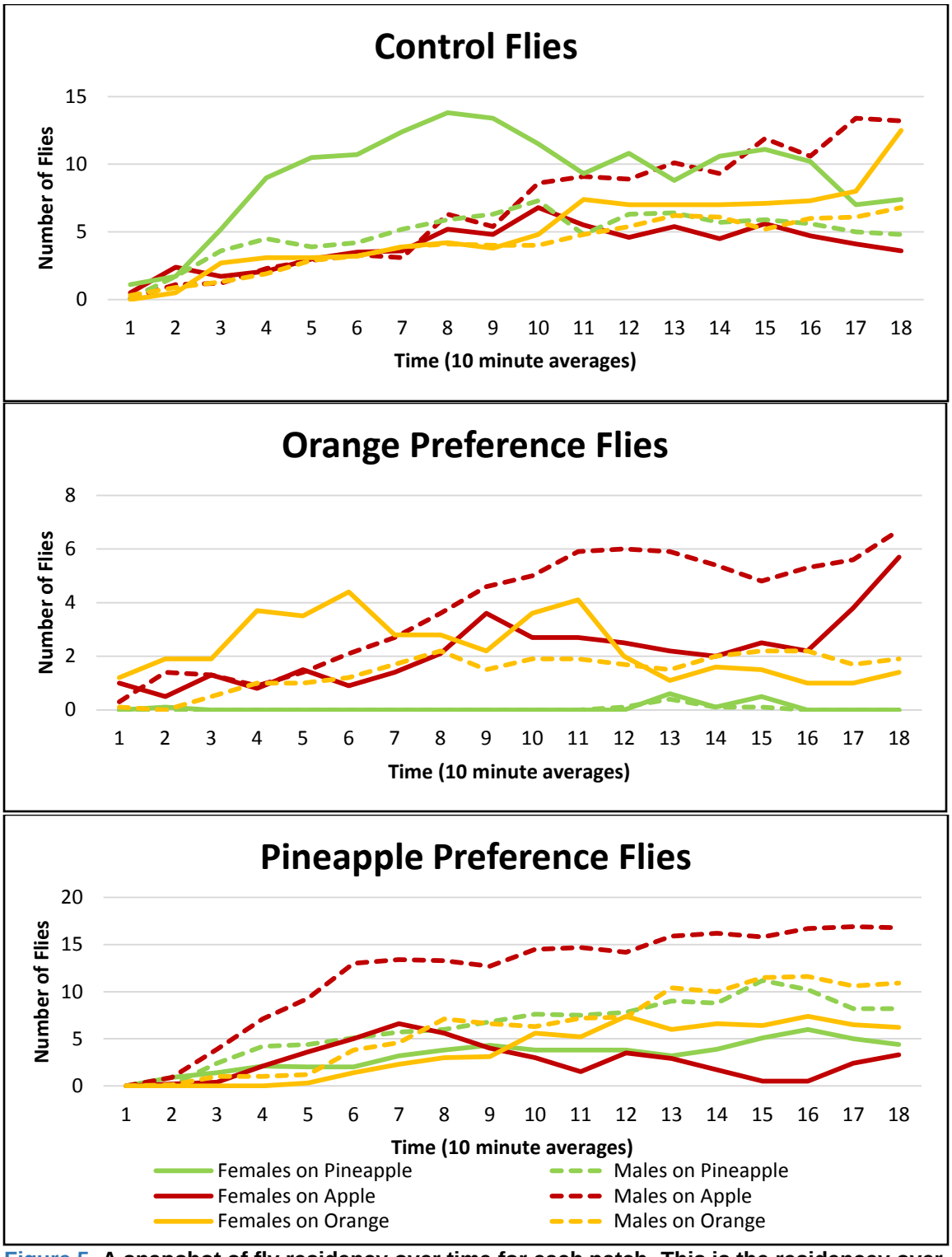


Figure 5. A snapshot of fly residency over time for each patch. This is the residencey over time data of a single triplicate (N=1). There is one graph for each of the preference and control lines within the triplicate. Each colored line represents one patch: green is the pineapple patch, red is the novel patch (apple), and gold is orange. Males are represented by dashed lines, and females, by solid lines. The values are total fly counts at 1 munute intervals were averaged over a 10 minute intervals. On the x-axis, minutes are represented as 1 time bin per every 10 minutes. Sample size: N=1.

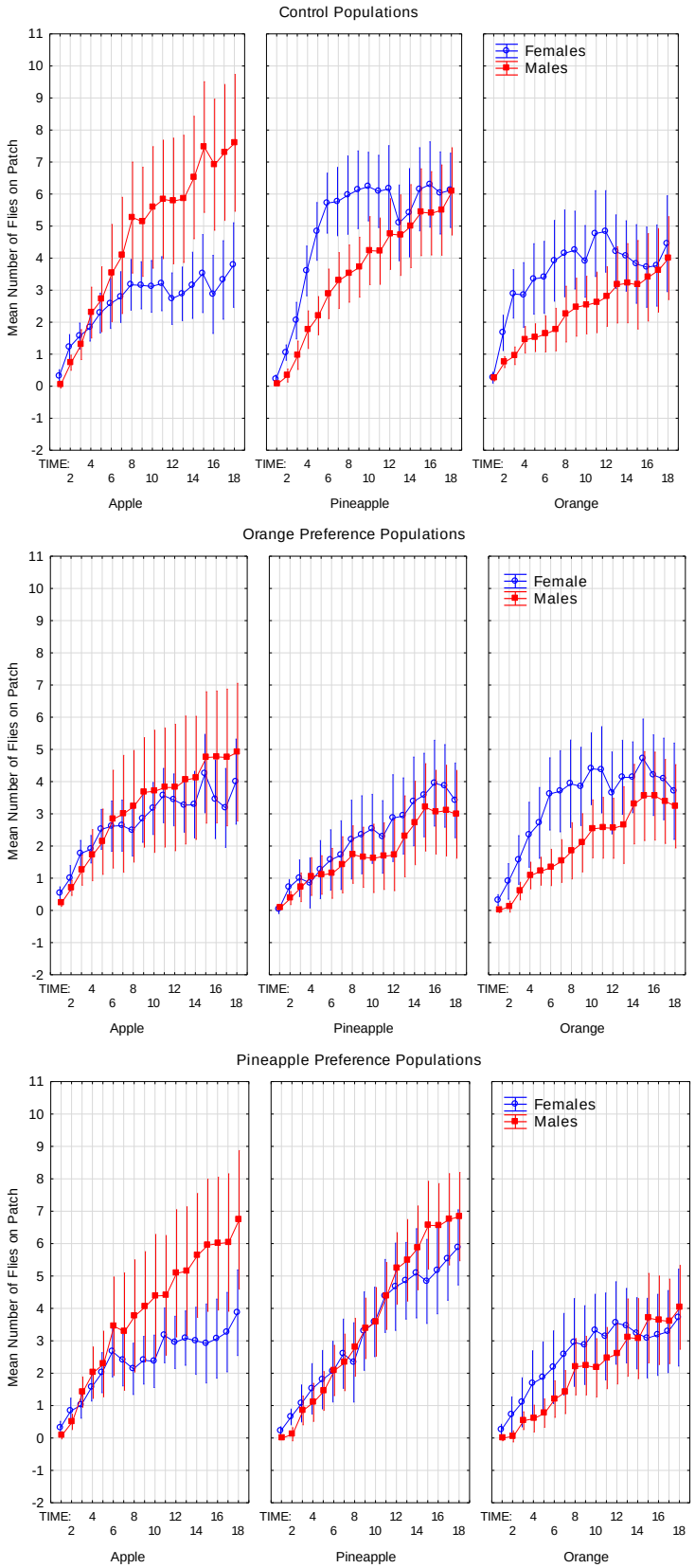


Figure 6. Fly residency for each patch over time. This is the residencey over time data for the entire dataset. There is one set of graphs for each of the preference and control lines.

Each patch is graphed seperately within the graph set. Males are represented by solid red squares, and females, by open blue circles. The values are average fly counts (between lines). The between line averages were taken from total counts at 1 minute intervals that were averaged over a 10 minute intervals. On the x-axis, minutes are represented as 1 time bin per every 10 minutes. Sample size: N=18 (6 lines per selection history).

Table 3. Repeated Measures Full Factorial ANOVA for Fly Residency over Time

Effect	SS	df	MS	F	*p*
Intercept	*18110.91*	*1*	*18110.91*	*62.67906*	*0.000001*
Selection History	361.46	2	180.73	0.62549	0.548390
Error	4334.20	15	288.95		
Sex	2.36	1	2.36	0.02613	0.873750
Sex * Selection History	70.35	2	35.17	0.38862	0.684626
Error	1357.63	15	90.51		
Patch Type	152.84	2	76.42	1.30562	0.285963
Patch Type * Selection History	294.78	4	73.70	1.25908	0.307755
Error	1755.95	30	58.53		
Time	*3442.30*	*17*	*202.49*	*48.70952*	*>0.000000*
Time * Selection History	126.48	34	3.72	0.89489	0.639428
Error	1060.05	255	4.16		
Sex * Patch Type	*488.70*	*2*	*244.35*	*5.10708*	*0.012335*
Sex * Patch Type * Selection History	103.25	4	25.81	0.53950	0.707852
Error	1435.36	30	47.85		
Sex * Time	*159.89*	*17*	*9.41*	*5.31284*	*>0.000000*
Sex * Time * Selection History	47.98	34	1.41	0.79712	0.783731
Error	451.42	255	1.77		
Patch Type * Time	105.58	34	3.11	1.03187	0.421245
Patch Type * Time * Selection History	141.66	68	2.08	0.69220	0.969501
Error	1534.86	510	3.01		
Sex * Patch Type * Time	*99.12*	*34*	*2.92*	*2.09683*	*0.000381*
Sex * Patch Type * Time * Selection History	40.25	68	0.59	0.42574	0.999983
Error	709.06	510	1.39		

Sample size: N=18 (6 lines per selection history).

DISCUSSION

Our flies were evolved for innate bias under a specific context: oviposition on either orange or pineapple. We aimed to test the generality of this bias. We found that oviposition preference does not always carry over to other contexts. In particular, there is a clear distinction between a female fly's oviposition

preference and her courtship preference. The differences in preference also carry into residency and to male choice. Males more frequently choose the novel option that the flies were never exposed to during the experimental evolution, whereas females did not display this preference. This difference in the preference of males may be influencing a number of aspects of female behavior.

Oviposition

The trend to prefer orange over other substrate types is consistent with previous natural history studies on *Drosophila melanogaster* for oviposition preference (Dweck et al. 2013). This is evident in the control flies, where they laid more eggs than expected on orange than pineapple. The orange flies laid eggs on orange more than expected, while they lay eggs on pineapple about as much as expected. The Pineapple flies laid more eggs on pineapple, but still lay the most eggs on orange. This fits with preference data in the selections, where pineapple flies started with a preference for orange and have moved their preference towards pineapple over time (see Methods section for preference scores). Across the treatments, flies do not prefer the novel substrate for laying eggs. The larger spatial scale of this test as well as the addition of a novel patch may be affecting the oviposition preferences of females, but the testing of these effects would require an additional experiment.

There is also the factor of patch discovery. While the fly's perception of citrus is known to be very simple, and involves a single gene, the fly's perception of pineapple is unknown (Dweck et al. 2013). This means that pineapple selected lines may differ in how these flies discriminate between odor cues over longer

distances. The gene sequence data for these flies and economics behind their choices are currently under study within the lab; however, further study on cue perception may be necessary to fully interpret these results.

Courtship

Contrary to the observed oviposition preferences, all selection histories seem to have shown a different pattern of substrate preference for courtship. Although, all flies seem to avoid the orange substrate more than expected for courtship, the orange-selected flies seem to prefer using the novel substrate over pineapple, which is an opposite trend to the other lines. This suggests that orange-selected flies may be influenced by some unknown factor. For instance, an innate bias for courtship substrate preference or a mechanism related to how that bias evolved (i.e. sensory bias) could have been inadvertently influenced during selection for these lines, but not the others. Because courtship was a much rarer and much more variable behavior across trials than oviposition, or fly residency, it is very difficult to make many conclusions from these data without a larger sample size.

Residency

In general, females were observed more frequently on the orange substrate, and males were more frequently observed on the novel substrate (Figure 4). The higher presence of females on the orange patches is paralleled by their use of orange substrate for oviposition (compare Figure 2). We expected this because the females of course need to be present on a given patch in order to lay eggs. We also expected an interaction between the patch type and the

flies' selection history; however, this interaction was not significant (Table 2: $F_{4,30}$=1.79, p=0.156). Patterns in patch use by males and females are also reflected in their patch residency over time (Figure 6). Males and females tend to find patches at the same rate. However, females tend to accumulate more on orange and pineapple patches, while males are more abundant on the novel apple substrate. This may be because the selective conditions for these lines was performed on female choices, and the context of their selection was not generalizable (i.e. it does not affect a broad sensory bias), thus the males did not conform to the evolved biases of the lines. However, one question remaining is why the males prefer the novel substrate. Perhaps it is the result of a decoy effect, or a difference between the choice context of three options in this assay versus the two options in the selections that the males are particularly susceptible to. There could also be some other unknown preference the males possess, such as an overlooked nutritional metric found in apples like a higher sugar concentration.

Issues of Sample Size and Variance

Fruit flies are a system that is notorious for its high variance (e.g. Mery and Kawecki 2003). This, among other factors such as their size, makes flies a challenging system in which to study behavior. On top of this, the data collected for this experiment have a small sample size. Since the 18 lines, or 6 comparable triplicates, were only tested once, unintended variance due to aspects like humidity or small differences in rearing can be higher than it would be with more replicates of each triplicate (N=18, 6 lines per selection history). This added noise

could mask important patterns, or even show patterns that do not actually exist. This is true of all aspects of the data. It is especially true of the courtship data. Some of the interactions, such as the interaction of patch type and selection history in Table 2, were expected to be significant, but are not. Further study and replication on these fly lines will be needed to confirm these surprising results.

Bringing It All Together

Although flies prefer orange or pineapple substrates for oviposition (Figure 2), we found a surprising use of the novel substrate. Notably, male flies spent the most time on the novel substrate (Figure 4, Figure 6). This also coincides with its notable use for courtship among the orange-selected lines (Figure 3). Additionally, examination of the videos showed that males tend to spend their time on one patch as females came and went. No quantitative data have been gathered to describe this phenomenon, but in combination with the other data, it suggests that females are coming to males to be courted. This is contrary to a general assumption that males would go to females to court. In other words, males seem to be deciding the place of courtship. The selection on these lines has been purely on females, so perhaps the discrepancies between oviposition and courtship preference is due, at least in part, to the actions of the males. Previous studies have noted that fruit flies utilize grouping pheromones (Bartelt et al. 1985). Flies produce a number of pheromones that can act in an aggregation function, and it is known that at least one compound produced by males can influence when females oviposit (Lin et al. 2015). The data we present here, in a novel patch choice context, suggest that males may be attracting females to

patches due to a chemically-mediated lekking type behavior.

Future Analyses and Directions

In order to strengthen the validity and breadth of this study, we will need to increase our sample size. The first step would be to add replicate trials of each line, to eliminate a more accurate series of measures. We could also add more selected lines. In our lab we have another set of strong preference lines. Their selection history is only slightly different, and their ovipositional preferences on orange and pineapple are identical. However, the lines would still be difficult to compare because of the differences in their selection. Further work with the current dataset will include traditional patch use metrics such as time of first arrival to patches and tracking individual fly residency. Flies with a strong innate preference should arrive at their preferred patch faster than other patches (Stephens and Krebs 1986). Additionally, individuals should remain on a patch longer if they prefer it (Stephens and Krebs 1986). Furthermore, while present study only considered flies as groups of males and females, further information can be gleaned by looking at how one individual, or a social group of individuals, is using patches.

Further study still needs to be done in order to understand large scale patch use in fruit flies. This is especially true for adult flies due to the fact most patch studies have been done on larvae (e.g. Dukas 1999; Scherer et al. 2003). Furthermore, more attention needs to be paid to the males' behavior, and how they influence the patch use of the entire population. And lastly, it is important to expand the study of fly patch use into even larger scales. Although this study

looks at patch use in a large flight cage, the cage in no way reflects the vast environment in which wild fruit flies navigate. Furthermore, despite their extensive use in genetics, fruit fly field and natural history studies are sorely lacking. Their size and a lack of practical field tracking technology may be partly to blame, but field studies on these surprisingly under-explored animals would greatly improve our understanding of how flies and other animals use patches.

Chapter 3

Effect of Predators on Patch Use and Innate Preference in *Drosophila melanogaster*

INTRODUCTION

Survival until one is able to reproduce is a key to an animal's fitness. One factor that can influence an animal's survival is a predator. Predators kill and consume other animals, thus terminating that animal's future ability to reproduce. However, animals cannot avoid predators completely and still be able to maximize their fitness. In order to forage or find mates, an animal must expose itself to predators. This leads to a trade-off between feeding and foraging (Longland and Price 1991). These trade-offs are often managed behaviorally as they are in fear ecology, and result in antipredator actions such as avoidance (Ripple et al. 2001).

The fruit fly, *Drosophila melanogaster*, is a low trophic animal, and is thus prey to many generalist predators. As both larvae and adults, they eat rotting fruit (Jacobs 2003). Rotting fruit naturally appears patchily in a fly's native environment, and inevitably patches differ in the number of predators. The quality of a patch may vary not only in relative safety, but also in nutrition and preference. It is optimal for female fruit flies to choose the best place for their eggs between these patches of varying quality.

As I discussed in Chapter 2, there are several factors that can influence the oviposition preference in flies. In that chapter, I explored the effects of spatial scale and a decoy patch on innate preference by introducing flies into a large flight cage. These flies have regularly been tested in small scale boxes. There is a reason for this. Large scale patch decisions require higher search and sampling costs (see Stephens and Krebs 1986). This can bias or hide any subtle

patterns in decision making when animals choose a patch. This is why most patch use studies benefit from small scale assays. Additionally, other assays in our lab study the patch use of these flies on a small scale. By keeping this study in a small scale, I can more easily compare the results from this study to the others. Additionally, novel patches can also influence patch use decisions so only the two patches present in the flies' selection were used, not three patches like in Chapter 2. This also enables for an easier comparison of this study to the other two patch studies prevalent within our lab.

In addition to scaling and decoy effects, males and females can differ in how they use patches (see Chapter 2). This can also obscure patterns or weaken the predation effect. For example, if a predator can more readily capture males, the predation pressure will weaken on the females. This is why many researchers will test only males or females in one assay. Separating males and females will also change the behaviors of the flies. For example, females may spend more time being courted and less time laying eggs, thus biasing any oviposition preference data. This is another reason to test only female flies when testing for the effect of predation on oviposition preference.

In this experiment I tested the same experimentally evolved fly lines from Chapter 2, which have innate preferences for either orange or pineapple substrates. I examined how they might choose to adjust where they lay their eggs based on whether there is a predator over their preferred egg laying substrate or not. I hypothesize that: (1) flies will prefer to stay in the safe patch without predators, but (2) will risk laying more eggs on a patch with a predator if it

has their preferred substrate.

METHODS

Subjects and Predators

I used flies that have been experimentally evolved to have an oviposition preference for either orange or pineapple substrate (Dunlap and Stephens 2009). These are the same selected lines described in Chapter 2, but approximately 12 generations later. These lines are experimentally evolved for an innate preference for laying eggs either on orange or pineapple substrates. These lines include a total of 12 selected lines (6 orange-selected and 6 pineapple-selected) plus 6 control lines. Each line has been blocked as a triplicate with one selection type line each. These triplicates have always been selected and assayed together to allow for better comparison between selection treatments. One of each type has been selected together to form a triplicate. Each of the 6 triplicates was assayed together.

Eggs from each line were collected into 4 vials of 80 eggs each from standard fly food within a few days of a selection. These flies were reared in an incubator at 24°C in a 24-hour light cycle. Within four days of emerging as adults, and only a few hours prior to the assay, I sexed approximately 100 females. There were some issues with getting all of the eggs to hatch in a vial for the flies to reach maturity, so the exact number of females varied, but there were usually 100 flies with one case with only 51 flies (range=49, median=100). I sexed the flies with a vacuum suction tube. Occasionally a male would be accidentally

sucked up the tube, and a few males were included. I ensured that there were no more than three males in the entire group by watching for mistakes. Due to limited time, it was impractical to re-sex the flies to remove one to three males, but if there were more than three males, I re-sexed the flies to remove the males. Sexing errors were rare, so there were several assays without males. The sexed flies were kept in a glass vial without food until the assay.

For the predator in this experiment, I used Chinese mantids (*Tenodera sinensis*) that were just the right size to be interested in fruit fly prey. This is after about the second molt, where the mantid measures approximately 25mm, +/- 5mm, in length from head to the tip of the abdomen). Mantids were housed singly to prevent cannibalism. Additionally, a small twig was placed in each cage to give the mantid a place to rest, and to prevent mis-molting. The mantids were fed around 7 flies every three days. They were sprayed with water at this time to prevent dehydration. In addition to being fed sparsely, they were kept in an incubator set to 16°C to stunt their growth so that they could be used for more assays. They were also kept on a 12-hour light-dark cycle in order to avoid any adverse effects of a 24-hour light cycle. Their incubator was also the same one in which the assays themselves were run. Mantids were used in assays when they had not been fed for 2 or 3 days.

Apparatus and Camera Setup

Assays were performed in specially designed custom built clear Plexiglas boxes, which are referred to here as the apparatus (see Figure 7). The boxes were divided into two chambers, and separated by a movable door that could be

raised or lowered with a string. The doorway was also partitioned with a mesh screen that was only permeable to the flies. The mesh was affixed to the right side of the doorway. Each chamber consisted of one patch. The patch on the left was always the predator patch, and the patch on the right was always the safe patch. The predator patch was assigned one of two patch substrate types: orange or pineapple. The safe patch always had the other substrate. For example, if the predator patch had orange, the safe patch had pineapple. The patch itself consisted of a square plate filled with 20 mL of orange or pineapple flavored agar. The agar substrate in each plate was prepared with frozen fruit juice with the same recipe that was used during the flies' selection (see Chapter 2). Each plate was placed on a removable tray, and the edges around the tray lip were sealed with a strip of parafilm. Each apparatus only contained one line of flies at a time.

The assays were recorded in digital HD movies with Sony Handycams (see Figure 8). The cameras were held above each apparatus with adjustable camera arms. The lens of the cameras was focused so that the entire apparatus was clearly visible. White paper was placed under the apparatus to improve visual contrast in the videos. The recordings started just prior to opening the door at the assay's start, and ended just after the door is closed at the assay's end. With a few exceptions due to technical difficulties, each video was approximately 2 hours long.

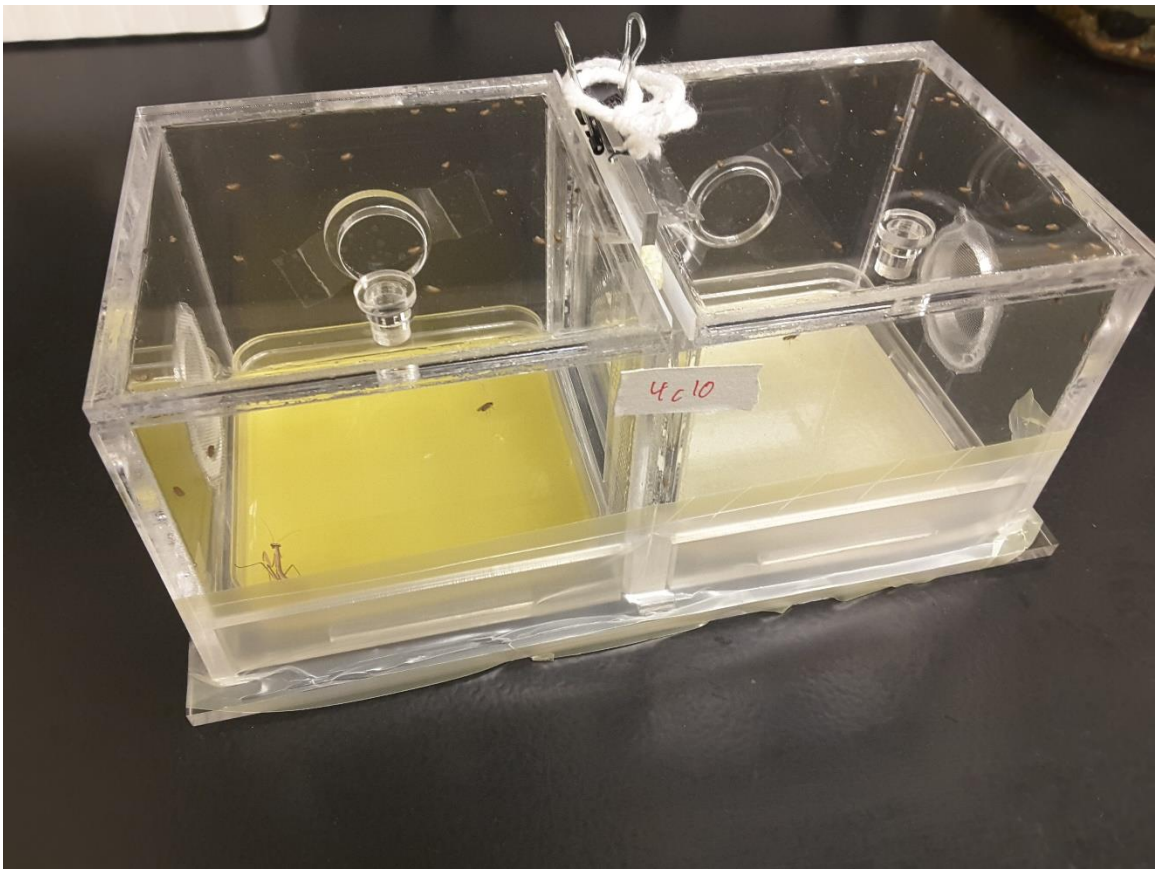


Figure 7. The apparatus. Here the assay has just ended and the door is closed. Note how the door is wedged into a slot in the wall that contains the opening. A fly-permeable only mesh has been affixed to the right side entrance to the door-way. Also note the coiled yarn with a clip that is used to hold up the door when it is open. Prior to the assay, flies were "knocked" into the right side (safe patch) with a funnel via a small hole that is plugged here with a clear plug. Now that the assay has been run, the flies have been trapped in either chamber with one of the patches. The chamber on the left (predator patch) always holds the mantis. The patch substrate flavor on either side is chosen randomly, but the flavors are switched when the same line is assayed again. The patches are removable by trays, which are sealed here with a parafilm strip.

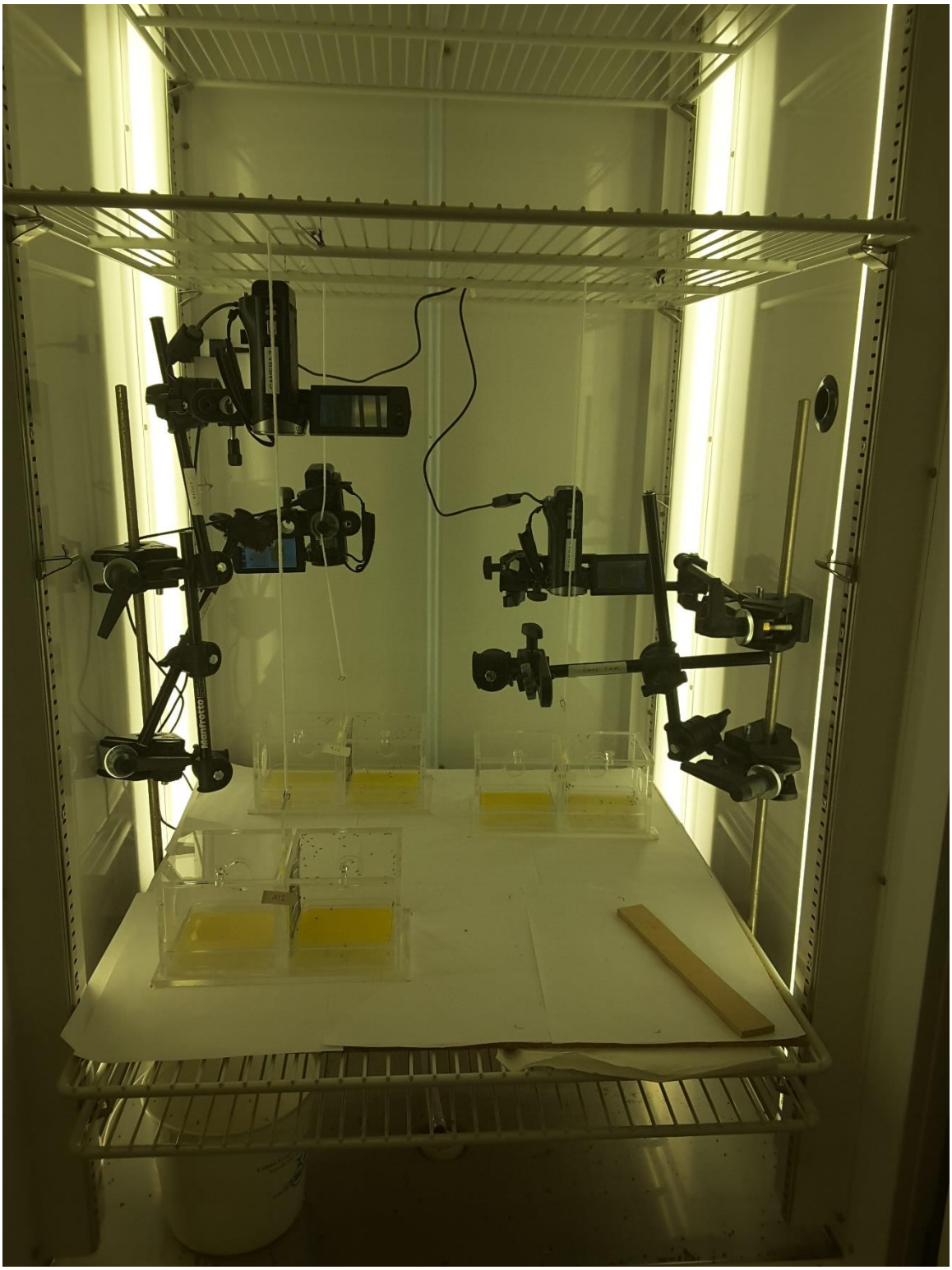


Figure 8. The camera set-up. Here is a single triplicate that has just started its assay. Note how the cameras are positioned above each patch, and how the yarn is holding the doors open with a clip affixed to the shelf above.

Experimental Design and Procedure

All three lines in a triplicate were always tested simultaneously. Each triplicate was tested for 2 hour-long assays either in the late morning (approximately 10:30 to 12:30) or the afternoon (approximately 12:30 to 14:30). Female flies for each line were sexed just prior to the assay, and were “knocked” into the assay apparatus. “Knocking” is done by gently tapping flies in a vial into a small hole at the top of the apparatus with a funnel. Flies were always placed into the safe patch chamber, which was opposite to the predator patch chamber with the mantid. The door between the chambers remained closed except for the duration of the assay, when flies could move freely between the patches. Because flies were often still able to get around the edges of the door, the flies were “knocked” into the apparatus just seconds prior to the assay’s start.

For the assay, the entire apparatus was placed in the mantid incubator, which was set at 25°C for the duration of the assay. During this time, the cameras recorded the activities of the flies in the apparatus for later analysis. At the end of the assay, the flies and mantids were knocked out with cold and counted. Mantids were placed back into their cages for use in a future assay.

Data Collection and Analysis

After the assay was completed, the remaining flies that the mantis had not eaten were counted. These counts were acquired manually by counting out the flies after they had been knocked out. I counted flies in both the safe and predator patch chambers. The doorway was part of the predator patch, but was counted separately from the rest of the patch. This was done just in case there

was another trend that could be observed with these data, but for the results below, it was treated simply as another part of the predator patch.

The agar plates were scanned with an EPSON scanner so the eggs from both the safe and predator patches could later be counted for oviposition data using the Multipoint tool in ImageJ. Egg plates were counted according to their plate number, so that the counting procedure was blind to the plate's identity as a predator or safe plate, and to the line's selection history. This minimized any counting bias.

Data were not extracted from the videos, but there are several measures that can be taken. These include fly mortality, individual decision making over time and residency. Further data can also be obtained from the egg plates, such as the special placement of eggs. I will elaborate upon these measures at the end of the discussion.

Both the oviposition and residence raw counts were used to calculate the preference index. I used the same formula as is described for the flies' selection assays (see Chapter 2), but with the predator patch as the focal patch instead of the orange substrate patch. For oviposition, the change makes the formula as follows: (eggs on predator patch- eggs on safe patch)/ (eggs on predator patch + eggs on safe patch). Likewise, for residency it is: (flies on predator patch- flies on safe patch)/ (flies on predator patch + flies on safe patch). As with patch flavor preference, a 1 indicates a strong preference for the predator patch, and a -1 indicates a strong preference for the safe patch. In this case, a value of -1 can be described as a strong aversion to the predator patch. Oviposition and residency

preference values were analyzed using STATISTICA in a repeated measures ANOVA. This type of ANOVA accounts for repeated measures on a single unit, which is each population in this case. Here the factors included selection history and predator patch. For a more detailed explanation of the repeated measures ANOVA statistical values, see the methods section of Chapter 2.

RESULTS

Flies showed a universal preference for the safe patch. Or in other words, flies were universally averse to the patch with the mantid, regardless of their selected preferences. This held true for both fly residency and oviposition as evident by the prevalence of all negative preference values, which indicate a preference for the safe patch (see Figures 9 and 10 respectively). This is not to say the flies avoided the predator patch entirely, but all egg and fly residency counts tended to be less on that side. The average value for fly residency on the predator patch was 34 flies, and the average number on the safe patch was 54 flies. Some differences were observed between how much the flies preferred the safe patch when comparing the two patch flavor types with a predator present.

Residency

Some trends may be observed in fly residence (Figure 9). The control flies seemed to show a similar degree of safe patch preference to the selected flies. Control and orange-selected lines also both seem to show similar discrepancies between their aversion to orange and pineapple predator patches. Both treatments may be more averse to visit orange patches with a mantid than similar

pineapple patches. As with oviposition, pineapple-selected flies seem less averse to orange patches with a mantid than equivalent pineapple patches. Despite what may be viewed as potential trends, this data set does not have any significant values or interactions (see Table 4).

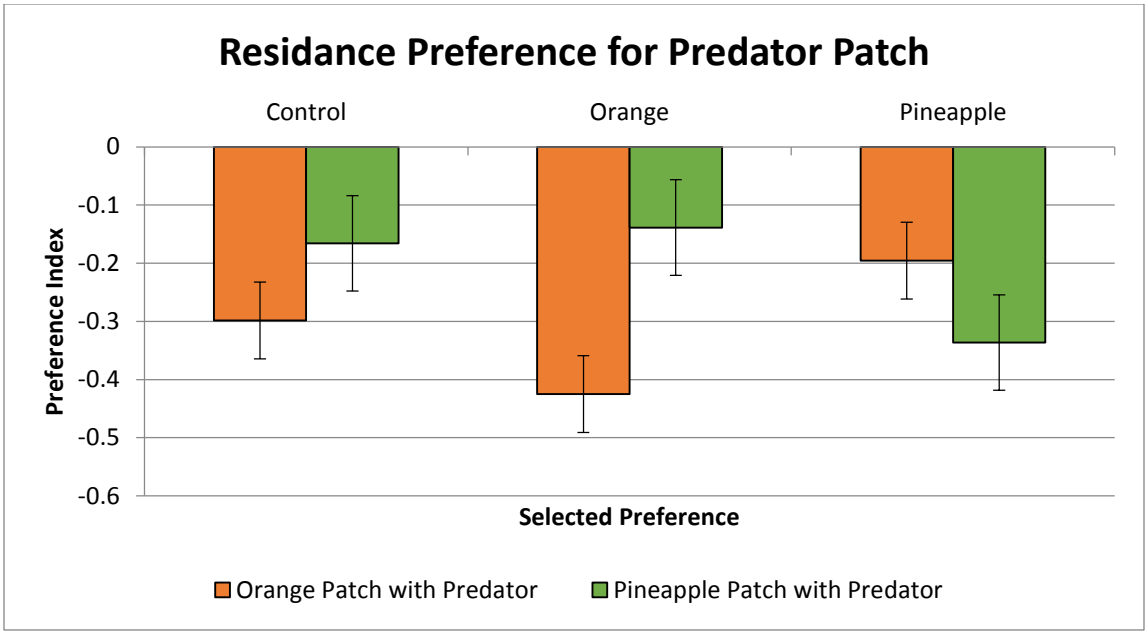


Figure 9. Residency preference in the presence of a predator. Presented here are the between line averages for each selection history's preference for residency on the predator patch. Selection histories are shown along the x-axis. A value of 1 indicates a strong preference for the predator patch, and a value of -1 indicates a strong preference for the safe patch. Values inbetween show a gradient of the degree of preference with zero indicateing that there is no preference. Predator patches could have an orange substrate (orange bars), or a pineapple substrate (green bars). Sample size: N=6 lines per selection history with 1 trial per focal predator patch type; standard error for orange patch with predator=0.066057582 and pineapple patch with predator=0.08209686.

Table 4. Repeated Measures ANOVA for Residency

Effect	SS	df	MS	F	*p*
Intercept	*2.313654*	*1*	*2.313654*	*29.28084*	*0.000072*
Selection History	0.045030	2	0.022515	0.28494	0.756038
Error	1.185239	15	0.079016		
Mantis Location	0.043592	1	0.043592	0.34007	0.568452
Mantis Location * Selection History	0.244645	2	0.122322	0.95427	0.407274
Error	1.922756	15	0.128184		

Sample size: N=6 lines per selection history with 1 trial per focal predator patch type.

Oviposition

In general, control lines in this data set may be less averse, or more risk-taking, to the predator patch than the selected lines in terms of oviposition. This can be observed by an apparently weaker preference for the safe patch in oviposition when compared to residency (Figure 10, Table 5). Additionally, when comparing treatments to their aversion to orange or pineapple predator patches, both control and orange-selected lines seemed to treat either flavor with similar degrees of aversion within treatments. Both treatments may be more averse to laying their eggs on orange patches. However, the pineapple-selected lines showed a seemingly less pronounced discrepancy in their aversion to either flavor. When given an orange patch with a mantid, pineapple-selected flies seemed to not prefer the two patch types differently, but may be potentially less averse to laying on orange predator patches. There seem to be trends; however, there are no statistically significant values to support any of these possible trends (see Table 5).

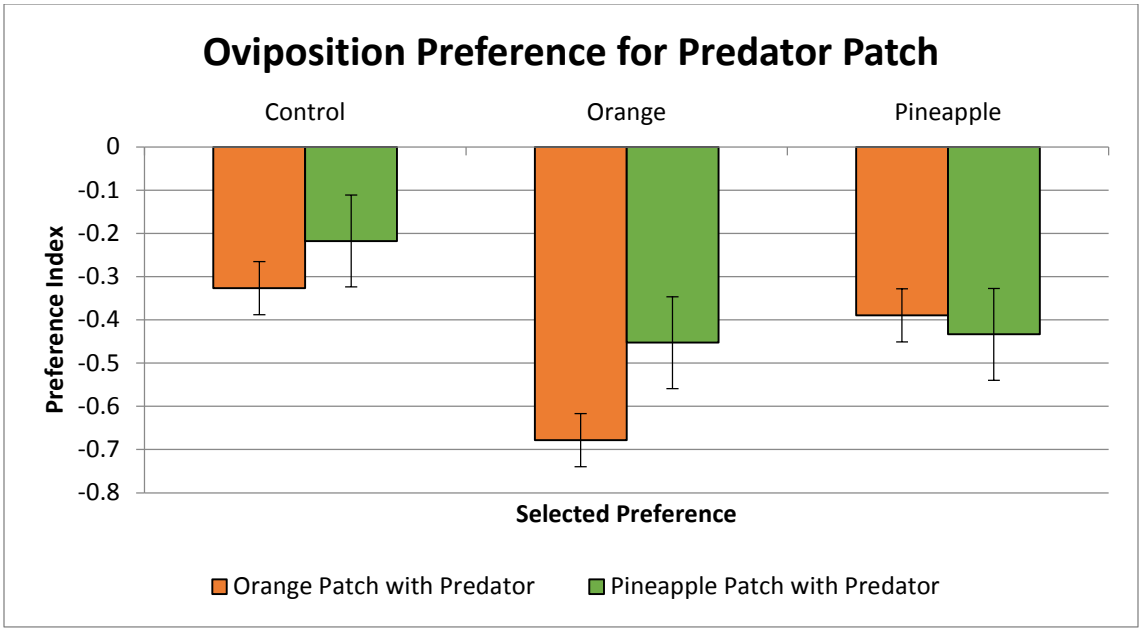


Figure 10. Oviposition preference in the presence of a predator. Presented here are the between line averages for each selection history's preference for oviposition on the predator patch. Selection histories are shown along the x-axis. A value of 1 indicates a strong preference for the predator patch, and a value of -1 indicates a strong preference for the safe patch. Values inbetween show a gradient of the degree of preference with zero indicateing that there is no preference. Predator patches could have an orange substrate (orange bars), or a pineapple substrate (green bars). Sample size: N=6 lines per selection history with 1 trial per focal predator patch type; standard error for orange patch with predator=0.061411045 and pineapple patch with predator=0.106262919.

Table 5. Repeated Measures ANOVA for Oviposition

Effect	SS	df	MS	F	*p*
Intercept	*6.244440*	*1*	*6.244440*	*45.38696*	*0.000007*
Selection History	0.516147	2	0.258073	1.87578	0.187459
Error	2.063733	15	0.137582		
Mantis Location	0.225168	1	0.225168	1.80927	0.198581
Mantis Location * Selection History	0.021786	2	0.010893	0.08753	0.916660
Error	1.866793	15	0.124453		

Sample size: N=6 lines per selection history with 1 trial per focal predator patch type.

DISCUSSION

As I expected, flies have a strong aversion to the patch with a predator. They are most certainly aware of the predator's presence. The mode of their awareness is most likely varied, but previous studies indicate it is probably by the predator's shadow, a vibration caused by their movement, or some olfactory cue such as volatiles released with conspecific death (Iliadi 2009). For the purposes of my study, the mode of detection may or may not explain the observed patterns.

Residency

One would expect that flies may prefer to take fewer risks on orange substrate regardless of selection history. As the data in Chapter 2 suggest, flies may lay eggs on orange substrates, but they do not prefer it for doing other behaviors. Additionally, previous studies show that flies have an oviposition specific preference for citrus (Dweck et al. 2013). One would expect that the flies would show a weaker preference for safe patches with pineapple substrate, and be more risk-taking on them when a predator is present. This is what the data suggest is happening with the orange and control lines. However, the pineapple-selected lines for fly residency appear to be more likely to take risks on orange patches than on their preferred oviposition substrate of pineapple (Figure 9). The statistical power here is still weak, maybe less insignificant as the oviposition data (Table 4). This suggests that there is something going on even if there is too much noise to see it.

Oviposition

Even if the flies are not spending time on their preferred egg laying substrate, it is still optimal for them to lay eggs on it. The orange and control lines should lay eggs on orange, but might do so by sneaking over to an orange patch, then sneaking back. The same is expected for the pineapple preference lines laying on pineapple. However, the data suggests a reversal of innate oviposition preference in the presence of predators. Control lines seem to have a stronger preference for orange safe patches when compared to pineapple safe patches, when it should show the opposite (Figure 10). Orange preference lines also may show this same difference in preference between the two patch types. They also seem to prefer safe patches more strongly. Pineapple lines may also have less preference for going to their preferred oviposition substrate of pineapple when it has a predator; however, this pattern seems to be weaker or non-existent. Instead, pineapple lines may have no particular difference in how they prefer orange or pineapple predator patches. If there really is a preference reversal, this is surprising and contrary to my initial hypothesis. The data may suggest flies essentially taking more risks on patches that they do not innately prefer for oviposition. However, with the weak statistical power of the current data set (Table 5), this trend is unconfirmed, and I am left to speculate.

Issues of Sample Size and Variance

As discussed in Chapter 2, the data set studied here has limited statistical power due to a small sample size (6 lines per selection history with 1 trial per focal predator patch type) and large variance. As with that study, additional

replicates with each line may reduce the variance resulting in, along with the increased sample size, stronger statistical power. This would make any significant trends, especially the possible interaction in the fly residency data, emerge.

Bringing It All Together

In the light of no statistically significant effects, it is difficult to say what any trends might be; however, I will postulate trends that seem to be hinted at in the data. Perhaps predator cues, at least in part, drive the trends observed in this study. Certainly predator cues drive the flies' general aversion to the predator patch. This drive may also hold for the possible trends in oviposition and fly residency. One way a cue might be important to determining the flies' actions might be due to the curious response by flies to conspecific death smell. In small concentrations, the volatiles released by flies as they are being killed or as their bodies decay can actually attract other flies, but in large concentrations it deters them (Iliadi 2009). It would be interesting to see if the flies exhibit a preference reversal for the entire two hours, or if their preference varies over time. It is possible that, if counted at various time steps, one could witness an alteration in preference. Perhaps before conspecific death cues accumulate, the flies' preference follows their selected innate bias. Maybe low concentrations lessen the flies' aversion to the predator patch, and then increase it after the odor accumulates. These possibilities could be investigated with the video data collected in this experiment, and further studies.

In addition to the overall trend for flies to avoid the mantid, the trend

seems to remain consistent across the two contexts of oviposition and patch residence. This could simply be due to the fact that all the flies are females, save one or two males that may have been added by mistake. As adults, these females are probably mostly choosing where they want to lay their eggs. Because they need to visit a patch to lay their eggs, there may just be a strong correlation between fly residency and oviposition. The oviposition preferences of these flies are not general across contexts (see Chapter 2). Thus there is no reason to think that the similarities between the contexts are due to their selected preference; however, as in fear ecology, prey universally aim to avoid predators (Longland and Price 1991; Ripple et al. 2001). The flies' overarching aversion to the predator patch in both oviposition and residency seems to suggest that the correlation is driven by the flies' preference to avoid predators.

If we assume the possible trends we observe in this data hold, the two contexts differ mostly in the oviposition preferences of the control lines and the extremity of the evasion between the two patch substrates for all treatments. As for the control lines, perhaps the fact that these lines were not under active oviposition preference pressure contributed to their weaker aversion, although the reason for this remains unclear. Furthermore, wild type flies have a pre-existing bias for laying eggs on oranges (Dweck et al. 2013). Work on understanding how these preferences manifest across contexts in the selected lines is still being evaluated by others in the lab.

Future Analyses and Directions

I am currently working to strengthen the current dataset with further

replicates and triplicates. This will help solidify what is actually happening when these flies are choosing patches for where they will spend their time or lay eggs. I am also collecting more data to add additional factors I can examine. By adding more metrics, I will be able to see more of what the flies are actually doing when encountering a predator. In particular, I hope to shed more light on the unexpected observation that flies seem to me taking more risks on substrates they do not prefer for oviposition. Analysis of the videos from the assays will provide data on fly mortality, and individual fly residency and movement over time. I would also like to take a closer look at exactly where flies are laying eggs in the patches. Several studies have shown that animals will vary their spatial use of foraging patches (e.g. Cassini et al. 1991; Longland and Price 1991; Ripple et al. 2001). This raises the possibility that flies may position their eggs differently on the predator patch than the safe patch. They may in fact be laying them just on the other side of the door from the safe patch, where it is easiest to make an escape from the predator.

Outside of this study, there is a general need for more work to be done on fruit flies and predation. The lack of published studies is disappointing. Although there is some work on larvae (Dukas 1999), there is a striking lack of adult studies. Additionally, most fly work focuses on genetics, but sadly neglects the natural history of these animals. Behavioral studies done on flies in a lab environment have already revealed that flies live rich lives, and it is well worth it to come to understand how this rich life came to shape the genes we have come to know through the fruit fly.

References

Bartelt RJ, Schaner AM, Jackson LL.1985. cis-Vaccenyl acetate as an aggregation pheromone in *Drosophila melanogaster*. Journal of Chemical Ecology. 11: 1747.

Bateson M, Healy SD. 2009. Comparative evaluation and its implications for mate choice. Trends in Ecology & Evolution. 20(12):659-664.

Bennett AM, Murray DL. 2015. Carryover effects of phenotypic plasticity: embryonic environment and larval response to predation risk in Wood Frogs (*Lithobates sylvaticus*) and Northern Leopard Frogs (*Lithobates pipiens*). Canadian Journal of Zoology. 93:867-877.

Bishop JA. 1972. An experimental study of the cline of industrial melanism in *Biston betularia* (L.) (Lepidoptera) between urban Liverpool and rural North Wales. Journal of Animal Ecology. 41:209-243.

Brown JS, Kolter BP. 2004. Hazardous duty pay and the foraging cost of predation. Ecology Letters. 7:999–1014.

Brunel-Pons O, Alem S, Greenfield MD. 2011. The complex auditory scene at leks: balancing antipredator behaviour and competitive signaling in an acoustic moth. Animal Behaviour. 81:231-239.

Burnham TC, Dunlap AS, Stephens DW. 2015. Experimental evolution and economics. SAGE Open. 5:1-17.

Cassini MH, Kacelnik A, Segura ET. 1991. The tale of the screaming armadillo, the guinea-pig and the marginal value theorem. Animal Behavior.

39:1030-1050.

Chivers DP, Smith RJF. 1998. Chemical alarm signaling in aquatic predator-prey systems: a review and prospectus. Ecoscience. 5:338-352.

Dukas R. 1999. Ecological relevance of associative learning in fruit fly larvae. Behavioral Ecology and Sociobiology. 45:195-200.

Dunlap AS, Stephens DW. 2009. Components of change in the evolution of learning and unlearned preference. Proceedings of the Royal Society B. 276:3201-3208.

Dweck HKM, Ebrahim SAM, Kromann S, Bown D, Hillbur Y, Sachse S, Hansson BS, Stensmyr MC. 2013. Olfactory preference for egg laying on *Citrus* substrates in *Drosophila.* Current Biology. 23:2472-2480.

Ferrari MCO, Chivers DP. 2009. Temporal variability, threat sensitivity and conflicting information about the nature of risk: understanding the dynamics of tadpole antipredator behavior. Animal Behaviour. 78:11-16.

Gibson WT, Gonzalez CR, Fernandez C, Ramasamy L, Tabachnik T, Du RR, Felsen PD, Maire MR, Perona P, Anderson DJ. 2015. Behavioral responses to a repetitive visual threat stimulus express a persistent state of defensive arousal in *Drosophila*. Current Biology. 25:1401-1415.

Hackland T, Schausberger P. 2014. Learned predation risk management by spider mites. Frontiers in Ecology and Evolution. 2, doi: 10.3389/fevo.2014.00058.

Heithous J, Frid A,Wirsing AJ, Dill LM, Fourqurean JW, Burkholder D, Thomson J, Bejder L. 2007. State-dependent risk-taking by green sea turtles

mediates top-down effects of tiger shark intimidation in a marine ecosystem. Journal of Animal Ecology. 76:837-844.

Hossie TJ, Sherratt TN. 2014. Does defensive posture increase mimetic fidelity of caterpillars with eyespots to their putative snake models? Current Zoology. 60:76-89.

Huffaker CB. 1958. Experimental studies on predation: dispersion factors and predator–prey oscillations. Hilgardia: A Journal of Agricultural Science. 27:795-834.

Huheey JE. 1975. Studies in warning coloration and mimicry. VII. Evolutionary consequences of Batesian-Müllerian spectrum: a model for Müllerian mimicry. Evolution. 30:86-93.

Iliadi KG. 2009. The genetic basis of emotional behavior: has the time come for a *Drosophila* model? Journal of Neurogenetics. 23:136-146.

Iverson JB. 1991. Patterns of survivorship in turtles (order Testudines). Canadian Journal of Zoology. 69:385-391.

Jacobs, SB. 2003. Vinegar flies factsheet. Pennsylvania State University. Revised 2013. (http://ento.psu.edu/extension/factsheets/vinegar-flies) [Easiest access to source is by internet.]

Kauffman MJ, Brode J, Jules ES. 2010. Are wolves saving Yellowstone's aspen? A landscape-level test of behaviorally mediated trophic cascade. *Ecology.* 91:2742-2755.

Keene A, Waddell S. 2007. Drosophila olfactory memory: single genes to complex neural circuits. Neuroscience. 8:341-354.

Lartviere S, Messier F. 1996. Aposematic behavior in the striped skunk, *Mephitis mephitis*. Ethology. 102:986-992.

Latty T, Beekman M. 2011. Irrational decision-making in an amoeboid organism: transitivity and context-dependence preferences. Proceedings of the Royal Society B. 278(1703):307-31.

Lin C, Prokop-Prigge KA, Preti G, Potter CJ. 2015. Food odors trigger *Drosophila* males to deposit a pheromone that guides aggregation and female oviposition decisions. eLife.4:e08688, doi: 10.7554/eLife.08688.

Longland WS, Price MV. 1991. Direct observations of owls and heteromyid rodents: can predation risk explain microhabitat use? Ecology. 72:2261-2273.

Marchinko KB. 2008. Predation's role in repeated phenotypic and genetic divergence of armor in threespine stickleback. Evolution. 63:127-138.

Mendoza E, Colomb, Rybak J, Pfluger HJ, Zarz, et al. 2014. *Drosophila* FoxP mutants are deficient in operant self-learning. PLOS ONE. 9(6):e100648.

Mery F, Kawecki TJ. 2002. Experimental evolution of learning ability in fruit flies. Proceedings of the Natural Academy of Sciences U.S.A. 99:274-279. (doi:10.1073/pnas.222371199)

Mery F., Kawecki T.J. 2003. A fitness cost of learning ability in *Drosophila melanogaster*. Proceedings of the Royal Society B. 270:2465-2469.

Mery F, Kawecki TJ. 2004. The effect of learning in experimental evolution of resource preference in *Drosophila melanogaster*. Evolution. 58:757-767.

Martin J, Lopez P, Cooper WE. 2003. Loss of mating opportunities influence

refuge use in the Iberian rock lizard, *Lacerta monticola*. Behavioral Ecology and Sociobiology. 41:311-319.

Petrusek A, Tollrian R, Schwenk K, Haas A, Laforsch. 2009. A “crown of thorns” is an inducible defense that protects *Daphnia* against an ancient predator. Procedings of the National Academy of Sciences U.S.A. 106:2248-2252.

Relyea RA. 2007. Getting out alive: how predators affect the decision to metamorphose. Oecologia. 152:389-400.

Ripple WJ, Larsen EJ, Renkin RA, Smith DW. 2001. Trophic cascades among wolves, elk and aspen on Yellowstone National Park’s northern range. Biological Conservation. 102:227-234.

Rohner N, Jarosz DF, Kowalko JE, Yoshizawa M, Jeffery WR, et al. 2013. Cryptic variation in morphological evolution: HSP90 as a capacitor for loss of eyes in cavefish. Science. 342:1372-1375.

Ruehl CB, Trexler JC. 2015. Reciprocal transplant reveals trade-off of resource quality and predation risk in the field. Oecologia. 179:117-127.

Scherer S, Stocker RF, Gerber B. 2003. Olfactory learning in individually assayed *Drosophila* larvae. Learning & Memory. 10:217-225.

Shafir S, Waite TA Smith BH. 2002. Context-dependent violations of rational choice in honeybees (*Apis mellifera*) and gray jays (*Perisoreus canadensis*). Behavioral Ecology & Sociobiology. 51:180-187.

Sih A, Giudice MD. 2012. Linking behavioral syndromes and cognition: a behavioral ecology perspective. Philosophical Transactions of the Royal Society B. 367:2762-2772.

Skelhorn J, Rowland HM, Ruxton GD. 2010. The evolution and ecology of masquerade. Biological Journal of the Linnean Society. 99:1-8.

Stankowich T, Cambell LA. 2016. Living in the danger zone: exposure to predators and the evolution of spines and body armor in mammals. International Journal of Organic Evolution. 70:1501-1511.

Stephens DW, Dunlap AS. 2011. Patch exploitation as choice: Symmetric choice in an asymmetric situation? Animal Behaviour. 81:683-689.

Stephens DW, Krebs JR. 1986. Foraging Theory. Princeton, New Jersey: Princeton University Press.

Thomson RL, Forsman JT, Mönkkönen M. 2011. Risk taking in natural predation risk gradients: support for risk allocation from breeding pied flycatchers. Animal Behavior. 82:1443-1447.

Vincent JFV. 2002. Survival of the cheapest. Materials Today. 5:28-41.

Walsh MR, Cooley F IV, Biles K and Munch SB. 2015. Predator-induced phenotypic plasticity within- and across-generations: a challenge for theory? Proceedings of the Royal Society B. 282:20142205.

Westneat D, Fox C, eds. 2010. Evolutionary Behavioral Ecology. Cary, NC: Oxford University Press. 1st ed.

Yamamoto A, Zwarts L, Callaerts P, Norga K, Mackay TFC, Anholt RRH. 2008. Neurogenetic networks for startle-induced locomotion in *Drosophila melanogaster*. Procedings of the National Academy of Sciences U.S.A. 105:12393–12398.

Zuk M, Rotenberry JT, Tinghitella RM. 2006. Silent night: adaptive

disappearance of a sexual signal in a parasitized population of field crickets. Biology Letters. 2:521-524.