

Introduction

Alterations to a population's ecology can contribute to adaptation events by promoting mechanisms that provide the greatest fitness in an environment. These aforementioned promotions occur in the form of molecular modification, specifically through some form of gene expression, and can ultimately have effects on behavioral responses. This process of gene expression depends on environmental conditions, thus similar genes can undergo varying intensities of expression depending on the ecology in question. Long term adaptation events within ecology diverged populations may eventually result in reproductive isolation mechanisms—restricting gene flow and thus preventing hybridization. Once populations have lost the ability to successfully reproduce with one another, they have been said to have undergone a speciation event and can be classified as different species with respect to the biological species concept. Ecology-dependent speciation, otherwise known as ecological speciation, evaluates the barriers to gene flow between populations due to ecologically-based divergent selection (Rundle & Nosil 2005). This approach provides an exceptional explanation for the effects of speciation on insect populations that make use of host plants, such as *Drosophila mojavensis* (*D. mojavensis*).

The *Drosophila* genus serves as an ideal biological model due to their short generation time and ease of handling. They also vary in geographic distribution and host plants, which over millions of years, has resulted in a specialized genetic disposition for species within a given region (Markow & O'Grady 2008). Of the *Drosophila* genus, the species *mojavensis* is a cactophilic fly that inhabits the Southwestern portion of North America and Northwestern Mexico. The species has undergone an ecologically based population divergence and is represented by four different populations that each makes use of a different host cacti for

sexual reproduction and feeding purposes. The cacti are geographically isolated (Fig. 1) and are each a different species, thus are composed of different molecular structures.

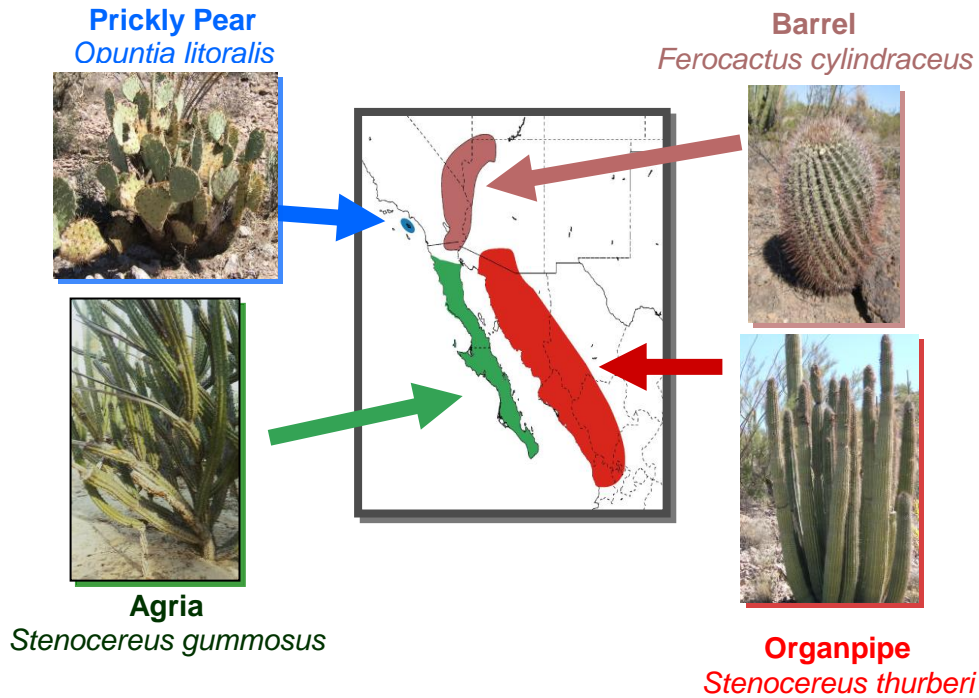


Fig 1. Map of the Southwestern USA and Northwestern Mexico with geographic distribution of host cacti for *D. moj.* Population, host plant pairings are as follows: Catalina Island – Prickly Pear; Mojave – Barrel; Baja – Agria; Organpipe – Organpipe.

Some overlap exists between organpipe and agria within the Baja peninsula, however, the host plant concentration consists mostly of agria.

Adaptation from Matzkin et al. 2012.

The *D. mojavensisavensis* populations have recently diverged from its neighboring sister species *Drosophila arizonae*. It has been estimated that the Baja California population, which feed and reproduce atop *Stenocerus gummosus* (agria cactus), diverged from an ancestral species native to the Sonoran desert around 230,000-270,000years ago (Smith et al. 2012). Divergence within this mainland Sonora-Arizona population has been estimated to have occurred sometime later, about 117,000-135,000 years ago (Smith et al. 2012, Matzkin 2014). From these phylogenetic analyses it has been deduced that Baja California is the ancestral *D. mojavensisavensis* population and agria the ancestral host.

D. mojavensisavensis do not make use of the living cactus tissue; rather, they only interact with the necrotic tissue of a naturally rotting cactus (Fig 3). This necrosis provides a suitable environment for *Drosophila* larva and a food source in the form of fermenting yeast.

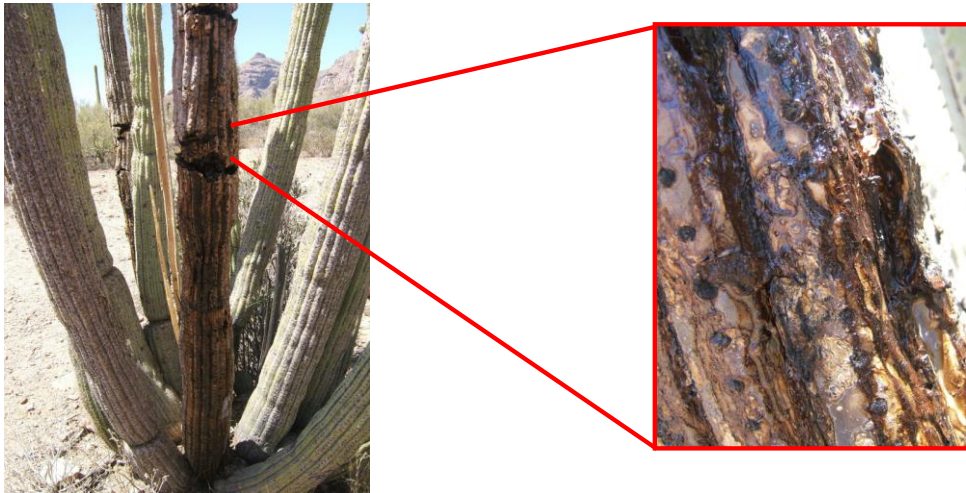


Figure 3. Rotting organpipe cactus, host of *D. mojavensis* Sonora

The cactus necrosis poses threats to insects and requires massive amounts of enzymatic adaptation by the *D. mojavensis* populations to become usable as a host plant (Matzkin 2005). Additionally, the four cacti have differing molecular compositions and thus have facilitated differing adaptation events within the respective *D. mojavensis* populations (Heed 1978, Matzkin 2005, Matzkin et al 2006, Matzkin 2008, Matzkin 2014). The difference in adaptation events has caused each respective population to become specialists to their own cactus, thus they display high levels of fitness for it and relative diminished fitness for other population's host cactus (Matzkin 2005). Host specialization has made it imperative that the respective populations locate their own host cactus' rot in order to maximize individual fitness. A combination of ecological specialization and local adaptation also drives sexual isolation. Reproductively capable individuals, for a given population, are more likely to seek out their respective cactus than that of another populations; thus an individual is more likely to mate successfully on its own cactus instead of another population's cactus.

Individual *D. mojavensis* use olfactory cues to locate their host plants. Necrotic tissue yields a large concentration of short-distance olfactory stimuli in the form of alcohols produced by fermenting yeast and other microorganisms (Heed 1978, Matzkin 2014). Most rots are not

limited to a single alcohol but contain multiple alcohols. Differences in alcohol concentrations exist between the rot of agria and organ pipe, and it is assumed that the other two host cacti have unique olfactory cocktails (Fig. 2, Heed 1978). Due to the reproductive importance of locating a host cactus, it has been suggested that the *D. mojavensis* populations have adapted to prefer the smell of their own cactus' necrotic olfactory cues and navigate their way to it (Newby & Etges 1998). In this study, we attempted to quantify differences in behavioral responses between three of the four populations with respect to individual alcohol solutions. While the flies are probably subject to a combination of alcohol cues at once, looking at response

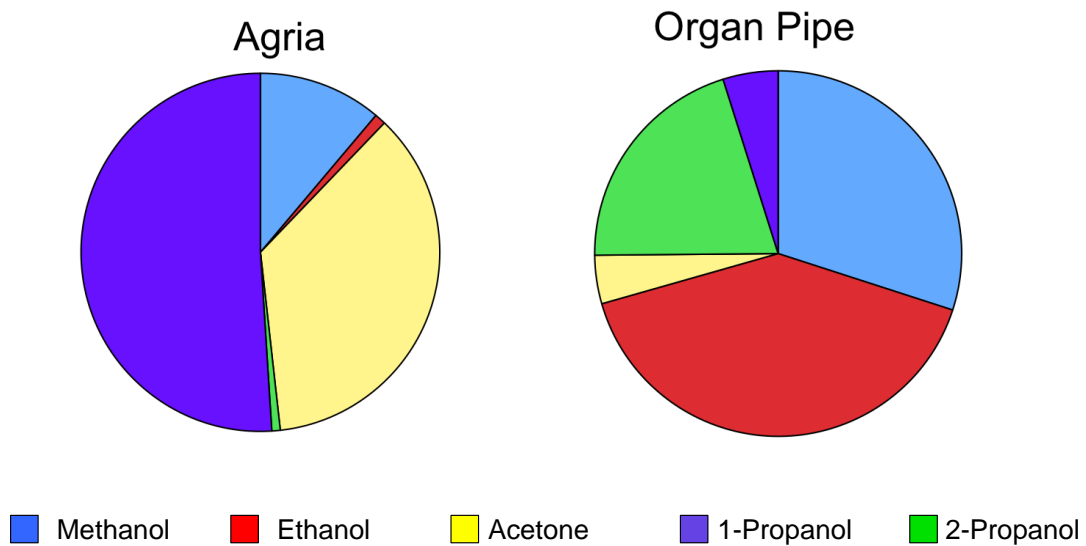


Figure 2. Volatile composition in two necrotic cactus host of *D. mojavensis* (Heed 1978).

differences for a single compound

can give an idea about the up-regulation of individual receptor proteins – the determinants of neuronal activity and ultimately, behavioral actions. Due to the relatively recent divergence time, it is unlikely that the structure of the receptor proteins would have changed to accommodate

for behavioral differences. Differing amounts of receptor protein can cause slight differences in action potential signaling between primary sensory neurons and interneurons (the latter of which can define preference and response patterns), thus behavioral differences may occur from protein regulation.

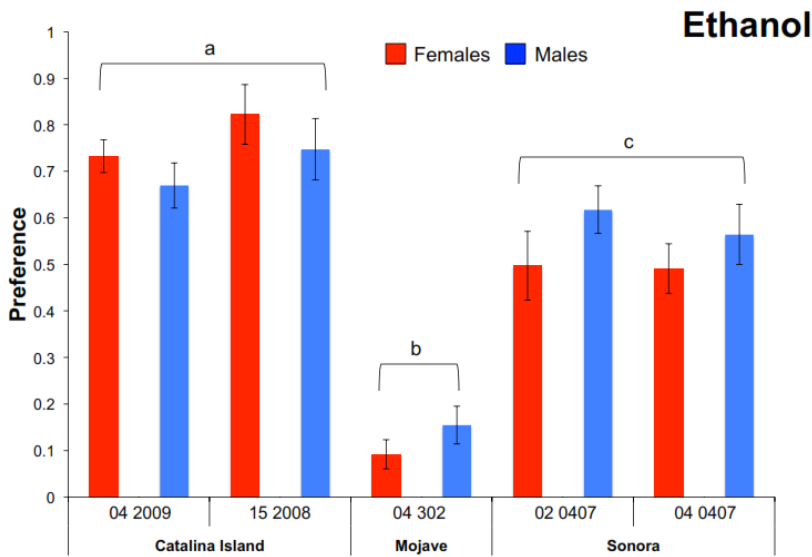
Results

Behavioral Preference for Alcohol Solutions

The necrosis of the four *D. mojavensis* population's host cacti are believed to house varying concentrations of Ethanol, 1-Propanol, 2-Propanol, Acetone, and Methanol, so we studied the attraction individual flies have for these isolated compounds (Heed). While the flies are expected to respond to a combination of olfactory cues in nature, differences in behavior for individual compounds should indicate alterations to the olfactory receptor mechanisms between populations. For the purposes of this experiment, Ethanol, 1-Propanol and 2-Propanol were used as olfactory candidates. These three alcohols showed the greatest difference of rot concentration between agria and organ pipe samples, and thus may have contributed a great deal to olfactory cue recognition. We conducted a two choice experiment using a behavioral "T-maze" assay (Fig. 7). *D. mojavensis* candidates were subject to a 10% solution of one of the three aforementioned olfactory cues for a period of 24hours in a temperature-controlled environment. Preference was determined through the percentage of flies that remained in an olfactory containing chamber after the end of the 48 hour period.

Preference among olfactory cues varied between the three populations for all tested alcohols. Overall, the populations showed the highest preference for 2-propanol and mixed preferences to ethanol and 1-propanol. No differences due to sex were recorded nor were

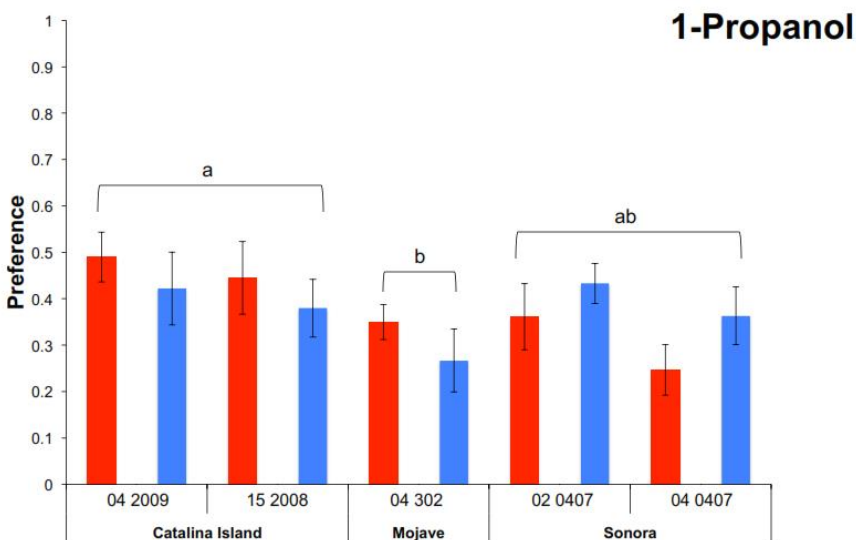
differences between isofemale lines for the populations, suggesting that changes with respect to olfactory cue attraction reflect a general representation of the population's behavioral responses. While sex-related preferences were not observed in this study, prior literature suggests that behavioral responses differ between male and female *D. mojavensis* (Date 2013). This may be due to a compounding issue from the interaction of multiple olfactory cues.



Source	DF	MS	F Ratio
Sex	1	0.084	1.465
Population	2	4.933	85.8 ***
Line[Population]	2	0.138	2.409
Sex x Population	2	0.158	2.758
Sex x Line[Population]	2	0.004	0.068
Error	106	0.057	
Total	115		

Table 1. Result of ANOVA for arcsine square root transformed ethanol preference. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

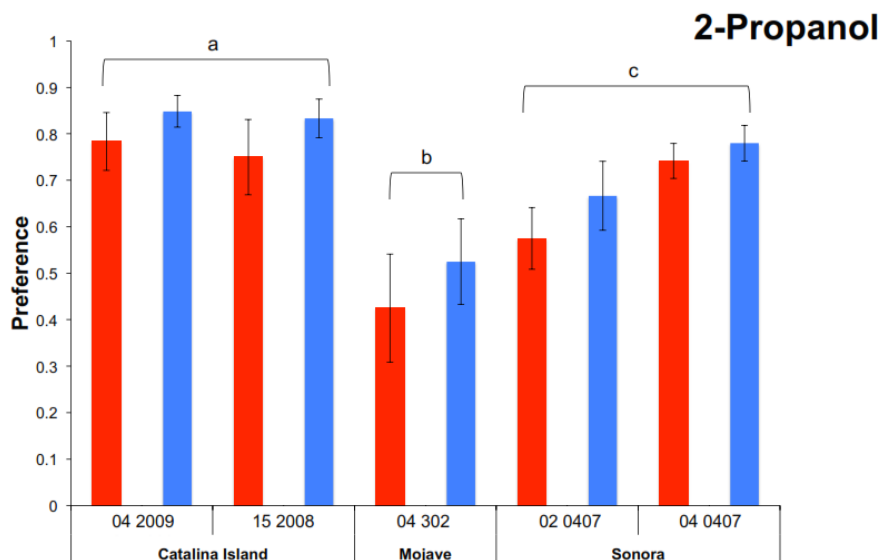
Figure 4. Mean and std. error for Ethanol preference for each isofemale line tested. Results of a *post hoc* pairwise Tukey test between populations is shown by the letters above each population (differences in behavior were found between all populations, $P < 0.05$).



Source	DF	MS	F Ratio
Sex	1	0.042	0.612
Population	2	0.220	3.288 *
Line[Population]	2	0.088	1.267
Sex x Population	2	0.209	2.998
Sex x Line[Population]	2	0.003	0.046
Error	109	0.069	
Total	118		

Table 2. Result of ANOVA for arcsine square root transformed 1-propanol preference. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 5. Mean and std. error for 1-propanol preference for each isofemale line tested. Results of a *post hoc* pairwise Tukey test between populations is shown by the letters above each population (differences in behavior were found between Mojave and Catalina Island, $P < 0.05$).



Source	DF	MS	F Ratio
Sex	1	0.20	2.22
Population	2	1.17	13.16 ***
Line[Population]	2	0.15	1.64
Sex x Population	2	0.00005	0.001
Sex x Line[Population]	2	0.015	0.166
Error	102	0.325	
Total	111		

Table 3. Result of ANOVA for arcsine square root transformed 2-propanol preference. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 6. Mean and std. error for 2-propanol preference for each isofemale line tested. Results of a *post hoc* pairwise Tukey test between populations is shown by the letters above each population (differences in behavior were found between all populations; Mojave and Catalina, $P < 0.001$; Catalina and Sonora, $P < 0.05$; Sonora and Mojave, $P < 0.01$).

Methods

Behavioral Assay

The free walking behavioral assays consisted of ten flies placed onto a cotton plug platform, which was inserted into a glass vial, within an inverted 50mL Falcon tube. The funnel end of the Falcon tube was drilled out and attached to a plastic aquarium tubing t-joint. Either end of the t-joint lead into a glass vial that contained a prepared cotton ball (Fig 7). Flies were test 6-8 days post-eclosion and were allowed to mate normally prior to testing. Assays were performed in a 25°C temperature controlled environment with an alternating 14/10hr L/D cycle for 48hrs. The maze contained 1mL of vehicle control with or without odorant. All odorant-primed vials were prepared in 10% solutions – odorants were obtained from Fisher science at highest available purity. Responses were calculated by dividing the number of flies that were located in the odorant-primed vial by the total number of starting flies. Eight replicates per sex, population, isofemale line, and odorants were conducted. Statistical analyses were conducted

using ANOVA followed by a Tukey-Kramer post-hoc test. Statistical analyses were conducted within a given sex and two choice testing using ANOVA. All analyses were done using JMP 9.0 software.

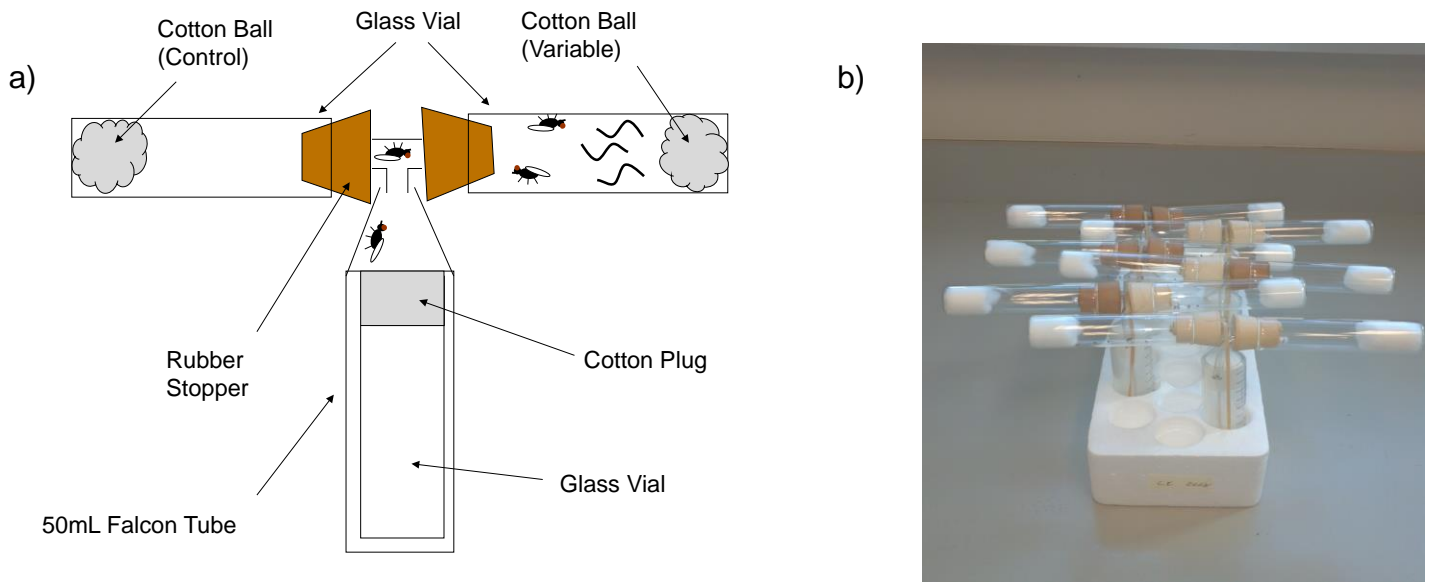


Figure 7. a). Diagram of Matzkin Olfactory Neuromodulation Chambers (MONCs). Provides a choice between aversive behavior and attractive behavior for a given volatile compound. Cotton balls within collection vials are aliquoted 1mL of an alcohol solution. Groups of *D. mojavensis* begin the experiment on the cotton plug loading dock. Entire apparatus was placed in a 25°C chamber on a 14/10 hour light/dark cycle. Movement towards a scented cotton ball after a 48 hour period of exposure was recorded. *Apparatus is secured with a rubber band (not shown). b). Experimental set-up.

Drosophila Stocks

All flies were obtained from the *Drosophila* Species Stock Center or were obtained from the field by Dr. Luciano Matzkin and are as follows: Baja California population, mainland Sonoran population, and the Mojave desert population. All flies were reared on a banana agar medium at 25°C, 50% humidity under a 14/10 hr L/D cycle.

Discussion

Behavioral Responses to Synthetic Compounds

We analyzed the behavioral responses of three *Drosophila mojavensis* populations with respect to preference for 10% Ethanol, 10% 1-Propanol and 10% 2-Propanol solutions and found that behavioral differences exist between populations from the Mojave desert, Catalina Island and the Sonora desert. These differences suggest genetic differences at chemosensory genes between host populations and may have input on higher order neuronal processing. These results are not supported by similar studies, which suggest that no differences in chemosensory behavior occur for individual odorants and this may be due to experimental methodology (Date et al 2013, Etges 1998). No differences due to sex effect were reported, suggesting that both males and females exhibit similar preferences for single compounds. Arguably, females play a larger role than males in the mating process and thus should have a larger drive to locate their host cactus. This may be a fundamental issue with testing for individual compounds, as prior studies have shown a sex effect for combinations of synthetic odorants within *Drosophila mojavensis* (Reed et al 2013, Etges 1998). Little variation existed between tested isofemale lines, pointing to general changes throughout the *D. mojavensis* populations. The lack of between line differences indicates that mutations to the olfactory receptor mechanism may have a relation to host-plant adaptation with respect to individual populations instead of line-specific random mutations.

Evolution of Drosophila mojavensis Olfactory Preferences & Future Work

The difference in olfactory preferences across populations does suggest that some evolution has occurred in the olfactory receptor mechanism for *D. mojavensis*; however, in nature the flies are responding to complex olfactory cues that encompass both long distance

and short distance signals. A more ecologically relevant approach that correlates cactus specific volatiles to population preference will be conducted to test this theory.

In addition, the factors that lead to the alterations of olfactory receptor mechanisms (and thus a change in preference) are poorly understood. More work must be put into determining what environmental and social factors can determine gene regulation. We are in the process of updating the behavioral assay and determining what external forces may influence olfactory preference.