

TESTING THE EFFECTS OF GENETIC ADMIXTURE BETWEEN POPULATIONS ON
FITNESS IN A NOVEL ENVIRONMENT

By

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This is dedicated to my mom and dad for their support and encouragement.

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Genetic admixture between previously separated populations can have several effects on how those populations adapt to new environments. Some argue that admixture will result in outbreeding depression by displacing locally adapted genes and disrupting co-adapted gene complexes, thereby lowering fitness. Others have shown that admixed populations have greater population mean fitness because of their higher effective population size and additive genetic variance in a population. This may be the reason that invasive species formed from multiple introductions are often more successful than a single introduction.

The goal of this work was to test the effects of genetic admixture. Populations of *Drosophila melanogaster* were collected and reared in the lab. The populations were introduced into a novel environment of high ethanol concentration in their food in three different treatments. The first treatment was a single introduction, the second was multiple introductions from one source, and the third was multiple introductions from multiple sources. Multiple introductions, especially from multiple sources, had higher mean population fitness following the second introduction.

Next we tested the hypothesis that admixture of individuals from populations adapted to different environments could produce novel combinations of adaptive traits, making them better at adapting to novel environments than flies from any single population. We obtained populations with high ethanol tolerance or high desiccation tolerance. These populations were combined and the offspring were reared in an environment with combined ethanol and desiccation stresses. I show that the admixed population has intermediate levels of adaptation to each stress.

Finally, to test a larger range of parameters, an individual-based model was created that simulated the introduction and evolution of populations in a novel environment with or without admixture. Results showed that immediately following a secondary introduction there is a decrease in fitness caused by diluting of locally adapted genes. In subsequent generations, there is an increase in mean population fitness of the admixed populations.

The results suggest that admixture can be both beneficial or harmful to a population in a novel environment depending on the conditions. This has implications for many fields including conservation and evolutionary biology.

CHAPTER 1 GENERAL INTRODUCTION

Migration and Adaptation

A common occurrence during the movement of organisms from one environment to another is genetic admixture among different populations, often having important consequences for the evolution of these populations (Dieckmann et al.1999).

Population dispersal and migration events to novel environments have regularly occurred throughout the history of life on Earth, often from the effects of tectonic plate movement or when they were forced to flee unsuitable habitats during global climate change events (Vermeij 1991). However the increasing impact that humans are having on the planet is resulting in a far greater rate of biotic introductions than ever before. Species are being forced to alter their natural ranges due to habitat destruction and global climate change (Parmesan 2006, Pereira et al.2010), while modern transportation has resulted for the introduction of invasive species across the globe (Pimentel et al. 2005). Because these patterns are likely to stay the same or intensify in the near future, a better understanding of population migration, the resulting population admixture, and its evolutionary and ecological consequences is greatly needed.

Individuals from populations that are locally adapted will experience a loss in fitness during immigration to a novel environment (Joshi et al.2001, Leimu & Fisher 2008, Kawecki and Ebert 2004). Fitness in this case is defined as the ability to survive and reproduce and therefore determines an individual's ability to contribute genes to future generations of a population. Most populations introduced to novel environments become sink populations, such that the mean fitness is too low for them to persist and they deterministically decline to extinction, preventing most introductions from being

successful. Only recurrent immigration or genetic adaptation will rescue such a population and result in its persistence (Holt 1997). Most exotic species introductions go extinct (Williamson 1996), presumably because the individuals are not adapted to the conditions of the introduced environment. An inability to adapt to the local environment has been cited as a major reason why parasitoid populations introduced for biological control fail to establish (Stiling 1990). Nonetheless, the widespread prevalence of prolific invasive species demonstrates that introduced populations can be successful without continuous immigration, indicating that post-introduction adaptation may be playing a large role in establishment and spread.

Adaptation is characterized by a genotypic change in a population that results in a phenotype of greater mean fitness in a particular environment. How introduced populations adapt to novel environments has been the subject of much theoretical work. While current models have proven useful, they fail to answer certain questions and are based on some unrealistic assumptions (which are outlined below), making their sufficiency for fully explaining adaptation limited (Orr 2005). More importantly, controlled empirical tests of such theories are limited. However many insights have come out of the established theories of adaptation and so a review of them is useful.

Traditional population genetic models of adaptation to novel environments have mostly considered the effect of new beneficial mutations arising in the population that are then driven to fixation by selection as opposed to selection on standing genetic variation (Orr 2005). The first such proposed framework was Fisher's geometric model (Fisher 1930), which states that fitness is determined by n characters and the state of these characters can be represented in an n -dimensional phenotypic space with the

local optimum at the origin. A population that is not adapted to the environment exists in the phenotypic space at some point away from the optimum. The population can change positions through random mutations represented by vectors in the phenotypic space. Mutations that move the population closer to the origin of the space are favorable while those moving it away are deleterious (Orr 2005).

The next major advance in modeling adaptation did not come until the introduction of DNA sequence based models, in particular Gillespie's mutational landscape model (Gillespie 1984). Here, a genotype is modeled as a segment of DNA in which, due to a recent change in the environment, one or more favorable alternative sequences exist. The sequence adapts via point mutations that are brought to fixation when beneficial. This assumes that while the current genotype is not at the local optimum, it is relatively close. While both of these models have been widely used and have provided us with many insights, both only consider mutational variation and ignore standing genetic variation (Orr 2005).

The role of selection on standing genetic variation in adaptation has been considered by others more recently (Przeworski et al. 2005, Hermisson and Pennings 2005, and Orr and Betancourt 2001). Adaptation from standing genetic variation is expected to be more efficient than from new mutations. Alleles already in the population are likely to be present in more than a single copy, making them much less likely to be lost due to genetic drift. Additionally, while new mutations arise completely random with respect to their effect on fitness, standing alleles in the population have already proven to be beneficial (or at least not too harmful) in at least some historical environment. They have been filtered by selection in the ancestral environment and, assuming the

new environment has some commonality with the source environment, they will be more likely to increase individual fitness than new mutations (Barret and Schluter 2007), which are largely unconditionally deleterious (Dobzhansky 1970).

Gene Flow as an Adaptive Force

While selection has long been seen as the main driving force behind adaptation, gene flow can be a powerful force as well and may be more important in determining the likelihood of adaptation (Arnold 1992). Little consideration has been given to the role that gene flow or admixture between genetically divergent populations (a concept related to and sometimes referred to in the literature as outbreeding, outcrossing, introgression, transgression or intraspecific hybridization) has on the standing genetic variation and how this affects the rate of adaptation. Admixture can be very common in populations that are dispersing or have been affected by human activities because geographic barriers are often removed or bypassed in these cases. It can dramatically affect populations' genetic structure and their ability to evolve in a new environment in several of ways, having both positive and negative consequences for population mean fitness and their ability to persist (Slatkin 1987, Verhoeven 2010).

Here I will outline these consequences and reconcile their differences. The first set of effects genetic admixture has result from the increase in effective population size experienced from gene flow. The effective population size, a concept first proposed by Wright (1931) is defined as the size of an ideal population that would experience the amount of genetic drift as what is observed. Populations of small effective population size are particularly vulnerable to the loss of genetic diversity over time due to the random fixation of alleles. Additionally in populations of low effective size, the force of selection is inefficient compared to drift, making beneficial alleles less likely to increase

in frequency and more likely to be lost (Lande and Barrowclough 1987, Ellstrand 1993). But one of the most detrimental effects of small effective population size is that it increases the amount of homozygosity, resulting in greater levels of inbreeding depression. Inbreeding depression is a loss in individual fitness that results from the expression of deleterious recessive alleles that are normally at low frequencies and thus present only in heterozygotes; or because decreased heterozygosity precludes overdominance (East 1909, Roman and Darling 2007). Introducing unique alleles can dramatically decrease the incidence of inbreeding depression in a population and has been shown to increase the likelihood of persistence (Drake 2006). Populations recently introduced to a new environment are expected to have small effective population sizes and genetic admixture can be important in alleviating all of these processes (Verhoeven 2010).

Besides increasing the effective population size, population admixture can also increase the additive genetic variance found in the population for quantitative, fitness related traits. Fisher's fundamental theorem of natural selection states that the rate of increase of fitness in a population is equal to its genetic variance for fitness (Fisher 1930). Selection for beneficial alleles in the introduced environment can result in transgressive segregation, or the production of offspring with more extreme phenotypes than either parental line (Dlugosh and Parker 2008). Additionally, population admixture can result in novel combinations of traits through adaptive trait introgression that may be beneficial in the introduced environment, further increasing the probability of population persistence (Verhoeven 2010).

Conversely, gene flow and admixture have been known to disrupt local adaptation via the process of outbreeding depression. If a population has already become adapted to a set of local conditions, then introducing additional genetic material from another environment can dilute the locally adapted genes. This is known as migration load or gene swamping (Lenormand 2002). Gene swamping is expected to be particularly prominent when an introduced population crosses with a native population or when there is recurrent gene flow into the new environment from the ancestral environment. In these situations, the benefit of increasing effective population size and genetic variance may not outweigh the effect of having locally adapted alleles. However, with an invasion consisting of a small number of discrete introduction events into an environment that is novel to all populations (often the case with invasive species), little local adaptation is expected; and hence gene swamping is not expected to be relevant.

Admixture in Novel Environments

Various scenarios exist where one would expect to find genetically divergent populations coming into contact with one another in a novel environment and producing offspring. While in some cases this can be attributed to natural dispersal events, most recent cases are human-induced, indicating that this mechanism of evolution was relatively rare until recently (but see Frank and McCoy 1991). The most commonly observed scenario is when there are multiple introductions of an invasive species to the same environment (Collins et al. 200; Maron et al. 2001; Novak & Mack 200; Lavergne & Molofsky 2006; Facon et al. 2008; Hufbauer & Sforza 2008 and reviewed in Dlugosh & Parker 2008). An invasive species is a population that has arrived to and successfully establishes outside of its native range, often spreading uncontrollably and resulting in great ecological, economic, and public health burdens. Global transportation has greatly

increased the frequency of invasive species in the last century and the problem is continuing to grow (Pimentel et al. 2005). The frequency of multiple introductions of invasive species is increasing as well and has been demonstrated in many studies of molecular variation (Kolbe et al. 2004; Fonseca 2001; Durka et al. 2005).

Other examples of anthropogenic population introductions exist as well. One example is biological control, where the natural predators or parasites of an invasive species are intentionally introduced to control the numbers of or alleviate the negative impacts of an invasive species (Müller-Schärer et al. 2004). Post-introduction adaptation can have a large impact on the probability of success of biological control attempts (Hopper 1993). Additionally, endangered species that have fallen to very low numbers or even become locally extinct have been the target of reintroduction to a previous home range. These reintroductions have been shown to benefit from post-introduction adaptive processes that increase genetic diversity (Invasson 2001). For example, to reduce the prevalence of inbreeding depression in Florida panthers (*Puma concolor coryi*), stocks of the Texas puma (*P. c. stanleyana*) were introduced to Florida and have since been shown to have a positive effect on the fitness of the now mixed panther population (Hostetler et al. 2010).

In an analogous practice in the field of agriculture, crops are often crossed with genetically divergent lines or wild progenitors in order to introgress certain desirable traits or to increase genetic variation. This can make crop populations less vulnerable to pests or diseases and can increase yields (reviewed in Wang and Chee 2010). Admixture can also be used in forestry to increase genetic variation, population fitness and promote forest conservation on managed lands (González-Martínez et al. 2005).

Even more evidence of the benefits of population admixture come from the study of interspecific hybrids. Although the amount of genetic differentiation is generally greater than with intraspecific admixture, various works have suggested that hybridization can result in adaptation (reviewed by Arnold 1992). In one example described by Lewontin and Birch (1966), they performed an interspecific cross experiment of two species of tephritid flies to show that hybridization could be a source of genetic variation that facilitated adaptation to extreme temperatures.

While the most admixture can be attributed to anthropogenic causes, it is well known that gene flow occurs during natural range expansions as well. Continental drift and climate change have always been prominent forces influencing the ranges of populations (Vermeij 1991). Populations that became isolated within glacial refugia can subsequently come into secondary contact during post-glaciation expansions (Keller and Taylor 2010). Natural range expansions can result in introgression between multiple expanding populations or between expanding and native populations (Excoffier et al. 2009). One example is given by a study that used mitochondrial and cytochrome b sequence haplotypes to show historical outbreeding of sable antelope populations in sub-Saharan Africa. Their results indicated that multiple previously isolated populations came into contact with each other during historical colonization events that resulted in gene-flow between them (Pitra et al. 2002). A similar event is believed to have resulted in genetic admixture between early *Homo sapiens* and *Homo neanderthalensis* after the human migration out of Africa between 44,000 and 30,000 years ago (Green et al. 2010). However many present population dispersal events that may appear natural are

actually the result of modern human-induced habitat disturbance or global climate change (see Midgley et al.2006, Morin et al.2008, & Hill et al.1999 for examples).

Despite the evidence for population admixture increasing the rate of adaptation to novel environments and the important consequences this adaptation can have for conservation efforts and economics, there has been little effort to test admixture's effects empirically. Most studies investigating this phenomenon have measured genetic diversity in introduced populations and compared it to that of the native population and attempted to correlate differences with changes in fitness (Kolbe et al.2007; Lavergne & Molofsky 2007; Facon et al.2008). One of the first studies to directly show the effects of multiple introductions and admixture on evolutionary potential was conducted by Facon et al. (2008). This study took advantage of a freshwater snail species, *Melanoides tuberculata* that has multiple populations, each with its own distinctive shell morphology. There have been multiple successive, well documented introduction events of *M. tuberculata* into the freshwaters of Martinique in the French West Indies. Using mitochondrial sequence data, they were able to determine five different origin populations of the invasive snails and show that some individuals possessed haplotypes formed through recombination between several ancestral populations. They also performed careful measurements of morphological and life history traits. Their results show that the largest component of both genetic and phenotypic variance was due to the recombination of genotypes from different sources. The variance components of phenotypes closely associated with fitness such as fecundity and juvenile size were much greater among populations than within populations with distinctive shell morphologies, likely conferring an increased ability to evolve advantageous

morphologies. In the introduced range, the admixed populations outcompeted their parental morphs, showing that the increased variance provided a selective advantage. While this and other studies provide examples of multiple introductions increasing population fitness, none have attempted to demonstrate the effect in a controlled setting with large scale replication.

The purpose of the following work was to test the hypothesis that population admixture can increase mean population fitness compared to single populations and lead to adaptation in a novel introduced environment using various methods that have not been previously applied to this question. These methods include artificial evolution experiments with a model organism and agent-based simulation modeling.

CHAPTER 2 THE EFFECT OF MULTIPLE INTRODUCTIONS ON A NON-NATIVE POPULATION'S ABILITY TO ADAPT TO NOVEL ENVIRONMENTS.

Introduction

Non-indigenous invasive organisms are one of the most serious threats to global biodiversity. Non-indigenous species that are released from the ecological constraints of their natural range often displace or outcompete native species and are a leading cause of species extinction (Gurevitch and Padilla 2004). They can also cause huge economic costs and spread harmful diseases (Pimentel 2001). Preventing such species from establishing is a high priority for conservationists as well as several industries and governmental agencies. It has been argued that most introductions are unsuccessful and go locally extinct rather than establish (Williamson 1996). Why some introductions result in a stable, invasive population while others fail to do so is poorly understood (Lee 2002).

Populations introduced into novel environments experience selective pressures to which they may not be well adapted. Non-native populations often result from the introduction of a small number of individuals, which further increases the probability of extinction (Shaffer 1981). This is evident both in the low rate of successful establishment of exotic species (Williamson 1996) and the low rate of success of biological controls used to manage invasive populations (Hopper 1993). Common theories for successful invasion cite ecological, physiological and life history factors including (1) release from predation or parasitism, (2) high fecundity, (3) high tolerance to stress, or (4) superior dispersal ability (Rejmanek & Richardson 1996; Sakai et al. 2001). However, these factors and studies of the traits associated with them do not fully explain or allow for accurate prediction of species invasions. Mounting evidence

suggests that genetic architecture and evolutionary dynamics following introduction could play an important role in determining which exotic species become invasive (Lee 2002).

For a population to persist, a novel environment must be within the ecological tolerance levels of the species, (“ecological niche”; Hutchinson 1957). Because most species are not able to easily change their niche on short time scales, the set of conditions under which a species is able to invade a region is usually limited (known as niche conservatism). For example, many non-indigenous species from warm tropical climates that have successfully invaded sub-tropical south Florida have failed to spread to the temperate regions of north Florida because they cannot tolerate the freezing temperatures that occur there (Wiens & Graham 2005). Although a species' ecological niche is often conserved and shaped in large part by its past evolutionary history, niches can evolve and result in a population adapting to a novel environment.

Population genetic theory says that gene flow and genetic variance are the primary factors that determine the rate of local adaptation of a population. High levels of gene flow into a population from a larger source population will introduce maladapted alleles and thus tend to hinder local adaptation (Lenormand 2002). This form of outbreeding depression can be approximated using an island model of migration, which consists of a source population (referred to as “mainland”), and one or more “islands” of much smaller area representing novel environments (Wright 1943). If migration is large enough ($m > s$, where m is the fraction of immigrants per generation from the source population and s is the selection coefficient against immigrant genotypes), alleles from the source environment will become fixed, even if they are not the most favored allele

(Ellstrand & Elam 1993; Holt 1996; Wiens & Donoghue 2004). Therefore the greatest potential for local adaptation should occur at low rates of gene flow (Holt & Gomulkiewicz 1997).

However, some genetic variance is needed for local adaptation. This may be particularly relevant in introduced populations, which may have been created from only a few founders. Immigration can increase additive genetic variance in quantitative traits by introducing new alleles or by changing the frequencies of existing alleles, increasing the population's potential to adapt to the new environment (Holt & Gaines 1992; Holt et al. 2003; Weber 1990). Finally, immigration may increase effective population size and heterozygosity, decreasing inbreeding depression (Newman & Tallmon 2001). Invading populations are expected to have particularly low levels of additive genetic variance and heterozygosity due to being established by few founders, sometimes a single fertilized individual (Pascual et al. 2001; Dlugosh and Parker 2007) so this may have an important effect on the population's ability to persist in the new environment.

Thus, gene flow must exist to provide sufficient genetic variance and prevent inbreeding; yet too much gene flow will swamp locally adapted genotypes with alleles from other populations. The amount of gene flow and potential for local adaptation is mostly determined by the number and magnitude of introduction events (Lockwood et al. 2005; Lenormand 2002) and the relative allelic frequencies of each population. Having multiple (but discrete), independent introductions of a non-indigenous species is believed to increase its chances of persisting in some cases (Sakai et al. 2001).

Many recent molecular studies have shown that a surprisingly many successful invasions have been the result of multiple introductions (Collins et al. 2001; Fonseca

2001; Maron et al. 2001; Novak & Mack 2001; Kolbe et al. 2004; Durka et al. 2005; Lavergne & Molofsky 2006; Dlugosh & Parker 2007; Facon et al. 2008; Hufbauer & Sforza 2008). Multiple introductions can increase the rate of increase of a population through transgressive segregation, the effect of combining additive alleles at different loci to produce a phenotype more extreme than that of either parental population (Reiseberg et al. 1999, Stelkens & Seehausen 2009). Transgressive segregation results from increased additive genetic variance in fitness-related traits such as fecundity and survival, leading to an increased ability to respond to natural selection (Fisher 1932). Multiple introductions can also permit recombination of traits that are beneficial in the new environment, resulting in invasive populations to expand into environments that contain novel combinations of selective pressures. Finally, multiple introductions can raise the mean fitness of non-native populations by decreasing the amount of inbreeding depression. Inbreeding depression, caused by homozygous deleterious recessive alleles and the action of overdominant alleles (Roman & Darling 2007), is of particular concern in bottlenecked populations, such as introduced species (Newman & Tallmon 2001).

Well-designed studies of the effects of multiple introductions have been conducted in the field (Facon 2008; Keller and Taylor 2010), but these studies have several shortcomings. They are fundamentally limited because they are only able to examine successful population introductions. Further, extensive replication of a system in the field is difficult. A different approach, which overcomes these challenges, is using model organisms in controlled laboratory settings. *Drosophila melanogaster* is an ideal organism for this purpose due to its short generation time, ease of maintenance, and

the accumulated genetic and biological knowledge on the species. *D. melanogaster* has been successfully used in studies of evolutionary and conservation genetics in the past (Frankham 2002). Here, we test the hypothesis that multiple introductions of genetically divergent populations to a novel environment followed by hybridization can improve the fitness of the population and hence the likelihood of a successful invasion. Our experiment utilized populations of *Drosophila melanogaster* to mimic a population invasion and compare population mean fitness after a single versus multiple introductions.

Methods

Fly Stocks

Three lines were established, each one from a single, wild inseminated female fly collected from the field (*i.e.* isofemale lines). These lines are expected to be genetically variable, because flies have sperm storage organs that retain the sperm of multiple males in the field or in the lab (Imhof et al. 1998). Further, because of the large population size of *D. melanogaster*, a large amount of genetic polymorphism is expected to be present within a small number of individuals, as was shown in *D. subobscura* (Pascual et al. 2007). The collection process nonetheless results in bottlenecked populations of effective population size that one might expect after an invasion event. These lines were collected in geographically separate locations that included Putnam County, Georgia (collected by K. L. Moody & M. L. Wayne); Wood County, Ohio (collected by R. Woodruff); and Los Angeles County, California (collected by S. V. Nuzhdin). All lines were established in 2008, one year before this experiment. In the time between collection and the onset of the experiment, the lines were kept in

the lab at large uncontrolled population sizes to maintain a large proportion of the genetic variance from the field. A fourth line used, the *yellow* line, was a laboratory mutant line that had a recessive yellow body color mutation (*y*; gift of J. V. Fry). This was a lab stock that had been maintained at uncontrolled density for at least twenty years, making its adaptive ability fundamentally different from the other populations. Although genetic diversity was never assayed, this population was expected to have a lower effective population size. The use of the yellow color mutation allowed for easy assessment of genetic contamination of this line with the others, as well as an indicator of genetic admixture within crossed lines. All lines were maintained in the lab at moderate, uncontrolled densities for approximately 25 generations before the experiment.

Stocks were maintained throughout the experiment in vials on a cornmeal-molasses medium at a constant density of ten male and ten female parents at each generation. This results in some amount of selection to laboratory conditions that is constant for all populations. They were kept in incubators at a constant temperature of 25° C with a 12:12-h light/dark cycle. To simulate a novel environment, we used a medium with a high concentration of ethanol (Fry 2001). After the prepared food cooled to 47° C, we added 95% ethanol to produce a 12% ethanol medium, which is much higher than what is experienced in the field (Gibson et al. 1981). Ethanol was chosen because it is an environmental stress commonly encountered by fruit flies and used as a resource to avoid parasitoids (Milan et al. 2012). The concentration found in nature varies, and *D. melanogaster* has been shown to adapt to this concentration in previous experiments (Fry 2001).

Experimental Design

The experimental procedure was designed to mimic the process of species invasions in nature with single or multiple introductions from the same and different sources. At the onset of the experiment, we established thirty-three replicate populations of each of the four lines (with ten pairs of non-virgin flies) on the ethanol medium and thirty-three on standard medium as a control. Although the numbers of founders in invasive populations varies, ten pairs is certainly not an unrealistic scenario in nature (Ross & Shoemaker 2008; Pascual et al. 2007, and Ficetola et al. 2008) and these lines had already been through the bottleneck of collection from the wild. After two generations in the novel environment, each line was divided among three treatments with eleven replicate populations per treatment (four lines x three treatments x eleven replicates = 132 populations per environment). The first treatment represented a single introduction and was simply a continuation of the conditions of the initial population, with ten males and ten females. The second treatment represented multiple introductions from the same source population, and was created with six males and six females from the previous generation plus four males and four females from a separate stock of the same line that had not been under selection. The final treatment represented multiple introductions from multiple source populations and was created with six males and six females from the previous generation plus one male and one female from stocks of each of the four lines that had not been under selection. Thus, all treatments had the same total number of founding animals and were representative of what we thought was a realistic introduction scenario.

From the third generation on (*i.e.* after setting up the three treatments), we maintained populations at a constant density by allowing flies to randomly mate for

several days after hatching and then transferring ten males and ten females to new vials of the same medium. Parents laid eggs for five days and then were removed from vials to maintain discrete generations.

We defined fitness as the ability of an individual to contribute genes to future generations. As a proxy for this, we use the proportion of flies surviving from egg to adult, also called the egg viability. Fecundity was not included in this measure because it is believed the novel environment would have little effect on this component of fitness. Egg viability was measured for each replicate population each generation. After laying eggs to establish the next generation, the parents were placed in an egg-laying chamber, which consisted of a plastic bottle with a 10mm x 35mm Petri dish covering the top. The dish was filled with medium (standard or ethanol depending on the home environment of the population being measured) and the bottle was inverted. After allowing flies to lay eggs for three to five hours, we removed the covers and took thirty eggs out of the dish and placed the eggs in a vial of the appropriate type of food. These flies were then allowed to develop for fourteen days and adults emerging were counted daily.

After generation fourteen, we switched half of the populations in treatment one from standard medium to ethanol-supplemented medium in order to determine the response to selection in the course of the experiment. We again measured egg viability as well as mean development time as a second fitness proxy for both selected and control populations in the ethanol environment.

Statistical Analysis

All data analyses were performed in the R Statistical Package (R Development Core Team 2009). Raw egg viabilities were arcsine transformed before being analyzed

to better approximate normality of residuals. To analyze the survival data, we used the linear model

$$s = \mu + e_i + t_j + g_k + l_m + e_{gik} + t_{gjk} + e_{tij} + e_{tgijk} + \epsilon_{ijkmn}$$

where s is survival, μ is the overall mean, e_i is the effect of environment i , t_j is the effect of treatment j , g_k is the effect of generation number k , l_m is the effect of line m , and ϵ_{ijkmn} is the effect of each replicate. Additionally all two-way and three way interactions of environment, treatment, and generation were included. Environment, treatment, and generation were treated as fixed effects while line was a random effect.

The ACF function was used to test for autocorrelation between egg viability of each population between successive generations. A small amount of autocorrelation was observed and corrected for using the `corAR1` function in R. We performed an analysis of variance (ANOVA) to determine the effect of each of the factors as well as the interactions between them (Table 2-1). Non-significant interaction terms ($P > 0.05$) were sequentially dropped from the model, resulting in a reduced model:

$$s = \mu + e_i + t_j + g_k + e_{gik} + l_{mij} + \epsilon_{ijkn}$$

The final measure of adaptation was analyzed using Student's t-test.

Results

Egg viability assay results are summarized in Figure 2-1. We used a linear analysis ANOVA with egg viability as the response variable, from which a significant effect of treatment (the type of introduction) was observed in the ethanol environment but not in the control environment, resulting in a significant interaction term (environment * treatment, $P = 0.0017$). The multiple introduction treatments had the highest egg viability, and the multiple introductions from multiple sources had greater egg viability than multiple introductions from a single source (treatment $P = 0.0350$). As

expected, environment and generation also had a significant effect on egg viability ($P < 0.0001$ for both), which is consistent with the ethanol environment being novel and adaptation to the novel environment occurring over the course of the experiment (i.e. the positive slope of each of the selected lines across generation). Rates of adaptation between treatments (generation* treatment interaction), were not significantly different from one another ($P = 0.8150$).

The final measurements of ethanol tolerance in adapted and control lines (shown in Figure 2-2) were compared using several one-tailed Student's *t*-tests. Three of the four selected lines show either greater egg viability or faster development time or both than their controls. The mutant *yellow* (*y*) line, which has been maintained in the lab for much longer than the other lines, showed the opposite trend for both measures after selection (lower survival and longer development time). When all four lines were combined and controls were compared to adapted lines, survival increased from 18.31 flies to 20.57 flies ($P = 0.0176$) and development time decreased from 32.13 hours to 29.5 hours ($P = 0.0159$).

Discussion

The multiple introduction treatments showed higher egg viability in the ethanol environment as well as in the control environment (though to a lesser extent), consistent with the hypothesis that multiple introductions can potentially make a population better able to survive in novel conditions. This can be explained either in terms of adaptation, or in terms of heterosis. We discuss these two alternatives in turn.

One possible explanation for the increased egg viability in the multiple introduction treatments is increased additive variation for quantitative fitness related traits, such as larval tolerance to ethanol. While both multiple introduction treatments

increased in egg viability, the treatment with multiple sources (rather than from a single source) had a greater increase (and rate of increase) as would be expected.

Results of the final fitness assays show that some adaptation to the novel environment did occur over the course of the experiment in all but one of the lines, as fitness increased over time. The single line that failed to show a response to selection was the inbred *yellow* mutant line that probably has lower standing variation than the other three wild caught lines (as seen in Figure 2-2). This lower variation probably limited the response to selection during the experiment.

Multiple introductions of the same and of different lines may also have resulted from positive heterosis, either by increasing population heterozygosity and reducing the effect of inbreeding depression, or by alleviating drift load (Whitlock et al. 2000). As flies are remarkable for their dispersal ability, the latter is less likely, though it is a possibility with lines that have been maintained in the lab for some time. Heterosis explains why the difference between treatments is noticeable, though not significant, in the very first generation after crossing, where response to selection on increased variation would not yet have occurred. Heterosis could also explain the trend in increasing egg viability of the control populations, as they are not under selection for adaptive variance but would still benefit from increased heterozygosity. Moreover, the slopes of the regressions with generation are not different between treatments (non-significant treatment * generation interaction) as would be expected if increased evolvability due to greater genetic variance were the only cause of fitness differences between treatments. Of course, in this experiment, as in real world situations, positive heterosis and higher evolvability are not mutually exclusive, and may well work in

concert to increase the colonization success that is sometimes seen with multiple introductions.

The results do not show evidence of outbreeding depression. Though we used a relatively large migration rate, the short time period between the primary and secondary introductions may have precluded adaptation to the novel environment, a pre-requisite for gene swamping.

The generality of these results to situations with different introduction parameters (number of individuals *per* introduction, number of introductions, *etc.*) is not evaluated with this study. In particular, the effect of multiple introductions might be greater with smaller founding populations, which would often be expected in invading populations. A greater lag time between introductions might have allowed for more local adaptation, and hence the detection of outbreeding depression. Mathematical modeling is one possible way to approach generality.

Insight into how non-native populations establish could be beneficial in designing management plans to prevent the establishment of or mitigate the harmful effects of invasive species. Many successful invasive populations currently being studied show evidence of multiple introductions followed by recombination of the different populations, and our results indicate this could be increasing their fitness in the introduced environment, either by increased adaptability or removal of inbreeding depression. This suggests that invasive populations founded from multiple introductions are likely to be more resilient and difficult to control. These populations should be closely monitored and efforts should be made to stem their proliferation.

Our model of population introductions can be applied as well to understand the establishment of biological control and endangered species reintroductions (Invarsson 2001). These (re)introduced populations have often been bred in laboratories or zoos for many generations, have very low effective population sizes, and must effectively adapt to a novel environment in the field. In each of these practices, a better understanding of the role genetics plays in how populations succeed in novel environments can help conservationists make informed decisions to maximize the probability of successful establishment.

Table 2-1 ANOVA results for initial linear model including all two-way interactions with F-values are based on Type III sums of squares. Environment is the ethanol versus control medium. Generation is which generation of the experiment the data was collected. Treatment is the different introduction patterns.

Source	F	p-value
Environment	224.4410	224.4410
Generation	18.1242	18.1242
Treatment	3.8528	3.8528
Env * Gen	9.8704	9.8704
Env * Trt	0.4227	0.4227
Gen * Trt	0.2048	0.2048

Table 2-2 Mean of transformed values of egg to adult survival rounded to the nearest hundredth for each of three treatments in both environments for each generation that data was collected. Data was not collected generations 4, 8, or 9.

Generation	1	2	3	5	6	7	10	11	12	13	14
	Standard Environment										
Single Intro	0.94	0.92	1.09	0.94	0.86	0.93	0.87	1.00	1.02	0.99	0.91
Mult Intro (S)	0.99	0.92	1.04	0.94	0.88	0.94	0.93	0.98	0.97	1.03	0.85
Mult Intro (M)	0.87	0.97	1.10	1.10	0.98	0.98	1.02	0.99	0.95	1.04	0.94
	Ethanol Environment										
Single Intro	0.59	0.60	0.64	0.54	0.55	0.70	0.63	0.61	0.56	0.73	0.71
Mult Intro (S)	0.59	0.72	0.59	0.61	0.59	0.73	0.60	0.59	0.69	0.76	0.77
Mult Intro (M)	0.61	0.64	0.74	0.64	0.71	0.83	0.69	0.65	0.71	0.82	0.82

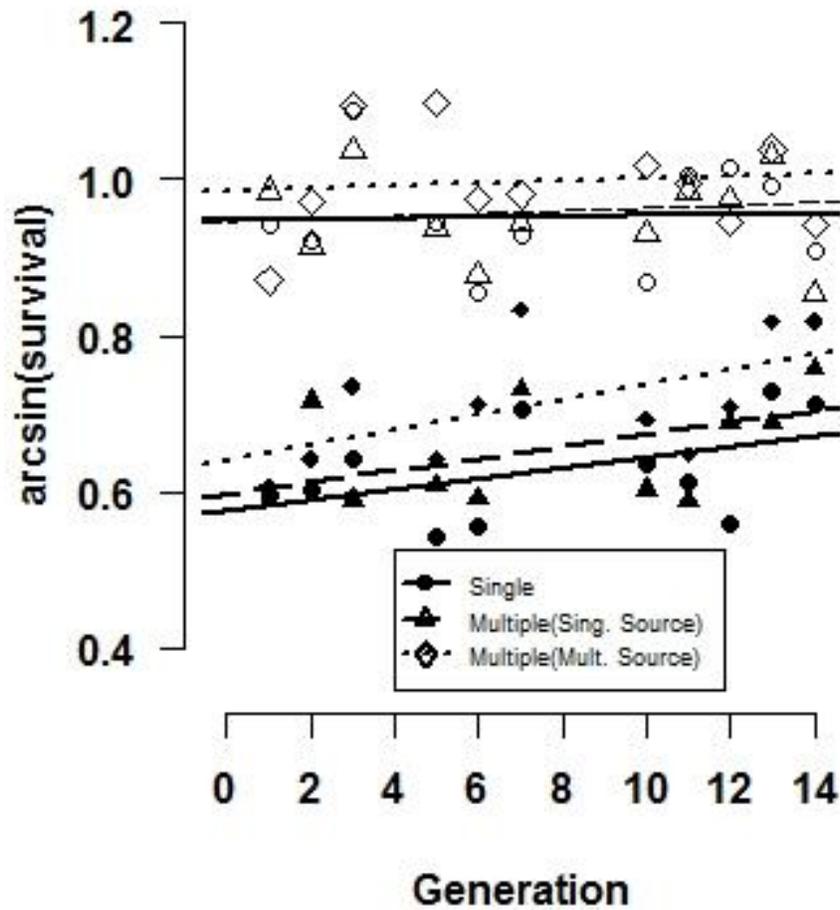


Figure 2-1. Transformed values of egg viability (a proxy for fitness) over fourteen generations for each environment by treatment combination. The upper three lines with unfilled points represent the standard environment while lower three lines with filled points represent the ethanol environment. Solid lines show treatment one (single introduction), dashed lines treatment two (multiple introductions from the same source), and dotted lines treatment three (multiple introductions from multiple sources).

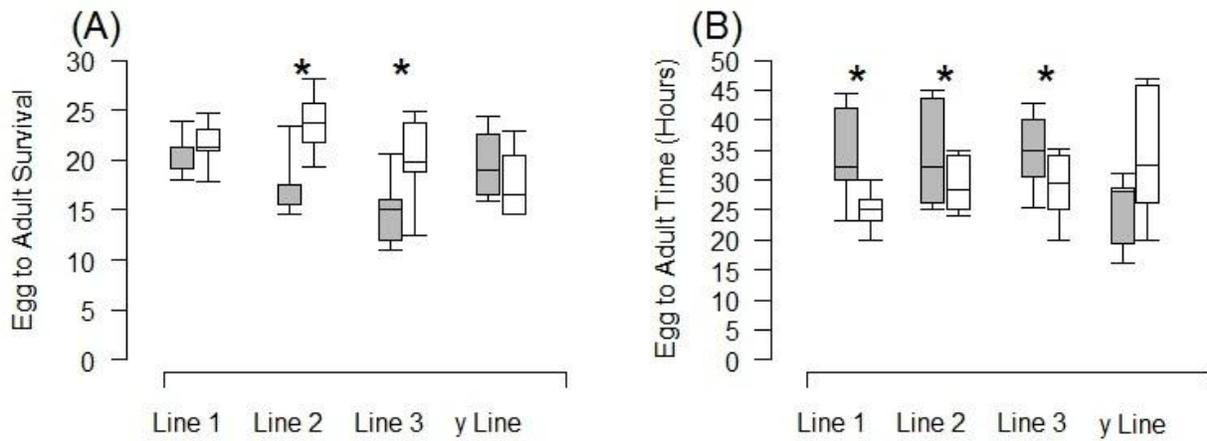


Figure 2-2. Final measures of egg to adult survival (A) and development time (B) in ethanol environment of each line used in the experiment. Gray boxes on left represent control populations not under selection. White boxes on right represent selected populations. All lines except the y line show evidence of adaptation to ethanol throughout the experiment. Significant differences between selected and control lines is indicated by a “*”.

CHAPTER 3
CREATING BENEFICIAL TRAIT COMBINATIONS IN A NOVEL ENVIRONMENT
THROUGH POPULATION ADMIXTURE WITH *DROSOPHILA MELANOGASTER*.

Introduction

Being able to predict how a population will adapt after being introduced to a new environment has long been of both practical and theoretical importance. For example, in predicting the success or failure of an invasive population to establish in its introduced range (Gomulkiewicz et al. 2010). The threat that invasive species pose to natural ecosystems, as well as their economic costs, has been well established (Pimentel 2002). The rate of biotic introductions is expected to rise. This has led to an abundance of studies attempting to better understand the establishment of exotics in a novel environment, using cases in the field as “natural experiments” (Sakai 2001). However attempts at predicting which invasive populations will establish and spread, and which will go extinct, have been generally ineffective (Peterson and Vieglais 2001).

Within the last decade, the importance of the evolutionary dynamics of invasive populations has become more apparent (Lee 2002). Genetic admixture (also known as gene flow), defined as the sharing of genetic material between two divergent populations, has been shown to be quite common in invading populations (many examples are reviewed in Dlugosh and Parker 2007). Multiple introductions of a species to a novel environment can result in gene flow that increases genetic diversity and effective population size, as well as contributing to heterosis. Multiple source invasions can result in contact between populations that have been geographically and genetically separated for long periods of time, and thus may contain distinct genetic variants, fixed either by drift or by local adaptation to distinct home environments.

Studies show that admixture can either reduce or increase population mean fitness, defined as an individual's ability to survive and reproduce, and thus reduce or increase the likelihood of species persistence depending on several factors. Admixture has been known to lower individual fitness through outbreeding depression, which usually results from the swamping of locally adapted alleles and disruption of coadapted gene complexes. Outbreeding depression is expected to occur when one or more of the multiple populations are locally adapted to the environment where contact between populations occurs, and admixture introduces locally non-advantageous alleles from another population. This situation is commonly seen when a conspecific member of a nonnative population invades an area and admixes with native populations (Huxel 1999). Outbreeding depression may also occur when there is continuous gene flow between populations that prevents local adaptation from occurring (Lenormand 2002; Holt & Gomulkiewicz 1997). This situation can be seen when there is a regularly open route of invasion, such as a common shipping route (Leuven et al. 2009).

In contrast to outbreeding depression, a rapidly growing body of evidence from the field claims that genetic admixture and multiple introductions tend to increase an invading population's mean fitness and thus the probability of successful establishment (Dlugosh and Parker 2007). Admixture is commonly believed to increase population mean fitness and/or adaptability by reducing inbreeding depression and increasing additive genetic variance (Verhoeven 2010; Dlugosh and Parker 2008). Studies have shown empirical evidence that both of these occur (Durka 2005; Lavergne and Molofsky 2007; Kolbe et al 2008). Another proposed mechanism of admixture increasing mean fitness that has been less studied is through the formation of novel combinations of

adaptive traits that are beneficial in the introduced environment. This mechanism is essentially an extension of the Fisher-Muller model (Fisher 1930, Muller 1932). The F-M model states that the advantage of sexual reproduction results from the ability to recombine beneficial mutations from separate lineages into single individuals. However, examples of the creation of novel adaptive combinations of traits have been sparse and relevance to population persistence remains uncertain.

The process of genetic admixture leading to novel combinations of traits is, however, commonly observed in studies of interspecific hybridization, where it is known as adaptive trait introgression (Kim and Rieseberg 1999). In a review, Arnold (2004) proposes that genetic exchange of adaptive traits between species can increase mean individual fitness in a population and allow for geographic range expansions into novel environments, as well as novel ecological niches within the home environment, that could not be exploited by the isolated parental populations. He reviews several studies of introgression occurring during natural hybridization events including three plants (*Iris*, *Helianthus*, and *Cowania/Purshia*), two animals (*Bactrocera* and *Anopheles*), and two microbes (*Trypanosoma* and *Haemophilus*). These examples show correlations between hybridization and fitness increases or range expansions across a wide range of taxa. *Helianthus* in particular has been used as a model to demonstrate increased adaptive potential of hybrids (Rieseberg et al. 2007).

Slightly modifying the term 'adaptive trait introgression', we refer to the pooling of traits by admixture of divergent populations of a single species as intraspecific trait introgression. This process can occur between genetically divergent populations of one species and can allow invading populations that are each adapted to different subsets

of parameters in the environment to form novel genotypes well adapted to the entire introduced range. It can also facilitate expansion into surrounding environments with novel selective forces. Populations that can take advantage of this recombination of traits are likely to exist if ancestral environments share some, but not all, properties with the environment the population is being introduced to. Through sexual reproduction or other forms of genetic recombination, beneficial traits from all ancestral environments can be pooled and the most useful suite of traits selected for in the novel environment. The delay between admixture and formation of beneficial trait combinations has been implicated by some as the cause of the commonly observed lag phase of species invasion (Ellstrand and Schierenbeck 2000). The lag phase is the time following the initial introduction in which the species persists in a small, localized, novel range, but is unable to spread beyond this area. At a later time, the population begins to multiply and to rapidly extend its range. It is usually at this point that the new species is noticed (Crooks and Soule 1999). This pattern is consistent with the initial introduction of a population lacking the necessary traits to be viable in surrounding environments, followed by subsequent introductions bringing in additional adaptive traits, which recombine with existing traits, allowing the admixed population to increase and spread.

Most studies of genetic admixture in invasive species have shown evidence of increased molecular genetic variation or have detected alleviation of inbreeding depression but few have attempted to characterize traits introgressed from ancestors that may have contributed to their ability to invade. One exception to this is a study on the invasive Brazilian peppertree (*Schinus terebinthifolius*) in Florida (Geiger et al. 2011). The Brazilian peppertree was introduced to Florida in two independent events

on the east and west coasts. Contact between the two spreading populations has since produced admixed individuals. The study was able to show, using a common garden experiment, that admixed individuals had a fitness advantage over either parent population. They measured specific traits including germination rate, 8-month survival, and biomass. Results showed that admixed plants had high germination rates similar to the western introduction and high survival and biomass measures similar to the eastern introduction.

Other existing evidence of intraspecific trait introgression contributing to successful invasion of new habitats is limited to simple cases of genetic exchange between populations of pathogens leading to multiple drug resistances. These examples are analogous to non-indigenous invasions where the pathogen represents an invading population and multiple drugs represent a novel environment for them to adapt to. Each population may have resistance to only a subset of drugs until genetic admixture recombines these resistance traits into individuals. In a study of *Streptococcus pneumoniae*, which undergoes recombination frequently, it was shown that populations that have a greater history of admixture were more likely to have acquired resistances to multiple antibiotics than single strains (Hanage et al 2009). Similarly, in a study on HIV, it was shown that recombination of mutations for resistance to zidovudine and resistance to the protease inhibitor SC-52151 found in different lineages could lead to fast acquisition of multiple drug resistance in recombinant strains and might be responsible for many more multi-drug resistant strains (Moutouh 1996). Though far from the macroscopic scale of most non-indigenous invasions, these offer a proof of concept of intraspecific trait introgression.

Other examples of advantageous admixture come from the practice of crossing domesticated crops with their wild relatives in order to introgress favorable traits, many of which are reviewed by Hajjar and Hodgkin (2007). Evolution has provided crop scientists with many diverse gene pools from which to draw traits for improving varieties. The most common reason for crossing crops with their ancestors is to confer pest and disease resistance genes as well as tolerance to other stresses, adaptations that are beneficial in the novel environment of modern agriculture.

In contrast to the model of intraspecific trait introgression, some have proposed that individual fitness is driven less by locally adapted traits and more by general stress reducing traits, for example chaperone proteins. This was demonstrated in one study where selection for increased starvation resistance in *Drosophila melanogaster* resulted in a correlated increase in resistance for ethanol, desiccation, and radiation. This suggests there is a common additive genetic mechanism that selection was acting on, such as metabolic rate, affecting all of these traits (Hoffmann and Parsons 1989). Genetic admixture can still result in transgressive segregation for these kinds of traits by increasing additive genetic variation in them. Unlike intraspecific trait introgression, the benefit of increasing variance for a single trait is less dependent on the specific environments and is more likely to be beneficial in any environment. Demonstrating local adaptation (i.e. that the traits involved are specific to a certain environment and not generally beneficial under stress) is needed to implicate intraspecific trait introgression as a source of adaptation.

Though theoretical work suggests that trait introgression can occur when multiple invading populations are introduced to a novel environment, it is difficult to empirically

test this hypothesis in the field. The goal of this study was to create a laboratory model to demonstrate intraspecific trait introgression and better characterize how it affects fitness. Using populations of *Drosophila melanogaster*, we performed an artificial evolution experiment to simulate genetic admixture of introduced populations. We used populations of fruit flies adapted to different environments introduced to a novel environment in both single introduction and admixed treatments. We looked for evidence of introgression of tolerance to two specific stresses (alcohol and desiccation), both of which are common in natural environments of *D. melanogaster*.

Methods

Fly Stocks

Six artificially selected lines, originally derived from independent field populations, were used for our experiment. The first three lines were derived from populations collected in the United States and put under a selection regime for larval ethanol resistance for approximately three years, hereafter referred to as E_S lines. The other three lines were derived from animals collected from the east coast of Australia and selected for adult desiccation resistance for approximately ten years (Telonis-Scott 2006). These lines are hereafter called D_S lines. For both sets of lines, an equal number of replicates were kept that were not put under the selection regime to act as control lines (hereafter E_C and D_C). The desiccation lines underwent relaxed selection for approximately one year, but fitness assays following this period (described below) confirmed that they were still well adapted to desiccation relative to their controls before the start of this experiment.

Flies were maintained throughout the experiment in vials on a cornmeal molasses medium at a constant density of ten male and ten female parents each

generation. They were kept in incubators at a constant temperature of 25° C with a 12:12-h light/dark cycle. The ethanol environment was created by allowing the prepared food to cool to 47° C and then adding 95% ethanol to produce a medium with 15% ethanol by volume (Fry 2001). Selection for ethanol resistance was performed by rearing flies on this mixed medium. Desiccation selection was performed by placing adult flies in a humidity-controlled environment with silica gel desiccant at approximately 25% humidity until one third of the flies had died. Survivors were used to set up the next generation.

Experimental Design

Each of the three E_S lines was paired with one of the three D_S line and crosses of these flies were performed (three different crosses in total) to produce admixed offspring lines, hereafter referred to as admixed or A_S lines. To prevent any assortative mating, and to maximize admixture in the first generation, ten virgin males of one line were crossed with ten virgin females of the other line in half of the replicates and the reciprocal cross was performed for the other half of the replicates. Similarly, E_C and D_C lines were crossed to produce an A_C line.

Each of the E_S , D_S , and A_S lines consisted of three different populations, and 10-15 replicates of each population was kept throughout the experiment, as well as an equal number of their unselected controls. For each subsequent generation following the cross, all populations were set up with ten males and ten females from the previous generation and underwent selection regimes for both ethanol and desiccation, representing a joint environment containing components of both ancestral environments. Flies laid eggs on an ethanol medium. The adult flies that emerged from

these eggs were subjected to the desiccation treatment until approximately two thirds remained alive. Ten males and ten females were selected haphazardly from all of the survivors to be the parents of the next generation. The control lines of unselected flies were treated identically throughout the experiment. A summary of the life cycle with selection regimes is given in Figure 3-1. This experimental design simulates the introduction of two populations, each adapted to a different environment, as well as an admixed population combining the two ancestral populations, to a novel environment so that their fitnesses could be compared.

Environment Tolerance Assays

Environment tolerance assays were conducted to determine the performance of the three different treatments, E_S , D_S , and A_S , in each of the three environments (ethanol, desiccation, and joint). As proxies of mena population tolerance we measured egg viability in ethanol and time of adult survival in desiccation then combined these into a total value. The ethanol assay involved placing flies in an egg-laying chamber, which consisted of a 300 mL plastic bottle with a 10mm x 35mm Petri dish covering the top. The dish was filled with ethanol medium and the bottle was inverted, i.e. resting on the Petri dish. After enough eggs had been laid, we removed the covers and collected thirty eggs out of the dish and placed the eggs in a vial of ethanol food. These vials were then allowed to develop for fourteen days and adult flies emerging were counted daily, giving us an estimate of egg hatchability. For the desiccation assay, adaptation was measured as the average amount of time that an adult fly would survive under desiccation stress. Adult flies were placed in vials with humidity permeable covers and were placed in a tank containing silica gel desiccant and maintained at 25-35% humidity. Surviving flies were counted at 6-hour intervals. As an additional measure of desiccation adaptation,

regression analysis was used to determine the rate of desiccation for each vial and the LD50 (time for half of the flies in a population to die), was extrapolated from the equation of the regression line.

To combine the measures of each stress adaptation into a single estimate of fitness in a joint ethanol/desiccation environment, we multiplied the average egg-laying time by the egg to adult survival to get a measure of viable egg laying potential (VEP). This model of total fitness makes the simplifying assumption that the rate of egg laying over the course of the time in the desiccation environment remains constant. All data analyses were performed in the R Statistical Package (R Development Core Team 2009).

Results

To test the effects of genetic admixture on a population's mean environmental tolerance and fitness, we examined both the ethanol tolerance and desiccation tolerance of each of the three treatments (E_S , D_S , and A_S lines). We then combined these values into a total mean fitness value for each treatment. The population's level of adaptation for each of these measures was determined as the average difference between the selected and unselected controls. This accounted for environmental variation between generations and genetic backgrounds. The results of the first generation of these data are shown in Figures 3-2 and 3-3 respectively. Measures in the following seven generations are summarized in Figure 3-4.

Environment specific adaptation

As seen in Figure 3-2, ethanol selected and desiccation selected lines each show significant levels of adaptation to their respective "home" environments (i.e., mean of selected lines is higher than mean of control lines: $P > 0.001$ for both; one-tailed t-test),

while neither shows a significant difference between selected and controls in the environment they had not previously been exposed to ($P = 0.20$ for D lines on ethanol and $P = 0.07$ for E lines on desiccation; two-tailed t-tests). Interestingly, the mean time of survival in desiccation environment of selected E_S lines is lower than that of control E_C lines, though the trend is not significant.

The progeny of the cross of the two populations (A lines) show significant adaptation to both environments as the selected lines had greater fitness than the controls ($P = 0.01$ for A lines on ethanol and $P = 0.02$ for A lines on desiccation; one-tailed t-test). Both of these were less adapted than the unadmixed lines on their ancestral environment.

Combined fitness measures (VEP)

Viable egg lying potentials (defined as the mean time for desiccation multiplied by the percent larval survival) for the first generation following the cross are summarized in Figure 3-3. The E_S lines do not show evidence of adaptation to the joint environment compared to their control lines ($P = 0.39$; one-tailed t-test). The selected lines of D and C both have significantly greater VEP than their controls ($P = 0.002$ and $P = 0.007$, one-tailed t-test).

Egg to adult survival and desiccation time was also measured for each population for eight generations following the cross event to determine if the patterns of adaptation over time differed between treatments. Figure 3-4 shows the difference between selected and control lines of VEP for each of the treatments (ethanol adapted, desiccation adapted, and cross) over all eight generations. These data show that the significant differences between treatments seen in the first generation disappear in subsequent generations. While selected lines remain greater than control lines, this

difference becomes smaller over time. There are no indications that mean differences in total VEP between treatments occur over the course of the experiment, i.e. there is no signature of differential adaptation. Variance in VEP across generations does not show any trends as well.

Finally, fecundity (as measured by the total number of eggs laid in a period of time) of each of the treatments was measured to rule out the possibility that either control or selected lines within any of the treatments showed any evidence of inbreeding depression gained during the selection process. There were no significant differences in fecundity between control and selected lines in any of the treatments.

Discussion

Conflicting observations of how the mean fitness of a population changes following genetic admixture has led to disagreement on the overall effect of admixture on adaptation. Our fitness measurements indicate that the effects of admixture are highly dependent on the source environment of the invading populations, the novel environment, the degree of similarity between the various environments, and the time span over which fitness is being observed.

Consistent with theories of local adaptation, we show that a population has the highest mean fitness in the environment that it has historically evolved in, and has the lowest mean fitness in the environment that it has never been exposed to. These data are consistent with the hypothesis that there are stress-specific alleles of genes that allowed each of these populations to adapt to their particular environment, rather than only general stress resistance alleles such as heat shock proteins being selected for that

increase tolerance to any stressful environment (though the work of Hoffmann and Parsons 1989 suggest these exist as well).

Desiccation adapted populations are well adapted to the desiccation environment, but in the ethanol environment have the same time of survival as the unselected controls. Interestingly, ethanol selected populations show lower adaptation to the desiccation environment than their unselected controls. This could indicate a trade-off of locally adapted alleles. While adaptation to one environment does not necessarily limit adaptation to another, this kind of local adaptation trade-off has been demonstrated in many studies (reviewed by Hereford 2009). Futuyma and Morena (1988) also suggest that interference between certain traits can result in these trade-offs. This could have important implications when generally assessing how genetic admixture will affect population mean fitness.

Crosses of ethanol and desiccation adapted populations show significant levels of adaptation to both environments, although not to the same extent as the uncrossed populations in their own ancestral environment. This suggests that some level of outbreeding depression does occur and this results in intermediate levels of adaptation to each specific environment. This intermediate level of adaptation to multiple stressful environments, while imposing a cost in stress-specific adaptation, could be beneficial in some novel environments. If either of the E_S or D_S lines was introduced to an environment containing high levels both ethanol and desiccation stress, both lines would have very low population mean fitness. The admixed population (A_S lines) employs a more generalist phenotype that may be more useful in a completely novel environment or an environment containing components of each of the ancestral ones. In

a real world introduction, many more environmental stresses likely play a role in adapting and avoiding extinction in a novel environment.

When the two fitness measures are combined to determine VEP, results show that the ethanol-adapted lines benefited from the admixture with desiccation-adapted lines (A_S had higher VEP than E_S), however there is no benefit to the desiccation-lines (A_S lines were not different in VEP than D_S). Although they are less well adapted to the ethanol environment than the A_S lines, the very high mean fitness that the D_S lines have in desiccation allows them to overcome this and have overall greater mean fitness in a combined environment. This further supports that the effect of admixture is highly dependent on the source environments.

Looking at the VEP of each of the lines across eight generations following the cross shows that the differences in VEP between the lines are only significant in the first generation following the cross. This suggests that it may be a transient effect due to the high amount of heterozygosity and segregation of alleles initially present. As random mating occurs in subsequent generations, this heterozygosity in the admixed population is expected to decrease. There is a noticeable pattern of the difference between selected and control lines decreasing in all lines which is likely due to adaptation of the controls over the course of the experiment. As this occurs, our measure of adaptation approaches zero and our ability to detect differences between selection treatments decreases.

The mechanism of creating useful combinations of traits via admixture of genetically disparate populations has seldom been used as an explanation for the increased ability to adapt to an introduced environment often found after multiple

introductions. However, this study demonstrates that genetic admixture can result in intermediate levels of multiple adaptive traits, effectively shifting the population from a specialist strategy towards a more generalist strategy that may be beneficial in an introduced environment containing many novel stresses.

This experiment modeled only two environments. Further investigation of the question of admixture with a greater number of environmental stressors (and/or multiple independently adapted lines to the same stressors as used here) is needed to better represent an actual population invasion. Additionally, the decision to keep population density constant throughout the experiment was made to avoid adding noise that would mask the effects of admixture. However in a natural population introduction, populations are not likely to be constant, but are changing rapidly. This will have a large effect on the level of gene flow that occurs during multiple introductions and how it affects fitness. The observed loss of fitness differences in later generations could be due to loss of heterozygosity. This would be expected to be much slower in larger populations.

In conclusion, our results provide evidence that interspecific trait introgression, observed often in microbial and agricultural populations, may occur in natural population invasions and could potentially play a role in establishment and spread. The use of artificial evolution experiments with model organisms is valuable for testing these hypotheses. More work is needed to make such artificial evolution experiments more closely model realistic scenarios in nature such as by increasing the amount of novel stressors.

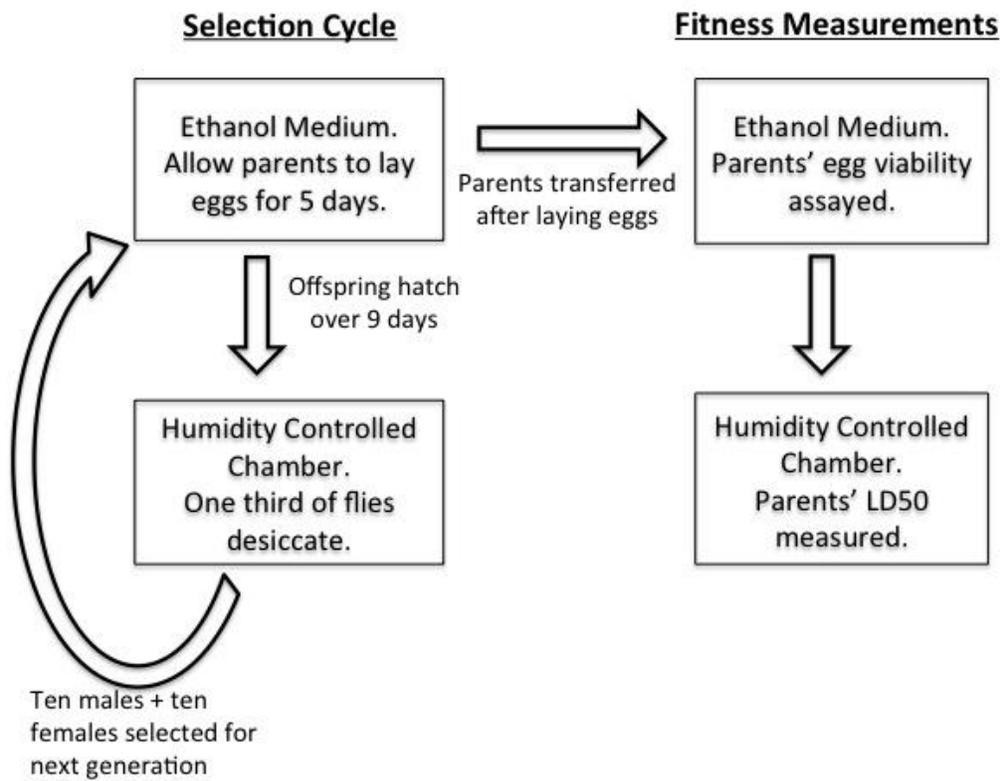


Figure 3-1. Diagram of selection regime and environment tolerance measurements used throughout the experiment. All populations underwent selection to both ethanol and desiccation each generation and measurements of stress-specific adaptation were taken.

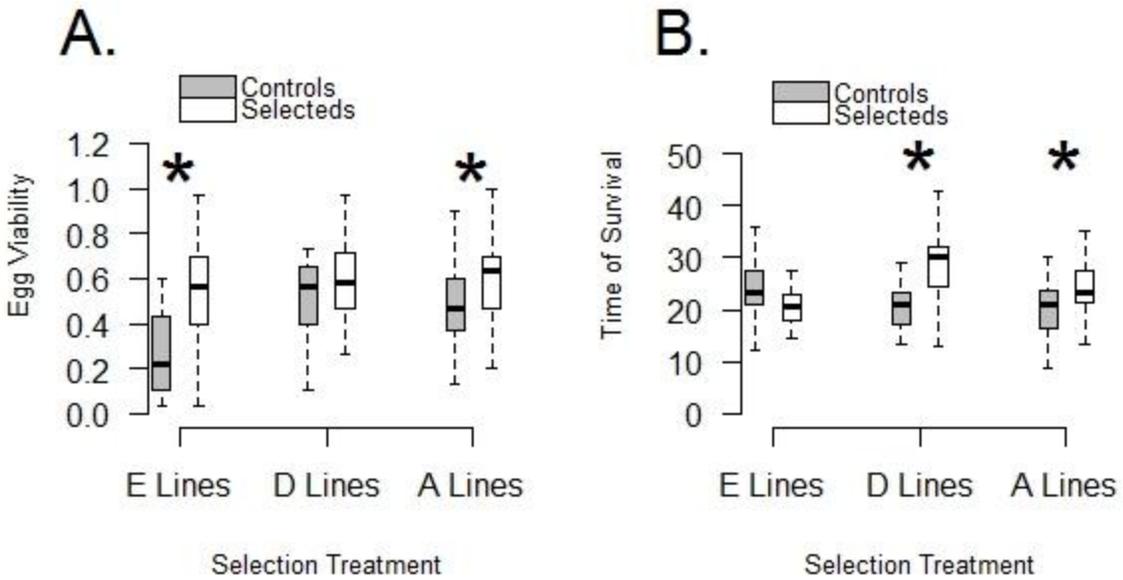


Figure 3-2. Admixed selected populations have significantly greater environmental tolerance than their controls (Adm.) for both measured stress-specific tolerance values while unadmixed selected lines are only adapted to their own ancestral environment one generation after crosses were performed. Treatments with a (*) indicates significant differences between selected and control populations which was used as a proxy to measure adaptation.

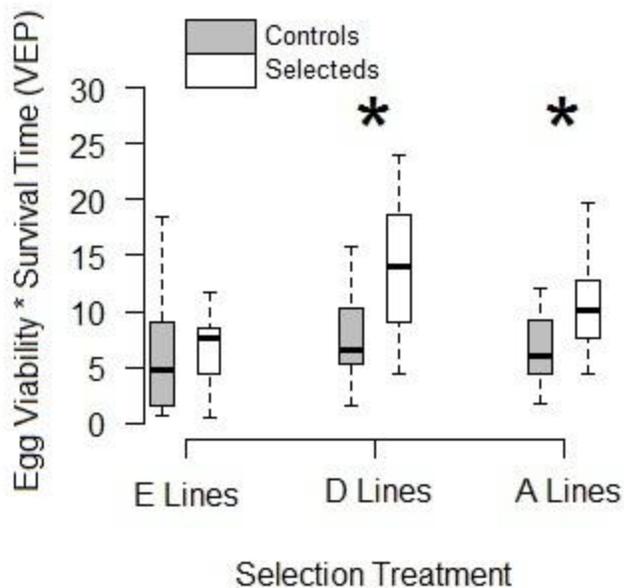


Figure 3-3. Admixed populations as well as desiccation selected (A_S and D_S) have significant greater viable egg lying potential (VEP) than respective control populations one generation after crosses were performed. Treatments with a (*) indicates significant differences between selected and control populations which was used as a proxy to measure adaptation. Level of adaptation was determined by comparing selected populations to their corresponding controls.

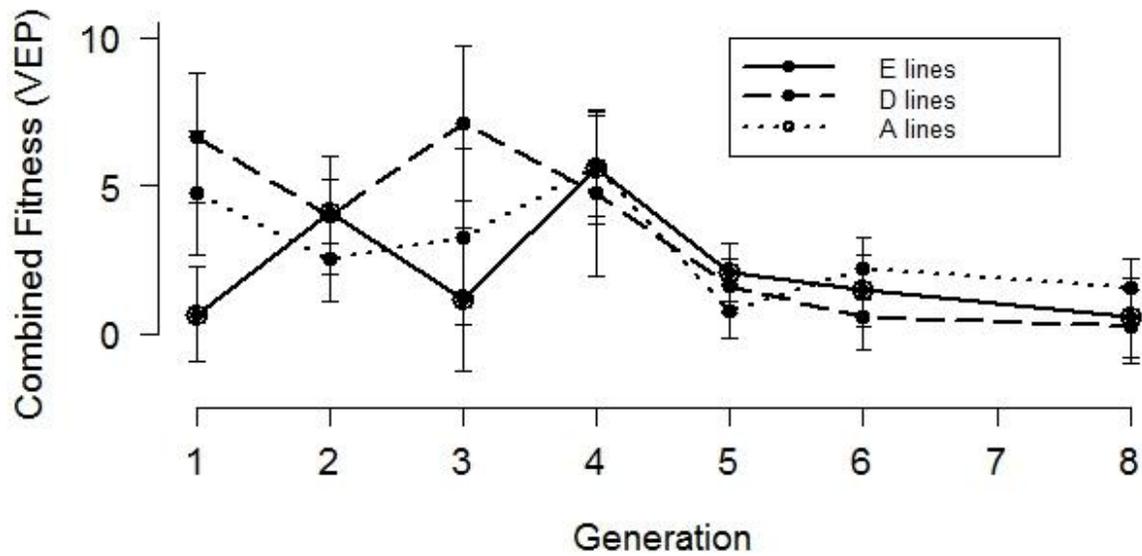


Figure 3-4. Viable egg laying potential (VEP) of all three treatments over the seven generations of the experiment show no significant difference amongst treatments after the first generation. Adaptation was defined as the difference in VEP for selected lines and control lines. A decreasing trend over time indicates adaptation of control populations during the course of the experiment.

CHAPTER 4
AN INDIVIDUAL-BASED MODELING APPROACH SHOWS THE EFFECTS OF
GENETIC ADMIXTURE UNDER DIFFERENT CONDITIONS AND AT DIFFERENT
TIME SCALES.

Introduction

Genetic admixture, or the exchange of genetic material between different populations, is ubiquitous in nature and can have a variety of impacts on fitness and therefore evolution (Vermeij 1991). Populations geographically separated from one another will tend to diverge genetically, a possible mechanism of allopatric speciation (Mayr 1963). However, barriers separating populations are often not completely impermeable; also, environmental changes allow for migration between these populations. Secondary contact can occur between genetically distinct populations before the onset of reproductive isolation. Genetic exchanges of this type have become dramatically more common as a result of human activities such as direct transportation of organisms, habitat destruction, and climate change (Parmesan 2006, Pereira et al 2010). How this exchange of genetic material affects the fitness of individuals, defined as their ability to reproduce and contribute genetically to future generations, in a population and its ability to adapt to a novel environment is unresolved because of the contradicting theory and evidence from the field.

There is much evidence that interbreeding, divergent populations will have reduced fitness, known as outbreeding depression (Edmands 1999). The most common explanation for this is that populations are already locally adapted to their ancestral environment (Kawecki and Ebert 2004). Thus, introducing genetic material from a foreign environment dilutes or swamps the locally adapted alleles (Lenormand 2002). In

addition, outbreeding could disrupt coadapted gene complexes, which would also lower mean fitness (Huff et al. 2011).

Alternatively there is a growing body of literature that says combining multiple populations will increase population mean fitness (Verhoeven et al 2010, Keller and Taylor 2010, Dlugosch and Parker 2008). The first explanation for this says that genetic admixture increases the additive variance of fitness-related traits. According to Fisher's fundamental theorem of natural selection, the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time (Fisher 1930), and so populations with more variance have greater potential for adaptation (but see Houle et al. 1996). Second, admixture between populations reduces the average homozygosity, which can alleviate any reduced fitness associated with inbreeding depression.

Inbreeding depression is caused by both the expression of recessive deleterious alleles, as well as by the loss of beneficial phenotypes of heterozygotes (evidence reviewed in Charlesworth and Willis 2009). This is particularly important in small populations that may be bottlenecked due to recent introductions and strong selection (Hedrick 2000).

Invasive species researchers have long struggled with the question of what makes some introduced populations establish successfully, while the vast majority goes extinct (Williamson 1996). Recent genetic work in this field has verified that many successfully introduced populations are the result of multiple introductions (Collins et al. 2001; Fonseca 2001; Maron et al. 2001; Novak & Mack 2001; Kolbe et al. 2004; Durka et al. 2005; Lavergne & Molofsky 2006; Dlugosh & Parker 2008; Facon et al. 2008; Hufbauer & Sforza 2008, Keller & Taylor 2010, Whitfield et al. 2006). This finding has led many to conclude that multiple introductions of a species from different sources

allow genetic admixture, which increases the probability of establishment and spread by increasing fitness. The overall effect that genetic admixture has on mean population fitness appears to be dependent on several parameters, including the amount of admixture that occurs (Lynch 1991), the amount of local adaptation in the source populations (Lenormand 2002), the extent of mismatch between the source populations and the new environment (Wiens and Graham 2005), the size of the introduced population, and the amount of genetic diversity within each source population (Roman and Darling 2007). These parameters are intrinsically difficult to manipulate in nature or even in a lab; further, simultaneously varying so many parameters is unrealistic. While many field studies and some empirical studies examining these questions exist, they tend to be limited in the range of parameter space that they are able to explore, vary only a subset of parameters, and have limited replication. A modeling approach thus seems a logical option for understanding systems of genetic admixture. Determining the region of parameter space in which admixture increases fitness and affects the rate of adaptation to a new environment can have many useful applications and will help in better understanding its consequences.

Agent-based or individual-based modeling is one approach that can be used that allows for the testing of the effects of changes in several different parameters (Parunak et al. 1998). Agent-based models simulate a collection of autonomous individuals called agents, for which certain characteristics are tracked through time. The agents exist in an environment and exhibit certain behaviors and interactions with one another based on a set of rules of that environment (Bonabeau 2002). Agent-based models are particularly useful when including many parameters that would make equation-based

modeling too complex, or when possible, unpredictable emergent behaviors of the population are of interest.

Presented here is an agent-based evolutionary model that simulates a single adapting population in a novel environment, as well as an admixed population formed from random mating of multiple populations. It was designed to imitate the introduction of a non-native species to a new environment followed by a secondary introduction to the same environment of a divergent population that can interbreed with the first, using realistic parameters for *Drosophila melanogaster* when needed.. The model is then used to address the question of how the fitness and persistence of this mixed population compares to those of a single introduced population (identical to the first of the admixed populations).

Methods

Overview

The model was programmed in the R statistical package. The model simulates a population, called A, of N diploid individuals with 20 unlinked loci that contribute to fitness. The population evolved in a simulated novel environment for a specified number of generations called the lag time (T). After the lag time, the population was copied and each copy underwent one of two treatments. In treatment one it continued to evolve as before (this copy is hereafter referred to as A'). In treatment two, a second population of size N called B was independently generated. Individuals from populations A and B were combined and N individuals were randomly selected. This population, hereafter referred to as AB, then continued to evolve. Both populations A' and AB evolved for a total of 40 generations after treatment and population mean fitness was compared

between them. Figure 4-1 summarizes the processes that occur throughout the simulation and within a single generation.

Initial conditions

The phenotype (or relative fitness of the phenotype) of each individual was defined by alleles at 20 diploid loci and a random component. Alleles took on one of two possible values at each locus. Since all loci had equivalent effects on phenotype, the possible alleles at each locus were labeled 0 and 1. Selection in the novel environment was directional, with allele 1 favored. The initial populations (A and B) were assumed to come from two different environments, where allele 1 was not necessarily favored. The population was started with N adults, whose alleles were randomly assigned, with the frequency of the 1 allele first randomly determined for each locus using a beta distribution. Then, each allele at that locus for the entire population was set to 1 with a probability equal to the locus's allele frequency (i.e., if a number chosen randomly from the distribution used was less than the chosen allele frequency). This simulated drawing individuals from a large source population. Actual allele frequencies of generated populations were not identical to the defined allele frequency for each locus because of random sampling (the initial number of 1 alleles at each locus was binomially distributed). In order to test how the initial frequency of the beneficial allele affected the model results, simulations were run under a range of different allele frequencies with the beneficial allele having a high, low, or intermediate initial frequency. A beta distribution was used for the allele frequencies because it is defined over the interval (0, 1), it has the flexibility to test a range of distributions, and has been used similarly to define allele frequencies for models (Falush et al. 2007; Hubisz et al. 2009; Zhivotosky 2009;). The initial frequency of 1 alleles was a proxy for the similarity

between the ancestral and introduced environments; the high frequency initial conditions represent cases in which the initial population was assumed to come from a habitat similar to the novel habitat, while for the low frequency case represents an initial habitat very different from the novel habitat. In the ‘intermediate frequency’ simulations, allele frequencies were drawn from a beta distribution that was equivalent to a uniform distribution from 0 to 1. These distributions are summarized in Table 4-1.

Reproduction and mutation.

Each generation, N random mating pairs were selected with replacement from the adult population (which also had size N , so on average each adult participated in two pairs). Individuals were hermaphroditic, so any two could form a mating pair. Each mating pair produced an equal number of offspring (F) to create a total of FN offspring. Offspring genotypes were determined by randomly and independently selecting one allele from each parent for each locus (no linkage). Genetic drift is inherently included in the model because of the random selection of parental pairs each generation and because parents may mate more than once or not at all.

Each allele within each offspring mutated with a probability μ , changing a 0 allele to 1 and vice versa. We used a value of $\mu = 5.8 \times 10^{-9}$ based on direct estimations of the single nucleotide mutation rate in *Drosophila melanogaster* (Haag-Liautard et al. 2007). We assumed that mutations of interest occur at only a single nucleotide per gene.

Novel environment and selection

After initialization, the alleles are under selection and fitness will tend to increase by increasing the frequency of the beneficial (1) allele. Only directional selection was used in the model. The total phenotype of each individual was calculated by summing all of the allelic effects as well as a random environmental effect (allelic effects and

environmental effects are described below). Natural selection was simulated by calculating the phenotype of each individual, ordering all of the offspring, and keeping only the N individuals of highest phenotype, which are used as parents for the next generation. This simulated competition for a limited number of mating sites.

Secondary introduction

After the lag time, a second population (B), also of size N , was generated. These individuals are assumed to be a sample from another large population generated in the same way as the first but using a different set of allele frequencies; these were drawn from the same beta distribution as population A. Population A at generation T was copied and split into two treatments. In the first treatment (A'), the population continued to evolve as before. In the second treatment, the population was combined with the newly generated population B, to make an admixed population of size $2N$. A total of N random individuals were selected from the collective population to become the second treatment (AB). Both A' and AB then proceeded to evolve independently. From this simulation, we can compare the fitness over time of population A' (un-admixed) versus population AB (admixed). Table 4-2 summarizes the variables in the model and the values we used in our simulations.

Allelic effects

The genetic contribution to the phenotype (relative fitness) was assumed to be the sum of the effects of each locus (i.e., there was no epistasis). Two different assumptions were made about dominance, which was very important in determining the effect of admixture. The two models of gene action were a beneficial-additive-alleles model (for which the relative fitness is related to the sum of all allelic values), and a deleterious-recessive-alleles model (for which the beneficial allele is dominant over the

deleterious allele). While an actual evolving population likely possesses a combination of these types of alleles, we chose to run simulations of each separately to be able to tease apart the effects of each. These models do not take into account differential fecundity of individuals and instead only selects adults with the highest phenotype to reproduce.

In the first model, the effect of the alleles at each locus on the phenotype was assumed to be additive (no dominance). There were two alternative alleles (0 or 1) at each locus. Allele 0 had no effect on fitness while allele 1 added $1/2L$ to the individual's fitness (so that the total genetic contribution to fitness was between 0 and 1). The mean fitness of all individuals in the population was calculated and tracked over time.

The second model simulated deleterious recessive alleles (i.e., that the beneficial allele is dominant), which are believed to play a large role in inbreeding depression (Charlesworth and Willis 2009). For this model, again there were only two alternative alleles. Dominance was assumed to be complete, such that a homozygous dominant or a heterozygous locus increased fitness by $1/L$ while a homozygous recessive locus had no effect on fitness (so that the total genetic contribution to fitness was again between 0 and 1). The mean fitness of all individuals in the population was calculated and tracked over time.

Environmental variance

To simulate realistic heritabilities, a parameter was added to the genetic contribution to the phenotype to simulate environmental variance and incorporate this into the phenotypic effect of an allelic substitution and thus into the selection response. The environmental effect for each genotype was drawn from a normal distribution with a mean of zero and variance of 0.1.

Parameters tested

The main parameters of interest were population size N and the time between primary and secondary introductions (lag time T) because they vary widely in actual introductions and are believed to have a major effect on how admixture changes fitness (Frankham 1996; Crooks & Soulé 1999). Simulations were run at eight different population sizes from a geometric scale (2, 4, 8, 16, 32, 64, 128, and 256 individuals) to cover a broad range of sizes that might be expected in a real introduction, with lag time kept constant at 10 generations. Simulations were then run at seven different lag times (0, 5, 10, 15, 20, 25, and 30 generations), with population size kept constant at 32. The simulations were allowed to run a total of 40 generations after the lag time. Allowing the model to run longer did not change the outcome. The average phenotypes of offspring after selection for both populations A' and AB were recorded a single generation after the lag time (hereafter referred to as initial fitness), as well as at the end of 40 generations (hereafter referred to as final fitness). For each of the $8+7=15$ sets of conditions tested, 300 repetitions of the simulation were conducted. The mean fitness from each of the two treatments was plotted over the range of parameters tested.

Following this, the model was run using the extreme values of population size and lag time. The initial allele frequencies were set to low frequency for the beneficial-additive-allele model and intermediate frequency for the deleterious-recessive-model because these showed the most dramatic effects in our main simulations. These extreme value simulations included low population size (2) and low lag time (0), low population size (2) and high lag time (35), high population size (256) and low lag time (0), and high population size (256) and high lag time (35). The model was run under

each of these four sets of conditions a total of 300 times. The agreement of these results with our main results was used to support our conclusions.

The model makes several assumptions including random mating, discrete generations, constant adult population density, no linkage, no epistasis and a constant mutation rate. The model is stochastic, with several processes depending on random probabilities. The initial allele frequencies are drawn from random probability distributions. Mating pairs are drawn at random within populations. For each locus within the genotype, one allele is drawn from each parent at random. Each allele is then allowed to randomly mutate to another allele with probability μ , which was set to 5.8×10^{-9} . Finally, the environmental effect on each individual's fitness is randomly drawn from a normal distribution with a mean of zero. Each of these stochastic processes results in variation of the outcome of the model.

Results and discussion

Beneficial-additive-alleles model

An effect of admixture in the additive alleles model was generally seen when the starting beneficial allele frequencies were low or intermediate unless the population size was very low (Figure 4-2); admixed populations (AB) had lower initial mean fitness values and greater final mean fitness values than the un-admixed population (A'). The lower initial mean fitness (Figures 4-2A and 4-2E) was due to the introduction of new alleles from the secondary population (B) after the first population (A) had already begun to adapt to the environment by increasing the frequency of beneficial alleles (gene swamping). The greater final mean fitness (Figures 4-2B and 4-2F) was due to the introduction of beneficial alleles by population B that were fixed for the deleterious allele in population A and were ultimately driven to high frequencies by selection. This

allows adaptation to occur much faster in the admixed population than in the unadmixed population, which can only introduce new alleles through mutation. This effect was not seen when initial allele frequencies of the beneficial allele were high (Figures 4-2C and 4-2) because the beneficial allele has already been driven to fixation before admixture at most loci. There are not enough unique beneficial alleles in each population for admixture to have an effect. At lower population sizes (2 to 4), genetic drift dominated and little adaptation occurred in either treatment for any initial allele frequencies. If the beneficial alleles were initially rare, there was a population size (of those that we tested, 32), for which the difference in mean fitness between treatments was largest and for which approximately half of the alleles were beneficial (Figure 4-2B). Below this size, drift lowers the efficiency of selection and mutation rates are very low; while above this size, the high frequency of beneficial alleles in both treatments reduces the difference between them.

The difference in initial mean fitness was not observed when lag time was either zero or greater than 20 generations when beneficial alleles are initially rare (Figure 4-3A). With no lag time there is no chance for population A to increase the frequency of beneficial alleles. With very long lag times, population A has fixed or driven to high fixation most of the beneficial alleles that it initially had. The single generation of selection before measuring mean fitness is enough to remove most individuals in population AB that are offspring of individuals from population B. As a result, very little if any admixture lasts in the population and population AB is essentially the same as population A'. Final mean fitness values reflect this as well (Figure 4-3B). As lag time increases, the effect size between treatments generally decreases because the amount

of admixture that subsists after selection decreases. The lag time had no apparent effect on the actual final mean fitness value that populations reached.

The data from the simulations run at the extreme conditions of the range of parameters we tested are summarized in Figure 4-6. At very low population sizes (2), little adaptation occurs in either treatment, regardless of lag time. With large population size (256) and no lag time, the AB populations have greater final mean fitness than the A' populations, as would be expected from our main results. There is no difference in the initial mean fitness, consistent with there not being time for local adaptation in the first introduction (A). When both population size and lag time are large (256 and 30 generations), initial mean fitness is lower in the admixed population, while final mean fitness is greater in the admixed population.

Deleterious-recessive-alleles model

When the deleterious recessive alleles are considered, the difference between the A' and AB populations is most pronounced when the starting allele frequencies of beneficial alleles have either intermediate or low starting frequencies (Figures 4-4 and 4-5). In the low frequency allele simulations, there is once again no effect observed at very low population sizes (2 to 4 individuals) due to the effect of drift. At higher population sizes, the admixed population (AB) has greater final mean fitness than the non-admixed population A' (Figure 4-4B). The difference between these treatments increased with population size probably due to larger populations' ability to maintain high levels of heterozygosity for longer time. However there is generally no difference between treatments in initial mean fitness (Figure 4-4A).

When the alleles in the original population are uniformly distributed (Figures 4-4E and 4-4F) there is an interesting pattern of admixed populations having greater initial

mean fitness at low population sizes and non-admixed populations having greater initial mean fitness at higher population sizes, with the switch occurring near a size of 16. This is likely because at small sizes, homozygosity in population A before admixture is very high and admixture alleviates inbreeding depression while in large population sizes more heterozygosity is maintained and we see the same pattern as before of admixture swamping an adapted population with deleterious alleles. The final mean fitness here (Figure 4-4F) shows the same pattern as when initially the deleterious allele is common except that it eventually reaches a plateau where mean fitness is high with or without admixture and no difference between treatments exists.

When lag time is varied, the treatment effect is again present in final mean fitness for the simulations where the beneficial allele is rare or intermediate (Figures 4-5B and 4-5F), though the lag time did not appear to change the effect size. There is no difference in initial mean fitness between treatments for most lag times in the common alleles model (Figure 4-5A). But in the intermediate distribution simulations there is a positive effect of admixture when there is no lag time but a negative effect size when the lag time is 15 generations or more (Figure 4-5E). This is because with no lag time, no purging of deleterious alleles occurs in population A before admixture and admixture only increases mean fitness by decreasing homozygosity. But when a lag time exists, admixture reverses the effect of purging by re-introducing deleterious alleles.

The data from the extreme conditions are summarized in the second half of Figure 4-6. In agreement with the other results, they show that at low population sizes (2), little adaptation occurs. At high population sizes (256) with no lag time, there is an increase in mean fitness from having multiple introductions. At high population sizes

and long lag times (256 and 30 generations), there is lower mean fitness in the multiple introduction treatment for initial mean fitness and a greater final mean fitness in the admixed populations.

The results of our individual-based model study could be useful in providing a more comprehensive framework to explore the fitness effects of population admixture. The effect of admixture is dependent on many particular parameters and assumptions in the model, but this study provides many important insights that could be used when assessing specific situations. This model may allow for more reliable prediction of the effect of genetic admixture between populations, which can help in controlling invasive species or promoting the introduction of beneficial populations to environments.

Table 4-1. Summary of parameters used in our model. Population size and lag time were varied while the others were kept constant.

Frequency	Distribution	Parameters
Low	Beta	Alpha = 1, Beta = 10
High	Beta	Alpha = 1, Beta = 1/10
Intermediate	Beta (Uniform)	Alpha = 1, Beta = 1

Table 4-2. Summary of distributions used to determine initial allele frequencies in our simulations.

Variable	Symbol	Value used
Population size	N	2, 4, 8, 16, 32, 64, 128, 256
Lag time	T	0, 5, 10, 15, 20, 25, 30, 35
Number of loci	L	20
Offspring per pair	F	3
Mutation rate	μ	5.8×10^{-9}

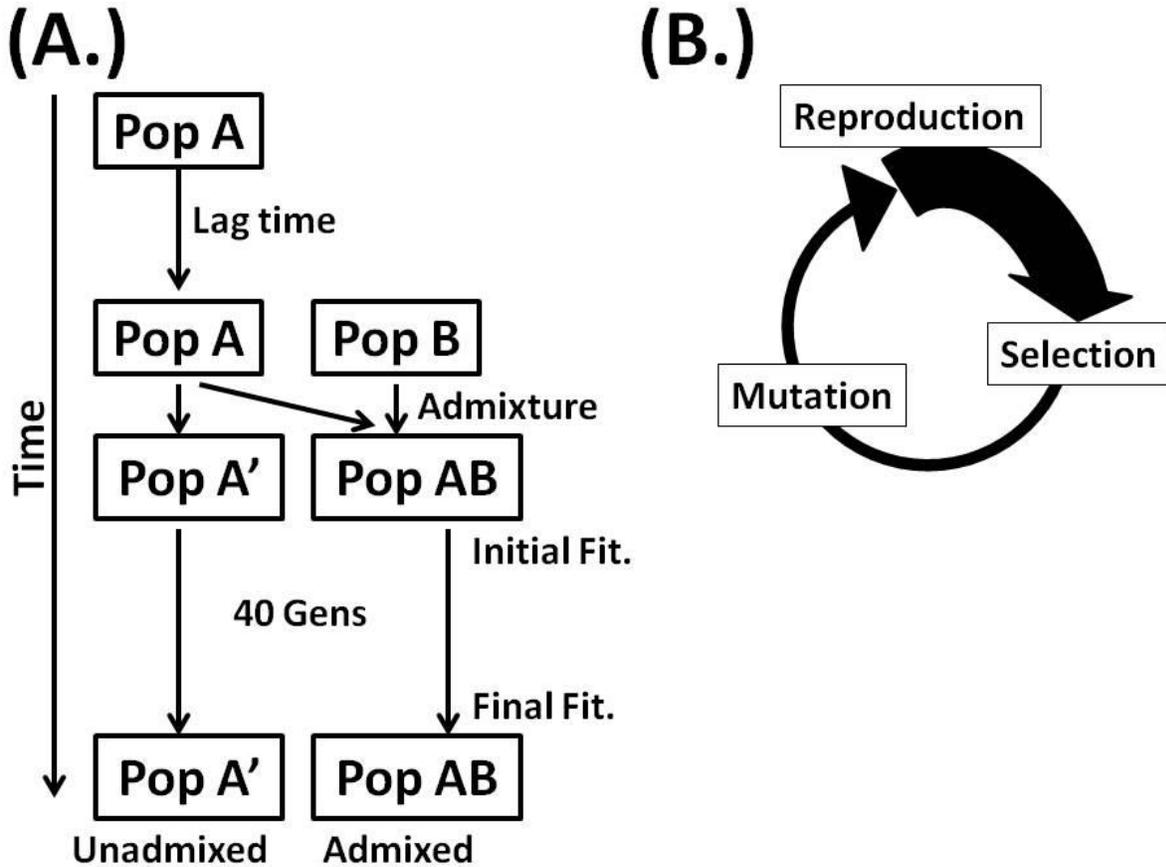


Figure 4-1. Diagram of processes that occur in the individual-based model. (A.) Each run of the simulation begins with the creation of a population A, followed by a lag time, the creation of a population B and admixture between A and B to produce AB, and finally 40 generations of evolution. Mean fitness measures are taken one generation after admixture (Initial Fit.) and at the end of the simulation (Final Fit.). (B.) Each single generation of the simulation includes reproduction (where the population size is tripled), selection (where population size is reduced back to its original size) and mutation, where each allele can change.

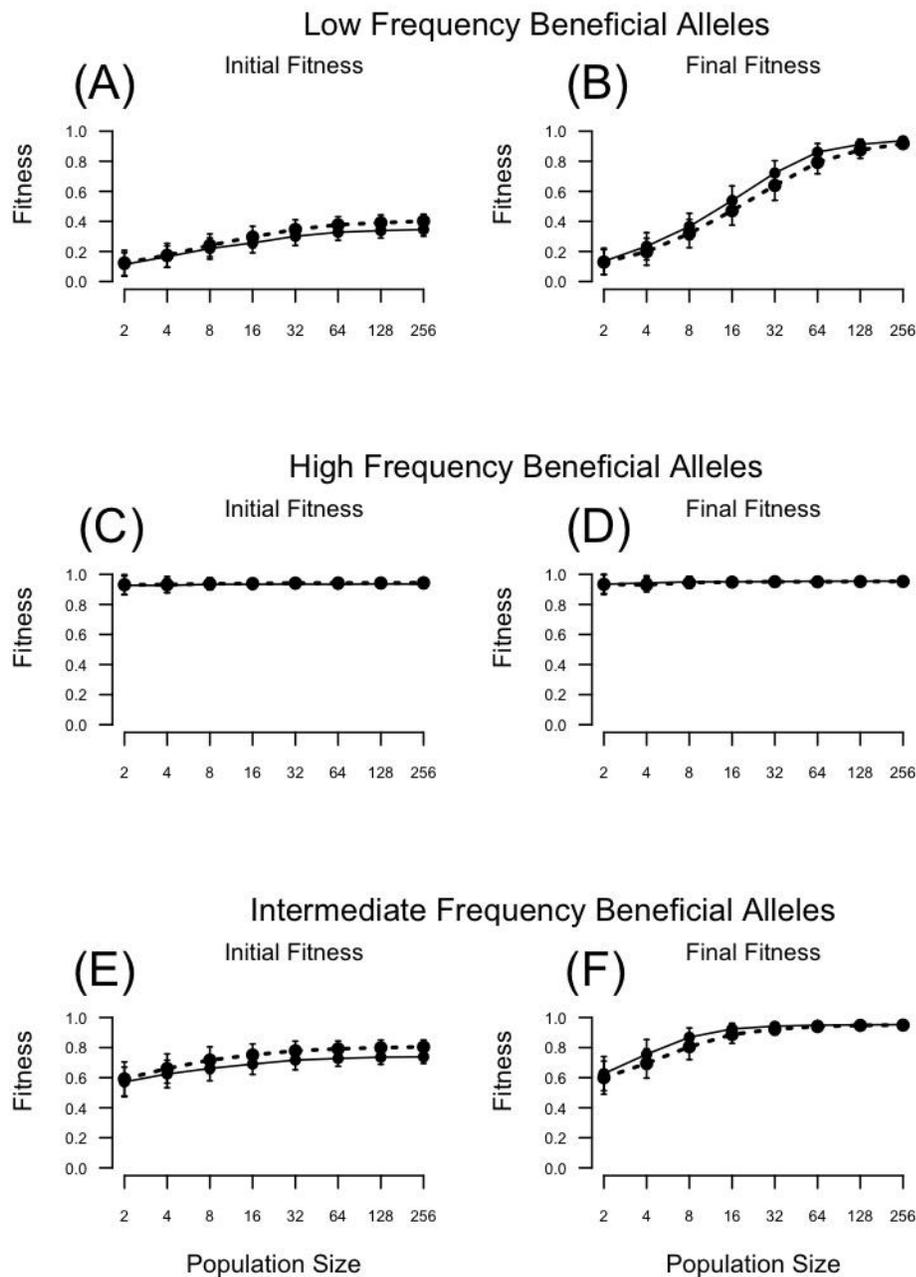


Figure 4-2. Initial and final mean fitness as a function of population size for initial populations having the beneficial allele rare, common or intermediate for additive alleles. Initial mean fitness was lower, while ultimate mean fitness was greater when beneficial alleles were rare and population size was not small. Lag time was 10 generations. Dashed lines represent non-admixed populations while solid lines represent admixed populations. Error bars represent one standard deviation.

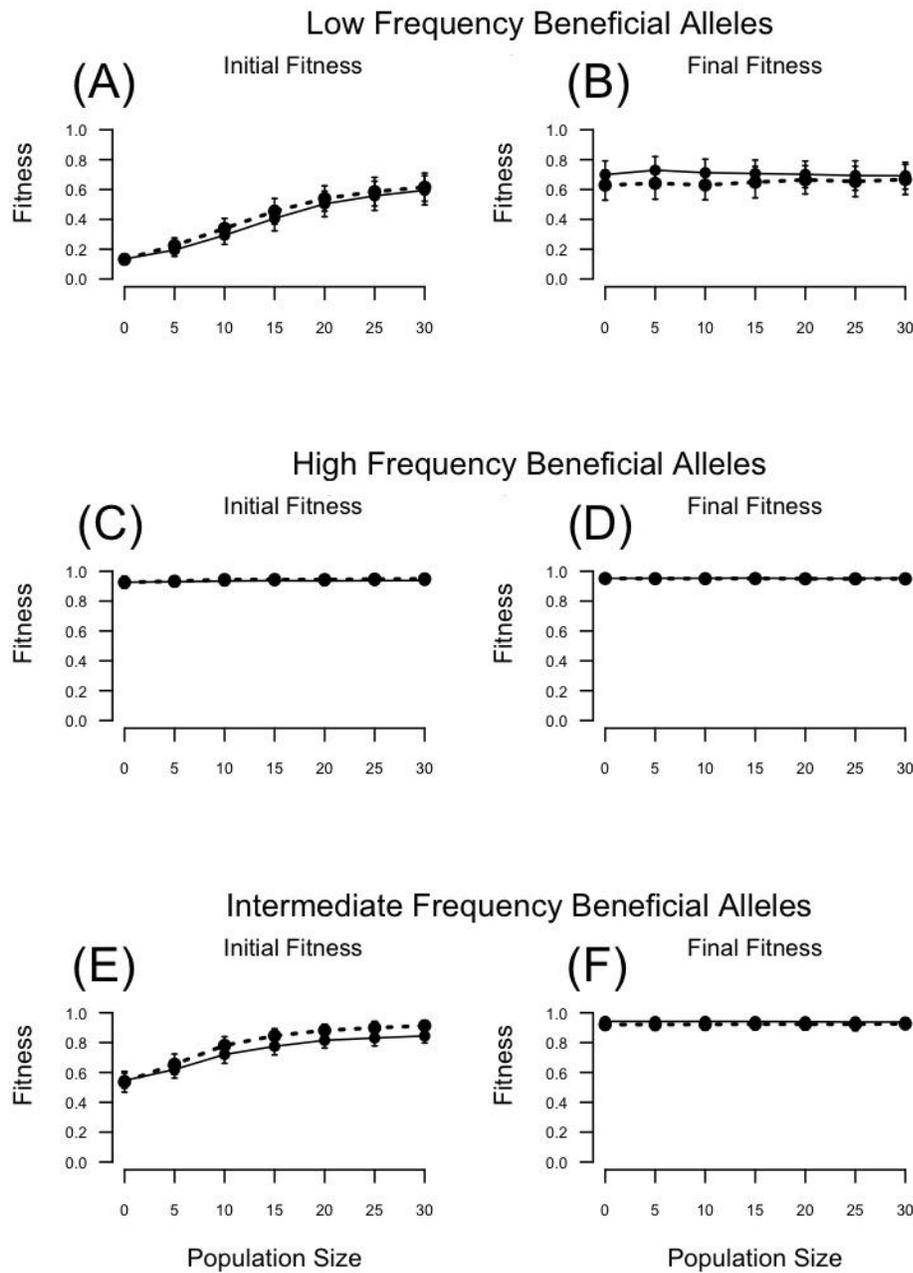


Figure 4-3. Initial and final mean fitness as a function of lag time for initial populations having the beneficial allele rare, common or intermediate for additive alleles. Initial mean fitness was lower, while ultimate mean fitness was greater, when beneficial alleles were rare and lag time was not too large. Population size was 32. Dashed lines represent un-admixed populations while solid lines represent admixed populations. Error bars represent one standard deviation.

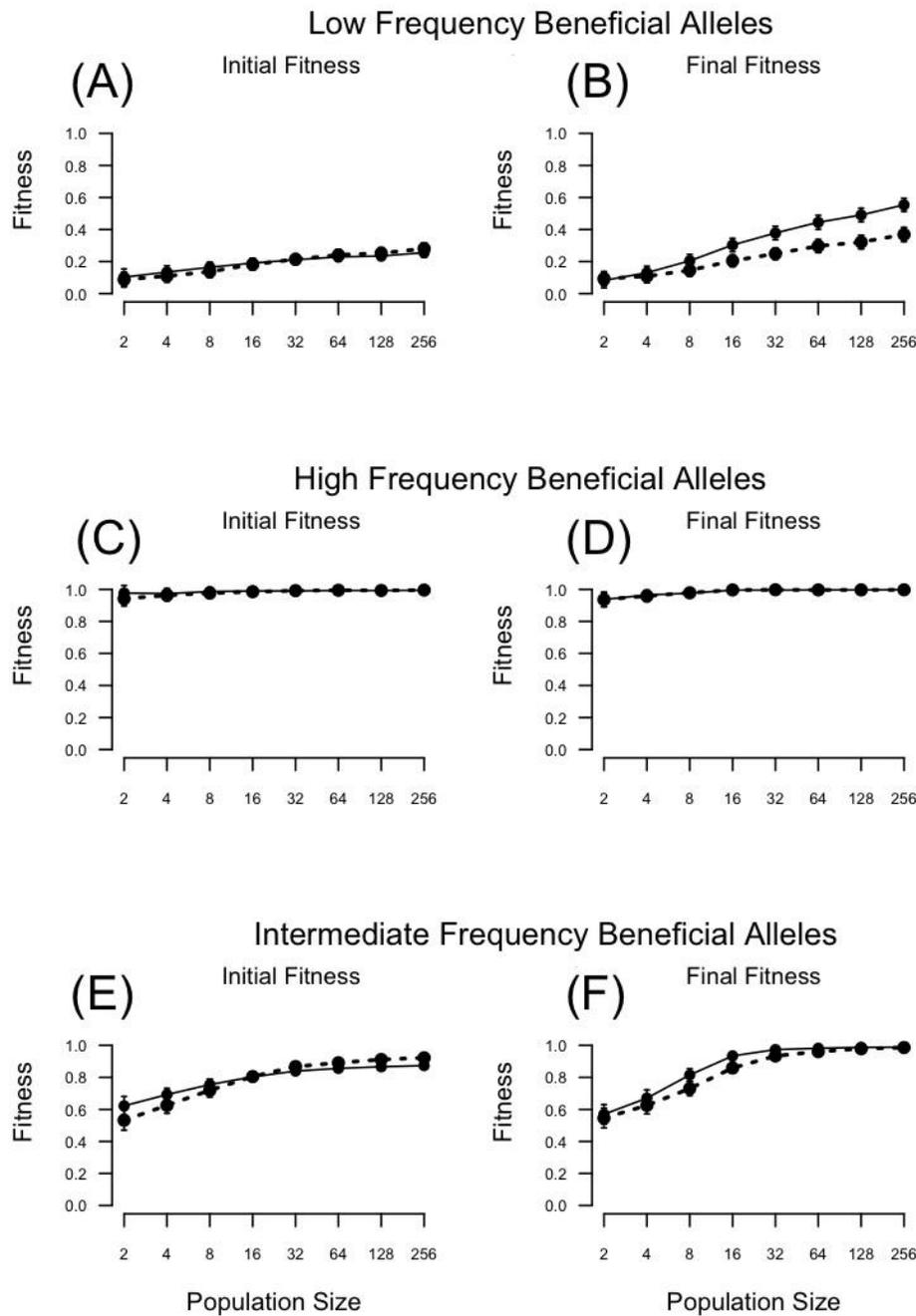


Figure 4-4. Initial and final mean fitness as a function of population size for initial populations having the deleterious allele rare, common or intermediate for deleterious homozygous recessive alleles. Initial mean fitness was lower, while ultimate mean fitness was greater, when deleterious recessive alleles were rare and population size was not small. Lag time was 10 generations. Dashed lines represent un-admixed populations while solid lines represent admixed populations. Error bars represent one standard deviation.

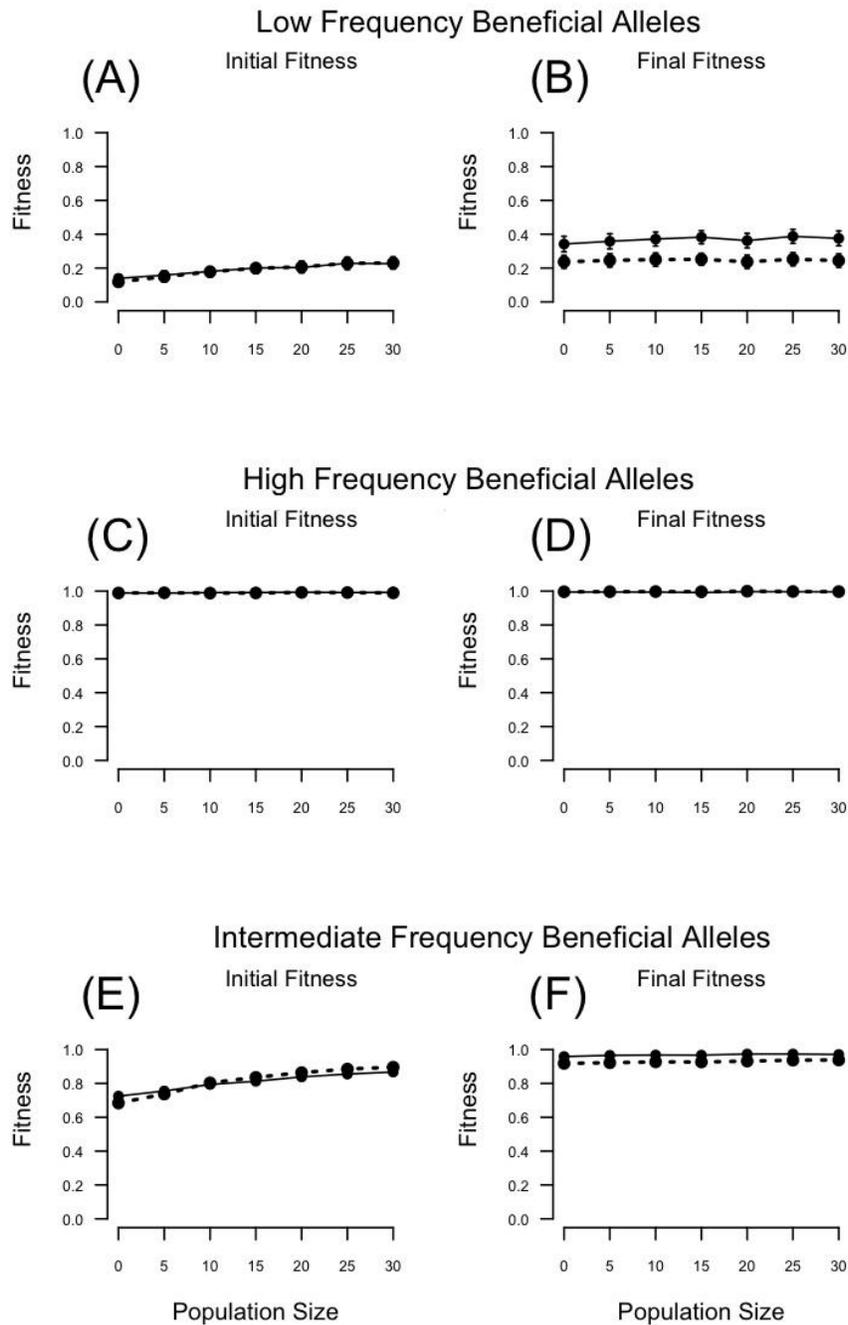


Figure 4-5. Initial and final mean fitness as a function of lag time for initial populations having the deleterious allele rare, common or intermediate for deleterious homozygous recessive alleles. Lag time was not important for deleterious recessive alleles, except for early evolution under the uniform distribution. When initial frequencies of the deleterious allele were high, admixture tended to decrease mean fitness immediately, but increased mean fitness after 40 generations. Population size was 32. Dashed lines represent un-admixed populations while solid lines represent admixed populations. Error bars represent one standard deviation.

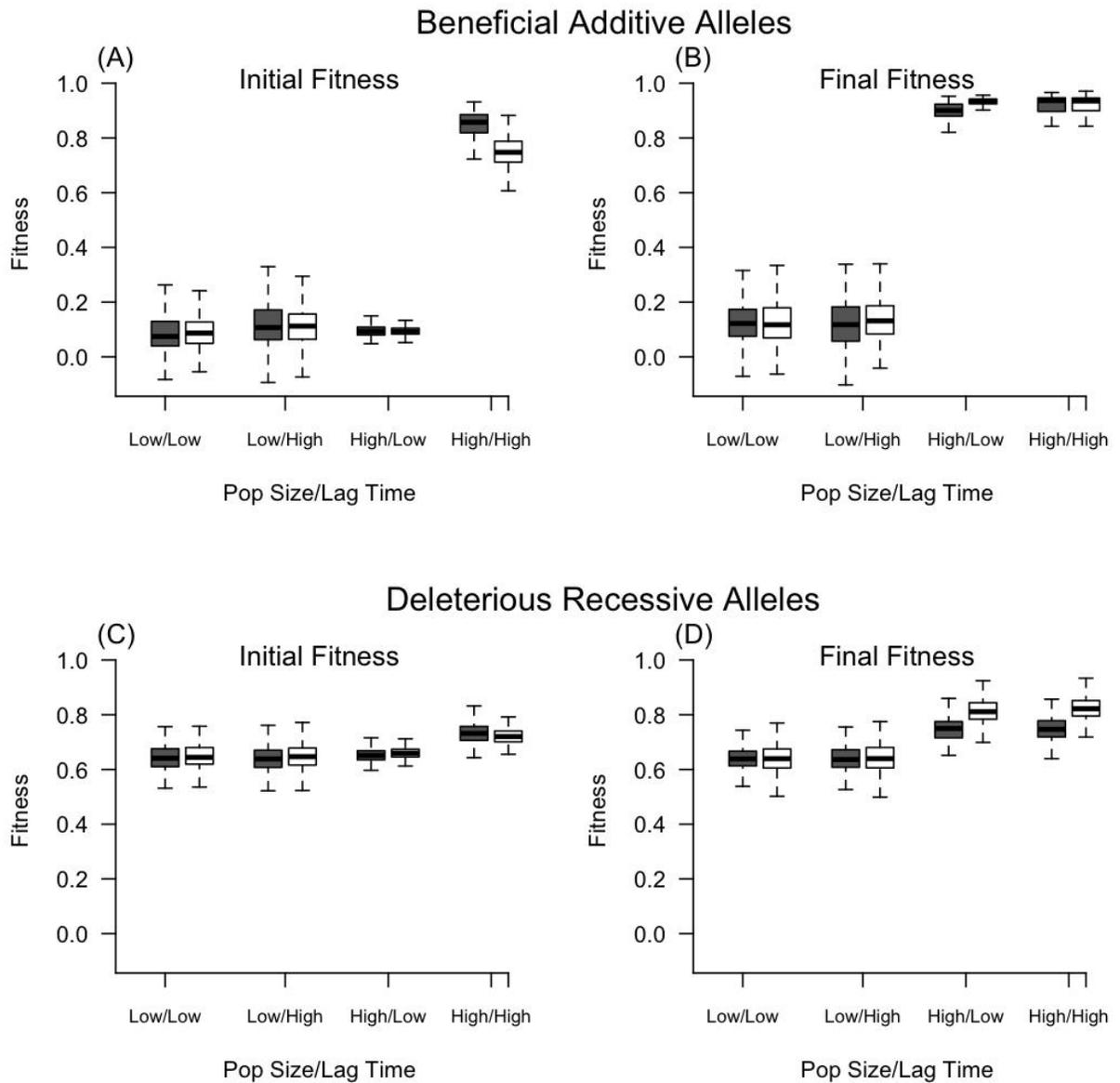


Figure 4-6. Results of model run at extreme values of the tested range. Initial values give the mean fitness of populations A' and AB a single generation after the lag time while final values give the mean fitness of populations A' and AB forty generations after the lag time. Initial allele frequencies were drawn from a beta distribution with low frequency for the beneficial-additive model and intermediate frequency for the deleterious recessive model allele in the deleterious recessive model.

CHAPTER 5 CONCLUSION

The effects of genetic admixture between divergent populations on their ability to establish, spread, and adapt to novel environments is of great importance to both applied conservation and evolutionary theory. Current population movements throughout the world often result in multiple populations of a species coming into contact and interbreeding. It is clear that population genetics plays an integral part in the establishment of some introduced populations, though to what extent and in what direction is still not clear. A better understanding of the genetics of admixture vis a vis adaptation will eventually allow us to make better conservation decisions, and will greatly add to our models of the evolution of populations. My dissertation has been an attempt to contribute to this understanding by providing empirical evidence from a model system, and simulated data from an individual-based model.

As shown by the *Drosophila* experiment described in Chapter 2, multiple introductions of species to a novel environment from different source populations followed by interbreeding does appear to result in both an immediate increase in population mean fitness and an increase in the rate of adaptation. This agrees with much of the evidence that has been gathered from genetic studies of invasive species in the field. The mechanism responsible for this fitness increase is difficult to determine currently, however it is believed to be a combination of increasing additive genetic variance and heterosis.

In Chapter 3, we attempted to demonstrate that combining adaptive traits in admixed populations could be particularly beneficial in a novel environment. These results show admixture of populations adapted to different environments generally

results in a population that has intermediate tolerance in each individual environment (lower than the pure adapted line but greater than the pure unadapted line). This essentially shifts the population from one of two specialist genotypes to a more generalist genotype. If admixture occurs in one of the ancestral environments, the pure ancestral line would be expected to have the greatest tolerance. If admixture occurs in a novel environment having components of both ancestral environments, the admixed population might have higher tolerance than either of the pure ancestral lines, even though the ancestors would outcompete the admixed line in their own ancestral environments. Additionally, the experiment also shows that there is a fitness increase one generation following admixture and this effect decreases in subsequent generations, likely due to a reduction in inbreeding depression that dampens as the amount of segregating loci lowers.

Much of the disagreement that has arisen about the effects of admixture on fitness was addressed in the stochastic individual-based simulation model that we present in Chapter 4. We tested the effects of population size as well as the time between the initial and subsequent introduction (lag time) on short-term and long-term fitness. These results reveal some interesting trends. One common finding was that if the lag time is long enough, the population will become partially adapted to the environment and then fitness immediately after an admixture event was temporarily lowered due to outbreeding depression or the swamping of locally adapted alleles. Despite this initial decline, however, final fitness of the admixed population often was higher than that of the unadmixed populations. Admixture appears to result in increased additive genetic variance and provides more beneficial alleles for selection to act on.

The initial frequency of these beneficial alleles was important. For additive alleles, the effect was strongest when the alleles were rare because different populations often had distinct beneficial alleles at different loci which could be combined in the admixed population. When alleles were deleterious and completely recessive, the effect of genetic admixture was strongest when the deleterious allele was common, because more homozygotes were present in the ancestral population and thus admixture resulted in an increase in heterozygosity and therefore a decrease in inbreeding depression. When deleterious alleles are rare and completely recessive, admixture is less important, because selection acts only against homozygotes, which are vanishingly rare in such cases. In very small populations, as expected, genetic drift tended to overpower selection. For very large population sizes, selection was often strong enough to drive both admixed and unadmixed populations to a very high fitness, resulting in an intermediate population size where the effect of admixture was strongest. A lag time between the first and second introduction was necessary to see the outbreeding depression effect, but the final fitness value was generally not affected by the time between introductions.

Summarizing all of our findings, it appears that genetic admixture can be either beneficial or detrimental to a population adapting to a new environment. Our results indicate that several factors will determine what the ultimate impact on population mean fitness is. The time scale of introductions seems to very important. It is common to observe a drop in mean fitness immediately following admixture between populations due to outbreeding depression, however an ultimate increase in mean fitness is generally seen as admixture increases genetic variance in the population, as was

observed in our first *Drosophila* experiment. How well either of the populations is adapted to the environment where admixture takes place is another important factor. If one population is native to or has had time to locally adapt to the environment, then a secondary introduction is more likely to result in outbreeding depression. When the environment is completely novel to both populations, admixture is more likely to increase mean fitness. This was clear when we measured adaptation to both to each single environment as well as estimated a total fitness measure by combining these measures in our second *Drosophila* experiment. One outcome of this is that longer lag times (time for local adaptation) often increase the effect of outbreeding depression, which was shown in our model. Population size is important because it determines the relative effects of selection and drift. At too low of sizes, drift dominates any change in allele frequencies and at too high of sizes both admixed and unadmixed adapt so quickly that admixture makes little difference. Finally, the frequency of alleles found in the populations before admixture strongly affects whether admixture is beneficial or detrimental, as seen in our model results.

A large amount of work still needs to be done in this field before a complete answer emerges. Currently, despite a good understanding of many of the parameters involved, determining how any given population will be affected by genetic admixture with another population is still very imprecise. One potential avenue that would be very beneficial in coming to a more predictive understanding would be to study in more detail the mechanisms that cause these fitness changes. Although theory says that admixture results in swamping local alleles, disrupting coadapted gene complexes, increasing additive genetic variance and decreasing inbreeding depression, it is difficult to

determine which of these are occurring and the relative impact of each process on total fitness. Genomic studies may be useful for determining mechanisms of evolution. Genome-wide analyses of heterozygosity or allelic diversity could potentially distinguish between heterosis and additive variance increase. Several methods of detecting selection could be used to determine specific genes that are beneficial in novel environments. Measures of expression differences in certain genes in novel environments could be used to determine the mechanisms of local adaptation, which could allow for testing of the local gene swamping hypothesis. The increasing availability of genome sequencing and expression measurement tools as well as annotated genomes will make this possible in a wide array of organisms where such studies would not have been previously achievable.

One of the major factors that this work does not address is how the amount of gene flow and a changing population size affects mean fitness of admixed populations. In both our empirical work and simulations we controlled the level of gene flow at a relatively high rate and kept population size constant. This high rate may seem unrealistic considering the initial population would often have time to begin expanding in the new environment before a secondary introduction would arrive. However it is believed that most population introductions do not grow very quickly and are in fact constrained by environmental stresses in the environment that they have not adapted to. Nonetheless, varying the amount of gene flow would likely reveal interesting patterns and would be a logical next step for this research. Allowing for expanding and contracting population sizes would better allow us to examine how demographics and genetic admixture interact with one another.

Our model, while a good starting point for figuring out the effect of different parameters, is far from complete. Additions of more parameters would make the model more likely to match the complexity of population introductions in the field and make thus increase utility. One change that would increase the similarity with actual invading populations would be to make populations sizes not constant but variable over time. Invading populations can often be expanding very rapidly and this likely affects how genetic admixture changes their fitness. Additionally, incorporating nonrandom mating patterns might make the model more applicable to a broader range of taxa in nature.

Finally, it should be noted that while this work modeled population introductions in nature and the effects of admixture on fitness, it does not explicitly test if these populations will be able to establish a stable population or spread in a novel environment. In both the empirical work and the simulation models, populations were not allowed to go extinct so that fitness could be measured and compared between populations. In a real world situation, there would be some threshold level of fitness below which the population would go extinct. If the effect of outbreeding depression following admixture is large enough then the population will not survive to be able to take advantage of the increased genetic diversity.

The findings of this study could be beneficial in making conservation decisions to help stem the tide of invasive species and their negative impacts. While genetic admixture can lower fitness over short time periods through outbreeding depression, as demonstrated in the agent-based model, this effect seems to reverse over longer time spans. The increased genetic diversity ultimately allows populations to have greater

fitness and adapt more efficiently. It would appear the avoiding genetic admixture between populations would be the most cautious course of action for land managers.

It is clear that genetic admixture can have both positive and negative effects on population fitness. These studies have demonstrated the value in using model organisms and computer simulation to clarify when each would be expected. As more empirical and theoretical studies are conducted, a more complete and predictive framework of how gene flow affects adaptation in new environments.

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BIOGRAPHICAL SKETCH

Frank Bouchard was born in Hollywood, FL in 1984. His interest in science and nature developed at an early age and motivated him to pursue it in college. Frank earned an Associate of Arts degree from Palm Beach Community College and then a Bachelor of Science degree in zoology from the University of Florida. He completed a wildlife biology internship at Pocosin Lakes National Wildlife Refuge in Columbia, NC before beginning graduate school. He was accepted into the inaugural class of University of Florida Genetics and Genomics graduate program in 2006 and received his Ph.D. in 2012.