June 2020

# THE ECOLOGICAL IMPORTANCE AND EVOLUTIONARY POTENTIAL OF PHENOTYPIC PLASTICITY IN NOVEL ENVIRONMENTS

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**Abstract:**

Phenotypic plasticity refers to a genotype's ability to produce different phenotypes in response to different environments. How organisms respond to environments through phenotypic plasticity can impact the fitness of individuals and thus the demography and even evolution of a population. Having environmentally relevant phenotypic responses could be especially important when a population encounters novel environments, where extinction risks are high such as at the edge of geographic ranges or when there are sudden environmental shifts. Although plasticity has been shown to facilitate the production of novel phenotypes in novel environments, it is less clear whether this leads to increased population survival. The first chapter of this dissertation addresses this question by investigating variation in phenotypic plasticity in a functional trait, alcohol dehydrogenase, and its effect on larval survival of *Drosophila melanogaster* in a novel alcohol environment. After a population colonizes a novel environment, the population often adapts to this new environment, and phenotypic plasticity has been proposed to facilitate trait evolution. I tested whether phenotypic plasticity could lead to increased fitness when organisms encounter a novel environment. The second chapter examines the genetic architecture of the functional trait alcohol dehydrogenase and its plasticity. Understanding the genetic architecture is important because it can influence the evolutionary response. Specifically, if the functional trait and its plasticity have shared genetic control, their evolution would be tightly linked, which could speed up the rates of evolution if selection on both the trait and plasticity was synergistic or constrain evolution if the direction of selection were divergent. Alternatively, if the trait and its plasticity had different genetic control, plasticity can evolve independently from the functional trait. I used quantitative trait loci mapping with the lines from the *Drosophila* Synthetic Population Resources to examine genetic architecture in historical and novel alcohol

environments. The first two chapters focused on plastic responses to abiotic environments, about which we have a wealth of theoretical and empirical understanding. Natural populations, however, almost never exist alone without interacting with other organisms. Biotic interactions are important drivers of species distributions and trait evolution and new interactions are analogous to novel environments. Biotic interactions are predicted to influence plasticity evolution, but this has been challenging to test and has received little empirical attention. The third chapter explores how biotic interactions may influence trait and plasticity evolution using synthetic yeast (*Saccharomyces cerevisiae*) communities. I chose to use yeast as a study system because yeast has a short generation time and can be used to form relatively simple replicate communities to isolate the effects of the interaction types. Specifically, I compared competition and mutualism, because they have very different effects on resource dynamics, and I expected them to influence trait and plasticity evolution very differently. I used experimental evolution with communities engaged in either no interspecific interaction, exploitative competition, and resource exchange mutualism. Taken together, this dissertation examines the evolutionary importance of phenotypic plasticity in novel abiotic and biotic conditions and demonstrates that plasticity can be important for both population survival and subsequent evolution in novel environments.

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by

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B.S., University of California, Davis, 2014

Dissertation

Submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Biology

Syracuse University

August 2020

**ACKNOWLEDGEMENTS:**

I could not have done this without all the support from my lab, my colleagues, my friends, and my family. I especially thank my advisor, Dr. David Althoff, for his continued support both academically and in life. The Althoff and Segraves lab members (Dr. David Althoff, Dr. Kari Segraves, Dr. Mayra Vidal, Dr. Clive Darwell, Haley Plasman, Thomas Anneberg, Anne Curé, and Laura Porturas) have helped me tremendously by being great friends, tolerated all my stubbornness, and had to review all my choppy writings and made them better. Dr Scott Pitnick, Dr. Steve Dorus, and Dr. John Belote shared their knowledge, equipment, and resources that enabled my fly work. Dr. Jannice Friedman and Dr. Matthew Rubin provided many great intellectual discussions about plasticity and QTLs. Dr. Kari Segraves trained and supported me to work with yeast which enabled me to perform the yeast experiment. Dr. Mark Ritchie and Dr. Jason Fridley listened to many of my scattered ideas and helped me sort the relevant from the random.

To all my friends: thanks for sharing the time and making it fun!

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# Chapter 1 : Phenotypic plasticity facilitates initial colonization of a novel environment

Wang, S. P. & Althoff, D. M. (2019). Phenotypic plasticity facilitates initial colonization of a novel environment. *Evolution*, *73*(2), 303-316.

## Abstract

Phenotypic plasticity can allow organisms to respond to environmental changes by producing better matching phenotypes without any genetic change. Because of this, plasticity is predicted to be a major mechanism by which a population can survive the initial stage of colonizing a novel environment. We tested this prediction by challenging wild *Drosophila melanogaster* with increasingly extreme larval environments and then examining expression of alcohol dehydrogenase (ADH) and its relationship to larval survival in the first generation of encountering a novel environment. We found that most families responded in the adaptive direction of increased ADH activity in higher alcohol environments and families with higher plasticity were also more likely to survive in the highest alcohol environment. Thus, plasticity of ADH activity was positively selected in the most extreme environment and was a key trait influencing fitness. Furthermore, there was significant heritability of ADH plasticity which can allow plasticity to evolve in subsequent generations after initial colonization. The adaptive value of plasticity, however, was only evident in the most extreme environment and had little impact on fitness in less extreme environments. The results provide one of the first direct test of the adaptive role of phenotypic plasticity in colonizing a novel environment.

## Introduction

Phenotypic plasticity, the capacity of a genotype to produce different phenotypes in response to different environments (Schlichting and Pigliucci 1998; Pigliucci 2001; DeWitt and Scheiner 2004), has long been assumed to facilitate population persistence in novel environments (e.g. Baldwin 1896; Morgan 1896; Williams et al. 1995; Price et al. 2003; Parsons and Robinson 2006; Morris 2014; Ragland et al. 2015; Colautti et al. 2017). The rationale for this assumption is that novel environments can be challenging for organisms if the ancestral phenotypes are poorly matched to the new conditions. Phenotypic plasticity, if adaptive, can allow a population to respond immediately to new environments, producing phenotypes that are better matched within a single generation. This reduces demographic stochasticity and can be critical for population persistence before local adaptation occurs (Lande 2009; Chevin et al. 2010; Coulson et al. 2017). Because adaptive plasticity is common in many organisms living in heterogeneous environments (Newman 1988; Gotthard and Nylin 1995; David et al. 1997), it is expected to be adaptive in response to novel environmental challenges (Sexton et al. 2002; Amarillo-Suarez and Fox 2006). Even though this idea is commonly invoked in the literature and there is a large body of indirect evidence, no selection analysis has shown plasticity to be positively selected during the initial stage of encountering a novel environment. This assumption needs tested directly, especially given the large amount of theory relying on this premise (West-Eberhard 2005; Chevin et al. 2010; Merila and Hendry 2014; Lande 2015; Scheiner et al. 2017).

Plasticity is thought to be a major mechanism by which populations can expand their geographic range (Otaki et al. 2010; Dawson et al. 2012; Doudová-Kochánková et al. 2012; Pichancourt and van Klinken 2012; Oplaat et al. 2015; Volis et al. 2015). As such, it has been suggested to be particularly important in invasive species (Richards et al. 2006; Caño et al. 2008;

Davidson et al. 2011; Liao et al. 2016), the ability of organisms to respond to climate change (Nicotra et al. 2010; Franks et al. 2014; Valladares et al. 2014; Volis et al. 2015) and the ability to colonize novel environments (Ghalambor et al. 2007). However, the role of plasticity for dealing with each of these challenges remains unclear (Godoy et al. 2011; Gratani 2014; Palacio-Lopez et al. 2015; Hendry 2016). For example, Palacio-López and Gianoli (2011) found similar levels of plasticity in invasive species and non-invasive species. In contrast, reviewing a somewhat different set of studies, Davidson et al. (2011) found plasticity to be higher in invasive species, yet this higher plasticity did not lead to higher fitness on average. Similar inconsistencies were found among studies of species affected by climate change. Some authors have argued that plasticity has limited impact on population persistence against future warming (Donelson et al. 2011; Gunderson and Stillman 2015), whereas others have shown that more plastic species were observed to have increased persistence in a warmer climate (Willis et al. 2008; Magozzi and Calosi 2015). Hendry (2016) pointed out that qualitative and quantitative reviews have reached different conclusions regarding the role of adaptive plasticity in colonization as well. The lack of a general pattern in the effects of plasticity has led to uncertainty about its importance for dealing with novel environments (Laland et al. 2014).

One reason for the inconsistencies in the adaptive role of plasticity in colonization is that studies need to test that a series of conditions regarding plasticity and its relationship to fitness have been met (e.g., Dawson et al. 2012). Hendry (2016) detailed five conditions under which plasticity is most likely to assist colonization. These conditions can be distilled down to two important ones: the trait needs to be important for fitness and the average plastic response of a population moves the population closer to the trait optimum in the novel environment. This point is critical because there is no *a priori* reason to expect that plasticity will move trait values in the

direction of selection (Dewitt et al. 1998; Valladares et al. 2007). Plastic responses are dependent upon the environment and there is little predictability of how a plastic trait will respond in a novel environment (Ghalambor et al. 2007; Schneider and Meyer 2017). Even though populations are more likely to exhibit adaptive plasticity if the novel environment resembles historical variation, adaptive plasticity in the ancestral environments does not necessarily predicate adaptive response to the novel environment (Yeh and Price 2004). A key component of any plasticity study should be to examine a direct measure of fitness and its relationship with plasticity of a given trait or suite of traits (Ghalambor et al. 2007; Dawson et al. 2012; Colautti and Lau 2015).

A second reason for the inconsistencies stems from confounding the ecological and evolutionary importance of plasticity (Gotthard and Nylin 1995; Forsman 2015; Levis and Pfennig 2016). Chevin et al. (2013) pointed out that initial plasticity can be detrimental on ecological timescales rather than beneficial if the novel environment is unpredictable; however, this initial maladaptive plasticity does not prevent a population from later evolving adaptive phenotypic plasticity in the novel environment. More importantly, theory suggests that selection of plasticity may not be temporally uniform as a population adapts to a novel environment. For example, Lande (2015) predicted that evolution would favor higher plasticity initially in a novel environment, but costs would cause plasticity to decrease towards ancestral levels over many generations. Many studies to date examining plasticity and colonization have compared plasticity between well-established descendant populations and ancestral populations thus confounding ecological and evolutionary timescales. (Richards et al. 2006; Davidson et al. 2011; Palacio-López and Gianoli 2011; Hua et al. 2015). Many of them also identified selection analysis as an

important future priority to disentangle roles of plasticity over ecological versus evolutionary time scales (Ghalambor et al. 2007; Dawson et al. 2012; Colautti and Lau 2015).

We are particularly interested in testing the role of plasticity at the initial stage of colonization. This is the time when plasticity could be the most important for population survival, whereas evolutionary changes will increasingly dominate as a population adapts to a novel environment. For example, adaptive physiological plasticity was shown to increase rapidly within 11 generations of colonization of the invasive plant *Polygonum cespitosum* (Sultan et al. 2013). Thus, the time at which a population is surveyed for plasticity has serious implications for determining whether it is adaptive or not during colonization (Agrawal 2001; Pigliucci and Murren 2003; Edgell et al. 2009). To the best of our knowledge, no study has tracked genotypes that differ in plasticity and measured their fitness in the first generation of colonization. Studies examining this crucial first step in a population's trajectory are needed to clarify the role of plasticity during the colonization process.

To directly test whether plasticity is important for the initial colonization of a novel environment, we conducted a manipulative experiment using the fruit fly, *Drosophila melanogaster*. We exposed family lines from a wild population to both historical (low) and novel (high) alcohol environments and then compared their physiological responses and survival. Environmental alcohol is an important ecological factor for *D. melanogaster* as the larvae naturally develop in decomposing fruits with varying alcohol concentrations (Atkinson and Shorrocks 1977; Gibson et al. 1981). The reported alcohol level in natural substrates varies substantially, ranging from 0.02 – 4% (Mckechnie and Morgan 1982; Oakeshott et al. 1982) in non-winery sites and 0.07-10.78% in winery seepage sites (Gibson et al. 1981). Compared to ecologically similar species, *D. melanogaster* is the only species found in abundance at the high

end of the alcohol range (McKenzie and Parsons 1972). The fruit fly's superior alcohol tolerance is a major ecological innovation promoting its global ecological success (McKenzie and Parsons 1972; Holmes et al. 1980; Dickinson et al. 1984; Markow 2015). The detoxifying enzyme alcohol dehydrogenase (ADH) is pivotal for alcohol tolerance (David et al. 1976; Cavener 1979; Hickey and McLean 1980; Geer et al. 1985; Heinstra 1993). Both adults and larvae upregulate ADH expression in response to alcohol exposures (Geer et al. 1985; Malherbe et al. 2005) and the ADH gene has experienced a faster rate of evolution in the *D. melanogaster* lineage compared to other closely related species (Aquadro et al. 1986; McDonald and Kreitman 1991; Akashi 1996). *D. melanogaster*'s existing adaptation to variable, but low amounts of environmental alcohol makes it particularly suitable to test our question because we can expect populations to harbor some adaptive plasticity when encountering novel alcohol environments. Here, we examined the functional link between ADH activity and plasticity of ADH activity to larval survivorship in the first generation of exposure to novel alcohol environments. The goal was to directly test if plasticity was a key factor facilitating larval survival and population establishment in novel environments.

## Methods

### *Experimental Population*

We collected over 120 wild, mated females of *Drosophila melanogaster* from Syracuse, NY, USA during the summer of 2015 and 2016 to form a stock population. Flies were collected from compost piles that were low in alcohol (likely less than 4%). We did not expect the population to be pre-adapted to high alcohol content, because there is no winery within a 24 km radius around

the site of collection and extreme alcohol tolerance tends to decay in the mere distance from the cellar to the outside (McKenzie and Parsons 1974; Briscoe et al. 1975). The population was maintained on a standard cornmeal-molasses diet at >1000 flies per generation for at least 3 generations. The stock population was maintained at 25°C and 24 h dark with overlapping generations with fresh food supply every 2 weeks.

*Experimental setup*

To measure selection on phenotypic plasticity during the initial encounter of a novel environment, we experimentally exposed related individuals to two historical and one novel alcohol environments. Related individuals were generated using a half-sib mating design. Each male was mated to multiple, non-overlapping females, giving rise to nested half-sib/full-sibs in the offspring generation. This design allowed us to estimate plasticity on families of related individuals, because we could not have exposed single individuals to multiple environments due to our destructive sampling. The families of offspring were randomly split into different treatment environments, in which we measured their ADH activity and survival through the larval stage.

The three treatments were 0%, 10%, and 16% alcohol in the larval food substrate. These values were chosen to represent the range of natural variation found in larval substrates (0 to 10.78%) and a value that was more extreme (16%). This extreme alcohol content is unlikely but possible in nature, because yeast can generate up to 12-15% alcohol in their substrate, beyond which point their own growth becomes inhibited (Dudley 2000). Ethanol (100%) was added after the cooked media had cooled below 70°C to minimize evaporative loss. To control for nutritional content and media firmness, 16% of the water was removed before cooking, then the same amount of liquid, 16:0, 6:10, and 0:16 parts water: 100% ethanol to 84 parts cooked media, was

added back for the three different treatments respectively. All food was stored at 4°C and used within 4 days after preparation to ensure freshness.

The parental generation was collected from the stock population immediately post eclosion, with individuals from the first day of eclosion discarded. Virgin males and females were aged in separate vials in groups of 10 or less with fresh food supplied daily for 3 days before mating. At day 4 post eclosion, each male was transferred to a fresh vial with 4 females for mating. After 30 hours, the males were removed and frozen and the females transferred to laying dishes. Females were allowed to lay individually on fresh laying dishes for 16 hours every day for the subsequent three days. They were kept on fresh media with yeast supplement for 8 hours between each laying. Only three of the mated females per male were used for egg collection. These females were chosen at random unless one of the four failed to produce eggs on the first day of laying. Eggs from each laying dish were transferred manually and divided equally to three vials for each of the three alcohol treatments. We recorded the number of eggs transferred to each rearing vial, averaging 13 ± 4. For analyses related to plasticity, we pooled the eggs from the three days (three independent vials) in each environment for each female, and this lead to about 36 eggs per treatment per female. The experiment was conducted in three sets (different days) due to logistical constraints of handling the collection and transferring of eggs. A total of 34 males and 107 females were used, with about 11 male families per set. We collected a total of 11,862 eggs across 911 rearing vials.

*Larval collection and survival assay*

Larvae were collected for ADH activity assays at the third instar stage, after developing at 25°C in the dark. Vials were arranged haphazardly in an incubator and were moved to a different shelf twice daily. Because flies had delayed development at higher alcohol levels, larvae of the

16% alcohol treatment were collected two days after the 0% alcohol and one day after that of the 10% alcohol treatments. The sequence of the larval collection within each treatment per day was haphazard to mitigate differences in developmental time. Larvae were collected by gently washing off the soft, top layer of medium, and then removed with a fine paint brush. All individuals were washed again in DI water and dried by gently rolling on an absorbent surface. We counted the total number of surviving larvae in each rearing vial. Larvae from each rearing vial were then pooled and weighed using a microbalance. If more than 6 larvae were collected from a single vial, they were split evenly into two samples. Each sample was frozen at -80 °C for later ADH enzyme analysis.

*ADH activity assay*

Standard procedures slightly modified from Malherbe et al. (2005) were used to quantify ADH activity. Samples from each tube were homogenized in 50 µl of cold buffer (50 mM glycine-NaOH, 1 mM EDTA, pH 9.5) times the number of larvae so that differences between samples depended only on the enzymatic activity of the samples and not the number of larvae per sample. Homogenates were centrifuged at 11,000 rpm for 5 minutes and then kept on ice. To quantify ADH activity, 10 µl of the supernatant was added to a reaction mixture containing 170 µl buffer at room temperature and 20 µl of cold reagent buffer (glycine-NaOH buffer with 5 mM $NAD^+$ and 200 mM 2-propanol). After 30 s of mixing, the rate of reaction was measured by quantifying the extinction of $NAD^+$ at 340 nm every 3 s for 60 s using a spectrophotometer (BioRad Smartspec). Each sample was measured twice, and the mean was used for analysis. ADH activity was calculated as the mean slope of NADH production over time divided by the average weight of the larvae in that sample.

*Plasticity of ADH activity*

To calculate plasticity, we used the means of full-sib families (Scheiner and Lyman 1989), because we were unable to expose a single larva to multiple environments. We analyzed the plastic response to an ecologically possible environment (10%) separately from the response to a novel alcohol environment (16%). We calculated plasticity as the phenotypic difference in ADH activity between 0% to 10%, between 0% to 16%, and between 10% to 16% alcohol. This approach gave us three plasticity measures per full-sib family, referred to as P0-10, P0-16 and P10-16, respectively. We chose these combinations because these scenarios are ecologically relevant. Plasticity from 0% to 10% encompasses the natural range of alcohol content for fruit flies, thus P0-10 might be under selection in both 0% and 10% alcohol environments. Additionally, all three of the plasticity measures might be important as the measures represent scenarios of flies encountering the extreme environment (16%) from historically different alcohol levels.

*Selection on ADH activity and plasticity of ADH activity*

We used larval survival (egg to third instar) as the fitness measure for all of the selection analyses, because larvae of the fruit fly are usually confined to their habitat patch due to limited mobility. We pooled the data from all vials for every female and every treatment, so our unit of replication was families rather than individuals. This was the case because both plasticity and survivorship need to be calculated on groups of individuals.

To understand selection on ADH activity, we first fitted a full model to test whether selection on ADH activity was different across the three different environments. The model tested the interaction effect between ADH activity and environment on larval survival. We standardized our estimates by scaling the phenotypic data to have a mean of zero and standard

deviation of one and the fitness measures by dividing survival rates by the population mean (Conner and Hartl 2004).

To further understand selection in each environment, we estimated the fitness functions of ADH activity in each treatment environment separately using multiple regression (Lande and Arnold 1983). The fitness functions had relative fitness as the response variable and ADH activity as the predictor variable. Data were standardized within each environment. We first tested whether there was any variance selection by fitting the data with polynomial models for each treatment. When the quadratic coefficients were not statistically significant, we reduced the models to contain only linear components and reported only the result from the linear model. The slope coefficients of these models represent linear selection differentials on ADH activity.

Using the same approach, we also estimated the fitness functions of ADH activity plasticity. We estimated selection on P0-10 in both 0% and 10% alcohol, because P0-10 might be under selection at both ends of the historical range. Additionally, we estimated selection on all three plasticity measures in the 16% alcohol environment, because any one of them could be important for colonizing the novel environment. We did not formally test whether selection on plasticity was different across environments, because plasticity measures were not independent across environments.

In addition to linear selection differential, we also estimated selection gradients for ADH activity and ADH plasticity jointly, because selection could affect the two traits simultaneously. We used multiple regressions with ADH activity and ADH plasticity as predictor variables and obtained the slope coefficients associated with each trait. Similar to estimating the selection differentials, we first fitted regression models with quadratic terms for both ADH activity and plasticity. None of the quadratic terms were significant, so we reduced the models to contain

only linear components. To help interpret the selection gradients, we also calculated Pearson's correlation coefficients between ADH activity and ADH plasticity in each environment for each model as well as among the different plasticity measures.

*Genetic Variation*

We tested whether there was genetic variation of ADH activity and its plasticity by using model comparisons. We compared models including sib-ship relationships to models excluding these factors. Sib-ship was represented by the combined effect of sire and dam in a linear mixed model. Our null model was that ADH activity or plasticity was only affected by alcohol treatment, and our alternative model included also sire and dam effects. If the alternative model matches the data better, there is support for genetic variation in ADH activity or plasticity. For all the models, we treated ADH activity as the response variable, alcohol treatment (TRMT) as a categorical fixed effect, sire, dam, and sire*TRMT as random effects. All linear mixed models were fitted with the maximum likelihood method, and model comparison was done using a Chi-square test. The above comparisons tested whether there was genetic variation across all the environments, but we were particularly interested in potential future adaptation in the novel environment, simulated by the 16% alcohol treatment. Thus, we estimated narrow-sense heritability of ADH activity and plasticity in each environment using variance components from linear mixed models (Lynch and Walsh 1998).

All statistical analyses were done using the statistical software R (R Core Team 2017). Mixed models used for examining the relationships of ADH activity and plasticity to survivorship and fitness were fitted using the package "lme4" (Bates et al. 2015). All figures were produced using the package "ggplot2" (Wickham 2016).

## Results

### *Response to alcohol in larval substrate*

Alcohol in the larval feeding substrate significantly decreased larval survivorship ($F_{2,887}$ = 643.5, $P$ < 2e-16). Mean population survivorship was the highest at 0% alcohol (84.87% ± 1.21%) and decreased significantly with increasing alcohol concentration (59.99% ± 1.76% at 10% alcohol and 20.25% ± 1.05% at 16% alcohol) (Fig. 1-1). In response to the presence of alcohol, larval alcohol dehydrogenase activity (weight corrected) increased significantly with increasing alcohol concentrations ($F_{2,807}$ = 230.5, $P$ < 2e-16, Fig. 1-1). Activity nearly doubled when larvae developed in 16% alcohol compared to 0% alcohol (from 0.455 ± 0.013 to 0.897 ± 0.019 AU/s/mg). The majority of full-sib families responded plastically to alcohol concentrations (Fig. 1-2). Most full-sib lines showed an increase in ADH activity when larvae developed in 10% alcohol and a further increase in activity at 16% alcohol. However, the response was nonlinear for many of them, and some full-sib lines even showed decreasing ADH activity from 10% to 16% alcohol. There was substantial variation among full-sib lines in constitutive ADH activity and plasticity (Fig. 1-3)

### *Relationship of ADH activity and plasticity to larval survival*

Across all environments, the selective pressure on ADH activity through larval survival differed among the alcohol treatments (Table 1-1; Fig. 1-4), indicated by the significant interaction effect between ADH activity and environment on fitness (F = 3.675, P = 0.026). Across historical environments, selection of ADH activity also differed (Table 1-1; Fig. 1-5). At 0% alcohol, there was selection for reduced ADH activity (ß = -0.025 ± 0.012, n = 283, P = 0.041), suggesting a cost of high constituent ADH activity. But at 10% alcohol, ADH activity

had no effect on survival (ß = -0.014 ± 0.023, n = 283, P = 0.535). Similarly, there was no evidence for selection on plasticity in either environment.

In the novel 16% alcohol environment, however, ADH activity was under selection to increase (ß = 0.118 ± 0.054, n = 240, P = 0.028), and selection on the different plasticity measures was variable (Table 1-2; Fig 1-6). Plasticity calculated across the historical range (P0-10) had no effect on fitness in the novel environment. In contrast, the other two plasticity measures did matter for larval survival, albeit in different ways. P0-16 was under stabilizing selection (quadratic coefficient = - 0.12 ± 0.051, n = 102, P = 0.02), while P10-16 was under positive directional selection (ß = 0.158 ± 0.064, n = 102, P = 0.015). Interestingly, when ADH activity and ADH plasticity were analyzed simultaneously for selection gradients in 16% alcohol, only P1016 had a positive relationship with fitness (ß = 0.169 ± 0.071, n = 102, P = 0.002). The difference between the selection differentials and selection gradients was likely due to the positive correlations between ADH activity and all three plasticity measures.

To better understand the different patterns of selection for the three plasticity measures in the 16% alcohol environment, we calculated the correlations among the plasticity measures. The Pearson's correlation coefficients between P0-10 & P0-16 and P0-16 & P10-16 were positive (r = 0.45 and 0.68 respectively). The somewhat strong correlation between P0-16 & P10-16 suggested that these two plasticity measures were largely driven by what happens in the 16% alcohol environment. On the other hand, P0-10 & P10-16 was negatively correlated (r = -0.35), indicating that plasticity was regulated differently depending on the environmental ranges used.

*Genetic variation in ADH activity and plasticity*

The positive selection on both ADH activity and ADH plasticity in the 16% alcohol environment demonstrated that these traits could contribute to adaptation in the most extreme alcohol environment, given the traits were heritable. Our experimental design allowed us to calculate both broad sense and narrow sense heritability for ADH activity and plasticity. There was significant genetic variation for both ADH activity (chi-square = 249.99, *df* = 2, P < 2.2e-16) and ADH plasticity (chi-square = 32.175, *df* = 5, P = 5.487e-06) across all environments. In addition, the narrow-sense heritability of both traits in 16% alcohol was quite high, $h^2 = 0.557 \pm 0.296$ for ADH activity and $h^2 = 0.776 \pm 0.443$ for ADH plasticity (Fig. S1-1, S1-2), indicating that selection could act effectively in this population for increased ADH activity, both constitutively and through plasticity.

**Discussion**

Phenotypic plasticity has been widely argued to facilitate population survival in novel environments (Ghalambor et al. 2007; Davidson et al. 2011) for two reasons. First, plasticity may allow more colonizing individuals to survive and establish a population that can then become locally adapted to the new environmental conditions (Crispo 2008; Muschick et al. 2011; Morris 2014). Second, plasticity itself may evolve quite quickly in the first generations to facilitate local adaptation (Price et al. 2003; Schlichting 2008; Wund et al. 2008; Lande 2015). The scarcity of direct evidence for either of these roles for plasticity, however, has led to intense debates over the last 30 years about the importance of plasticity (Laland et al. 2014; Morris 2014). Furthermore, novel environments can perturb normal development resulting in nonadaptive plasticity (Steinger et al. 2003; Tuomainen and Candolin 2011; Mazzarella et al. 2015), again calling into question the role of plasticity in colonization. To address this issue, we simulated colonizing a novel environment in the lab using wild *D. melanogaster* and raised them across

historical and novel alcohol environments. Our goal was to experimentally test how flies would respond to novel concentrations of alcohol in larval substrates during the initial encounter. The ability to tolerate alcohol in larval substrates is a key trait in *D. melanogaster*, and populations have naturally colonized the seepage from wineries that contain much higher levels of alcohol than rotting fruits in nature (McKenzie and Parsons 1974; Hickey and McLean 1980).

We focused on the first generation of encountering novel environments because this is a critical time point that has received little attention, in comparison to a wealth of studies that have investigated the evolution of plasticity in novel environments after establishment. Early laboratory studies demonstrated that plasticity can respond to both direct and correlated selection in novel environments (Waddington 1953; Scheiner and Lyman 1991). Later research built upon this finding by showing divergence in plasticity across environmental gradients in many natural systems, such as in *Impatiens capensis* (Donohue et al. 2001), field bindweed *Convolvulus arvensis* (Gianoli 2004), the common frog *Rana temporaria* (Lind and Johansson 2007), *Geranium carolinianum* (Bell and Galloway 2008), threespine stickleback *Gasterosteus aculeatus* (McCairns and Bernatchez 2010), whitefish *Coregonus spp* (Lundsgaard-Hansen et al. 2013), Trinidadian guppies *Poecilia reticulata* (Torres-Dowdall et al. 2012), and *Senna candolleana* (Lzaro-Nogal et al. 2015). Even though plasticity as an adaptation is both an intuitive and logical explanation for many of these examples, pinpointing the source of selection on plasticity can be prohibitively hard for most natural systems. This is problematic because, in addition to adaptive plasticity, non-adaptive and maladaptive plasticity can also drive patterns of evolution (Ghalambor et al. 2007; Ho and Zhang 2018). For example, very plastic traits such as gene expression plasticity evolve mostly through accommodating initially mal-adaptive plasticity rather than enhancing adaptive plasticity in a novel environment (Ghalambor et al. 2015). Thus,

divergence in plasticity does not indicate that plasticity was adaptive during the initial stages of colonization. Another concern is that as the focal trait itself evolves, plasticity may become unimportant (Pigliucci and Murren 2003; Lande 2015). This has been observed both in the lab and in the wild. Waddington's (1953) classic selection study on cross-veins in laboratory populations of *Drosophila* exposed to a novel temperature led to the evolutionary loss of plasticity. These results highlight the importance of dissecting the role of plasticity during the initial colonization event.

For plasticity in a trait such as ADH activity in *D. melanogaster* to have a role in colonization, theory suggests that plasticity should be adaptive in a novel environment when the plastic trait is important for fitness, and plasticity moves the population towards the phenotypic optimum in the new environment (Morris 2014; Hendry 2016). For *D. melanogaster*, individuals naturally respond to alcohol exposures by up-regulating the detoxifying enzyme ADH, and ADH activity is known to be critical for surviving natural levels of alcohol (Geer et al. 1985; Barbancho et al. 1987; Vandelden and Kamping 1988; Malherbe et al. 2005). Thus, ADH activity is a trait important for fitness. When a *D. melanogaster* population first encounters a high alcohol food substrate as a possible habitat, plasticity of ADH activity could be important, especially for larvae that cannot escape the high alcohol environment. Increased ADH activity is likely to be a new phenotypic optimum in this environment. We mimicked *D. melanogaster* encountering novel alcohol environments by exposing eggs to two novel alcohol environments: media containing 10% and 16% alcohol. Our population was composed of individuals collected from compost piles in the city of Syracuse, NY, USA. Such sites have reported alcohol levels ranging between 0.02-4% (Mckechnie and Morgan 1982; Oakeshott et al. 1982), thus our population was not expected to be pre-adapted to high alcohol content. This was evident in that

flies suffered increased mortality in the alcohol treatment environments (Fig. 1-1). Although there were no wineries within 24 km of our collection site, central New York has many wineries and it is possible that some individuals collected had experienced higher levels of alcohol (up to 10.78%) in their evolutionary past. However, the 16% alcohol treatment environment is truly a novel environment for larvae of *D. melanogaster* with respect to alcohol concentration.

If plasticity were important for colonization, we expected plasticity to increase ADH activity in the novel alcohol environment and that families with increased ADH activity would have greater survival. Indeed, most full-sib families were plastic in ADH activity (Figs. 1-2, 1-3), and families with greater plasticity generally had higher ADH activity in the high alcohol environments. This is also shown by the moderately strong correlation between ADH activity and plasticity in 10% and 16% (P10-16) alcohol environments (Table. 1-1; Table 1-2). High alcohol content reduced larval survivorship, especially the 16% alcohol treatment which imposed a strong selective force on the experimental population (Fig. 1-1). Plasticity of ADH activity measured between the 10% and 16% alcohol environments was associated with higher relative fitness as measured by larval survival rate in 16% alcohol (Table 1-2; Fig. 1-6). Even though other traits likely contributed to the differential survival, families with higher plasticity had higher survivorship in 16% alcohol, highlighting the important role of ADH plasticity. Together, these results demonstrate that plasticity can be initially adaptive in a novel environment when the trait is important to fitness and the mean response aligns with the direction of selection (in this case, ability to process alcohol). There were also significant amounts of additive genetic variance for both ADH activity and plasticity (0.56 and 0.78, respectively) that would facilitate further local adaptation after the initial colonization event. Plasticity not only influenced colonization through survivorship, but it might also be instrumental in further local adaptation.

The effects of plasticity in the novel 16% environment, however, depended on the range of environments used to measure it. Even though plasticity measured between 10-16% was positively selected, the other two plasticity measures made between 0-10% (P0-10) and between 0-16% (P0-16) were not under strong positive selection in the 16% alcohol environment (Table 1-2; Fig. 1-6). This suggests that different plasticity measures could have different predicative powers. Indeed, the plastic responses of many maternal lines were non-linear (Fig. 1-2), and the correlation between P0-10 & P10-16 was even negative. This suggest that the plastic responses across different alcohol ranges might be under different genetic control. This made P0-16 a poor predictor of survival in the high alcohol environment. Even though P0-16 is positively correlated with high ADH activity at 16%, families with the highest ADH activity at 16% had both high constituent ADH activity at 0% alcohol and high ADH plasticity (Fig. 1-3). The different patterns with different plasticity measures highlight the need to study plasticity across environments that are relevant to both the biology of the organism and the questions being asked.

Based on the existing ecological information for *D. melanogaster*, we expected plasticity to increase ADH activity in both 10% and 16% alcohol environments, which should facilitate alcohol tolerance (Briscoe et al. 1975; Mercot et al. 1994; Malherbe et al. 2005; Anderson 2012). Although plasticity played an adaptive role in 16% alcohol, it had no effect on relative fitness in 0 and 10% alcohol (Table 1-1). One potential reason for this difference is that ADH activity is a costly trait in lower alcohol environments. For example, there was a slightly negative relationship between relative fitness and ADH activity when no alcohol was present. Only when selection was strong enough did the benefit of high levels of ADH activity outweigh the costs. Furthermore, ADH activity is only one key component of alcohol detoxification and other physiological processes are also important for alcohol tolerance, including alcohol absorption

and tolerance to high cellular alcohol concentrations (Geer et al. 1985; Heinstra 1993; Montooth et al. 2006; Morozova et al. 2006; Fry 2014). Even though the importance of the ADH protein has been clearly demonstrated using knockout experiments (Vandelden and Kamping 1988), other studies have found limited fitness effect of ADH in environments containing 10% or lower alcohol (Malherbe et al. 2005). Thus, the natural variation in ADH activity and its plasticity might be only important to fitness in more extreme alcohol environments. For this study, the 16 % alcohol environment reduced survivorship by 65% and was a potent selective force that coupled ADH activity and plasticity with larval survivorship.

The lack of an effect of ADH activity and plasticity in the 10% alcohol environment highlights the pitfalls of using information collected in historical environments to understand responses to novel environments. Selection is well known to depend on the environment in which it is measured (Grant and Grant 2002), thus the function of a trait in any environment is best determined in that particular environment. If a trait is not functionally important in a novel environment, then trait plasticity cannot be a direct target of selection in that environment. In addition to our results, Schaum and Collins (2014) found that evolutionary response in photosynthesis counters the direction of plasticity in a marine alga, even though the ancestral plasticity correlated positively with growth rates. Because ADH activity itself was not a target of selection in the 10% alcohol environment, ADH activity plasticity could not be adaptive in this environment.

By taking an experimental approach, we investigated the adaptive value of phenotypic plasticity in the first generation of encountering a novel environment, when plasticity is argued to be the most important, and before local adaptation can alter its relationship with fitness. Although *D. melanogaster* larvae would likely never experience 16% ethanol in nature, our

results simulate what it might have been like for flies that first were able to colonize higher alcohol environments as *D. melanogaster* spread with humans across the globe. Our experimental work on initial colonization complements other studies that suggest the importance of phenotypic plasticity during colonization in natural populations, such as in dark-eyed juncos (Yeh and Price 2004), great tits (Charmantier et al. 2008), three-spined sticklebacks (Kim et al. 2017), Trinidad guppies (Torres-Dowdall et al. 2012), sculpins (Whiteley et al. 2009), and emmer wheat (Volis et al. 2015). Additionally, our method of introducing natural populations to novel environments experimentally allowed us to simultaneously assess the genetic variation in plasticity as well as examine how selection acts on this variation during initial colonization. Whether plasticity would continue to play adaptive roles as a population adapts to novel environments is an interesting avenue for future research. This can be achieved by comparing selective pressures on plasticity in populations at different stages of colonization. Three conclusions emerged from our results. First, plasticity was adaptive in the most extreme novel environment; more plastic families had higher survival. Second, the adaptive value of plasticity varied depending on the environment, and plasticity was especially important when the trait itself (ADH activity) was important to fitness. Third, the results with *D. melanogaster* also provide support for theoretical studies that suggest plasticity is likely to be more important under large and abrupt environmental changes compared to small or gradual changes (Scheiner et al. 2017).

Although we were able to demonstrate selection for phenotypic plasticity in a novel environment, whether our results can be extended to other kinds of plastic traits remains to be tested. Plastic responses can be reversible or irreversible and can happen with or without significant time lags (Scheiner 1993). ADH activity is a physiological trait that responds to environmental changes with little time lag and reversibly (Lockett and Ashburner 1989). This

kind of plasticity may be more likely to play adaptive roles in novel environments than irreversible plastic traits, such as the development of alternative morphs that have a significant time lag. This is because a novel environment can be different in both the mean value of an environmental factor and/or its predictability. If the novel environment is unpredictable, an adaptive response to a cue at an earlier time point can still result in phenotypes that mismatch the environment at the time of trait expression (Laubach et al. 2018). Additional tests with different types of plastic traits are needed.

*Conclusions*-- Most of the theoretical arguments about the role of plasticity focus on evolutionary dynamics during colonization, and often assume that trait plasticity is adaptive (Waddington 1953; Price et al. 2003). We provide the first empirical evidence supporting the direct fitness benefits of plasticity within a population colonizing a novel environment. By examining colonization of a range of environments, we also demonstrated that the adaptive value of plasticity depended on the specific novel environment. The role of phenotypic plasticity in influencing successful colonization of novel environments may be more restricted than anticipated, yet plasticity may be pivotal in some of the most extreme environments in which initial mortality is high. To move forward in our understanding of the role of plasticity in colonization we need more direct experimental tests of the conditions in which plasticity will be adaptive (Scheiner and Lyman 1991; Hendry 2016).

Table 1-1:

Selection estimates (mean estimate ± SEM) on ADH activity and plasticity for larval D. melanogaster developing across the historical alcohol range, 0% and 10% alcohol. P010 (ADH activity in 10% alcohol minus ADH activity in 0% alcohol) is used as the plasticity measure for all models presented here.

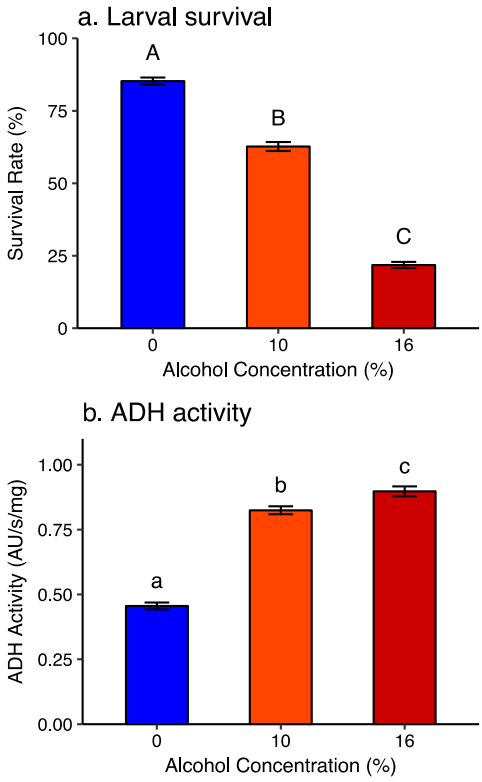
| | | **0% Alcohol** | **10% Alcohol** |
|---|---|---|---|
| **Linear selection differential** | *ADH activity* | - 0.025 ± 0.012 * | - 0.014 ± 0.023 |
| | *ADH plasticity* | - 0.004 ± 0.016 | - 0.064 ± 0.035 |
| **Selection gradient** | *ADH activity* | -0.007 ± 0.016 | 0.001 ± 0.043 |
| | *ADH plasticity* | -0.028 ± 0.017 | - 0.064 ± 0.043 |
| *Correlation between ADH activity and plasticity* | | -0.11 | 0.56* |

Table 1-2:

Selection estimates (mean estimate ± SEM) on ADH activity and plasticity for larval D. melanogaster developing in a novel alcohol environment, 16% alcohol. All models involving ADH activity used the activity measure from the 16% alcohol environment.

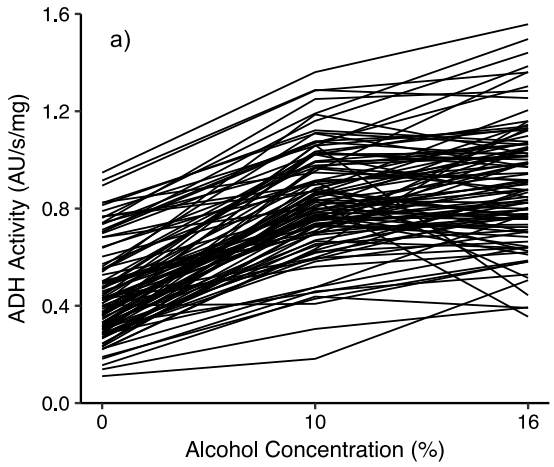
| | | **P010** | **P016** | **P1016** |
|---|---|---|---|---|
| **Linear selection differential** | *ADH activity* | 0.12 ± 0.054 * | | |
| | *ADH activity plasticity* | -0.093 ± 0.064 | 0.081 ± 0.064 | 0.16 ± 0.064 * |
| **Quadratic selection differential** | *ADH activity plasticity* | NA | - 0.12 ± 0.051 * | NA |
| **Selection gradient** | *ADH activity* | 0.076 ± 0.068 | - 0.005 ± 0.083 | - 0.027 ± 0.071 |
| | *ADH activity plasticity* | - 0.11 ± 0.067 | 0.08 ± 0.083 | 0.17 ± 0.071 * |
| *Correlation between ADH activity and plasticity* | | 0.28* | 0.62* | 0.42* |

Figure 1-1



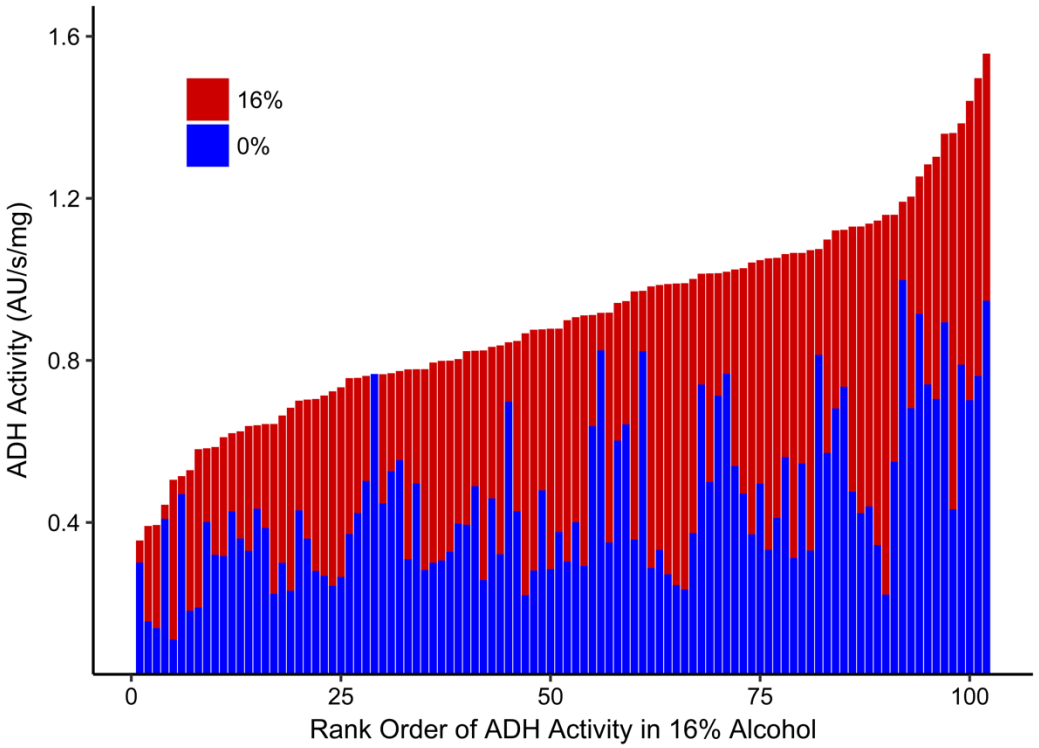

a) Average larval survival rate and b) average larval alcohol dehydrogenase (ADH) activity for *D. melanogaster* in three alcohol environments. Error bars are standard errors and different letters signify statistically significant differences among treatments. Increasing alcohol concentrations decreased larval survival and increased ADH activity.

Figure 1-2



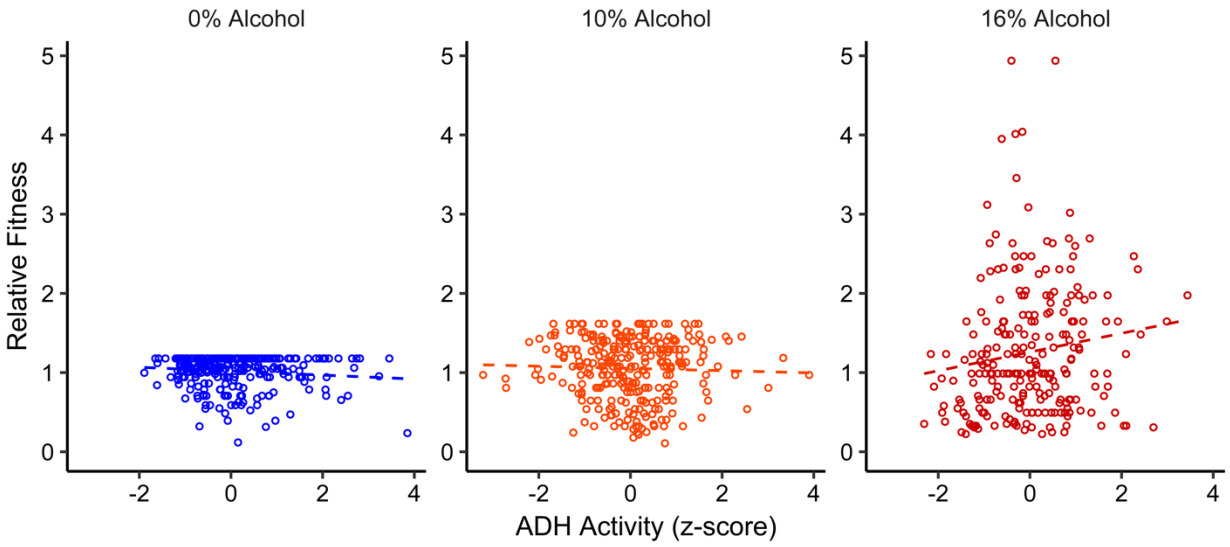

Reaction norms of larval alcohol dehydrogenase (ADH) activity for all maternal lines of *D. melanogaster* across three alcohol environments. All lines exhibited an increase in ADH activity from 0% to 10% alcohol in the larval feeding substrate, but there was variation in the ADH activity response from 10% to 16% alcohol.

Figure 1-3



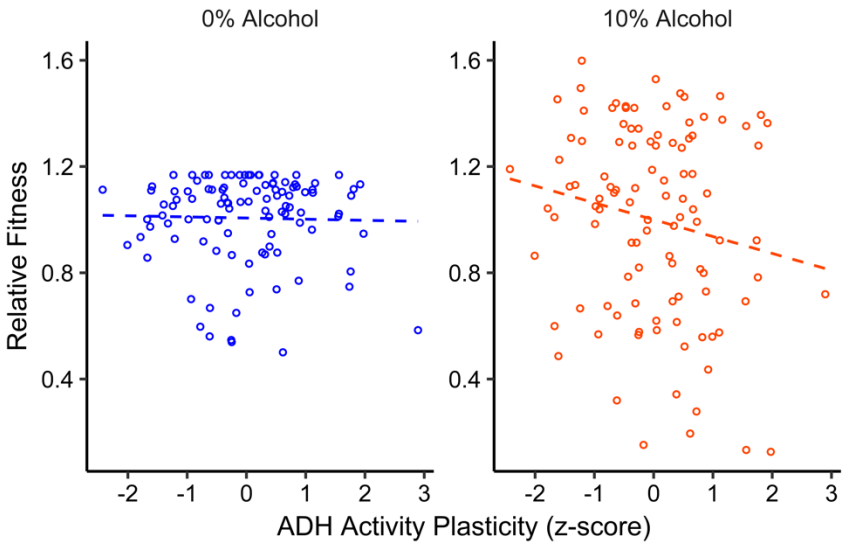

Ranked variation in total dehydrogenase (ADH) activity among maternal lines of larval *D. melanogaster*. The total height of each column represents ADH activity in the 16% alcohol and the lines are ranked by ADH activity in the 16% alcohol environment. Stacked columns indicate baseline ADH activity (blue) when larval lines developed in 0% alcohol and the plastic increase in ADH activity from 0% to 16% alcohol (red). All lines had an increase in activity in the 16% alcohol environment except one that showed no difference.

Figure 1-4



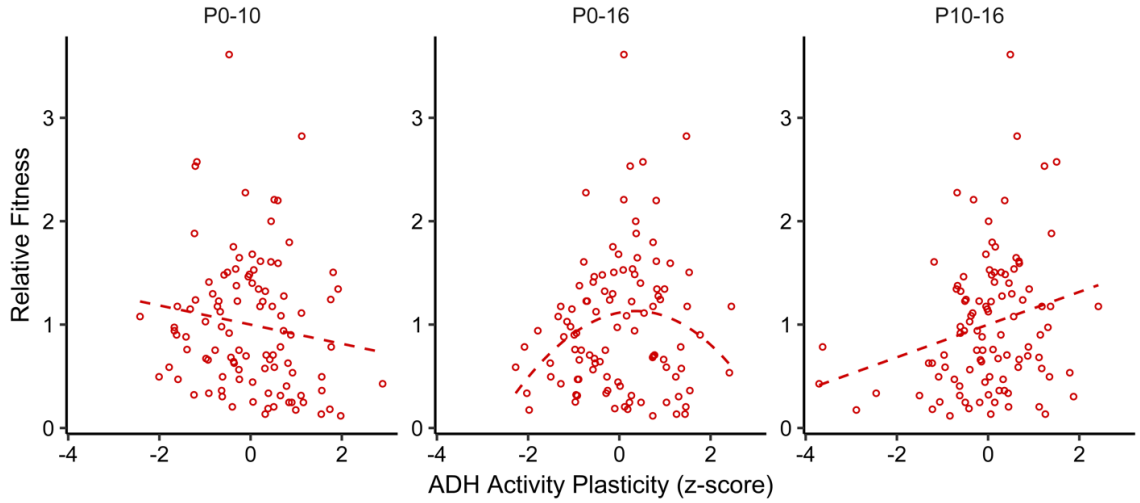

Relationship between relative fitness as measured by *D. melanogaster* larval survival rate and alcohol dehydrogenase (ADH) activity in three alcohol environments. There is a slightly negative relationship between relative fitness and ADH activity when there is no alcohol present in the larval feeding substrate ((ß = -0.025, P = 0.04), no relationship in 10% alcohol environment ((ß = -0.014, P = 0.5), but a significant positive relationship in the 16 % environment ((ß = 0.12, P = 0.03). Each dot represents a maternal line.

Figure 1-5



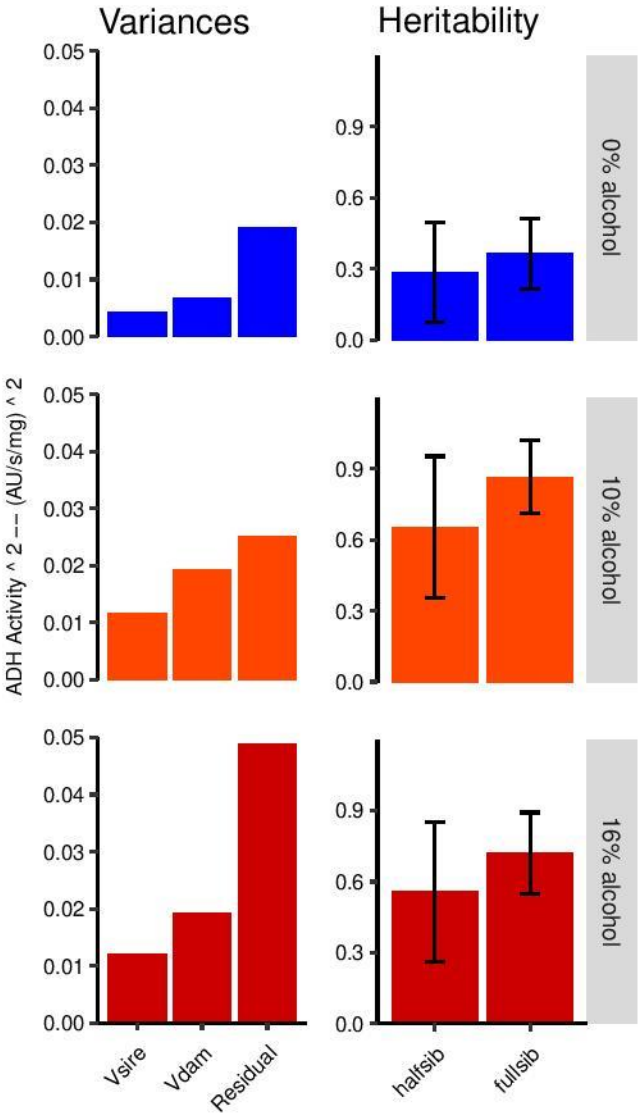

Relationship between relative fitness of larval survival rate of D. melanogaster and alcohol dehydrogenase (ADH) plasticity across historical alcohol environments. ADH plasticity in both panels is measured between 0% and 10% environments. Plasticity had no effect on relative fitness in the either the 0% ((ß = - 0.004, P = 0.8) or the 10% alcohol environment (ß = -0.064, P = 0.07). Each dot represents a maternal line.

Figure 1-6



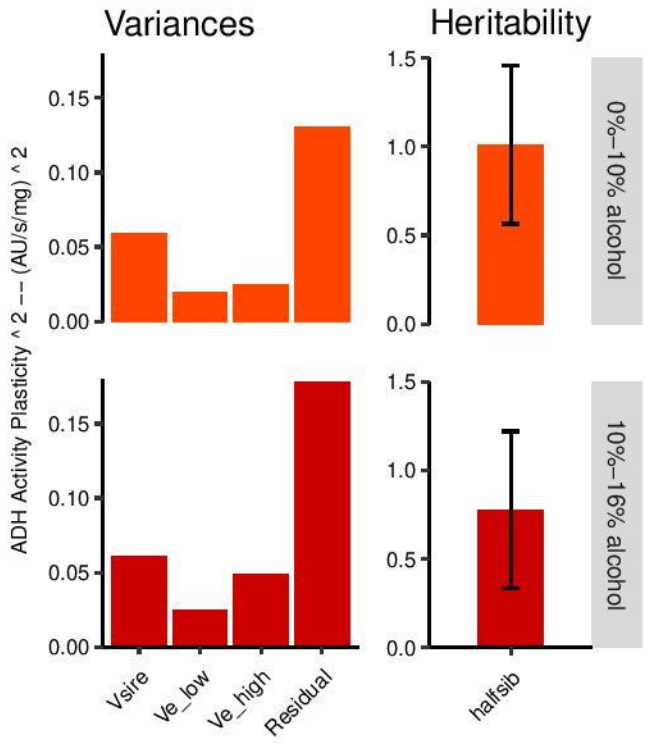

Relationship between relative fitness of larval survival rate of D. melanogaster and alcohol dehydrogenase (ADH) plasticity in the novel 16% alcohol environment. From the left to the right panels, ADH plasticity is measured between 0% and 10%, 0% and 16%, and 10% and 16% alcohol respectively. P0-10 had no effect on relative fitness (ß = - 0.093, P = 0.2). But P0-16 was under stabilizing selection (ß2 = -0.12, P = 0.02), while P10-16 was positively selected (ß = 0.16, P = 0.02). Each dot represents a maternal line.

Figure S1- 1




Variance components and heritability estimates of ADH activity in the treatment environments. "Vsire" is the variance component associated with sires, equivalent to additive genetic variance. "Vdam" is the variance component associated with dams. "Residual" is the residual variance. Heritabilities was estimated using both sire variances and sire + dam variances, resulting in half-sib and full-sib estimates.

Figure S1- 2




Variance components and heritability estimates of plasticity of ADH activity across two environmental ranges, 0%-10% alcohol and 10%-16% alcohol. The layout is similar to Fig S1, except there are two more variance estimates. Ve-low and Ve-high represent residual variances of ADH activity itself in the two alcohol environments across which plasticity is measured. We Added these additional variances to the residual variances when estimating phenotypic variances, because the residual variances alone underestimate true within environment variances when plasticity is calculated as mean differences of full-sibs.

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## Chapter 2 : Different genetic basis for alcohol dehydrogenase activity and plasticity in a novel alcohol environment for *Drosophila melanogaster*

Wang, S. P., & Althoff, D. M. (2020) Different Genetic Basis for Alcohol Dehydrogenase Activity and Plasticity in a Novel Alcohol Environment for *Drosophila melanogaster*. *Heredity.*

### Abstract:

Phenotypic plasticity is known to enhance population persistence (Wang and Althoff, 2019), facilitate adaptive evolution (Levis et al., 2018), and initiate novel phenotypes in novel environments (Levis and Pfennig, 2016). How plasticity can contribute or hinder adaptation to different environments hinges on its genetic architecture. Even though plasticity in many traits is genetically controlled, whether and how plasticity's genetic architecture might change in novel environments is still unclear. Because much of gene expression can be environmentally influenced, each environment may trigger different sets of genes that influence a trait. Using a quantitative trait loci (QTL) approach, we investigated the genetic basis of plasticity in a classic functional trait, alcohol dehydrogenase (ADH) activity in *D. melanogaster*, across both historical and novel alcohol environments. Previous research in *D. melanogaster* has also demonstrated that ADH activity is plastic in response to alcohol concentration in substrates used by both adult flies and larvae. We found that across all environments tested, ADH activity was largely influenced by a single QTL encompassing the *Adh* coding gene and its known regulatory locus, *delta-1*. After controlling for the allelic variation of the *Adh* and *delta-1* loci, we found additional but different minor QTLs in the 0% and 14% alcohol environments. In contrast, we discovered no major QTL for plasticity itself, including the *Adh* locus, regardless of the environmental gradients. This suggests that plasticity in ADH activity is likely influenced by many loci with small effects and that the *Adh* locus is not environmentally sensitive to dietary alcohol.

**Introduction:**

Phenotypic plasticity, the ability of an organism to alter its phenotype according to its environment, is argued to play important roles in many ecological and evolutionary processes, including range expansion, niche shift, population divergence, speciation, and population persistence under anthropogenic environmental changes (DeWitt and Scheiner, 2004; Merila and Hendry, 2014; Pigliucci, 2001; Schaum and Collins, 2014; Schlichting and Pigliucci, 1998; West-Eberhard, 2003). The first step in each of these processes depends on a population's ability to persist and eventually grow under new environmental conditions. During the initial encounter, plasticity can allow a population to respond to the novel environment directly and produce better matching phenotypes before genetic changes have time to occur (Snell-Rood et al., 2010; Yeh and Price, 2004). Thus, plasticity can be initially adaptive. As a population continues to locally adapt, plasticity itself can also be selected to increase and may be instrumental in maintaining the fit between phenotype and the environment over generations (Lande, 2009; Nussey et al., 2005; West-Eberhard, 2003).

Whether and how plasticity will evolve in novel environments relies on its underlying genetic architecture: the amount of genetic variation and the genetic correlations with other traits under selection (Blows, 2007; Gomulkiewicz et al., 2010; Kopp and Matuszewski, 2014; Via et al., 1995). If plasticity is controlled by a few loci with large, additive effects, we can expect rapid evolutionary responses in plasticity and fixation at these loci (Jain and Stephan, 2017). Alternatively, if plasticity is controlled by many loci with interacting effects, its evolutionary trajectory may be much slower, as well as less predictable. A complex network of interacting loci might constrain changes in plasticity due to the large number of simultaneous changes that need to occur. As these changes accumulate over time, there might be new plastic phenotypes

that arise because the effects of a locus can depend on the allelic variation at other loci under selection, which can change as evolution proceeds (Badyaev, 2005; Lande, 2009; West-Eberhard, 2003).

Additionally, the correlation between plasticity and other traits under selection can also influence the rate and speed of plasticity evolution. For example, positive genetic correlations can accelerate the rate of adaptation if the direction of selection aligns between plasticity and other traits under selection (Chevin, 2013; Schluter, 1996). Genetic correlations can arise when plasticity shares causal loci with other traits under selection, or when different causal loci are physically linked on a chromosome. Thus, elucidating the genetic architecture of phenotypic plasticity is critical for understanding the role of plasticity in the evolutionary potential of populations experiencing novel environments.

Growing evidence has shown that phenotypic plasticity is genetically controlled and can correlate extensively with other traits (Hangartner and Hoffmann, 2016; McCairns and Bernatchez, 2010). Two alternative genetic mechanisms have been proposed for phenotypic plasticity. In the allelic sensitivity model, the same genes control both phenotypic variation in each environment and variation in plasticity (Via et al., 1995). Alternatively, in the plasticity genes model, plasticity is an outcome of differential regulatory control of gene expression in different environments (Scheiner, 1993). In reality, these mechanisms are non-exclusive and can jointly influence the plasticity of a single trait (e.g., Wei and Zhang, 2017). When plasticity is mostly determined by allelic sensitivity, variation in plasticity would be highly correlated with trait values. Alternatively, plasticity based on plasticity genes would allow for separate and possibly independent evolutionary fates of trait values and their plasticity.

Although both the evolutionary importance and the genetic architecture of plasticity has received growing interests among both theoretical and empirical studies, we know much less about the evolutionary potential of plasticity in novel environments (Levis and Pfennig, 2019). In order to understand past evolutionary patterns, many studies focus on historically relevant environmental gradients when studying the genetics of plasticity, and both the genetic structure of plasticity and its correlation with other traits can vary substantially, depending on the range of environments investigated (Callahan and Waller, 2000; Fischer et al., 2016; Husby et al., 2011; Lacaze et al., 2009; Tonsor and Scheiner, 2007). The genetic architecture of plasticity in novel environments, however, may further differ from historical environments, thus whether we can predict the effects of plasticity in novel environments is still unclear (Chevin and Hoffmann, 2017).

One way to explore the genetic architecture of plasticity is through quantitative trait mapping (QTL), especially in model systems with a sequenced genome and known gene functions. Identified QTLs provide a starting point for determining the potential causal loci with the largest effects on phenotypes. QTLs regions can be further mined for genes of known functions that may provide insight in their mechanisms of action on plasticity. For example, regulatory genes may be less constrained than protein coding genes to evolve without disruptive outcomes, and small changes in regulatory genes can have large impacts on a phenotype.

We used QTL mapping to compare and contrast the genetic basis of alcohol dehydrogenase (ADH) activity and plasticity across a range of historical and novel alcohol environments using the model organism *Drosophila melanogaster*. Adaptation to alcohol environments through the ADH protein has been a critical evolutionary force in many *Drosophila* lineages, especially in *D. melanogaster*, which spread around the globe by colonizing high alcohol environments associated with human activities such as agriculture (Markow, 2015; Mercot et al., 1994). Some

populations have even colonized winery waste with over 7% alcohol content (Gibson et al., 1981). In addition, Wang and Althoff (2019) demonstrated that *D. melanogaster* can develop in substrate with even higher alcohol content (16%) well outside the range of natural alcohol conditions experienced by larvae. Part of *D. melanogaster's* ability to handle alcohol is due to two well characterized alleles at the ADH locus, fast and slow (Geer et al. 1989), that differ in the rate at which they catalyze alcohol (McDonald et al. 1980; Thompson and Kaiser 1977). Siddiq et al. (2017; 2019) experimentally demonstrate this by using transgenic flies that contain one or the other allele, but also caution that these two alleles alone cannot explain all the variation in alcohol tolerance observed among populations. Thus, there are likely additional loci and other factors that are important in alcohol tolerance.

One reason for *D. melanogaster*'s exceptional alcohol tolerance is that larvae can use the alcohol as an energy source, 90% of which is processed through the ADH pathway (Geer et al., 1985). In larvae, the ADH protein catalyzes the first two steps of the pathway: Alcohol is converted to acetaldehyde and then to acetate, which is finally converted to acetyl-CoA and enters the TCA cycle (reviewed in Geer et al., 1993). Large amounts of the carbon from alcohol is then converted to glycerol and fatty acids for storage (Heinstra et al., 1990), and ADH activity has been shown to control the flux from alcohol to fatty acids (Freriksen et al., 1991). Dietary alcohol can induce increased ADH activity through increased transcription (Geer et al., 1985; Mckechnie and Geer, 1984), but this plasticity is not controlled by variation in the *Adh* coding gene. Using mutant strains, Kapoun et al. (1990) showed that two regions close to the transcription start site can regulate the transcriptional plasticity of *Adh*. However, what natural genetic variation exist for ADH plasticity is not well understood.

To investigate the genetic basis of ADH activity and plasticity, we took advantage of an established mapping resource, the *Drosophila* Synthetic Population Resource (DSPR). DSPR has both fine-mapping power and encompasses global genetic variation, because each mapping population was initiated by crossing 8 genetically distinct founder lines from different parts of the world (King et al., 2012). Using the DSPR, we investigated the genetic basis of ADH activity and plasticity in larvae developing across different alcohol environments that includes the range found in natural conditions (0% and 7% alcohol) and a novel environment (14%). We chose these concentrations because they encompass the environment in which the DSPR is raised (0%), the most extreme concentration (7%) for natural populations (wineries), and a value that is outside the historical range of alcohol concentrations (14%). These three values allow us to compare and contrast the genetic basis of ADH activity and ADH plasticity as alcohol environment changes.

## Methods

### *Mapping population*

We used an established mapping population of *Drosophila melanogaster*, the pB2 subpopulation of the *Drosophila* Synthetic Population Resources (DSPR) for this experiment (King et al., 2012). The DSPR consists of four subpopulations of partially sequenced (using restriction site associated DNA sequencing) recombinant inbred lines (RILs) (King et al., 2012). Each pair of the subpopulations was initiated with 8 genetically diverse founders and were maintained for 50 generations of random mating before generating the RILs, giving the mapping population fine-mapping power (King et al., 2012). We obtained data from 289 of the RILs from the pB2

subpopulation and 5 of its founder lines. All stocks were maintained on standard cornmeal-yeast-molasses medium in growth chambers at 25°C and 24 h darkness.

*Experimental setup*

We measured alcohol dehydrogenase (Bhatia et al., 2014) activity larvae exposed to both historical (0% and 7%) and novel (14%) alcohol environments. For each RIL, the parental generation was raised and kept on food substrate with 0% alcohol. Once eclosed, the adults were allowed to mate freely among individuals with the same genotype for three days, so their offspring (used for the experiment) represented the inbred genotype. Mated females were transferred to fresh media for 16 hours to lay eggs. The eggs were then manually transferred to six rearing vials, two for each treatment: 0%, 7%, and 14% alcohol (ethanol) in the food substrate. Each rearing vial contained 20 eggs at the maximum to keep the larval density low. If the rearing dish contained less than 60 eggs, only three rearing vials were used, one for each treatment. We setup two vials of larvae for some RILs to ensure that enough larvae survived for subsequent analyses, but only collected larvae haphazardly from one of the vials.

All rearing vials were maintained at 25°C and 24 hr darkness until the larvae reached the third-instar stage and were collected for subsequent analyses. Given the slower development at higher alcohol concentrations, larvae in different treatments were collected at different times such that they were all collected at a similar developmental stage based on size (3 days for 0%, 5 days for 7%, and 5.5 days for 14%). We collected larvae by removing the top layer of the food substrate, washing them twice in clean DI water, and used a paintbrush to gently roll them on a clean piece of Kimwipe to dry. Groups of five larvae (or as many as alive if less than five, 3.74 ± 1.58) were collected from one rearing vial per treatment and weighed together on a microbalance. They were then flash frozen on dry ice and stored at -80°C before assayed for ADH activity.

*Phenotyping*

We measured the ADH activity of larvae using spectrophotometric assays following established protocols (modified from Malherbe et al., 2005). To extract ADH from the samples, each group of larvae were homogenized and suspended in an extraction buffer (50 mM glycine-NaOH, 1 mM EDTA, pH 9.5). We scaled the amount the buffer with the number of larvae in each sample, using 50µl of extraction buffer for each larva. The suspension was then centrifuged for 5 minutes at 11,000 rpm, and only the clear supernatant was used for the spectrophotometric assay. All samples were kept on ice during preparation and stored at -80 °C before assaying. To measure ADH activity, we mixed 170 µl of extraction buffer with 20 µl of supernatant and 10 µl of reagent buffer (extraction buffer + 5 mM NAD + and 200 mM 2-propanol). After 30 seconds of mixing, we measured the absorbance at 340 nm every 3 seconds for 60 seconds using a spectrophotometer. The absorbance indicated the amount of NADH produced from 2-propanol and NAD+, catalyzed by the ADH protein, and the rate of NADH production corresponded linearly to ADH activity. For each assay, we used the slope coefficient from a simple linear model (absorbance ~ time) to calculate the average rate of absorbance change. Each sample was measured twice as technical replicates, and we used the mean as the activity measure for that sample. We also assayed a common standard sample prepared at the same time as the experimental samples, and this allowed us to correct for the slow deterioration of ADH activity and the reagent buffer over time. We further corrected all ADH activity measures by the average weight of the larvae in each sample.

*Plasticity measures*

We calculated three different plasticity measures for each RIL: the change in ADH activity between 0% -7% (p07), between 0% - 14% (p014), and between 7% - 14% (p714) alcohol

environments. We used the discrete intervals rather than using parameters from a linear fit, because many RILS responded non-linearly across the different intervals. Since we had no prior expectation which interval was the most biologically relevant, we used all three possible plasticity measures for subsequent QTL analyses. We also calculated the Pearson's correlation coefficients and their significance between all pairs of traits: ADH activity in each environment and the three plasticity measures. These phenotypic correlation coefficients gave us the upper limits of genetic correlations among these traits.

*Quantitative Trait Loci Analysis*

We mapped QTLs for ADH activity in all three treatment environments and all three plasticity measures separately using the R packages "DSPRqtl" and "DSPRqtldataB" that were developed in concert with the DSPR (King et al., 2012; R Core Team, 2017). We visually inspected the data for normality, and none of the traits appreciably deviated from normal distributions. Each QTL analysis produced a list of LOD scores for a set of genetic locations at regular intervals across the genome, and the LOD scores indicate how strongly variation at a genetic location associated with variation in the trait values. To determine which of the genetic locations contained significant QTLs, we performed 1000 permutation tests to determine the appropriate whole-genome LOD score thresholds, with a significance value threshold of 0.05. We then used the conservative 2-LOD support intervals as the confidence intervals. To extract all annotated genes in each QTL region, we used the gene map table from FlyBase (FB2019_05 Thurmond et al., 2018), and we extracted the list of genes in each interval using R (R Core Team, 2017). We further explored these genes' function and their stage specific expression patterns through GBrowse on FlyBase (Thurmond et al., 2018).

Previous studies have shown that allelic variation in the *Adh* protein coding gene and the cis-acting *delta-1* region had large effects on ADH activity in flies, and existing genetic information allowed us to control for their effects in our QTL analyses (Laurie and Stam, 1994). Two alleles for *Adh* and for *delta-1* were found among the founders of our population, the fast and slow alleles of *Adh* and the high and low alleles for *delta-1*. The 8 founders fall into three genotypic classes, fast and high, fast and low, and slow and low. We assigned each RIL to one of the genotypic classes as the founder with the highest hidden Markov model probabilities at the genomic locations where *Adh* and *delta-1* resided. For any of our previous analyses that showed significant QTLs overlapping the *Adh* and *delta-1* region, we performed additional analyses using residuals from models accounting for allelic variation in these two genes. These additional analyses allowed us to map QTLs that were not associated with *Adh* and the *delta-1* loci with greater statistical power.

**Results**

For ADH activity expressed in the 0% alcohol environment, there was a single significant QTL on the left arm of the second chromosome (Table 2-1; Fig. 2-1). This region contained the *Adh* coding gene and its known regulatory loci, *delta-1*, both of which have been established to affect ADH activity in flies (Laurie and Stam, 1994). The same QTL region was also detected for ADH activity in both the 7% and the extreme 14% alcohol environments (Fig 2-1). No other significant QTL was detected for ADH activity.

Because allelic variation in both *Adh* and *delta-1* are well understood to affect ADH activity in flies, we did additional QTL analyses after controlling for their effects. We fitted simple linear

models with ADH activity as the response variable and the allelic variation as the predictor variable, and we used the residuals from the models (separately for each environment) for the additional mapping analyses. This procedure allowed us to pinpoint whether the know allelic variation in *Adh* and *delta-1* loci are the causal loci in the single QTL revealed in the previous analyses. Additionally, controlling for this know allele variation gave us more power to detect additional QTLs. These analyses revealed two additional QTLs for ADH activity, one in the 0% and the other in the 14% alcohol environment (Table 2-2; Fig. 2-2). Neither of these new QTLs overlapped with the QTL encompassing the *Adh* gene, suggesting that allelic variation in *Adh* and *delta-1* was the causal variants for the mapping analyses before controlling for their effects. In the 0% alcohol environment, the additional QTL was located on the left arm of the second chromosome 1.9 Mb downstream from the *Adh* gene. This region contains a large genomic region and 143 annotated genes. Given the large number of genes covered by the region, we only explored the 100 Kb region surrounding the peak location. There were 12 genes in this restricted region, including 7 protein-coding, 3 non-protein-coding, one microRNA, and one small nucleolar RNA genes. Only two of these genes have been observed to have peak expression during the larval stage: *dachshund* and snoRNA:Me28S-C1237. In the 14% alcohol environment, the additional QTL encompassed a total of 12 genes, all but one that code for proteins. Only four of the protein-coding genes are known to have high expression during larval stages. Among these four, only one has any annotated function: CG8223 encodes for a protein that can bind to histones and is involved in nucleosome assembly. The different QTLs mapped in the 0% and 14% alcohol environments suggested that their effects depended upon what alcohol environments larvae experienced. This pattern is further supported by the intermediate correlations among ADH activity in the three environments (Table 2-3).

The RILs showed substantial plasticity in ADH activity (Fig. 2-3). The majority of lines had increased ADH activity when larvae were exposed to higher alcohol concentrations in the larval substrate. There were several lines, however, that exhibited decreased ADH activity in response to increasing alcohol (Fig. 2-3). In contrast to ADH activity, we did not detect QTLs for any of the three plasticity measures (Table 2-1). This was also true when controlling for the allelic effects at the *Adh* and *delta-1* loci. There were several peaks that were close to the global significance threshold, but none that crossed the significance threshold. Interesting, the QTL containing the *Adh* and *delta-1* loci did not contribute to plasticity. Given that some RILs had contrasting patterns of plasticity, we redid the QTL analysis with just the lines that exhibited increasing ADH activity with increasing alcohol separately for each plasticity measure. If different loci were important for increasing or decreasing plasticity, we felt that this might have influenced the significance threshold for any one QTL locus across all RILS. No QTLs were identified for this subset of RILs either.

None of the correlations between ADH activity and plasticity measures were very high (Table 2-3), supporting the pattern that ADH activity and plasticity have somewhat separate genetic basis. The correlations between the three plasticity measures were also intermediate, suggesting somewhat independent genetic architecture for different plasticity measures. Notably, the correlation between p07 and p714 was negative, capturing the substantial nonlinearity in plastic responses to the range of historical to novel alcohol environments.

**Discussion**

Using quantitative trait loci (QTL) analysis, we investigated the genetic architecture of a functional trait, ADH activity, and its plasticity across historical and novel alcohol environments

in *Drosophila melanogaster*. Our goal was to determine if genetic architecture is the same across environments and whether plasticity is controlled by environmentally sensitive loci that code for a functional trait, other loci, or a combination of both. Across all three environments, ADH activity in fly larvae was largely controlled by a major QTL encompassing the *Adh* coding gene and its regulatory loci, *delta-1* (Table 2-1; Fig. 2-1). The effects of *Adh* and *delta-1* have been well characterized for their effects on ADH activity in flies, and the results from this study demonstrate that their importance is not attenuated by differences in alcohol environments. The positive correlations observed between ADH activity measured in the three environments also supported that the genetic basis for ADH activity is strongly consistent across environments (Table 2-3). The sequencing coverage information for the founder lines and existing knowledge in the allelic variation in *Adh* allowed us to test whether the allelic variation at *Adh* and *delta-1* loci was the causal factor in the single QTL (King *et al.* 2012). There are two major alleles for *Adh*, fast and slow, producing ADH proteins that are different in amino acid sequence and specific activity (Kreitman, 1983). Even though the amino acid change does not cause changes in protein quantity, the fast allele is in linkage disequilibrium with other loci in and around the *Adh* gene that leads to higher protein quantities, including a sequence length polymorphism in the first intron, *delta-1* (Corbin and Maniatis, 1990; Laurie and Stam, 1994). Because the fast and slow alleles are correlated with *delta-1* in our mapping population, we controlled for their effects simultaneously. After controlling for this allelic variation, the same QTL region showed little correlation with ADH activity in all three alcohol environments, suggesting that variation in the *Adh* coding gene is the most important cause of differences in ADH activity regardless of the alcohol environments.

Controlling for allelic variation in *Adh* also allowed us to map two additional QTLs for ADH activity, one in the 0% and the other in 14% alcohol environments (Table 2-2; Fig. 2-2). In the 0% alcohol, the new QTL overlapped with a QTL previously discovered in adults raised without alcohol exposure, suggesting that its effects were independent of the developmental stages of the fly (King *et al.* 2012). There were 12 annotated genes in this region, but the molecular function is known for only a few of them. For example, the *dachshund* gene has broad regulatory roles in eye, leg and brain development (Martini et al., 2000). In the 14% alcohol environment, a different QTL was found on the 3R chromosome. In this region, few of the genes annotated have known functions related to alcohol metabolism. The third chromosome has previously been shown to affect alcohol tolerance. Fry (2014) compared the third chromosome from a temperate population with that from a tropical population after crossing either chromosomes into a common African genetic background, and adults with the temperate third chromosome had higher survival under alcoholic conditions than flies with the tropical third chromosome. There was no additional QTL discovered in the 7% environment, suggesting that different genes or the same genes had different effects in the different alcohol environment. Together, the different results from the different alcohol environments indicated that different genetic elements partly control trait variation within historical and across historical and novel environments. Thus, evolution in novel environments may not be entirely constrained by past evolution in historical environments. This is consistent with a large body of evidence from other systems supporting the importance of cryptic genetic variation in evolution in novel environments (Dlugosch et al., 2015; Hamilton et al., 2015; Paaby and Rockman, 2014).

Even though the QTL containing *Adh* and *delta-1*, and the two additional ones found in 0% and the 14 % alcohol environments, explained considerable variation in ADH activity (34 - 49%),

none of them showed any significant association with ADH plasticity. The RILs had strong plastic responses to alcohol concentrations in the larval feeding substrates (Fig. 2-3), but we found no QTLs of major effect that explained variation in plastic responses (Table 2-1). Our ability to map some QTLs in ADH activity suggests that we had enough sample sizes to discover QTLs with large effects, and this suggests that plasticity in ADH activity was not controlled by a few genes with large effects. The lack of plasticity QTLs does not, however, suggest that plasticity is under less genetic control than functional trait values, because plasticity could be controlled by many QTLs with small effects. We did not, however, had enough power to detect QTLs with small effects, and it remains unclear whether plasticity in ADH activity is controlled by similar set of genes in historical versus novel environments.

The contrasting results for ADH activity and its plasticity suggest that somewhat different genes control for ADH activity in different environments and for plasticity. Similarly, Zhou et al. (2012) found little overlap of genetically based and phenotypically plastic transcript expressions in *D. melanogaster*, suggesting a different genetic basis for plasticity. This is consistent with other studies investigating genetic structure of plasticity in other systems across historical environments. For example, trait values and plasticity were found to associate with largely different sets of genetic markers for growth and phenology traits in willows (Berlin et al., 2017; Hallingback et al., 2019), for morphology, phenology and reproductive traits in maize (Kusmec et al., 2017), and for growth traits in yeast (Yadav et al., 2016). Even though QTLs for trait values and plasticity can differ greatly in some organisms, it is not uncommon to find plasticity genes to co-locate with trait QTLs, such was found for biomass in hybrid willows (Berlin et al., 2017) and for phenology in maize (Li et al., 2016). These examples and our data highlight that

plasticity can evolve independently from trait values in some environments, and this pattern is robust across both historical and novel environments.

In conclusion, we found that the genetic basis of ADH activity was largely determined by a QTL containing the gene *Adh* and its regulatory locus, *delta-1*. This QTL is important in ADH activity across both historical and novel concentrations of alcohol in larval substrates. We also found that the *Adh* coding gene is the most important factor influencing variation in ADH activity, but different minor QTLs were important in different alcohol environments. These patterns indicate that ADH activity could evolve unconstrained by trait evolution in either historical or novel environments. Although fly RILs expressed plastic responses to alcohol concentrations, plasticity is likely controlled by many loci with medium to small effects and is largely controlled by separate genetic elements than those controlling ADH activity. Studies in additional systems are needed to determine if this is a common pattern for functional traits and their plasticity. As demonstrated in this study, established mapping resources could be used for detailed genetic dissection of functional traits and plasticity.

Acknowledgement:

The authors thank J. Belote, S. Dorus, and S. Pitnick for providing facilities to rear the flies. J. Friedman, S. Pitnick, and K. Segraves also provided valuable discussions throughout the course of the study. We thank three anonymous reviewers for their insightful comments that greatly help refine the manuscript. Funding for the research was provided NSF 1556568 and 1655544 to D.M.A.

Table 2-1

Results of QTL analysis for ADH (alcohol dehydrogenase) activity and plasticity of ADH activity in larvae of Drosophila melanogaster developing at different alcohol concentrations in the larval feeding substrate.

| ADH Trait | Substrate Alcohol | Chromo-some | Peak location(Mb) | LOD | Confidence interval | Effect on phenotype | Genes |
|---|---|---|---|---|---|---|---|
| **activity** | 0% | 2L | 14800000 | 36.6 | 2L:14,380,000..14,960,000 | 49.07% | Adh |
| **activity** | 7% | 2L | 14840000 | 32.2 | 2L:14,430,000..14,950,000 | 45.59% | Adh |
| **activity** | 14% | 2L | 14470000 | 20.4 | 2L:14,320,000..14,940,000 | 34.78% | Adh |
| **P07** | | no significant QTL detected | | | | | |
| **p014** | | no significant QTL detected | | | | | |
| **P714** | | no significant QTL detected | | | | | |

Table 2-2

Results of QTL analysis for ADH (alcohol dehydrogenase) activity in larvae of *D. melanogaster* after controlling for *Adh* and *delta-1* loci effects.

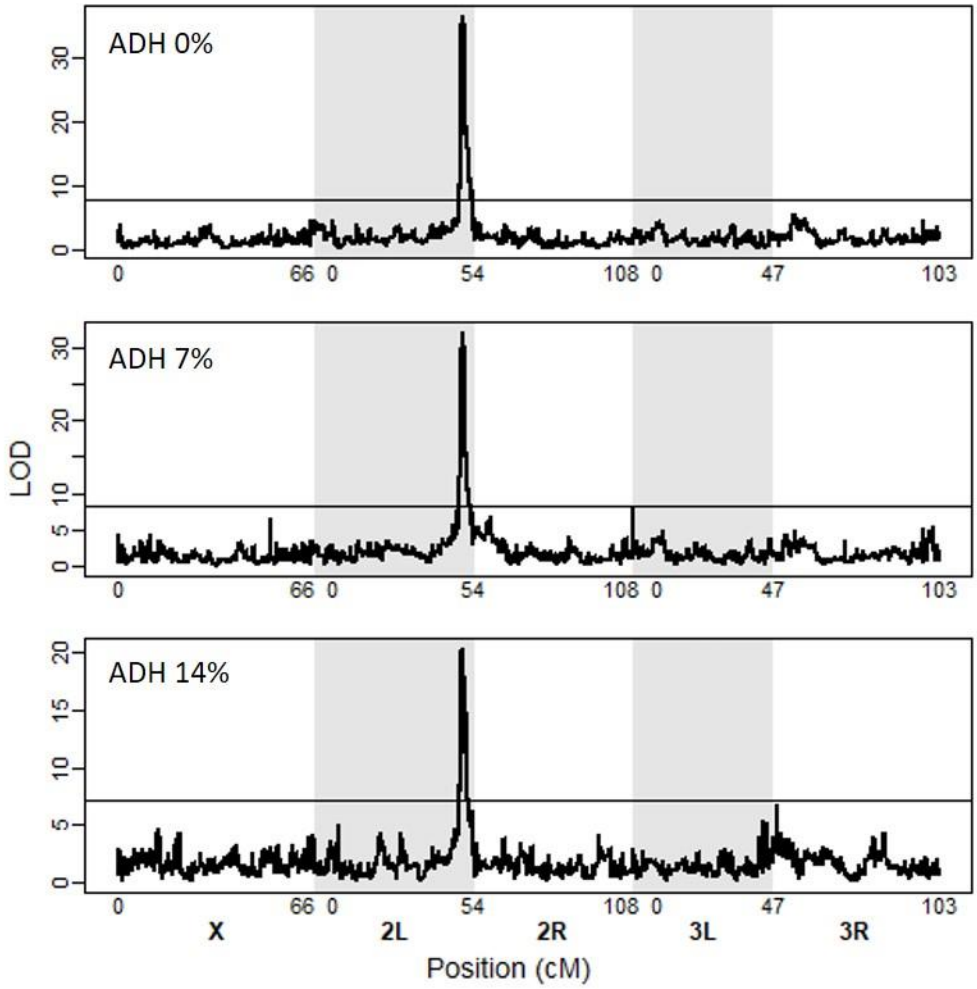
| ADH Trait | Substrate Alcohol Conc. | Chromosome | Peak location (Mb) | LOD | Confidence interval | Effect on phenotype | Genes |
|---|---|---|---|---|---|---|---|
| **activity** | 0% | 2L | 16510000 | 8 | 2L:16,180,000..17,480,000 | 13.93% | unknown |
| **activity** | 14% | 2L | 4590000 | 9.6 | 3R:8,744,278..8,794,278 | 18.41% | unknown |

Table 2-3

Pearson's correlation coefficients between alcohol dehydrogenase (ADH) activity and its plasticity across differing concentrations of alcohol (0, 7, and 14 %) in the feeding substrate of larval *D. melanogaster*. Plasticity is indicated by p07, p014, and p714 which indicates environments across which plasticity was estimated. Significant coefficients are marked by * ($p < 0.05$).

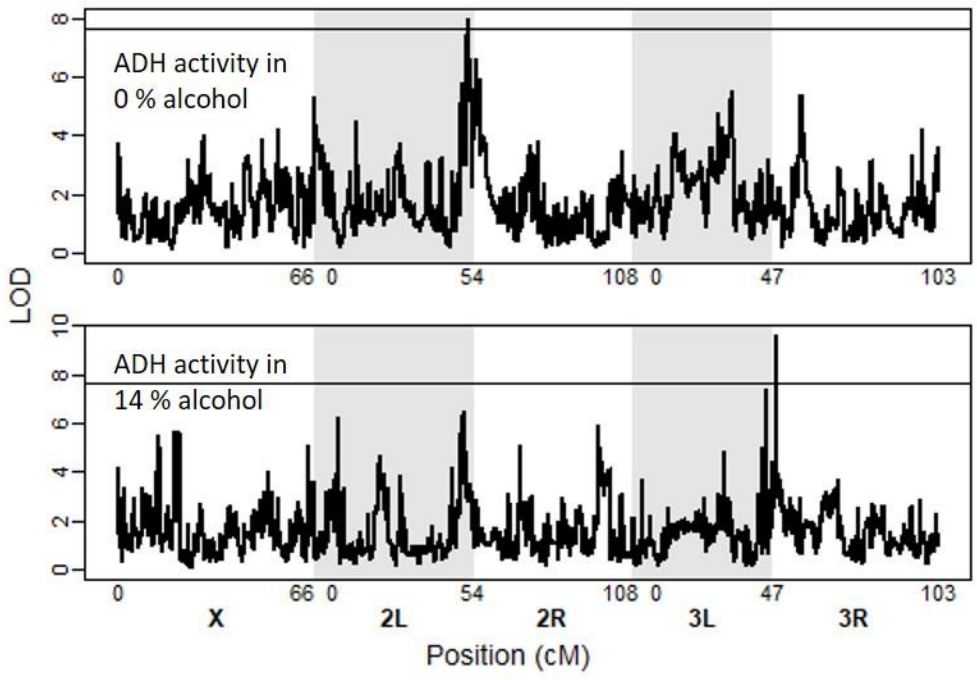
| ADH Traits | ADH 0% | ADH 7% | ADH 14% | p07 | p014 | p714 |
|---|---|---|---|---|---|---|
| **activity 0%** | 1 | ------ | ------ | ------ | ------ | ------ |
| **activity 7%** | 0.57* | 1 | ------ | ------ | ------ | ------ |
| **activity 14%** | 0.47* | 0.46* | 1 | ------ | ------ | ------ |
| **p07** | -0.44* | 0.48* | 0.011 | 1 | ------ | ------ |
| **p014** | -0.41* | -0.027 | 0.61* | 0.41* | 1 | ------ |
| **p714** | -0.038 | -0.45* | 0.58* | -0.44* | 0.64* | 1 |

Figure 2-1



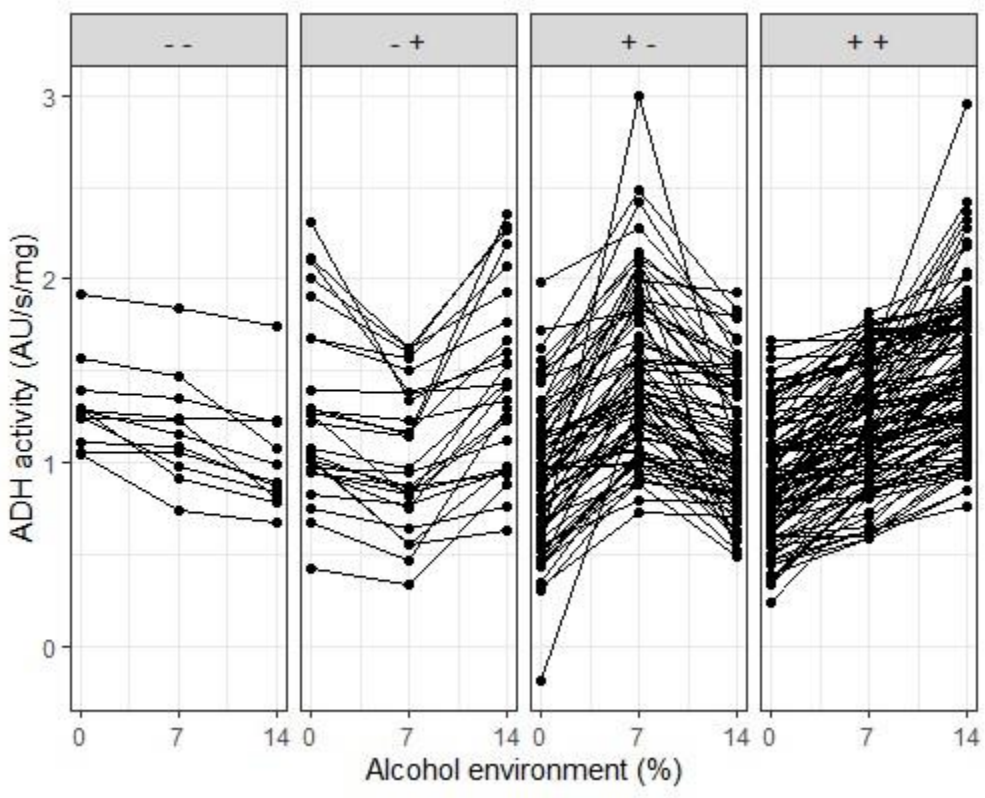

QTL identified for ADH (alcohol dehydrogenase) activity in larval *Drosophila melanogaster* developing in substrate with 0%, 7% and 14% alcohol (top, middle, bottom panel, respectively). Black line represents the 95% LOD score thresholds using 1000 permutation tests. The identified QTL contains the *Adh* gene and its regulatory locus, *delta-1*.

Figure 2-2




QTL identified for ADH (alcohol dehydrogenase) activity in larval *Drosophila melanogaster* developing in substrate with 0%, and 14% alcohol (top, bottom panel, respectively) after controlling for allelic variation in Adh and delta-1 loci. Black line represents the 95% LOD score thresholds using 1000 permutation tests.

Figure 2-3




Reaction norms for ADH (alcohol dehydrogenase) activity in *Drosophila melanogaster* larvae developing in different concentrations of alcohol in the larval substrate. Each line represents a RIL from the pB2 subpopulation from the *Drosophila* Synthetic Population Resources. Symbols in each panel represent the sign of ADH plasticity for the 0-7% and 7-14% alcohol ranges.

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**Chapter 3 : Contrasting effects of competition and mutualism in evolving yeast populations**

Wang, S. P., & Althoff, D. M. Contrasting effects of competition and mutualism in evolving yeast populations. In prep.

**Abstract:**

Phenotypic plasticity, the ability of a genotype to express alternative phenotypes in different environments, can determine how an organism performs when facing abiotic and biotic environmental variations. Although we have a relatively advanced understanding of when and how phenotypic plasticity can evolve in response to changes in abiotic environments, we know much less about how biotic interactions influence plasticity evolution. We experimentally tested how two distinct types of interactions- competition and mutualism- affected trait and plasticity evolution using brewer's yeast (*Saccharomyces cerevisiae*). We quantified evolutionary changes in growth rate, growth plasticity, resource use efficiency (RUE), and RUE plasticity in strains evolved alone, with a competitive partner, and with a mutualistic partner. Compared to their ancestors, strains evolved alone had lower resource use efficiency and RUE plasticity. We also found an evolutionary tradeoff between changes in growth rate and RUE in strains evolved alone, suggesting a selection for increased growth rate. Assayed in monocultures, similar to strains evolved alone, strains evolved with a competitive partner had higher growth rates, slightly lower RUEs, and a stronger tradeoff between growth rate and efficiency. In comparison, mutualism had opposite effects on trait evolution. Strains evolved with a mutualist partner had slightly lower growth rates, higher RUEs, and a weak evolutionary tradeoff between growth rate and RUE. One reason for this difference was that unlike competition, mutualism weakened resource limitations and temporal heterogeneity in limiting resources over time. Despite their different effects on trait evolution, competition and mutualism had little effect on plasticity

evolution in either growth rate or RUE, suggesting that abiotic factors could be more important than biotic factors in generating selections for plasticity. Our results demonstrated that competition and mutualism had very different effects on trait evolution, while only abiotic factors influenced trait plasticity.

**Introduction:**

Species interactions such as competition, predation and mutualism are important drivers of evolution, exemplified by the phenotypic diversification of Darwin's finches (Grant and Grant 1995), widespread aposematic coloration in butterflies (Mallet and Joron 1999), and active pollination behaviors in yucca moths and fig wasps (Pellmyr et al. 1996; Machado et al. 2001). These remarkable examples of adaptation are caused by both direct selection from the interactions and indirect selection pressure due to altered resource dynamics (Svanbäck and Bolnick 2007; Martin and Pfennig 2012; Caruso et al. 2017; Reznick et al. 2019). Given the natural variability in resource availability and interacting partners across time and space, many species have evolved plastic phenotypic expressions in response to both abiotic and biotic factors (e.g. Ayrinhac et al., 2004; Dorn et al., 2000; Scoville & Pfrender, 2010). Even though the importance of biotic interactions on trait evolution has been demonstrated repeatedly (Cosetta and Wolfe 2019; Miller et al. 2019; Moreira-Hernández and Muchhala 2019), we are just beginning to understand how these interactions influence the evolution of phenotypic plasticity (Agrawal 2001; Scheiner et al. 2015).

Adaptive phenotypic plasticity has been shown to be important for many types of biotic interactions. For example, spadefoot toad larvae can develop either omnivore or carnivore morphs depending on prey densities (Pfennig 1990), and *Daphnia* individuals can plastically produce helmets that protect them from predators (Tollrian 1990). Temporal and spatial heterogeneity in interaction types and strength can select for evolutionary increases in phenotypic plasticity (Sultan and Spencer 2002), but we know very little about how biotic interactions differ in their effects on plasticity evolution. A notable exception is a simulation study by Scheiner et al. (2015), which showed that biotic interactions led to reduced plasticity

when species are simultaneously adapting to abiotic and biotic conditions. Their results also showed that different types of biotic interactions varied in their effects on plasticity evolution, and competition led to reduced plasticity in response to abiotic variation while mutualism increased plasticity. Additionally, these two types of interactions presumably change resource availability for the interacting species in opposite ways, making competition and mutualism ideal starting points for investigating how biotic interactions impact plasticity evolution.

We expect competition and mutualism to have different effects on plasticity evolution, partially through their differential effects on resource dynamics. Exploitative competition can reduce the availability of limiting resources for the competing strains, while resource-exchange mutualism can provide limiting resources for each other. The effects of these interactions, however, are mitigated against the backdrop of resource input and its temporal variability in the environment. When resources are pulsed into the environment, competitive interactions will deplete these resource pulses quickly. Under this type of resource cycling from high to low, we expect plasticity in resource use traits to evolve higher. In contrast, when living in a competitive environment in which resources in the environment do not arrive in pulses, resource availability will remain low and we expect the organism to evolve increased competitive ability by increasing resource uptake and/or by reducing resource requirements (Tilman 1982; Helling et al. 1987). This could lead to less sensitivity to the abundance of limiting resources and select for decreased plasticity. Mutualistic interactions can reduce temporal resource heterogeneity by providing a continual input of resources that would reduce the magnitude of resource pulses or provide a stable level of resource input in the absence of pulses. Reduced resource heterogeneity may lead to reduced plasticity in traits related to resource use. However, whether competition

and mutualism have contrasting impacts on the evolution of phenotypic plasticity remains empirically untested.

The lack of empirical evidence partially stems from the challenge of monitoring plasticity evolution in species engaged in different types of interactions. To directly test the effects of the interactions, similar species need to consistently engage in different types of interactions such that the evolution of plasticity can be monitored, which is extremely difficult in natural systems. One good model system that satisfies this need is microbial communities. Microbes can engage in diverse types of interactions, and their rapid generation time allows for monitoring of evolutionary changes in short time periods (Elena and Lenski 1997). Although competition is the predominant interaction in some microbial communities (Foster and Bell 2012), other types of interactions such as mutualism are also widespread (Crespi 2001; Morris et al. 2013). For example, syntrophic metabolism or microbial cross-feeding has been observed across many different habitats ranging from marine sediments to freshwater, and from thermal springs to permafrost (McInerney et al. 2008). Additionally, phenotypic plasticity has been shown to evolve rapidly in microbes in response to abiotic environments (Schaum and Collins 2014). Thus, experimental evolution with microbes is an accessible system to test the effects of species interactions on plasticity evolution.

We quantified evolution of two phenotypes, growth rate and resource use efficiency (RUE), as well as their plasticity in yeast (*Saccharomyces cerevisiae*) strains evolved with a competitive or a mutualist partner. Microbes are considered efficient in resource use when they are able to convert the same amount of resources into more biomass, thus sustaining higher population density with the same amount of resources (Bachmann et al. 2013). We also evolved all strains alone to establish a baseline of evolution caused by the culturing conditions. In the absence of

biotic interactions, we expect evolution of rapid growth, because growth rate is a direct measure of fitness in our culturing method- serial propagation (Bachmann et al. 2013; Lin et al. 2020). Fast growing cells are more likely to be represented in each transfer, but these cells are usually less efficient and typically have a lower yield for a given amount of resources than slower growing cells (Frank 2010; Bachmann et al. 2016; Manhart et al. 2018). We expect this tradeoff to drive the evolution of reduced resource use efficiency in evolved strains. Additionally, the cycling of resources depletion from abundant to extremely limited due to population growth after each transfer should select for increased plasticity in resource acquisition in order to maintain high growth rate across all resource availabilities, leading to reduced plasticity in growth rate. Competition and mutualism can modify the resource dynamics and are expected to change how traits and their plasticity evolve. Because competition can cause increased rates of resource decline, we expect strains evolved with a competitive partner to respond similarly to strains evolved alone but have more pronounced evolutionary response. In contrast, the mutualistic interaction can provide a continual input of limiting resources, and the more stable resource environment should select for slower growing, more efficient cells, and a reduction in plasticity.

To construct competitive and mutualistic communities, we combined yeast strains with different mutations in key metabolic genes (Fig. 3-1). Specifically, the competitive strains are deficient in production of one essential nutrient, either adenine (Ade) or lysine (Lys), but were self-sufficient with the other nutrient ($Ade^{WT}Lys^{-}$ and $Ade^{-}Lys^{WT}$), while the mutualists strains are similarly deficient in producing one of the nutrients but overproduced the other nutrient ($Ade^{OP}Lys^{-}$ and $Ade^{-}Lys^{OP}$). We evolved all strains in standard medium with reduced adenine and lysine content (24mg/L of adenine; 54mg/L of lysine). We chose these concentrations such that a competitive pair needs to compete for resources other than adenine and lysine, and that a mutualist pair

benefits from each other's adenine and lysine production while also competing for other resources. After 4 weeks of evolution, we compared the ancestral and evolved strains in their growth, RUE, as well as their phenotypic plasticity in response to resource availability. We quantified plasticity using two environments: the resource environment in which they evolved (the 'high' environment) and an environment with reduced adenine and lysine (the 'low' environment, 4mg/L of adenine; 9mg/L of lysine). Using this design, we addressed three specific questions: (1) How does evolution in isolation from interspecific partners influence the evolution of traits related to fitness and their plasticity? (2) How does evolving with a competitor change the pattern of evolution as compared to evolving alone? (3) Does evolving with a mutualistic partner produce results similar to competition, or are there different evolutionary outcomes?

**Methods:**

*Community setup*

We used eight genetically engineered and reproductively isolated strains of *Saccharomyces cerevisiae* to form two types of communities dominated by either mutualistic or competitive interactions (Fig. 3-1). The differences in the strains' ability to produce essential nutrients determine how they interact. The competitor strains are self-sufficient with either adenine or lysine but are deficient in producing the other nutrients (CA: $Ade^{WT}Lys^{-}$ and CL: $Ade^{-}Lys^{WT}$), thus, in medium with lysine and adenine available, they interact competitively for all other essential nutrients. In contrast, the mutualist strains provide complementary benefits to one another, and the partners overproduce either adenine or lysine and are deficient in making the other nutrient (lysine and adenine respectively) When the two types of mutualist strains (MA:

$Ade^{OP}Lys^{-}$ and ML: $Ade^{-}Lys^{OP}$) grow in a co-culture, they benefit from each other's overproduction. We used additional genetic markers—histidine and leucine deficiency—to differentiate interacting strains within co-cultures using positive selection. We created strain pairs of all possible combinations of interaction types and marker types to account for potential markers effects. In total, we used four pairs of interacting strains to test the differences between mutualistic and competitive communities: MA his- with ML leu-, MA leu- with ML his-, CA his- with CL leu-, and CA leu- with CL his-. Since these strains are reproductively isolated and can perform different ecological roles, we treat them as different species.

*Experimental design*

We used experimental evolution to investigate the effect of biotic interactions on the evolution of phenotypic plasticity. We set up three treatment levels of biotic interactions: communities initiated with a single strain (evolved alone with no interspecific interactions), competitor pairs, and mutualist pairs, giving a total of 12 unique community types. The communities with strains evolved alone allowed us to test for the selective effects of the culturing environment. The culturing medium was modified from a standard medium (0.15% (w/v) Difco yeast nitrogen base without amino acids or ammonium sulfate, 0.5% ammonium sulfate, 2% (w/v) dextrose, with supplemental amino acids), and we reduced the adenine and lysine content by 40%. This reduced level of adenine and lysine allowed all experimental communities to sustain growth while the mutualistic communities reached higher total biomass than competitive communities.

Experimental communities were maintained in 2 ml of medium in 48-deepwell plates. The plates were covered with aluminum foil punctured once with a sterile needle for aeration and placed on a rotating wheel at 30°C under 24h darkness. We obtained data on a total of 166

communities (75 single, 56 mutualistic, and 35 competitive). All cultures were initiated at a low population density of 0.1 $OD_{600}$ with equal starting densities for strain pairs. The clones used to initiate the experiment were flash frozen in 25% glycerol and stored at -80°C. These were designated as the 'ancestral' strains. Cultures grew for two days before the first transfer and were then transferred to fresh medium daily. We used a standard volume transfer of 5% (100 µL). We allowed the experimental cultures to evolve for four weeks, and at the end of the four weeks, the cultures were frozen as above. These cultures were designated as 'evolved'.

*Growth measurements*

We assayed growth features of the ancestral and evolved strains in two assaying media using monocultures. We first revived the ancestral and evolved strains by plating the frozen cultures onto selective agar plates where only one type of strain can grow (four types of selective plates with standard medium: -Ade-Leu for $Ade^{WT/OP}His^{-}$, -Ade-His for $Ade^{WT/OP}Leu^{-}$, -Lys-Leu for $Lys^{WT/OP}His^{-}$, and -Lys-His for $Lys^{WT/OP}Leu^{-}$). To ensure that all replicates had independent evolutionary histories for the evolved strains, we haphazardly picked one colony per strain type per community to be grown for two days (with daily transfer from low density, determined visually) in the same liquid medium that was used during experimental evolution. The two days of growth helped standardize subsequent trait measurements, because it allowed all the inoculating cultures to reach similar and relatively high population sizes prior to setting up the assay cultures for growth measurements. We used the day two cultures to inoculate two assay cultures in two nutrient environments: low and high adenine and lysine (4mg/L of adenine and 9mg/L of lysine for the low environment, and , 24mg/L of adenine and 54mg/L of lysine for the high environment) starting from a low density (0.1 $OD_{600}$). This setup allowed us to assess both trait evolution in the original evolutionary environment and trait plasticity. We grouped strains

by their initial strain type for these growth assays; thus, measurements of ancestral and evolved strains from each treatment were directly comparable. Each group of strains contained one evolved strain from all possible treatments and two colonies of the ancestral strain. When possible, we assayed similar mutualist and competitor strains at the same time, determined by their genetic markers, such as MA his- with CA his-. This further allowed us to directly compare differences between strains evolved with a mutualistic partner and ones with a competitive partner. All cultures were initiated as 2ml cultures in 48-deepwell plates as described above and were aerated on a rotating wheel in a dark room at 30 °C.

We measured growth rate of the assay cultures during the exponential phase of population growth and yield at 24 hrs. To measure these parameters, we measured population density (as determined by $OD_{600}$) at 4, 6, and 24 hours. We calculated growth rate, *r*, as the number of doublings during exponential growth between 4-6 hours:

$$r = \ln(OD_{600}^{t1} / OD_{600}^{t0}) / \ln(2)$$

We used the $OD_{600}$ value at 24 hours as the measure of yield. We chose this time frame because this was the duration between transfers for the evolution experiment and represented the greatest population size attainable before transfer.

*Resource Use Efficiency measures*

We quantified resource use efficiency (RUE) by using yield as a proxy. Our yield measures can be interpreted as the biomass produced given the amount of resources in the media. In both the low and high assay environments, yield was limited by either adenine and lysine and represented adenine or lysine use efficiency. Thus, within an assay environment, more efficient strains

achieve higher population yield. The yield measures in the two assay environments were not directly comparable as efficiency measures, because the high environment contained six times more adenine and lysine than the low environment. We divided the yield measures from the high assay environment by 6 to standardize them to the low nutrient assay environment, making all RUE measures represent the amount of biomass produced with 4mg/L of adenine and 9mg/L of lysine.

*Plasticity measures*

We calculated plasticity of growth and resource use efficiency for each clone as the difference in the trait value between the low and the high adenine and lysine assay environments. We calculated the growth plasticity as growth rate measured in the high minus the low environment. For efficiency plasticity, however, we used efficiency measured in the low minus the high environment for efficiency plasticity, because efficiency decreased with increasing environmental adenine and lysine.

*Data analysis*

We used the differences in trait and plasticity values between strains evolved alone and their ancestors to quantify effects of the abiotic environment (evolving under our culturing condition for 4 weeks), and we used the differences between strains evolved alone and strains evolved with either a competitive or mutualist partner to quantify the effect of the biotic interactions. For example, we calculated plasticity for an ancestral strain and a strain evolved alone, and then we subtracted the ancestral value from the evolved value to determine the change in plasticity. A negative value would indicate that plasticity evolved to be lower, i.e., the evolved strain was less responsive to nutrient concentrations. These trait and plasticity differences were calculated

between pairs of strains that were measured at the same time, which allowed us to remove some of the random variation caused by genotypes and measurement batches. Since each measurement batch contained only one evolved strain from each genotype and treatment, calculating the trait differences (evolved alone minus ancestors, and evolved with a partner minus evolved alone) did not compromise independence or replication while allowed us to simplify our models.

We used linear mixed models to analyze effects of the abiotic culturing condition (evolved alone minus ancestors) and effects of biotic interactions (evolved with a partner minus evolved alone) for growth rate, growth plasticity, resource use efficiency, and RUE plasticity. We analyzed changes in growth rate and RUE using measurements from only the high adenine and lysine environment, which was the same as the evolutionary environment. We fitted four models (one for each trait) to tested whether the evolutionary effect was different from zero, represented by the intercept in the model output (abiotic effects in Table 3-1). Similarly, we fitted another four models to test the effect of biotic interactions with the type of interaction as a fixed effect; thus, the intercept represented the effects of competition in comparison with strains evolved alone, and the slope represented the effects of mutualism in comparison with competition (marked as competition and mutualism in Table 3-1). All models had strain identity as a random effect. We first tested whether the genetic differences among the experimental strains were important using models containing the strain type (either $Ade^-$ or $Lys^-$) and the marker genotype ($His^-$ or $Leu^-$) and their interaction as covariates. These genetic effects were minimal for all models, so we simplified all the models to contain only strain identity as a random effect to simplify model interpretation.

We also investigated the evolutionary tradeoff between growth rate and efficiency using simple linear regression. We used the trait differences between pairs of strains describe above in growth

rate as the predictor variable and the trait differences in RUE as the response variable. A negative correlation between these two measures suggested an evolutionary tradeoff between growth rate and RUE.

All statistical analyses were done in the R environment (R Core Team 2016). We used the lme4 and lmertest packages for fitting the linear mixed models and the ggplot package for figures (Bates et al. 2014; Wickham 2016). For interpreting the model outputs, we used a significance threshold of 0.05.

**Results:**

*How does evolution in isolation from interspecific biotic partners influence the evolution of traits related to fitness?*

Strains evolved alone had similar growth rates than their ancestors (Table 3-1; Fig. 3-2a). Similarly, there was no change in growth rate plasticity across the low and high adenine and lysine media (Table 3-1; Fig. 3-2a). In contrast, evolved strains had 7.9% lower RUE than their ancestors (Table 3-1; Fig. 3-2b). A stronger reduction was also detected for RUE plasticity (-30.1%, Table 3-1; Fig. 3-2b). These comparisons provide the baseline expectations of evolutionary changes due to the culturing conditions used in our experiment—lower RUE and a decrease in RUE plasticity.

*How does evolving with a competitor change the pattern of evolution in growth, efficiency, and their plasticity?*

In comparison to strains evolved alone, strains evolved with a competitor had in general higher growth rate, lower RUE, and no difference in either plasticity measures (Table 3-1; Fig. 3-2c, 3-

3a). Growth plasticity was slightly higher for strains evolved with a competitive partner, although this effect was only marginally significant (Table 3-1, Fig. 3-2c, 3-3c). Resource use efficiency was 3.6% lower for strains evolved with a competitive partner (Table 3-1; Fig. 3-2d, 3-3b). There was little difference in growth plasticity or RUE plasticity between strains evolved with a competitive partner or alone (Table 3-1; Fig. 3-2d, 3-3d).

*Does evolving with a mutualistic partner produce similar results than with a competitive partner, or are there different evolutionary outcomes?*

Evolving with a competitive vs a mutualist partner had different effects on trait evolution but not plasticity evolution. Strains evolved with a mutualist partner had similar growth rates than strains evolved alone (Table 3-1, Fig 3-2c, 3-3a, 3-3c). In contrast to strains evolved with a competitor, strains evolved with a mutualistic partner had 5.6% higher resource use efficiency (Table 3-1, Fig 3-2d, 3-3b). Despite its effects on trait evolution, mutualism did not have an observable effect on the evolution of growth or RUE plasticity (Table 3-1, Fig 3-2d, 3-3d).

*Tradeoffs in growth and efficiency*

We found strong evolutionary tradeoffs between growth rate and resource use efficiency among strains evolved alone and strains evolved with a competitive partner but not strains evolved with a mutualistic partner. Comparing strains evolved alone with their ancestors, there was a strong negative correlation between evolutionary changes in growth rate and changes in resource use efficiency ($\beta = -3.4 \pm 0.9$, $p < 0.01$, Fig 3-4a). A similar pattern was observed in strains evolved with a competitor ($\beta = -4.6 \pm 1.3$, $p < 0.01$, Fig 3-4b). In contrast, this negative correlation was lower and not statistically different than zero for strains evolved with a mutualistic partner ($\beta = -1.5 \pm 1.3$, $p = 0.27$, Fig 3-4c).

**Discussion:**

The phenotype of an organism is shaped by the interaction between its genetics and the environment, i.e. its phenotypic plasticity. Phenotypes of interacting organisms determine the outcomes of their interactions, which then can influence their fitness. Furthermore, through biotic interactions, phenotypic plasticity can influence a population's ecological success and evolutionary trajectory (reviewed in Agrawal, 2001). Some studies have demonstrated that plasticity can evolve quickly in response to variation in biotic partners (Scoville and Pfrender 2010; Stoks et al. 2016); however, we know very little about whether different types of biotic interactions tend to impose similar or contrasting effects on phenotypic evolution. Using a synthetic yeast model, we found that different types of biotic interactions led to variable trait evolution but similar plasticity evolution. Specifically, competitive interaction led to the evolution of increased growth rate, reduced resource use efficiency (RUE), and strong tradeoff between growth and RUE. In comparison, mutualistic interaction led to increased RUE and no tradeoff between growth and RUE. Despite evolutionary changes in trait values, competition and mutualism similarly had little effect on the evolution of either growth plasticity or RUE plasticity. Together, these results show that different types of interactions can alter the environment in such a way to drive trait evolution in opposite directions in the interacting species, but biotic interactions were less important for plasticity evolution.

Although we expected growth rate and growth plasticity to evolve as a result of culturing conditions, strains that evolved alone did not show a significant change in either trait. We expected the evolution of increased growth rate in our evolved strains because growth rate should contribute positively to fitness in well-mixed cultures that were frequently transferred. Our transferring regime of daily nutrient input should have selected for rapid growth during the

period of resource abundance when most of the population growth happened (Bachmann et al. 2013). One potential reason for the lack of change in growth rate and its plasticity is that our serial propagation regime and the medium is very similar to the conditions under which the ancestral strains have been maintained. Thus, growth may have already been optimized and an additional 4 weeks of evolution was insufficient to improve growth or its plasticity drastically.

Evolution in the abiotic culturing environment, however, caused a reduction in resource use efficiency and an evolutionary tradeoff between growth rate and efficiency. Reduction in efficiency is a common response of microbes to serial transfer culturing methods and is driven by selection to obtain resources and reproduce as quickly as possible to have propagules that survive the next transfer event and population bottleneck (Jasmin et al. 2012; Lin et al. 2020). We also found an evolutionary tradeoff between growth rate and RUE (Fig 3-4a). Taken together, our results suggest that the culturing conditions selected for maintaining fast growth, but at the cost of reduced resource use efficiency as expected under intraspecific competition in serially transferred cultures.

The abiotic environment also had an effect on RUE plasticity evolution. Although evolved strains did exhibit plasticity in RUE (Fig 3-2b), plasticity evolved to be lower suggesting strains were less responsive to changes in resource concentration. The evolutionary change in RUE was not uniform when assayed in different resource environments. The reduction in efficiency of the evolved strains was greater in the low than the high assay environment, making RUE more similar across assaying environments (Fig. 3-2b). Because of this, evolved strains had lower efficiency plasticity compared to ancestral strains. One possible reason for the lower RUE of the evolved strains is that 4 weeks of evolution in the normal culturing environment pushed strains to maximize growth rate at the expense of efficiency, and they have reduced ability to shift to a

more efficient use of resources when resources were scarce. Overall, the results from strains evolved alone showed that our experimental setup was sufficient to monitor evolutionary changes in both trait and trait plasticity.

There were consistent evolutionary trends between strains evolved alone and ones evolved with a competitive partner, suggesting that intra- and inter-specific competition had similar effects on trait evolution in our experiment. We predicted that strains evolved with a competitor strain should have similar, but more pronounced changes in the measured traits as compared to strains that grew alone. Indeed, strains that evolved with a competitor had significantly higher growth rates than strains evolved alone (Fig. 3-3) and there was a slight increase in growth plasticity, albeit this effect was only marginally significant ($p = 0.08$, Table 3-1). The tradeoff between growth rate and efficiency was also more pronounced under competition, suggesting increased selection to forage for and use resources as quickly as possible (Fig. 3-4). Taken together, these results suggest that interspecific competition served to further increase the selection pressures that were present from the culturing conditions—to obtain resources and reproduce as quickly as possible.

Unlike competition, mutualism had distinct effects on trait evolution. Strains evolved with a mutualist partner had slightly lower growth rates and significantly greater efficiency compared to strains evolved alone (Table 3-1; Fig 3-3b). In terms of plasticity, however, these strains were not different from strains that evolved alone. Notably, the effects of mutualism for all four traits measured were opposite to the effects of competition (Table 3-1). The distinct patterns of trait evolution in mutualist versus competitive communities may have been driven by the different patterns of resource dynamics caused by these interactions. The results were consistent with a hypothesis that greater average resource abundance associated with mutualism may have favored

lower growth rates and greater efficiency. Specifically, in resource exchange mutualism in which mutualistic resources are freely available in the local environment, temporal heterogeneity in resource availability may be less pronounced due to the continuous input of resources by the mutualistic partners. In the mutualistic communities in this study, yeast strains continually added either adenine or lysine above and beyond the amount supplied in the medium. The addition of these resources by mutualists would serve to provide continual nutrient inputs that delays the point at which adenine and lysine are exhausted. Under these conditions, the culturing conditions become more similar to chemostat conditions that select for slower growth rates and more efficient use of resources that lead to higher yields (Bachmann et al. 2016). Additionally, the additionally nutrient input from the mutualism could shift resource limitation from adenine and lysine to other nutrients, thus altering patterns of evolution in the mutualistic communities. Our results supported these expectations, demonstrating the contrasting effects between competition and mutualism.

The different selective environment created by mutualism also changed the evolutionary tradeoff between growth and efficiency. Specifically, there was an evolutionary tradeoff between growth and efficiency among strains evolved alone and with competitive partners, but this tradeoff was not observed for strains evolved with mutualistic partners. In the competitive communities, resources were added in distinct pulses that were depleted rapidly by population growth immediately after. This strongly heterogeneous resource environment likely selected for rapid conversion of resources into offspring and favored fast growth at the cost of reduced efficiency, leading to a strong evolutionary tradeoff between growth and efficiency. Similarly, the daily transfers also produced resource heterogeneity in the mutualistic communities, where abundant resource conditions after each resource pulse favored rapid growth. However, After the initial

pulse was depleted, the mutualism would have maintained a low abundance of adenine and lysine that supported continual population growth, when selection would favor increased resource use efficiency. The two alternating situations in the mutualist communities favored growth and efficiency at different times, which probably served to attenuate the evolutionary tradeoff between growth and efficiency.

Although competition and mutualism had opposite effects on trait evolution, they both had little effect on plasticity evolution. This does not mean that competition and mutualism are similar in their effects on plasticity evolution. Although the effects of competition and mutualism on plasticity were not significant, their effects were opposite to each other for both growth rate plasticity and RUE plasticity. These findings are consistent with an existing theoretical study, in which Scheiner et al. (2015) found that although biotic interactions differed in their effects on plasticity evolution, biotic interactions in general led to reduced plasticity when interacting species are simultaneously adapting to abiotic conditions. Both their predictions and our results suggest that abiotic factors may exhibit stronger selection on plasticity in comparison to biotic factors such as competition and mutualism. However, the generality of this pattern is unclear as there are examples of the role of predation and herbivory driving induced defenses (Kessler and Baldwin 2001; Benard 2004). Future research should directly test the relative importance of abiotic and biotic factors on plasticity evolution across a range of species interactions and environments.

Another reason for observing less evolutionary change in plasticity could be that both growth rate and resource use efficiency closely associate with fitness in microbial populations. Plasticity in functional traits is generally argued to help maintain fitness across environments, and traits closely related to fitness are expected to be less plastic than other traits (Acasuso-Rivero et al.

2019). In environmentally variable environments, selection via plasticity should keep fitness high and relatively unchanged. However, support for this argument has been equivocal. For example, a meta-analysis by Acasuso-Rivero et al. (2019) demonstrated that life-history traits were equally plastic as less fitness-related, non-life-history traits. Additionally, the argument of less plasticity in fitness related traits is most relevant when there are gradients of environmental stress, in which maintaining fitness might be the best an organism could do (e.g., 'jack of all trades', Richards et al. 2006). The environments used in our experiment were favorable for growth and increases in fitness via plasticity can also be a successful strategy ('master of some', Richards et al. 2006). Our results support this latter strategy, as evolutionary changes in traits and plasticity were always concordant despite their difference in magnitude.

Although our experiment showed that biotic interactions are very important for trait evolution, our interpretations are limited to simple communities involving only two species. Natural communities, on the other hand, often involve many more species, such as in pollination networks, and can involve species with very different ecology, such as resource requirements, life history, and generation times. In addition to these biotic factors, temporal and spatial variation in abiotic environments can also modulate outcomes of biotic interactions and plasticity evolution. Although not addressing these potentially important factors, our results highlight the utility of using microbial experimental evolution to test how biotic interactions influence trait evolution.

**Conclusions:**

We found that different types of biotic interactions influenced trait evolution in drastically different ways but had little effect on plasticity evolution. Exploitative competition, either intra

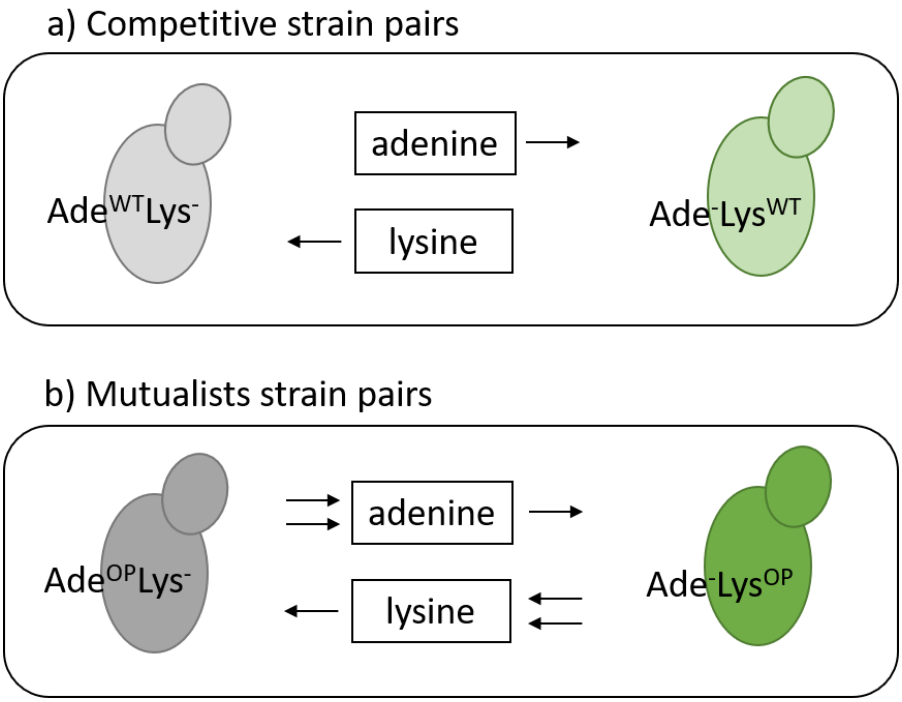
or inter-specific, led to a reduction in resource use efficiency. In contrast, resource-exchange mutualism selected for a different life-history strategy, that included selection for higher RUE. This contrast between competition and mutualism was potentially due to differences in temporal dynamics of resource availability in these communities. Unlike trait evolution, competition and mutualism had little effects on the evolution of either growth rate plasticity or RUE plasticity after 4 weeks of evolution. This finding suggests that abiotic factors may be more important than biotic factors in selecting for plasticity; however, this is likely context dependent. More research is needed to address the relative importance of abiotic and biotic factors on plasticity evolution and to provide a general framework for the role of species interactions in generating plasticity.

Table 3-1

Results of experimental evolution of *S. cerevisiae* with and without biotic interactions. Model outputs for models testing effects of evolution in response to the abiotic culturing condition (shaded) and for models testing effects of evolution with a competitive or mutualistic partner (no shading).

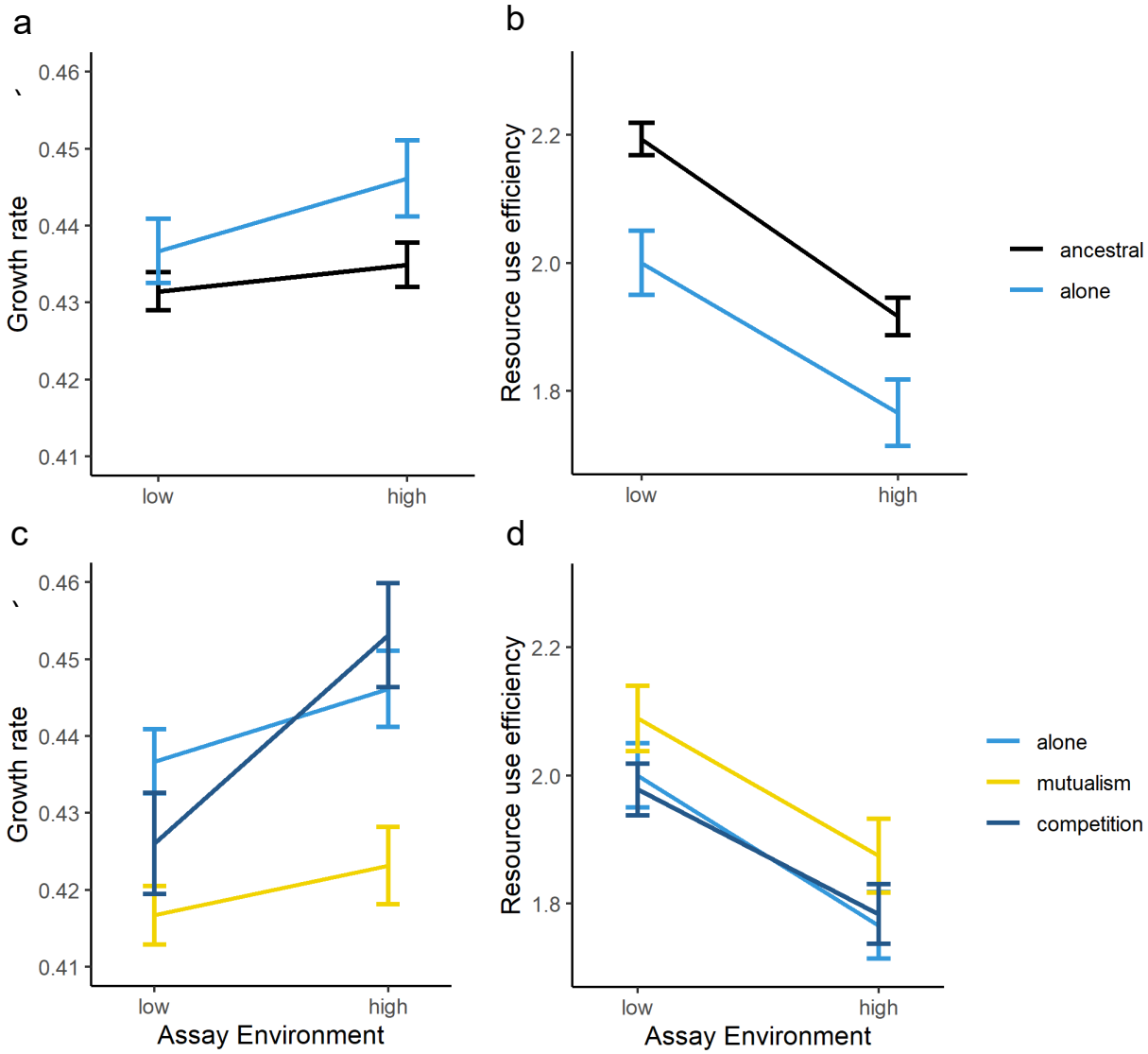
| | Effect type | Estimate | SE | p-value |
|---|---|---|---|---|
| **Growth rate** | Abiotic | 0.004 | 0.004 | 0.35 |
| | Competition | 0.015 | 0.008 | **0.05** |
| | Mutualism | -0.017 | 0.010 | 0.09 |
| **Growth plasticity** | Abiotic | 0.005 | 0.006 | 0.40 |
| | Competition | 0.016 | 0.009 | 0.08 |
| | Mutualism | -0.014 | 0.012 | 0.24 |
| **RUE** | Abiotic | -0.117 | 0.038 | **0.02** |
| | Competition | -0.116 | 0.060 | 0.06 |
| | Mutualism | 0.193 | 0.076 | **0.01** |
| **RUE plasticity** | Abiotic | -0.057 | 0.022 | **0.04** |
| | Competition | -0.018 | 0.041 | 0.68 |
| | Mutualism | 0.032 | 0.052 | 0.56 |

Figure 3-1



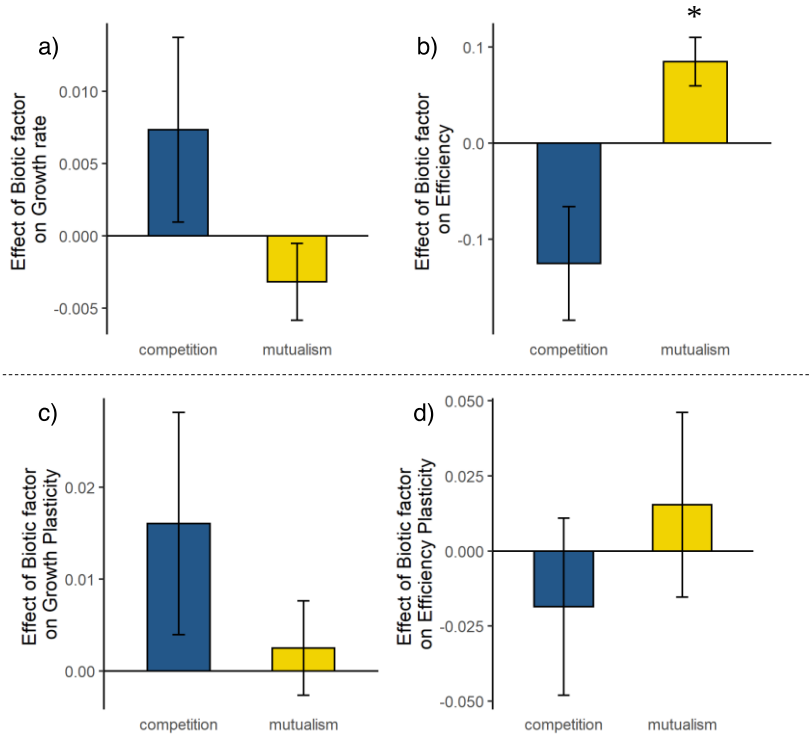

Schematic representing community combinations of *S. cerevisiae* strains that differ in production of adenine and lysine. Strains were paired with a competitor (a) or mutualist (b) to examine the effects of biotic interactions on evolution of growth rate, resource use efficiency, and their plasticity. The single arrows represent uptake from the environmental pool of adenine or lysine, and the double arrows represent overproduction of either adenine or lysine that is released into the environmental pool.

Figure 3-2



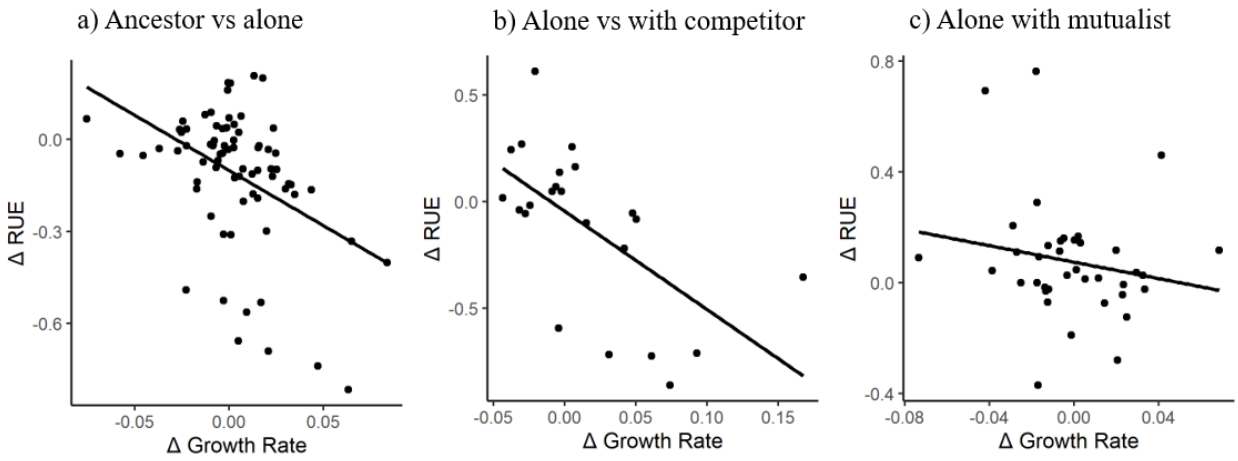

Reaction norms of growth rate (a, c) and resource use efficiency (b, d) for ancestral strains, strains evolved alone, strains evolved with a competitive partner, and strains evolved with a mutualistic partner.

Figure 3-3




Average effects of competition and mutualism on a) growth rate, b) resource use efficiency, c) growth plasticity, and d) resource use efficiency plasticity. Error bars represent standard errors. Values close to zero means that the trait values of strains evolved with a biotic partner are similar to strains evolved alone.

Figure 3-4




Relationships between Δ growth rate and Δ resource use efficiency between ancestral strains vs strains evolved alone (a), between strains evolved alone vs with a competitive partner (b), between strains evolved alone vs with a mutualist partner (c). Negative relationship represents evolutionary tradeoff between growth rate and efficiency.

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## Chapter 4 : Synthesis

Phenotypic plasticity, an organism's ability to change phenotypic expression in response to its environment, can determine whether an organism will survival against abiotic challenges and whether it might gain or lose in encounters with other organisms. The ability to express environmentally appropriate phenotypes can be especially important for organisms experiencing novel environments, such as at range edges, under anthropogenic environmental changes, and when encountering non-native species (Nussey et al. 2005; Morris 2014; Zenni et al. 2014). This dissertation tested the roles of plasticity in novel ecological and evolutionary scenarios.

In chapter one, I tested whether plasticity could facilitate population survival in a novel abiotic environment using a wild population of *Drosophila melanogaster* (Wang and Althoff 2019). I raised larvae across two historical and one novel alcohol environments and quantified physiological plasticity in a functional trait, alcohol dehydrogenase (ADH) activity. I found that the novel alcohol environment was stressful for larvae, but larvae with higher physiological plasticity survived better in the novel environment. This was the first direct empirical evidence supporting the role of plasticity in facilitating survival in a novel environment.

Survival is just the first step of colonizing a novel environment, and a population needs to adapt to the new habitat if it were to persist. Whether plasticity would facilitate or constrain the subsequent adaptation depends on its genetic architecture. Specifically, if plasticity is controlled by a few loci with large effects, evolution can proceed quickly and fix the beneficial alleles; alternatively, if plasticity is controlled by many loci with small effects, the rate of evolution might be slower. Additionally, genetic correlation between traits and their plasticity can further speed up or constrain adaptive evolution, depending on whether the directions of selection were similar for traits and their plasticity. In chapter two, I explored these questions by examining the

genetic architecture of a functional trait and its plasticity using quantitative trait loci mapping using an established resource, the *Drosophila* Synthetic Population Resources (King et al. 2012). I again used the functional trait ADH activity in larvae because its importance was already established in chapter one. I found that plasticity was not controlled by a few loci with large effects and that ADH activity and ADH plasticity were most under separate genetic controls. This suggested that the functional trait and its plasticity can evolve independently in a novel environment.

The first two chapters demonstrated the importance and evolutionary potential of phenotypic plasticity in novel, abiotic environments, but an organism's natural ecological environment consist of both abiotic and biotic factors. How plasticity evolves in communities with interacting species has received little systematic effort. Although adaptive phenotypic plasticity has been demonstrated in many organisms (Agrawal 2001), only one theoretical study showed that different types of biotic interactions influence plasticity evolution differently (Scheiner et al. 2015). I explored the effects of competition and mutualism on trait and plasticity evolution by evolving synthetic yeast (*Saccharomyces cerevisiae*) communities in the lab. I chose competition and mutualism because they have distinctive effects on organism fitness. Indeed, competition and mutualism led to distinctive resource-use strategies in the evolved yeast strains. Intra- and inter-specific competition led to increased growth rate and reduced resource use efficiency, but mutualism had the opposite effects. This contrast was likely driven by differences in temporal resource variation in the different communities. Despite the evolutionary changes in these traits, biotic interactions did not influence plasticity evolution.

Taken together, results from the three chapters raise the question whether abiotic or biotic environment is more important for plasticity evolution. Many theoretical and empirical studies

have established the importance of abiotic variation on plasticity evolution, and many examples exist for adaptive plasticity in response to biotic interactions (Pigliucci 2001; West-Eberhard 2003; DeWitt and Scheiner 2004). Results from this dissertation suggest that abiotic variation might be more influential to plasticity evolution, but more evidence from other systems and environments are needed before a general conclusion can be reached. In summary, I found that phenotypic plasticity can be an important driver for population survival and evolution in novel, abiotic environments, but plasticity may be less affected by variations in biotic interactions. Future studies need to test the role of abiotic and biotic variation simultaneously to compare their relative effects on plasticity evolution.

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Santos, M., Matos, M., **Wang, S. P.**, & Althoff, D. M. (2019). Selection on structural allelic variation biases plasticity estimates. *Evolution*, *73*(5), 1057-1062.

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**Manuscripts in Prep:**

**Wang, S. P.**, & Althoff, D. M. Contrasting effects of competition and mutualism in evolving yeast populations. In prep.

Vidal, M. C., **Wang, S. P.**, Rivers, D. M., Althoff, D. M., Segraves, K. A. (2020). Species richness and redundancy promote persistence of exploited mutualisms. *Science, in review*

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| | |
|---|---|
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| | |
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