DIET SPECIALIZATION AND GENERALIZATION TRADEOFFS IN THE MUSTARD HERBIOVRE *SCAPTOMYZA FLAVA*

By

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ABSTRACT

Evolutionary tradeoffs occur when the fixation of a beneficial trait reduces the effectiveness of another one. In a complex environment, a population with a highly variable mixture of traits may increase the mean fitness. Specialists on the other hand, may fix alleles optimal in one environment, and thereby give up the benefit of thriving in a different environment.
My senior thesis study aims to test whether the maintenance of variable traits is beneficial when the environment is variable and what, if any, tradeoffs arise as a result of specialization. I created replicated populations of a drosophilid fly species called *Scaptomyza flava* and evolved these in three different environments, two specialized and one generalized, for 10 generations. Emergence time, survival, and preference for environment were phenotypically tested for the different populations of flies in all environment types. Emergence time depended on both the environment in which they developed and on the population from which they came. This suggests that tradeoffs exist between specialized and generalized populations that affect their development on both types of environments.

INTRODUCTION

Experimental evolution

Two hypotheses address the evolutionary mechanisms responsible for the maintenance of genetic variation within populations. One posits that a substantial amount of genetic variation is adaptive and maintained for a functional purpose (Dobzhansky 1951; Gillespie and Langley 1974; Powell and Taylor 1979) while the other hypothesis states that most variation is neutral or slightly deleterious with respect to fitness arising via mutation but not removed by drift or selection (Kimura 1968; Lande 1976; Zhang and Hill 2005). This topic has been debated for decades and this project aims to test a scenario first proposed by Dobzhansky. I phenotyped two different environmentally specialized fly populations and one environmentally variable fly population. Following the Dobzhansky's theory, I predicted that the populations will rapidly evolve to maximize fitness when forced on a specialized environment if their preexisting genotype is maintained by environmental variation because they already possess the alleles to maximize fitness for that particular environment type. I also expected phenotypic evolution in each habitat to involve tradeoffs.

Ongoing Experiment

The experiment described here builds on a foundation of information linked to an ongoing experiment designed by Ph.D. candidate Andy Gloss in the Whiteman Lab, University of Arizona. In summary, a single population of *S. flava* flies originally collected from New Hampshire was divided into three categories with four

replicates in each category. One experimentally evolved population only develops and feeds on the *Turritis* plant, the second only develops and feeds on the *Barbarea* plant, and the last group is founded in each generation by 250 flies from one plant species and 250 flies from the other plant species. This last population type will be referred to as 'mixed'. The flies used in the experiment described below came from the twelve cage replicates of the 10th generation of this experiment. I helped maintain these populations beginning in January 2015 before beginning the phenotypic tests presented below.

METHODS

Organisms

Scaptomyza flava

S. flava is a drosophilid fly that feeds and develops as a leafminer in many cruciferous plants, including *Barbarea vulgaris* and *Turritis glabra*. *S. flava* detoxify mustard oils present in their bodies that are derived from their host plants (Gloss et al. 2014), although they are still harmed by their presence (Whiteman et al. 2012). The females use ovipositors to create oviposition sites on the leaves of plants where they lay eggs. The larvae develop, mine, and eventually pupate all within a living leaf. They then emerge and the cycle beings anew (Goldman-Huertas et al. 2015).

140 individuals of *S. flava* were collected in Dover, New Hampshire. These formed a founding colony which then led to evolving populations for three host plant conditions, four replicates per host plant condition. These populations were maintained at a population size of 500 flies for one year. Each *Turritus* host plant population replicate is given a unique name T1, T2, T3 and T4. *Barbarea* and mixed plant populations also have unique names; B1, B2, B3, B4 and M1, M2, M3, M4.

Mustard Species

Glucosinolates are secondary metabolites that create the bitterness found in mustards. For many insects, this toxin is a feeding deterrent. *Barbarea* and *Turritis* are co-occurring mustards in N. America. *S. flava* are found on these species in nature. *Turritis* leaves stay closely rooted to the main stem of the plant. *Barbarea* leaves come off of long stems (2-6 inches). These two species are not especially closely related phylogentically, so they likely have different glucosinolate profiles (Beilstein et al. 2008). This is meaningful because this difference will possibly affect the way the flies specialize on each plant type, and how they will perform on the plant type they are not specialized on during the experimental period.

These two plants that *S. flava* usually develop in and feed on were collected as wild seeds in Dover, New Hampshire. The seeds were planted onto Jiffy-7 42mm Peat Pellets, each one placed in an individual space of a 4-pack plastic planting container. Ten of these 4-packs were kept neatly arranged in a flat. They were then watered and covered to increase humidity, until the seeds germinated. After this they are uncovered and watered about every two days. To sustain the yearlong evolution

period and the phenotypic experimental period, this process was repeated about once a month.

Conditions
Flies: The live flies were kept in an isolated room with a 16:8 h light:dark cycle, provided by fluorescent lights, and a constant temperature of 21 °C. The developing flies were kept in a separate room under the same conditions.

Plants: The plants were grown in a separate isolated room kept at 21 °C during the day and 15 °C at night. Fluorescent lights provided a 16:8 h light:dark cycle.

Phenotyping
From the experimentally bred populations mentioned in the introduction, 60 *S. flava* were isolated from each population replicate. The mixed populations comprised of 30 *S. flava* bred on *Barbarea vulgaris* in the most recent generation and 30 bred on *Turritis glabra* in the most recent generation. Each replicate was allowed to mate within its replicate cage in an evenly mixed environment of the two plant species for exactly a week. Next, each group of 60 flies were placed in new cages each containing four *Barbarea vulgaris* and four *Turritis glabra* plants. This relatively large number of plants for 60 flies aims to reduce competition within cages. The plants of each species were grouped together and placed in separate dishes on either side of the mating cage. The flies were allowed to feed and oviposit in these cages for six light hours. The cages were rotated 180° half way through this time period. After this, each plant was given a specific ID based on its cage, plant type, and fly population. Then each plant was phenotyped for stipple and egg number for each leaf, within 48 hours of interacting with flies. These plants were bagged individually using a transparent micro-perforated plastic material to allow for airflow and light to enter. They were placed in a room under consistent light and temperature conditions. Their positions were rotated daily and two or three times a week the plants were watered. As flies emerge in the developing room, they are counted and compressed by hand within the perforated bags, effectively killing the flies. This was done twice daily, at 11 AM and at 7 PM. This process continued until each plant did not produce flies for 2 days.

The first model block, or round of the experiment, contained populations T1, B2 and M2. These flies mated for 8 light hours in cages containing 4 *Barbarea* and 5 *Turritis*, because *Turritis* were especially thin and comprised less biomass than *Barbarea*. Model block two for populations T3, B3, and B4, and model block three for populations T4, M3, and M4 are completed with no alterations. The stipple and egg numbers for model block four containing populations B1, M1, and T2, were not counted. Also, the developing flies in model block four were kept in the regular live fly room because the standard developing room was no longer available.

Statistical Analysis
Emergence time is modeled in R v. 3.0.2 using a generalized linear model (GLM) from the “stats” package. Model block is included in the model to account for any

differences between experimental rounds. The interaction term between model block and plant species is included, if significant, in the models to account for differences in plants among replicates, which might otherwise limit power to detect a relationship dependent on both the population the flies come from and the plants they are utilizing. "PlantSpecies" represents the plant type that the flies are developing on and "PopSpecies" represents the population that a fly is taken from.

```
emTimeMean ~ ModelBlock + PlantSpecies + ModelBlock:PlantSpecies
+ PopSpecies + PlantSpecies:PopSpecies
```

Number of stipples and eggs are modeled using a negative binomial generalized linear model from the "MASS" package.

```
NumStips ~ ModelBlock + PlantSpecies + PopSpecies
```

```
NumEggs ~ ModelBlock + PlantSpecies + ModelBlock:PlantSpecies +
PopSpecies
```

Survival is modeled using an arcsine transformation and a generalized linear model. Survival data is obtained from emerged fly count divided by egg count from each individual plant. Certain calculations were proportions >1, so it was assumed that the errors made in evaluating egg number were consistent across all plants. This was corrected by dividing all the proportion values generated from this by the proportion with the largest value to set the highest proportion equal to 1. These values were then transformed using the arcsine square root transformation. This is necessary to pull out the ends of the distribution so that values clustered and either 1 or 0, because of the strict bounds that proportions inherently create, are converted to a more normal looking distribution.

```
survtrans ~ ModelBlock + PlantSpecies + PopSpecies + NumEggs
```

Throughout, likelihood ratio tests are utilized to test for significance of model components. Non-significant independent variables were dropped from the model, except model block, plant species, and population species are kept in regardless of significance.

RESULTS

Table 1: The transformation, model distribution and significance value for each phenotypic test.

Phenotype	Transformation	Model distribution	P value for interaction term
Emergence Time	None	Normal	**0.01320**
Survival	arcsine	Normal	0.9478
Stipple Number	None	Negative binomial	0.27145
Egg Number	None	Negative binomial	0.24195

Emergence Times

In the emergence time model, the plant species and population species interaction term is statistically significant, p= 0.0132. This result indicates that the specific environment in which a fly's ancestors are bred on will affect the emergence time of those flies in different ways on each plant type. This is made visually obvious by the graph below. Interestingly, the *Barbarea* populations do not show much variation dependent on the plant species the fly uses to develop. Further, the mixed populations show the greatest variation.

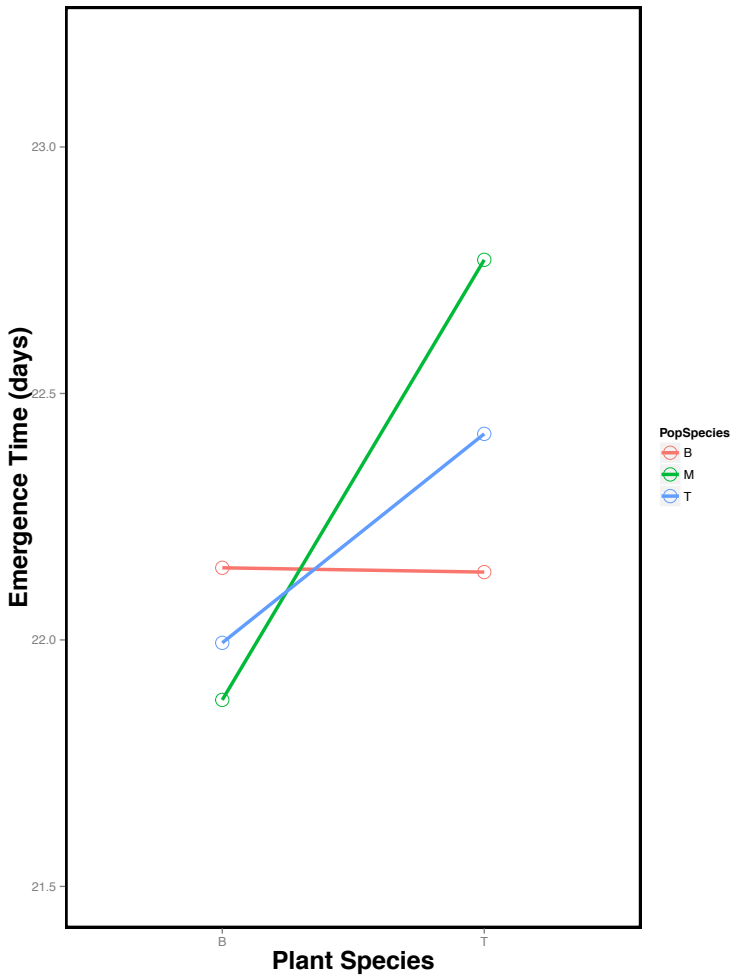

Figure 1: The average emergence times for each population type on either plant species. B = *Barbarea*. T= *Turritus*. M = *Mixed*.

Survival

The interaction term between the plant species and the population species is not statistically significant, p= 0.9478. There is however a statistically significant difference in survival rates between flies developing on *Barbarea* and ones developing on *Turritus*, p= 0.000171, but it is independent of the population the flies

came from (Figure 2). The mixed population appears to exhibit better survival, although this is not statistically significant, p=0.1086.

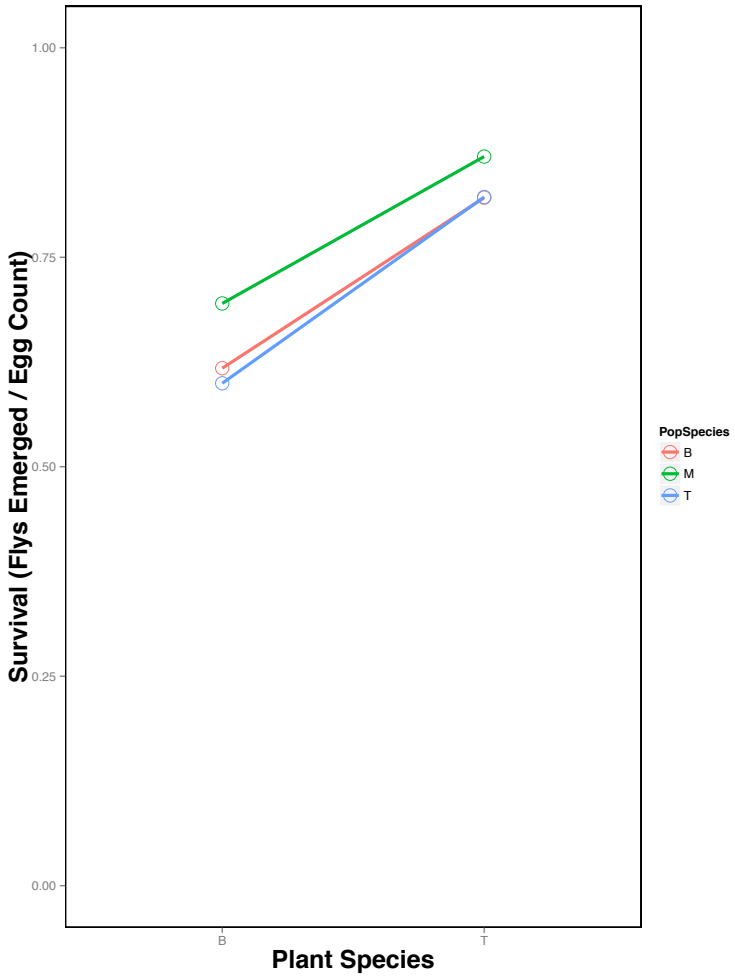

Figure 2: This graph shows the average survival rates as a proportion of emerging flies divided by eggs laid. Survival of each population condition is displayed for each plant species.

Number of stipples

To test if females exhibit a preference for either plant species, females were allowed to feed in a cage with an even mix of both plant species, and the number of feeding punctures were counted on each plant. The females show no significance preference for either plant type dependent on the fly population that their ancestors evolved on, p=0.27145.

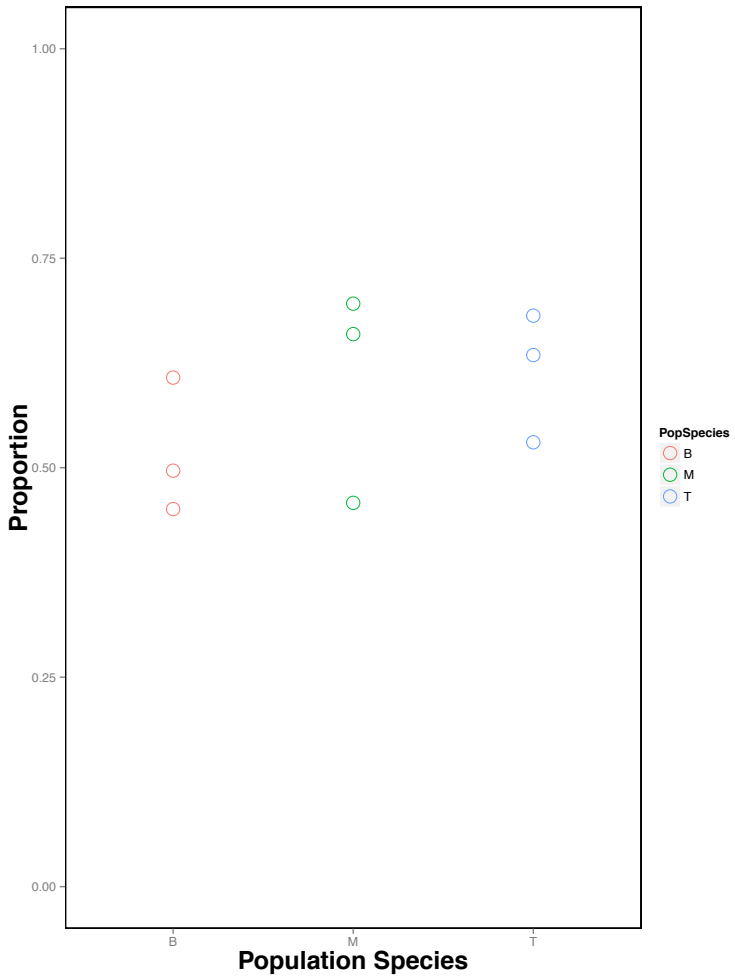

Figure 3: This graph shows the average proportions of stipples on *Barbarea* over the total number of stipples created (*Barbarea* + *Turritus*). The multiple points represent each model block.

Number of eggs

To test if females exhibit a preference for either plant species, females were allowed to feed and lay eggs in cages with an even mix of both plant species. The number of eggs laid was counted on each plant. The females show no statistically significant preference for either of the plant types that is dependent upon the population species they came from, p=0.24195.

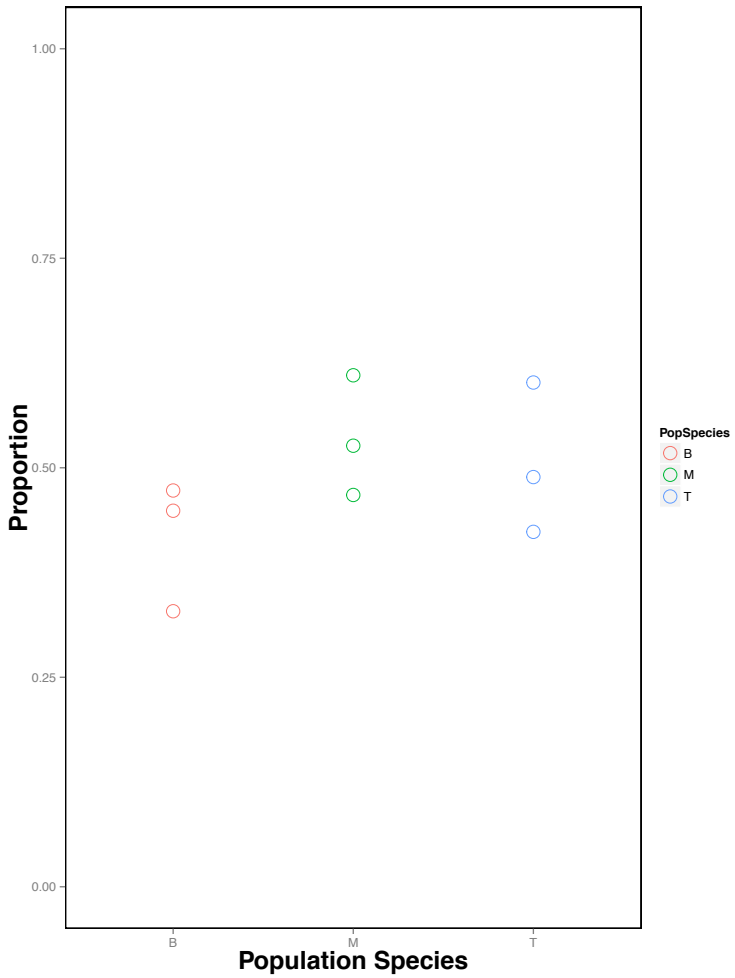

Figure 4: These graphs show the average proportions of eggs laid on *Barbarea* over the total number of eggs laid (*Barbarea* + *Turritus*). The multiple points represent each model block.

DISCUSSION

Emergence Times

The statistically significant interaction term for population species and plant species is a biologically meaningful finding. It suggests that the flies have physiologically adapted to the plants in the last 10 generations and that they develop in ways unique to each experimental treatment. This suggests that in each of the three treatments, there may be life history tradeoffs. S. *flava* are still able to develop and feed on either *Barbarea* or *Turritus*. The results of this experiment indicate that specialization could be possible, but that it might not be beneficial or else this would likely be the case in nature. Interestingly, the mixed populations exhibit the greatest degree of difference in emergence time when comparing flies developed on one plant to the other. This could mean that these flies are getting the most out of their developmental environments just as the flies would in nature. It is likely that *S. flava* are sometimes in an unevenly mixed environment of many plant types and may be

forced to lay eggs on one plant that does not maximize larval fitness. In other cases, plant availability may be ample and the flies can utilize the most beneficial plant. This supports the hypothesis that polymorphism is maintained within populations for a beneficial purpose in support of Dobzhansky's theory.

Survival

Survival is almost completely dependent on plant type while independent of the population that the flies came from. Specializing for 10 generations on one plant type does not affect larval survival on the other plant type. Survival is so essential in the genetic code that its likely these alleles are highly conserved and that 10 generations would not erase a larvae's ability to survive on a plant its actively been using for millions of years.

However, there are higher survival rates for eggs laid on *Turritus* regardless of the population they came from (p=0. 000171). This could be due to a combination of any number of factors including how much more or less frequently *S. flava* use *Turritus* in nature versus *Barbarea,* and what the unique glucosinolate makeup of each plant is.

Egg Number and Stipple Number

The flies did not exhibit a statistically significant preference for the plant that constituted their ancestor's specific environment. This result suggests that natural selection did not exert different selective pressures on host choice in the different experimental habitats. When considering that the flies were not faced with multiple plant options for the most recent ten generations, this makes sense. In the phenotypic tests, the flies likely fed and laid eggs wherever was most convenient because choosing between plants was not an option for their ancestors so they lack the genetic basis driving host plant choice behavior.

Possible shortcomings

This experiment may have been hindered by its mediocre sample sizes. Having more power to detect differences would allow for more confidence in reporting these findings. Additionally, this experiment only allows for results to be obtained through phenotypic testing. This, unfortunately, does not confirm what actual evolutionary mechanisms are at play. Specifics mentioned in this paper are largely determined by existing research and some informed speculation. Further genotypic research on these flies would likely reveal much more about what is shaping the changes that occurred within *S. flava*.

Opportunity for further research

Andy Gloss will sequence the genomes of the various populations of flies in the summer of 2016. He will compare the original outbred population to each of the evolved populations. This will provide data about what sorts of genotypic changes are happening and provide more conclusive insight about what evolutionary forces are at work. Hopefully, his conclusions will align with the phenotypic data from this report.

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