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Using *Drosophila melanogaster* to test the effect of multiple introductions on the ability of a non-native population to adapt to novel environments

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ABSTRACT

Question: How do multiple introductions, versus a single introduction, of the same species, and the number of source populations of those introductions, affect the fitness of a population in a novel environment?

Hypothesis: Multiple introductions will increase fitness by introducing additional additive variance on which selection may act, by creating new combinations of alleles, or by reducing inbreeding depression. Multiple introductions from multiple source populations will further increase this effect.

Methods: Using *Drosophila melanogaster* in a controlled laboratory setting, genetically divergent lines were introduced to a novel environment of high ethanol content medium using either single or multiple introductions from a single source or multiple sources and then fitness was measured for 14 generations.

Conclusions: Multiple introductions, particularly from multiple sources, have higher fitness in the introduced environment than do single introductions or multiple introductions from a single source.

Keywords: adaptation, effective population size, inbreeding depression, invasive species, multiple introductions, recombination, transgressive segregation.

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 result in major economic costs

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and spread harmful diseases (Pimentel, 2002). Preventing such species establishing is a high priority for conservationists as well as several industries and governmental agencies. However, 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. Exotic populations are often established by 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 of the 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 and 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).

To persist, a novel environment must be within the ecological tolerance levels of the species, its '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 (Wiens and Graham, 2005). Although a species' ecological niche is often conserved and shaped in large part by its past evolutionary history, niches can evolve to allow a population to adapt to a novel environment.

Population genetic theory states that gene flow and genetic variance are the primary factors that determine the rate of local adaptation of a population. High rates 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 favoured locally (Ellstrand and Elam, 1993; Holt, 1996; Wiens and Donoghue, 2004). Thus the greatest potential for local adaptation should occur at low rates of gene flow (Holt and Gomulkiewicz, 1997).

However, substantial 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 their potential to adapt to the new environment (Holt and Gaines, 1992; Holt *et al.*, 2003; Weber, 1990). Finally, immigration increases population effective population size and heterozygosity, decreasing inbreeding depression (Newman and Tallmon, 2001). Invading populations are expected to have especially low levels of additive genetic variance and heterozygosity, having being established by a small number of founders, sometimes a single fertilized individual (Pascual *et al.*, 2001; Dlugosch and Parker, 2007), and this may have an important effect on fitness.

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 rate of gene flow and the potential for local adaptation are in the main determined by the number and magnitude of introduction events (Lenormand, 2002; Lockwood *et al.*, 2005). Multiple (but discrete) independent introductions of a non-indigenous species are believed to increase its chances of persisting in some cases (Sakai *et al.*, 2001).

Many recent molecular studies have shown that a surprisingly large number of successful invasions have been the result of multiple introductions (Collins *et al.*, 2002; Fonseca *et al.*, 2001; Maron *et al.*, 2004; Novak and Mack, 2001; Kolbe *et al.*, 2004; Durka *et al.*, 2005; Lavergne and Molofsky, 2006; Dlugosch and Parker, 2007; Facon *et al.*, 2008; Hufbauer and Sforza, 2008). Multiple introductions can make an introduced population more fit through transgressive segregation, the effect of combining additive alleles at different loci to produce a phenotype more extreme than that of either parental population (Rieseberg *et al.*, 1999; Stelkens and Seehausen, 2009). Transgressive segregation results from increased additive genetic variance in fitness-related traits, leading to an increased ability to respond to natural selection (Fisher, 1958). Multiple introductions can also permit recombination of traits that are beneficial in the new environment, allowing invasive populations to expand into environments that contain different combinations of selective pressures. Finally, multiple introductions can raise the 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 and Darling, 2007), is of particular concern to bottlenecked populations, such as introduced species (Newman and Tallmon, 2001).

Well-designed studies of the effects of multiple introductions have been conducted in the field (Facon *et al.*, 2008; Keller and Taylor, 2010), but such studies have several shortcomings. They are fundamentally limited because they are only able to examine successful population introductions. Furthermore, 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. *Drosophila 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.

METHODS

Fly stocks

Three lines were established, each 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 (Imhof *et al.*, 1998). Furthermore, 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 and 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 the present experiment. A fourth line used, the *yellow* line, was a laboratory mutant line that had a recessive yellow body colour mutation [*y* (gift of J.V. Fry)]. This was a laboratory stock that had been maintained at uncontrolled density for at least 20 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 yellow colour 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 laboratory at moderate, uncontrolled densities for approximately 25 generations prior to 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. 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 that experienced in the field (Gibson *et al.*, 1981). Ethanol was chosen because it is an environmental stress commonly encountered by fruit flies. 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 33 replicate populations of each of the four lines (with ten pairs of non-virgin flies) on the ethanol medium and 33 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 (Pascual *et al.*, 2007; Ficetola *et al.*, 2008; Ross and Shoemaker, 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 11 replicate populations per treatment (4 lines $\times$ 3 treatments $\times$ 11 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 5 days and then were removed from vials to maintain discrete generations.

We estimated fitness as the proportion of flies surviving from egg to adult. Fitness 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 10 × 35 mm 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 3–5 h, we removed the covers and took 30 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 14 days and adults emerging were counted daily. Although these fitness measures were not conducted at the same density as the selection regime, we think a negative correlation between the two densities is unlikely.

After generation 14, we switched half of the populations in treatment one from standard medium to ethanol-supplemented medium to determine the response to selection in the course of the experiment. We again measured egg-to-adult viability as well as mean development time 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 survival proportions were arcsine transformed before being analysed to better approximate normality of residuals. To analyse the survival data, we used the linear model

$$s = \mu + e_i + t_j + g_k + l_m + eg_{ik} + tg_{jk} + et_{ij} + etg_{ijk} + \varepsilon_{ijkmn} \quad (1)$$

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. In addition, 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 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 1). Non-significant interaction terms ($P > 0.05$) were sequentially dropped from the model, resulting in a reduced model:

$$s = \mu + e_i + t_j + g_k + eg_{ik} + l_{mij} + \varepsilon_{ijkn} \quad (2)$$

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

RESULTS

Fitness assay results are summarized in Fig. 1. We used a linear analysis ANOVA with population fitness 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 fitness, and the multiple introductions from multiple sources had greater fitness than multiple introductions from a single source (treatment $P = 0.0350$). As expected, environment and generation also had a significant effect on fitness ($P < 0.0001$ for both), which is consistent with the ethanol environment being novel and adaptation to the novel environment occurring over the course

Table 1. Results of ANOVA for the initial linear model, including all two-way interactions

Source	*F*	*P*
Environment	224.4410	<0.0001*
Generation	18.1242	<0.0001*
Treatment	3.8528	0.0446*
Environment × Generation	9.8704	0.0017*
Environment × Treatment	0.4227	0.6628
Generation × Treatment	0.2048	0.8148

Note: *F*-values are based on Type III sums of squares.

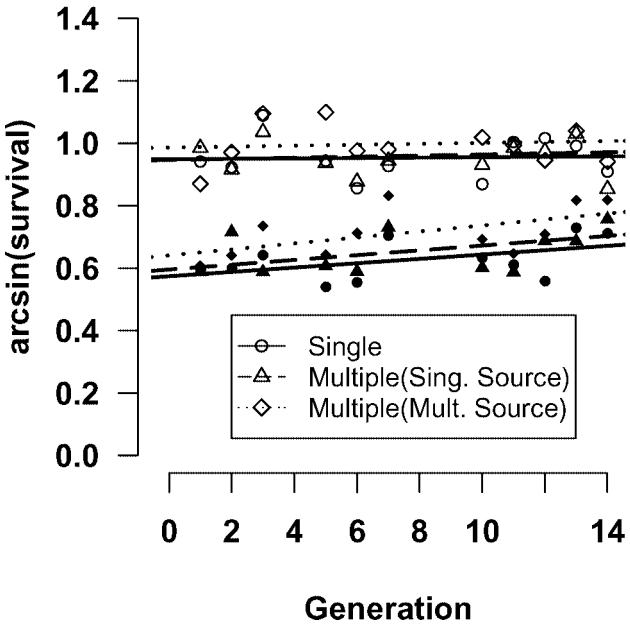

Fig. 1. Transformed values of proportion survival over 14 generations for each environment × treatment combination. The upper three lines with open symbols represent the standard environment, while the lower three lines with solid symbols represent the ethanol environment. Solid lines represent treatment 1 (single introduction), dashed lines treatment 2 (multiple introductions from the same source), and dotted lines treatment 3 (multiple introductions from multiple sources).

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 Fig. 2) were compared using several one-tailed Student's *t*-tests. Three of the four selected lines showed greater egg-to-adult survival or faster development time or both than their controls. The mutant *yellow* (*y*) line, which has been maintained in the laboratory for much longer than the other lines, showed the opposite trend for both measures after selection

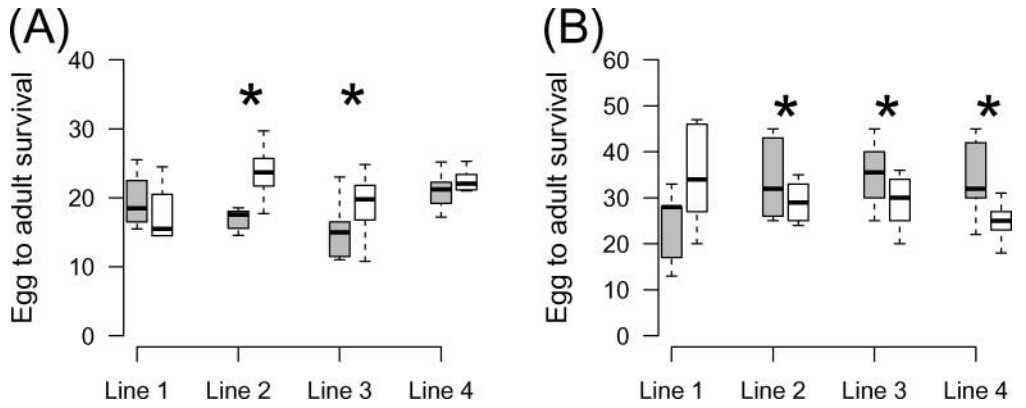

Fig. 2. Final fitness measures of both control and selection lines in the ethanol environment. Shaded boxes represent control populations and open boxes represent populations that had been in the ethanol environment for the course of the experiment. Samples were taken from the single introduction treatment. An asterisk denotes significant differences between control and selected lines. All but one of the lines (the *yellow* line) showed some evidence of significant adaptation to the ethanol environment during the experiment.

(lower survival and longer development time). When all four lines were combined and controls were compared with adapted lines, survival increased from 18.31 flies to 20.57 flies ($P = 0.0176$) and development time decreased from 32.13 h to 29.5 h ($P = 0.0159$).

DISCUSSION

The multiple introduction treatments showed higher fitness in the introduced environment as well as in the control environment (although 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 fitness in the multiple introduction treatments is increased additive variation for quantitative fitness related traits, such as egg-to-adult survival. While both multiple introduction treatments increased in fitness, the treatment with multiple sources (rather than from a single source) had a greater increase (and rate of increase) than would be expected.

Results of the final fitness assay 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. 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, although it is a possibility with lines that have been maintained in the laboratory for some time. Heterosis explains why the difference between treatments is noticeable 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 fitness 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 nature, 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. Although we used a relatively large migration rate, the short time between the primary and secondary introductions may have precluded adaptation to the novel environment, a prerequisite for gene swamping.

The generality of these results to situations with different introduction parameters (number of individuals per introduction, number of introductions, etc.) was not evaluated. 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 modelling 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 (Ingvarsson, 2001). These (re)introduced populations have often been bred in laboratories or zoos for many generations 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.

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