Population demographic history and evolutionary rescue: influence of a bottleneck event

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Demographic history and evolutionary rescue

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Abstract

Rapid environmental change presents a significant challenge to the persistence of natural populations. Rapid adaptation that restores positive growth rate, enabling populations in decline following severe environmental change to avoid extinction, is called evolutionary rescue. Numerous studies have shown evolutionary rescue can indeed prevent extinction. Here, we extend those results by considering the demographic history of populations. To evaluate how demographic history influences evolutionary rescue, we created 84 populations of red flour beetle, *Tribolium castaneum*, with three classes of demographic history: diverse populations that did not experience a bottleneck, and populations that experienced either an intermediate or a strong bottleneck. We subjected these populations to a new and challenging environment for six discrete generations and tracked extinction and population size. Populations that did not experience a bottleneck in their demographic history avoided extinction entirely, while more than 20% of populations that experienced an intermediate or strong bottleneck went extinct. Similarly, among the extant populations at the end of the experiment, adaptation increased fitness in the novel environment the most for populations that had not experienced a bottleneck in their history. Taken together, these results highlight the importance of considering the demographic history of populations to make useful and effective conservation decisions and management strategies for populations experiencing environmental change that pushes them towards extinction.

Keywords (max 6):
adaptation, genetic variation, inbreeding depression, heterozygosity, extinction, *Tribolium castaneum*

Introduction

In the Anthropocene, populations experience frequent and intense changes to their environments (Bellard *et al.*, 2012). If environmental change is severe enough, population growth may slow to the point that population size starts to decline. If dispersal capabilities are limited or suitable environments are no longer available (Schloss *et al.*, 2012), populations must adapt to challenging environments or risk extinction (Smith *et al.*, 1989; Holt, 1990). Adaptation that is rapid enough to reverse population decline due to a challenging environment and prevent an otherwise inevitable extinction is called evolutionary rescue (Gomulkiewicz & Holt, 1995; Carlson *et al.*, 2014). This process is characterized by a U-shaped curve in population size through time: initially, population size decreases due to maladaptation to the environment, then population size increases as adaptive alleles spread through the population (Gomulkiewicz & Holt, 1995; Orr & Unckless, 2014).

Theory and experiments with model organisms show that evolutionary rescue can avert extinction. Theory clearly shows that the probability of evolutionary rescue increases with genetic variation(Gomulkiewicz & Holt, 1995; Barrett & Schluter, 2008) and suggests that it decreases with mutational load and inbreeding depression (Barrett & Kohn, 1991; Ellstrand & Elam, 1993). Despite confirmation of these effects in experiments with model organisms (Bijlsma *et al.*, 2010; Agashe *et al.*, 2011; Lachapelle & Bell, 2012; Ramsayer *et al.*, 2013; Hufbauer *et al.*, 2015), evidence of evolutionary rescue in nature remains scarce, suggesting that the conditions that allow evolutionary rescue to occur may be limited (Carlson *et al.*, 2014). One condition that is particularly important to examine experimentally is the demographic history of populations. In the wild, populations are frequently subjected to various stressful events that can lead to a temporary reduction in size, such as hunting or

habitat destruction. These reductions in population size, called bottleneck events (Frankham *et al.*, 1999) can affect genetic variation, mutational load, and inbreeding levels in the populations that experience them (hereafter bottlenecked populations). Such bottleneck events, whether rare extreme events or repeated less-severe restrictions in population size, can reduce the future adaptive potential of populations (Nei *et al.*, 1975; Bouzat, 2010) and thus the probability of evolutionary rescue.

Bottlenecks influence the ability of populations to adapt to a future challenging environment in two main ways (O'Brien & Evermann, 1988; Frankham *et al.*, 1999; Reusch & Wood, 2007). First, standing genetic variation is lost by genetic drift during bottleneck events due to an increase of stochastic events associated with small population size (Hedrick, 2005). This reduction of genetic diversity decreases the evolutionary potential of populations since potential future adaptive alleles are lost (Futuyma, 1986). In general, adequate genome-wide genetic variation is important for the conservation of natural populations (Kardos *et al.*, 2021). In addition, when populations are small, selection is weak relative to genetic drift, thus reductions in genetic diversity can include increases in frequency and fixation of deleterious alleles (Lynch *et al.*, 1995), leading to populations with lower average fitness than populations that have not experienced a bottleneck. However, given the stochastic nature of which alleles go to fixation *versus* are lost, by chance, some beneficial alleles may be fixed as well, potentially leading some bottlenecked populations to have higher fitness (Bouzat, 2010). Thus, in addition to lower average fitness in bottlenecked populations, greater variation in fitness is expected across different bottlenecked populations compared to non-bottleneck populations.

The second main way that bottlenecks can influence the ability of populations to adapt is through inbreeding. After bottleneck events, inbreeding increases due to

increased homozygosity of alleles common by descent (Wright, 1977; Ralls *et al.*, 1979; Frankham *et al.*, 1999; Kirkpatrick & Jarne, 2000; Fox *et al.*, 2008; Charlesworth & Willis, 2009; Hedrick & Fredrickson, 2010). When homozygosity increases at loci with deleterious alleles, inbreeding leads to inbreeding depression, which can reduce fitness and increase the risk of extinction (Charlesworth & Charlesworth, 1987; Frankham, 2005a, 2005b; O'Grady *et al.*, 2006). Inbreeding in populations of intermediate size may also lead to purging of deleterious alleles (Crnokrak & Barrett, 2002; Glémin, 2007; Facon *et al.*, 2011). Thus, like for genetic drift, the inbreeding effects of bottlenecks can increase the variation in fitness among populations.

Despite the strong evidence for a positive effect of genetic variation (Hedrick, 2005) and a negative effect of inbreeding depression (Frankham *et al.*, 1999) on the rapid adaptation that is central to evolutionary rescue, it remains unclear what the consequences of past bottleneck events during the demographic history are for the future ability of a population to adapt to a new challenging environment. Given the role of bottlenecks in reducing genetic variation and increasing genetic load (Frankham *et al.*, 1999; Reusch & Wood, 2007), it is important to examine the effects of bottlenecks on evolutionary rescue experimentally. In a recent study, Klerks *et al.*, (2019) evaluated how population demographic history (bottlenecked or not) influenced the response of experimental populations to selection for increased heat tolerance. They found that bottlenecked populations had a 50% slower response to selection than normal populations. This suggests an important role for bottlenecks in the context of evolutionary rescue. Monitoring population dynamics was outside the scope of Klerks *et al.*, (2019), and so it is not clear if the U-shaped curve characteristic of evolutionary rescue occurred. Studying the population dynamics is,

however, necessary to understand the minimum population size that populations will reach without going extinct or how long it will take for populations of a given size to recover. In addition, the effects of bottleneck events in eukaryote populations are most commonly studied with two types of populations: bottlenecked or non-bottlenecked. The effect of bottleneck intensity has already been demonstrated in bacterial experimental evolution, where the adaptative pathways were different depending on whether the bottleneck was weak or intense (Vogwill *et al.*, 2016; Garoff *et al.*, 2020; Mahrt *et al.*, 2021). Furthermore, bottlenecks can be more or less intense in nature, which could influence subsequent population dynamics and response to selection.

Here we study how bottleneck events of two intensities influence the process of evolutionary rescue of populations in a severely challenging environment. We created populations of *Tribolium castaneum* that had experienced no bottleneck in their demographic history, an intermediate bottleneck, or a strong bottleneck, and monitored their dynamics in a challenging environment. We evaluate how the probability of extinction depends upon population demographic history as well as their estimated heterozygosity throughout the experiment, and whether surviving populations exhibit the U-shaped population trajectory characteristic of evolutionary rescue and how that depends upon population demographic history. Finally, we quantified adaptation to the new environment at the end of the experiment by measuring the growth rate of all rescued populations and evaluating the evolution of the rate of development, a life history trait that could be involved in adaptation (Berner *et al.*, 2004).

Methods

Model system

We used *T. castaneum*, the red flour beetle, to test the effects of demographic history on evolutionary rescue. *T. castaneum* populations were kept in 4 cm by 4 cm by 6 cm acrylic containers with 20 g of a standard medium (95% wheat flour and 5% brewer's yeast), which served as both habitat and a food source. We will henceforth refer to a container and medium together as a patch. Multiple patches were used to maintain each distinct laboratory population of *T. castaneum*, and single patches were used in growth assays. The life cycle of *T. castaneum* was constrained to mimic that of a seasonally breeding organism with non-overlapping generations and a discrete dispersal phase (Melbourne & Hastings, 2008), a life history found commonly among plants and animals. During laboratory maintenance, adult beetles were placed in groups of 50 individuals per patch with fresh standard medium for 24 hours to reproduce and start the next generation. At the end of the reproduction phase, adults were removed from the patches and the eggs were left to mature into adults for an additional 34 days for a total of 35 days per generation. During laboratory maintenance and the experiments, beetles were kept in incubators at 31°C with 54 ± 14% relative humidity. Several incubators were used, and patches were randomized among and within incubators once each week to prevent systematic effects of incubation conditions.

Creating populations with different demographic histories

A large diverse population was initiated from the multiple crosses of different stock strains ("GA1", "Lab-S", "Z2", "Z4" and "Estill" from USDA stock maintenance). These strains were originally all from samples from the US southwest, to minimize potential

genetic or cytoplasmic incompatibilities between strains. All possible pairwise crosses indicated no reduction in fitness between any given pair (data not shown). This diverse population was maintained and well mixed for approximately ten generations at a population size of 10,000 individuals, distributed in four temporal blocks with gene flow across the different temporal blocks. From this large and diverse population, we created 60 independent replicate populations experiencing bottlenecks in population size of two different intensities over two generations. During the first generation of the bottleneck event, all 60 populations were reduced to a size of two individuals. During the second generation of the bottleneck event, 24 populations were allowed to triple in size to six individuals to initiate the next generation (hereafter called “intermediate bottleneck”) and 36 populations were not allowed to grow, and experienced a second bottleneck of two individuals, to initiate the next generation (hereafter called “strong bottleneck”). Then these 60 populations were maintained for six generations in the laboratory to increase population size prior to starting the experiment (Figure 1). In contrast to the intermediate and strong bottlenecked populations, the diverse population was maintained as a single large population during those six generations to maintain high genetic diversity and was used to create independent replicate populations at the beginning of the experiment (hereafter called “diverse populations”). During the six generations, the population size of each bottlenecked population was recorded so that the individual population demographic history within each bottleneck category was known. This allowed us to calculate the heterozygosity remaining relative to the source population at the start of the experiment following Allendorf (1986). Heterozygosity is a good measure of genetic variation, and how it decreases during a bottleneck event is well understood

(Allendorf, 1986). The expected heterozygosity at generation *t* (He_t), is a function of expected heterozygosity and population size in generation *t*-1:

$$He_t = He_{t-1} - \left(\frac{1}{2N_{t-1}}\right) \qquad (1)$$

where He_{t-1} is the expected heterozygosity at the previous generation *t*-1 and N_{t-1} is the population size at the previous generation *t*-1. To calculate the proportion of expected heterozygosity remaining, expected heterozygosity of the source population at the time of the bottleneck is defined as 1, that is He_0= 1. By tracking population size each generation, we can calculate the proportion of expected heterozygosity remaining each subsequent generation. This also allows us to compare the strength of the bottlenecks imposed on our experimental populations with other bottleneck situations. For example, the intermediate bottleneck is comparable to a population remaining at a size of 50 individuals for about 37 generations, and the strong bottleneck is comparable to a population remaining at a size of 50 individuals for about 57 generations.

Evolutionary rescue experiment

To test the effect of demographic history on evolutionary rescue, we compared the probability of rescue of bottlenecked *versus* diverse populations. In total, we compared the evolution of 36 diverse populations, 24 intermediate bottleneck populations, and 36 strong bottleneck populations distributed over three temporal blocks. To start the experiment, 100 individuals from each population were housed in two patches connected via 2mm holes drilled in the sides. We challenged these experimental populations over six discrete generations with a stressful medium that consisted of 97% corn flour, 2.85% wheat flour, and 0.15% brewer's yeast, to represent a resource-limited environment similar to Hufbauer *et al.* (2015). Each

generation, the adults in each population were censused completely, then the individuals were put back equally in the two connected patches with fresh challenging media for the 5-week life cycle as described above. For each population, the proportion of expected heterozygosity was computed each generation (Eq. 1) until the end of the experiment or the extinction of the population. A population was considered extinct when the population size was 0 or 1 at census. At the end of the experiment, the growth rate of each population was measured as the number of offspring produced per 30 adults (replicated as much as possible depending on population size). To evaluate potential differences in development time among the populations that survived to the end of the experiment (hereafter, extant populations), the composition of different developmental stages was enumerated after 4 and 5 weeks of development by counting the number of larvae, pupae, and adults in each patch.

Due to a protozoan parasite outbreak in the lab colonies, 12 populations showing symptoms of contamination were excluded from the experiment (9, 1 and 2 populations for diverse-source, intermediate bottleneck, and strong bottleneck populations respectively). With these exclusions, 23 diverse-source populations, 23 intermediate bottleneck populations and 34 strong bottleneck populations were evaluated in the statistical analysis (Figure 1).

Statistical analysis

All statistical models were fitted in R version 3.4.2 (R Core Team, 2014). For all models, we estimated the significance of interactions and main effects by comparing a full model (i.e., with model terms of the same order) to a reduced model without the interaction or main effect of interest using a Likelihood Ratio Test (LRT).

To understand how the probability of extinction in challenging environments was influenced by demographic history, we analyzed the probability of extinction (binary outcome) as a binomial distribution. However, none of the diverse populations went extinct during the experiment. Thus, due to the separation problem caused by estimated extinction probabilities exactly equal to zero, the full model including this treatment could not be fitted (Albert & Anderson, 1984). To solve this issue, we used a method of penalized likelihood (Firth, 1993; Heinze & Schemper, 2002). Hence, we analyzed the probability of extinction using penalized logistic regression with Firth's correction using the package *logistf* (Heinze *et al.*, 2022). Explanatory variables in the model were demographic history (a categorical variable with three levels: "diverse", "intermediate bottleneck " and "strong bottleneck") and temporal block (a categorical variable with three levels), both as fixed effects. In addition, to test whether time to extinction was influenced by demographic history, for the populations that went extinct we analyzed the time to extinction (number of generations) using a generalized linear model with a Poisson distribution and log link. Explanatory variables in the model were the categorical demographic history and the temporal block, again as fixed effects. We alternatively performed these analyses using the expected heterozygosity (He_t) at the beginning of the experiment as a continuous measure of demographic history with comparable results (we present only the results using the model with the categorical variable).

A U-shaped curve in population size characterizes evolutionary rescue. To evaluate how the shape of rescue curves differ by demographic history, we focused on populations extant at the end of the experiment and analyzed size through time using a Generalized Additive Mixed Model (GAMM; Wood, 2017) with a negative binomial distribution to account for overdispersion using the package *mgcv* (Wood,

2022). Explanatory variables in the model were the categorical demographic history, generation (a continuous variable from 2 to 6) modeled as a 1-dimensional smooth effect, and the interaction between the two (thus allowing the smooth to vary by demographic history), along with temporal block (three levels) as fixed effects and population identity as a random effect. To model the shape of the rescue curve, the data started at generation 2, the peak in abundance before the decline.

In addition, to quantify the variability of responses and visualize whether outcomes of populations that experienced a bottleneck event are more variable than of populations that did not, we computed the coefficients of variation in population size across the three demographic histories across all generations for the extant populations. We used the package *DescTools* to compute coefficients of variation and their 95% confidence intervals (Signorell *et al.*, 2022). Considering the coefficient of variation instead of the variance allows us to compare variation while controlling for mean population size.

To test the influence of demographic history on adaptation, we evaluated growth rate under standard density and the distribution of developmental stages of extant populations. The growth rate (i.e., number of offspring divided by the number of parents) is commonly analyzed with a lognormal transformation. Due to the presence of 0 in our dataset, this direct transformation was not possible. To choose the best distribution to analyze our data, we compared the distribution of residuals from the growth rate analysis via an alternative lognormal transformation (log transformation of growth rate+1), a square root transformation, and without transformation. This preliminary analysis showed that analyzing the growth rate without transformation was the best option. Hence growth rate was analyzed using a linear model with a normal distribution. The development rate was evaluated by

comparing the presence of the different life stages (i.e. larva, pupa, or adult) weighted by the number in each box, using a cumulative linkage model for ordinal data to consider the order of appearance of each stage (larva, then pupa, then adult), using the *ordinal* package (Christensen, 2015). For the growth rate model and for the development rate model, explanatory variables in the model were the categorical demographic history and temporal blocks as fixed effects, with population identity included as a random effect to account for replication within populations. In addition, the observation time (4 weeks or 5 weeks) was also included as a fixed effect in the developmental rate model, as well as the replicate identity as a random effect. We alternatively performed these analyses using the expected heterozygosity at the end of the experiment as a continuous measure of demographic history with comparable results (we present only the results using the model with the categorical variable for the development stage analysis due to the absence of effect, see results).

Results

Extinction and heterozygosity

There was a significant effect of demographic history on the probability of extinction (LRT: χ^2_2 = 9.39, P < 0.001). Populations that had a history of a bottleneck experienced more extinctions in the challenging environment than diverse populations (22% and 24% of intermediate bottleneck and strong bottleneck populations went extinct, Fig 2). The absence of bottleneck events during the previous demographic history prevented extinction entirely (Fig. 2, left panel; 0% extinction among diverse populations; estimate of the probability of extinction = 0.004, 95% CI [0.001, 0.05]). Considering only the demographic histories that

experienced extinctions, the probability of extinction was not significantly impacted by bottleneck intensity during demography history (estimate of the probability of extinction for intermediate bottlenecked populations: 0.06, [0.01; 0.27] and for strong bottleneck populations: 0.06, [0.01; 0.28]). Among the populations that went extinct, the number of generations to extinction were similar regardless of demographic history, with largely overlapping confidence intervals (LRT: χ_1^2 = 0.21, *P* = 0.650; estimate of the time of extinction for intermediate bottlenecked populations: 4.8 generations, 95% CI [3.1; 7.0] and for strong bottleneck populations: 5.0 generations, [3.6; 6.7] using the model including the demography history effect). For populations that went extinct, there was not necessarily a gradual decrease in loss of expected heterozygosity that led up to extinction (Fig. S1). Instead, heterozygosity mostly declined markedly one generation before extinction (red-barred circles in Fig. S1), reflecting the sharp population declines.

Population size dynamics

Following introduction into the challenging novel environment, population sizes initially increased, due to the maternal effects from the high-quality environment the populations were in just prior to the experiment being initiated, and then dropped dramatically (Fig. 2). In extant populations, analysis of population size over time reveals a U-shaped demographic curve indicative of evolutionary rescue, graphed in Figure 3 with the predictions from the best-fit GAMM model where the shapes are the same regardless the demographic history reflecting the most parsimonious model (Figure 3A) and with the predictions from the full GAMM model allowing different shapes depending on the demographic history, best representing the experimental design (Figure 3B). These dynamics of rescue were similar regardless of demographic history (LRT on the interaction between demographic history and

generation allowing different smooths: χ^2_6 = 3.0, *P* = 0.42, meaning a lack of evidence that the smooth are different for each demographic history; this effect is present in model represented in Fig. 3A and is absent in the model represented in Fig. 3B). Although the overall U-shape was apparent for all treatments, the model estimate of population size was larger for the diverse population treatment than for the intermediate-bottleneck and strong-bottleneck treatments (demographic history effect: χ^2_2 = 15.43, *P* < 0.001; Fig. 3A confidence interval for the diverse populations never overlaps intermediate and strong bottleneck populations). The predicted mean population size of the intermediate-bottleneck populations was slightly higher than for strong-bottleneck populations, but confidence intervals overlapped for all generations (Fig. 3A). By the last generation, the mean population size of the extant populations was greater than the initial population size only for the diverse populations (confidence interval not including 100 for the diverse demography history; Figs. 2, 3A). Indeed, by the last generation, the diverse populations had reached a mean population size of 127.9 (95% CI [107.3, 152.4]) individuals, while the intermediate bottleneck and strong bottleneck populations had reached means of 86.8 [71.3, 105.7] individuals and 80.4 [67.3, 96.0] individuals respectively, according to the predictions from the best-fit GAMM model (lines in Fig. 3A). These predictions remain similar when using the full model that best represents the experimental design (population size at the last generation for diverse populations: 143.5 [116.3, 177.2], for intermediate bottleneck: 74.6 [58.0, 96.0] and for strong bottleneck: 76.8 [61.6, 95.6]; Fig 3B). The predicted mean of the extant diverse populations was always either above or overlapping the initial size (minimum of 96.0 individuals at 4.7 generations but with a confidence interval including 100 individuals). This was not the case for the bottlenecked populations where the population size fell to a mean of

65.2 individuals and 60.3 individuals for the intermediate bottleneck and strong bottleneck respectively (confidence interval not including 100 for generations 4 and 5 in Fig. 3A). These predictions remain similar when using the full model that best represents the experimental design (minimum of the population size for diverse populations: 99.8 [84.7, 117.6], for intermediate bottleneck: 62.0 [50.7, 75.8] and for strong bottleneck: 58.4 [69.5, 49.0]; Fig 3B).

Coefficients of variation for population size across the three demographic histories for almost all generations had overlapping confidence intervals (Fig 4). For most generations, diverse populations had the lowest coefficient of variation, and at generation 6, population sizes of the extant diverse populations were less variable than the extant intermediate bottleneck and strong bottleneck populations ($CV_{diverse}$ = 0.29 [0.22, 0.41]; $CV_{intermediate}$ = 0.59 [0.42, 1.07], CV_{strong} = 0.51 [0.38, 0.77]; Fig. 4). These results remained unchanged even when considering all populations, including the populations that went extinct as having a population size of zero (results not shown).

Growth rate and development time

Most of the extant populations for the three demographic histories had, on average at the end of the experiment, a growth rate higher than 1, which means that more descendants were produced than there were parents (Fig. 5). At the end of the experiment, the growth rate was significantly different depending on the demographic history (LRT for difference among histories: χ^2_2 = 15.64, P < 0.001). The growth rate was higher for the diverse populations (estimate: 2.57, 95% CI [2.20, 2.94]), than for bottlenecked populations (estimate: 1.70 [1.26, 2.13] for intermediate and 1.87 [1.50, 2.23] for strong bottleneck populations). Overall, the

growth rate increased significantly with the heterozygosity of the populations (slope estimate: 1.76 [0.85, 2.68]; LRT: χ_1^2 = 12.62, *P* < 0.001).

The demographic history of rescued populations, had little to no influence on developmental timing. The proportions of larvae, pupae, and adults were similar among the three demographic histories (Fig. S2) and there was insufficient evidence for a difference (LRT: χ_2^2 = 0.13, *P* = 0.94). These proportions varied significantly only with respect to timestep, i.e., between 4 weeks and 5 weeks (LRT: χ_1^2 = 2035.1, *P* < 0.001).

Discussion

In this study, we evaluated how the demographic history of populations influences the probability of extinction and adaptation to a challenging environment. We found that populations that experienced a bottleneck event in their demographic history were more likely to go extinct, and, if they survived, were less well adapted to the challenging environment than populations that had not experienced bottleneck events.

Extinction associated with loss of heterozygosity for bottlenecked populations

While all diverse populations persisted throughout the six generations of the experiment, multiple bottlenecked populations went extinct. These extinctions often occurred in the generation following a strong decrease of the heterozygosity of the population. However, our experimental design did not allow us to dissociate the effect of losing heterozygosity from the effect of drop in population size. Nonetheless, extinction only occurred in populations that underwent a bottleneck event, which implies that decreases in heterozygosity (long- or short-term) can strongly affect extinction.

Except for one population, none of the populations that experienced an initial drastic drop in population size, decreasing their estimated heterozygosity below 0.45, were able to avoid extinction. This may be due to an extinction vortex, a process in which small populations remain small due to a reinforcing negative feedback loop that makes them vulnerable to extinction due to demographic stochasticity (Gilpin & Soulé, 1986). The diverse populations did not decline to as small a size as the bottlenecked populations, and perhaps this enabled them to avoid falling into an extinction vortex. In the future, it would be interesting to know if diverse populations that decline more sharply, due to stochasticity or experimental manipulation, can escape more easily via rapid adaptation than populations that have experienced bottleneck events.

Dynamics of rescue for extant populations

All treatments exhibited the U-shaped poplation size trajectory characteristic of evolutionary rescue, when considering extant populations. The group of diverse populations showed a higher level of adaptation, quantified by population growth rate, than the bottleneck populations. These results confirm that rapid adaptation is compromised following a bottleneck event (Bouzat, 2010). Our findings are consistent with those of Klerks *et al.*, (2019) who showed that bottleneck populations had a 50% slower response to selection for an increased heat tolerance than normal populations. Further investigation should consider whether this difference is due to a reduction of genetic variation in bottlenecked populations or an increase of inbreeding depression. This information would facilitate efficient management of populations where genetically variable and unrelated individuals are often not available. For instance, it would increase the probability of success of a translocation effort by targeting the individuals to be introduced (Ralls *et al.*, 2020). If the effects of

bottlenecks are primarily driven by a lack of genetic variation, individuals evolving in different environments, even if they have a recent common ancestor, should be considered for introductions to natural populations, to maximize the probability of having a large pool of different alleles. However, if the effects of bottlenecks are primarily driven by inbreeding depression, we suggest that unrelated individuals should be introduced, even if they are all adapted to the same environment with all the adaptive alleles to their environment potentially fixed and thus lacking genetic variation in those traits.

Similar patterns of evolutionary rescue for intermediate and strong bottleneck populations.

The evolutionary responses in terms of extinction, rescue, adaptive dynamics, and traits involved were similar for populations that experienced intermediate bottlenecks and populations that experienced strong bottlenecks. Three alternative hypotheses can explain the general congruence between these treatments. First, the difference in expected heterozygosity between intermediate and strong bottleneck events at the start of the experiment was smaller than that between bottlenecked and diverse populations (see Figure S1; averages at the start of the experiment: $He_{diverse} = 0.99$, $He_{intermediate} = 0.68$, $He_{strong} = 0.54$). Both types of bottleneck reduced heterozygosity substantially, but still some heterozygosity remained. It may be that in this range of expected remaining heterozygosity, evolutionary responses to the environment are similar. Second, populations that experienced a strong bottleneck event may have purged deleterious alleles (Kirkpatrick & Jarne, 2000), reducing the negative fitness effects of the bottleneck and making phenotypic outcomes more similar to the intermediate bottleneck. The process of purging has been demonstrated in natural populations. For instance, a recent analysis of the genomes of wild populations of

Alpine ibex that had undergone severe bottlenecks revealed a purge of highly deleterious alleles (Grossen *et al.*, 2020). In our study, the level of adaptation to the challenging environment may be comparable in intermediate and strong bottlenecks, despite differences in heterozygosity, due to potential purging of deleterious alleles in strong bottlenecks. Third, even if the phenotypic patterns seem similar, the adaptive pathways used by these two types of populations may be different. For instance, Vogwill *et al.*, (2016) found that antibiotic resistance evolved through highly beneficial mutations for bacterial populations that experienced a strong bottleneck event, whereas it evolved through a greater diversity of genetic mechanisms in populations that experienced an intermediate bottleneck event. Differences in the adaptive pathways may have consequences on the future evolutionary potential of these populations.

Variance of evolutionary trajectories may depend on the demographic history of populations.

Evolutionary responses were more variable in strong and intermediate bottlenecked populations than in diverse populations but the confidence intervals overlapped throughout the experiment, except for the last generation. Theoretical expectations predict higher variance for bottlenecked populations due to sampling of the genetic composition of each replicate population during a bottleneck event (Bouzat, 2010). The lack of support for differing coefficients of variation at the beginning of the experiments might be due to the fact that all populations were introduced into a new environment and experience it in a similar way. In addition, contrary to theoretical predictions and experimental results (Vogwill *et al.*, 2016; but see Windels *et al.*, 2021), we did not observe significantly higher coefficients of variation for strong

bottlenecks than for intermediate bottlenecks (see above for hypothetical explanations).

Life history traits involved in the adaptation process

To better understand the different levels of adaptation between treatments, we examined the proportions of individuals in different life stages at two time points at the end of the experiment, to search for potential differences in development time. We found that these proportions were on average very similar among demographic histories. Life history traits other than development time may thus be responsible for adaptation to the challenging environment. For instance, Agashe *et al.*, (2011) demonstrated an increase of fecundity and egg survival, as well as a higher rate of egg cannibalism, associated with adaptation to a new corn environment for populations of *Tribolium castaneum*.

Conclusion and perspectives

Our findings have implications for conservation in our changing world. Managers often have been concerned with maintaining adaptation to local environments of the populations they manage (Dalrymple *et al.*, 2021). In the Anthropocene, where environments are changing extremely rapidly, a focus on supporting sufficient genetic variation for ongoing adaptation to shifting conditions is important. The reduced ability of bottlenecked populations in this study to adapt to altered conditions, however, suggests that more active management may be needed for populations that have experienced bottlenecks. While genetic rescue (i.e., process of alleviating inbreeding depression and restoring genetic diversity of inbred populations through gene flow, Ralls *et al.*, 2020) involves additional avenues for evolutionary rescue that were not addressed in this study, our work further supports

the substantial literature on this topic where immigrants are introduced to small populations largely to reduce inbreeding depression but also to facilitate adaptation.

Data Archiving Statement

Data for this study are available at: to be completed after manuscript is accepted for publication. The data and R scripts for our analyses are available at: https://github.com/olazlaure/AnalysisEvolRescueExp

Author contributions

Conceptualization and funding acquisition: L.O. and R.A.H.;

Data acquisition: B.L., L.O. and S.D.;

Statistical analyses and writing: L.O. with inputs of B.L., S.D., L.F.D, B.A.M. and R.A.H.

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Figures

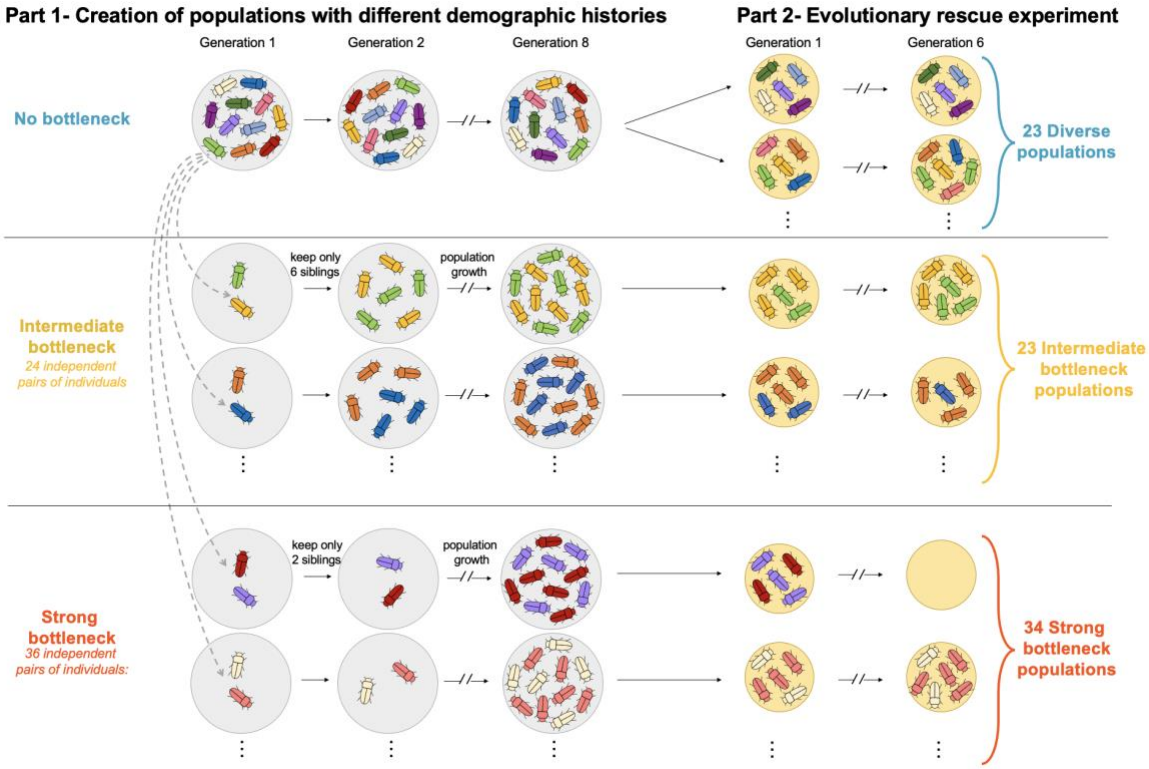

Figure 1. Experimental design

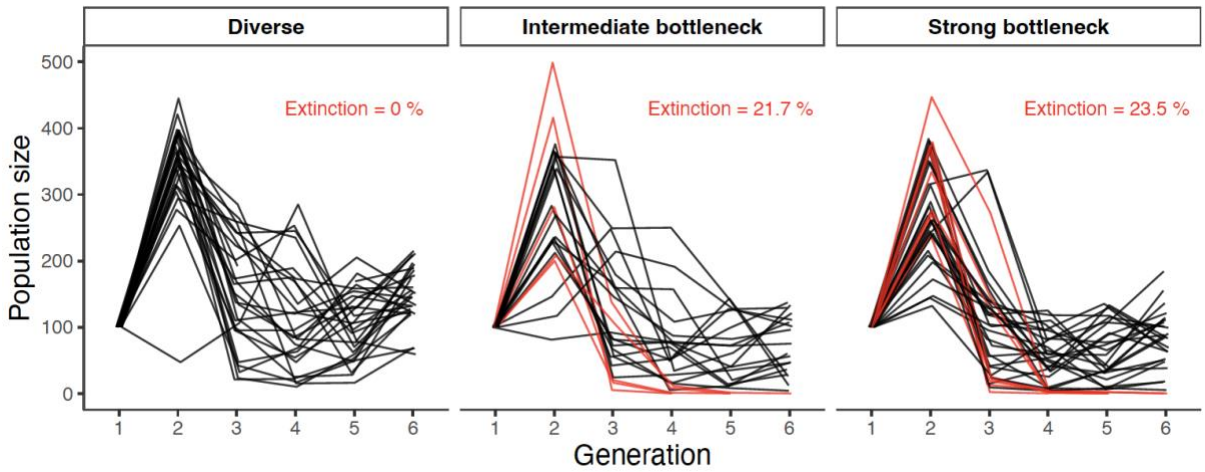

Figure 2. Population size of diverse, intermediate bottleneck, and strong bottleneck populations through time. *Red lines indicate populations that went extinct.*

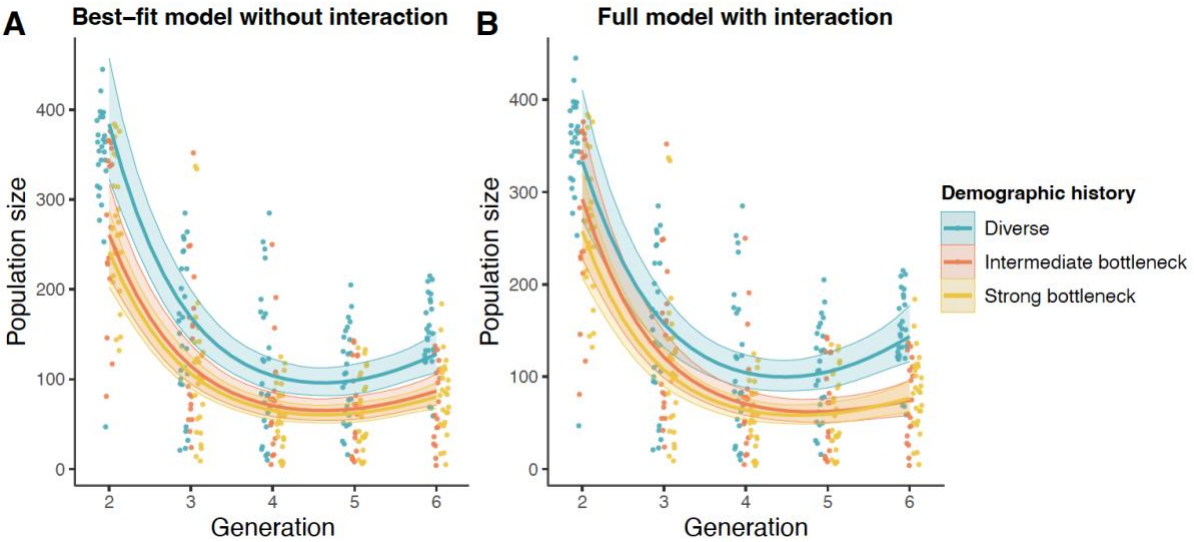

Figure 3. Population size of extant diverse populations (blue), intermediate bottleneck populations (red) and strong bottleneck populations (yellow) through time predicted (A) by the best-fit GAMM model or (B) by the full GAMM model including the interaction between time and demographic history. *Each population is represented by a small dot. The lines represent the model estimate of population size for the three demographic history levels predicted by the GAMM model, from which generation 1 and the extinct populations data were excluded, and considering an average over the three temporal blocks for this representation. The best-fit GAMM model does not include an interaction between the demographic history and the time, which means that the shape of the predictions cannot differ by demographic history. The full GAMM model does include an interaction between demographic history and time, which means that the shape of the curve can differ by demographic history.The shaded and colored area around each line represents the 95% confidence interval. Points in different treatments are staggered slightly and jittered on the generation axis to reduce overlap.*

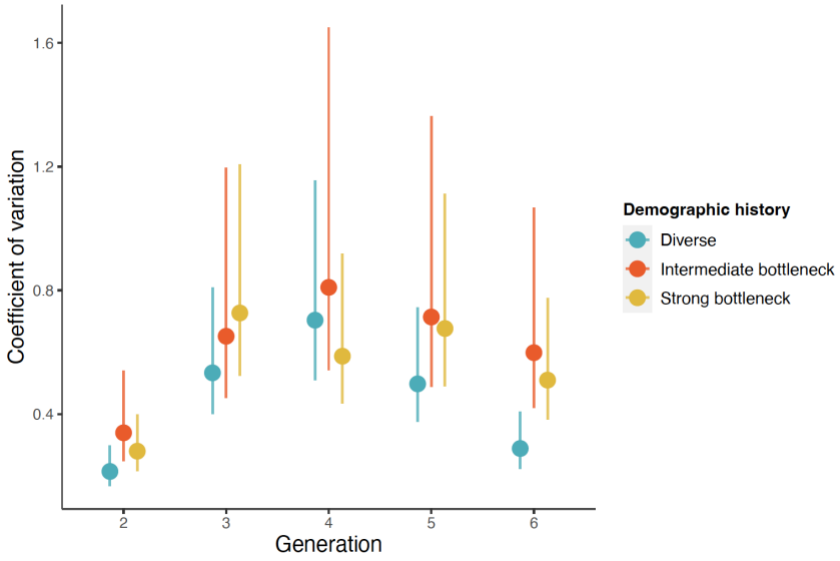

Figure 4. Coefficient of variation of population size through time. *The coefficient of variation for each demographic history is represented by a point, with the 95% confidence interval represented by an error bar. Extinct populations were excluded but results were similar when they are considered. Points in different treatments are staggered slightly on the generation axis to reduce overlap.*

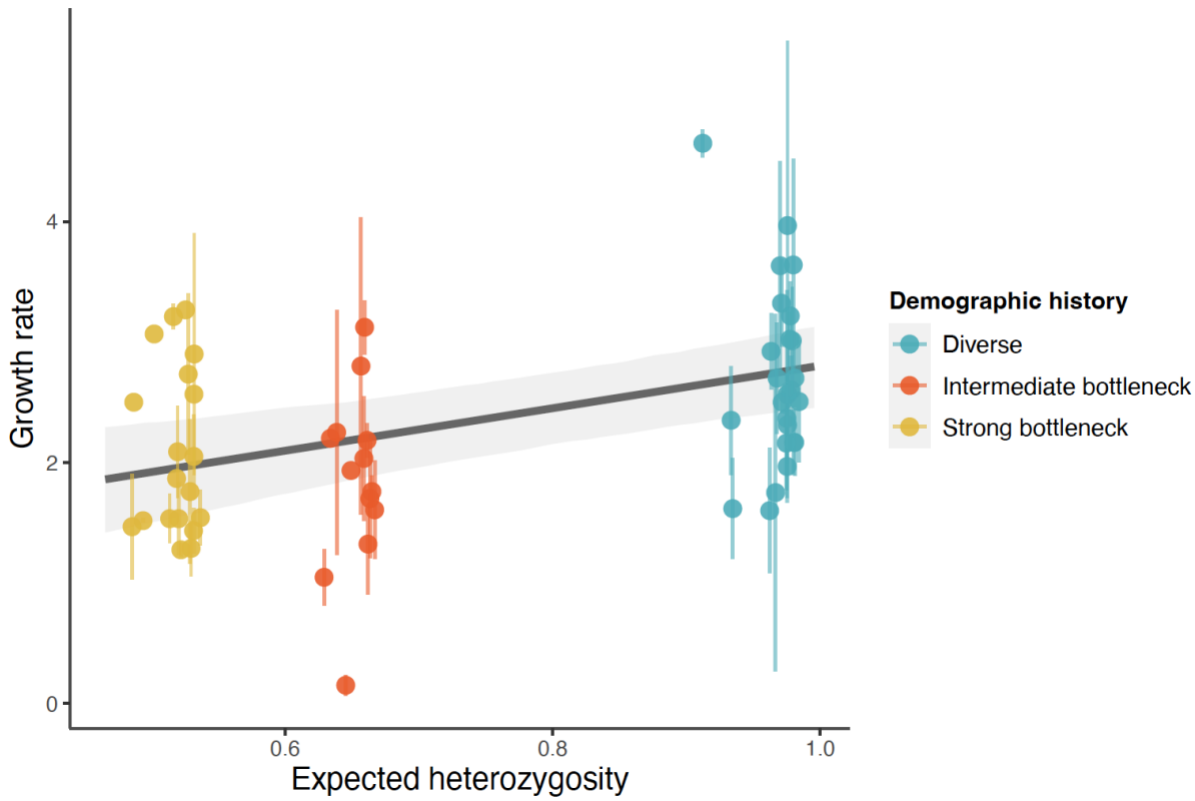

Figure 5. Growth rate assays for each extant population after the rescue experiment depending on its final heterozygosity. *The mean growth rate across assays of each population is represented by a point, with the standard error represented by an error bar. The points without error bars are from populations where there was only one replicate assay to estimate the mean. The solid gray line is the estimated mean relationship from the linear model. The shaded area around the line represents the 95 percent confidence interval.*