Density dependence impedes evolutionary rescue

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Abstract (200 words)

Evolutionary rescue is adaptation that enables populations to persist in the face of severe environmental change. It is well studied in conditions of density independence, but the effects of density on the probability of rescue are less well understood, including competition and other types of negative density dependence. The few theoretical studies of evolutionary rescue that address density dependence are contradictory, with density dependence either increasing or decreasing extinction while potentially increasing selection strength to facilitate adaptation. Here, we test empirically how density dependence influences evolutionary rescue, subjecting experimental populations to a stressful environment for six generations under density dependence or independence. Density dependence suppressed population size and increased extinction risk, while density independence enabled rapid population growth, particularly for populations with high genetic diversity. The evolution of the intrinsic fitness during and realized fitness after the experiment suggests density-dependent populations that persisted adapted more than density-independent populations, particularly if they had high genetic diversity. These findings help reconcile theoretical results, as they are compatible with adaptation being favored despite higher extinction rates for populations evolving in density dependence. Our results emphasize that it is crucial to consider density dependence when developing management strategies for populations facing detrimental environmental change.

Key words

Adaptation; eco-evolutionary dynamics; population dynamics; bottleneck; stress; demographic history

Main

Human-altered environments are leading to drastic declines in biodiversity globally[1,2]. Conservation interventions can be effective[3], but are unlikely to be sufficient on their own[4]. In addition to the need for fundamental changes to human resource use[2], it is vital that we also understand the conditions under which species will be able to adapt to human-altered conditions.

Evolutionary rescue is adaptation that enables populations to persist and grow following habitat alteration severe enough that it otherwise would cause extinction[5]. Evolutionary rescue leads to a characteristic U-shaped curve in population size with populations declining after environmental change, and then increasing again if they are able to adapt rapidly enough to achieve a positive growth rate before they go extinct. Theory and multiple experiments have demonstrated that evolutionary rescue can enable population persistence, with the probability of rescue increasing with genetic diversity[6–12]. However, in most of that research, density-independent growth is assumed or created experimentally. Thus, even if population size increases with adaptation, population density remains low. This means that, unlike in nature, populations in these theoretical and experimental studies can largely grow exponentially without experiencing intraspecific competition for resources or other forms of negative density dependence.

Conditions where population growth is density independent, or only affected weakly by density dependent processes, can occur in nature. For example, biological invasions typically occur under conditions close to density-independence as the new geographic range represents vast as yet uncolonized habitat. Biological invasions also provide many examples of rapid adaptation[13,14]. However, populations commonly experience density dependence. Habitat size is often a constraining factor leading to negative density dependence for populations of conservation concern in the modern era of environmental degradation and habitat fragmentation[15]. Thus, the findings that rapid adaptation can lead to evolutionary rescue when habitat size does not impose a strong constraint on population growth[e.g.,8,9,16] cannot be confidently applied to many situations. When habitat size is constrained, adaptation that

increases population growth rates may also increase population density exposing populations to stronger effects of negative density dependence[17,18].

Several theoretical models have started to address this knowledge gap by comparing outcomes between populations experiencing density independence and those experiencing density dependence, typically negative density dependence[11,19–23]. However, their findings are contradictory. Several studies show that density dependence hinders rescue through its effects on demography, as it renders populations more vulnerable to extinction due in part to a faster initial decline and slower recovery in population size[11,21–23]. For instance, Nordstrom et al. (2023) used stochastic individual-based simulation to show that density dependence can reduce the probability of rescue and increase the probability of extinction even in large populations generally expected to have a low risk of extinction. However, others find that density dependence can reduce extinction risk through ecological release that leads to an increase in fecundity or decrease in mortality when population size drops[19,20]. These contradictions among theoretical predictions stem from differences in model assumptions, including whether models start with equivalent intrinsic fitness[e.g.,11,21–23] or equivalent realized fitness[19,20] of populations under density dependence and density independence. Data from empirical systems will be useful to shed light on how density dependence alters extinction risk in nature.

Most of these theoretical studies focus on these demographic effects of density dependence on the probability of rescue (or its inverse, extinction) shortly after environmental change, but not on the evolutionary effects on fitness of the surviving populations after rescue. However, fitness following evolutionary rescue is crucial to understanding how populations might respond in the long term to environmental change in nature. Density dependence imposes selection that could influence fitness following rescue. In theory, if density dependence and the change in the environment select for similar phenotypes, density dependence will increase the strength of selection, which can then increase the speed of adaptation and the eventual fitness achieved in the new environment[24]. Fitness after rescue of replicate populations may additionally be higher for populations that evolved in density-dependent conditions as those that had lower fitness and were not able to adapt

rapidly enough went extinct[23]. Thus, even if density dependence reduces the probability of rescue by increasing extinction risk, it may also benefit persisting populations through increasing fitness of populations that adapt and persist.

These effects of density dependence on extinction and fitness may be modulated by genetic variation. For example, populations evolving under density dependence could be particularly reliant on starting with sufficient genetic diversity to respond rapidly to environmental change, and those with low diversity might not be able to adapt rapidly enough.

It is critical to test empirically how population density affects the probability of evolutionary rescue to understand the conditions under which rapid adaptation can support populations of conservation concern. Furthermore, it is important to understand how population density affects rescue for diverse populations relative to bottlenecked populations, given the strong positive relationship between genetic diversity and rescue. Finally, it is vital to be able to quantify the degree to which evolution drives the effects of population density given the feedback loops between these eco-evolutionary processes.

Here, we experimentally test for the first time how density-dependent population dynamics affect evolutionary rescue following sudden environmental change. We use *Tribolium castaneum* as a model system[25]. Our experiment compares replicate populations in habitats of fixed size where fluctuations in population abundance lead to fluctuations in density (hereafter, “density-dependent”), to populations in habitats that increase in size as abundance increases, thus maintaining low and constant density (hereafter, “density-independent”). Thus, in the density-dependent treatment, population density varied naturally, so that both positive and negative density dependence could occur, with negative density dependence being the dominant process when populations adapted to the new environment and reached high density. In the density-independent treatment, population density was held low and constant (10 individuals per patch), such that both positive and negative density dependence would be weak, to experimentally produce an environment approaching density independence. Experimental populations were initiated from either diverse or bottlenecked sources, allowing us to

disentangle the effects of density dependence from genetic diversity. We also implemented a control that prevented adaptive evolution, by replacing beetles from a non-adapted source population each generation, allowing us to distinguish whether population dynamics are due to both adaptive and demographic effects, or to demographic effects alone. In total, we tracked the dynamics of 188 experimental populations evolving in an environment made harsh by a pesticide[26] for 6 generations. At the end of the experiment we measured population realized fitness to evaluate how well surviving populations adapted to the new environment. Hence, we address the following question: How do density dependence, bottlenecks, and evolution influence extinction, population dynamics, and adaptation?

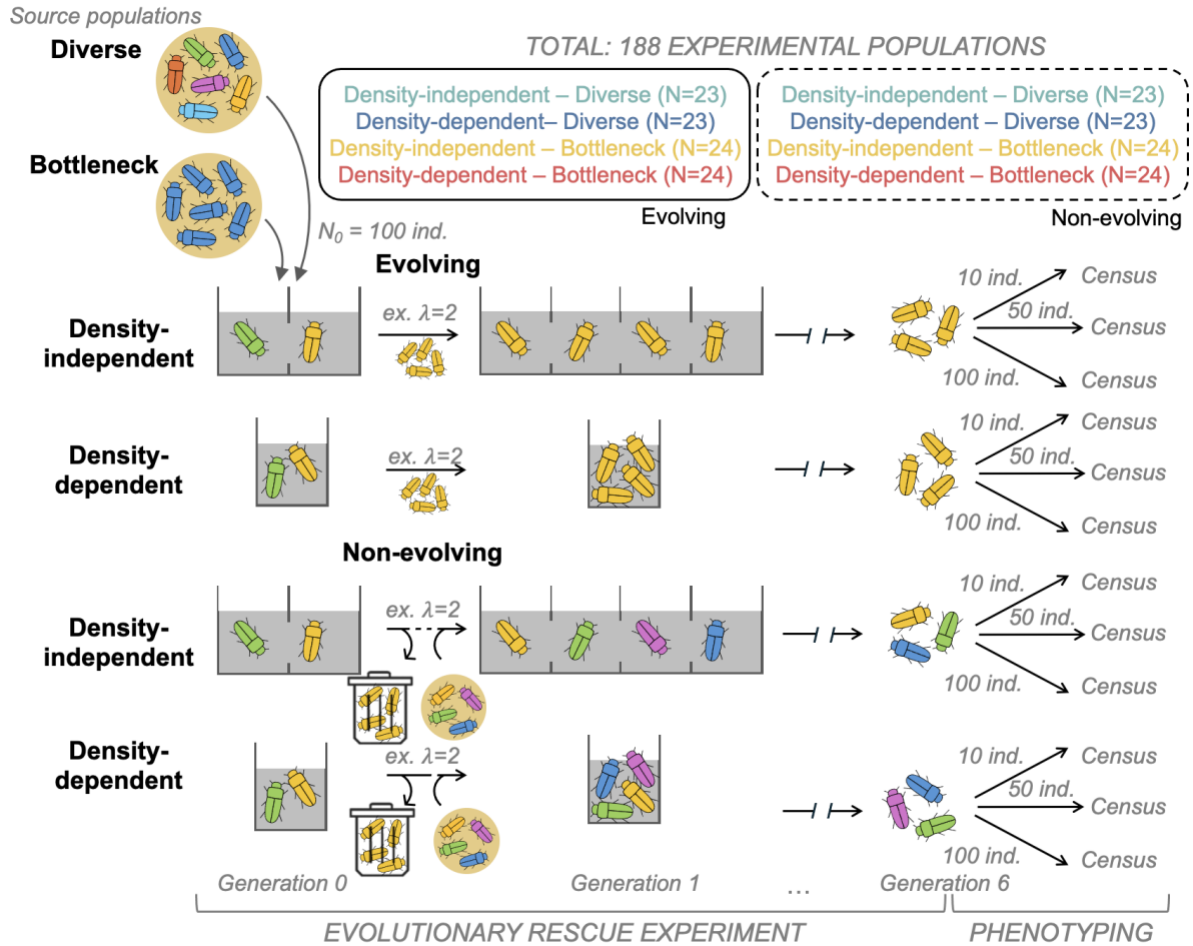

Figure 1. Experimental Design. *The multi-factorial experiment of the three variables, i.e., genetic diversity (diverse vs. bottleneck), density dependence (density independent vs density dependent), and evolution (evolving vs. non-evolving), corresponded to eight treatment combinations (2 x 2 x 2). The 188 experimental*

populations were initiated with 47 source populations (23 diverse and 24 bottlenecked populations) of T. castaneum*, over four temporal blocks.*

Results

Probability of extinction increased in density-dependent and bottlenecked populations

Density dependence and a bottleneck both increased the probability of extinction, and their effects were not additive (Fig. 2, $LRT_{density\ x\ bottleneck\ interaction}$: χ^2 = 10.22, *P-value* = 0.001, Fig. S1). Density dependence more than doubled the probability of extinction for diverse populations (Fig. 2a), but had a smaller effect on bottlenecked populations (Fig. 2b), likely because bottlenecked populations remained small, so the effects of density dependence were weaker. We did not detect an effect of evolution on the probability of extinction or of its interactions with density dependence or bottleneck status (LRT *P-values* > 0.05).

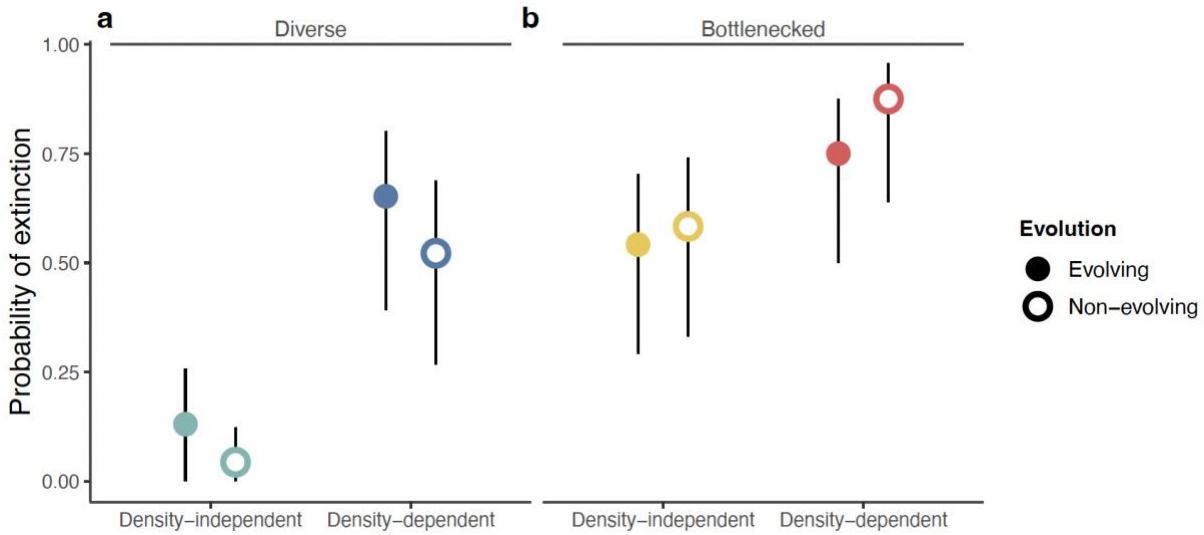

Figure 2. Probabilities of extinction showing that extinction was higher in bottlenecked and density-dependent populations. *The probabilities of extinction were estimated using a Cox proportional-hazards model of survival including all the treatments and their interactions, which fully represents the experimental design. Error bars are 95% confidence intervals for the probabilities.*

Populations grew larger under density independence

We provide two visualizations of population dynamics: raw abundance data and mean abundances from a generalized additive mixed model. The raw data, presented on two scales, illustrate clearly that density dependence, bottleneck status, and evolution each shape population size through time (Fig. 3). Density independence enabled population growth, particularly in diverse, evolving populations (Fig. 3a-i, 3a-ii). These populations grew rapidly to large size, so much so that continually expanding the size of the habitat became impossible as the habitats became too large for our climate chambers. At this point, the density-independent treatment of 10 individuals per patch was maintained as previously, but the population size was capped at 500 individuals. Given constant density, we could then project future population size from the growth rate while populations continued to adapt for the following two generations (blue points and lines in Fig. 3 represent these projections in generations 5 and 6, Fig. S2). These projections are conservative, as larger populations that had not been truncated, might have been able to adapt even more quickly. Diverse, density-independent, non-evolving populations (Fig. 3e-i, 3e-ii) grew much less than their evolving counterparts (Fig. 3a-i, 3a-ii), showing that adaptation made a key contribution to population growth in density-independent, diverse populations. At generation 6, diverse, density-independent, evolving populations reached a projected mean size of 5,563 individuals while their non-evolving counterparts had a mean size of only 283 individuals. These data also show that a previous bottleneck in population size, combined with density dependence, restricted population growth substantially (Fig 3, looking by row, compare the last column with the previous three columns).

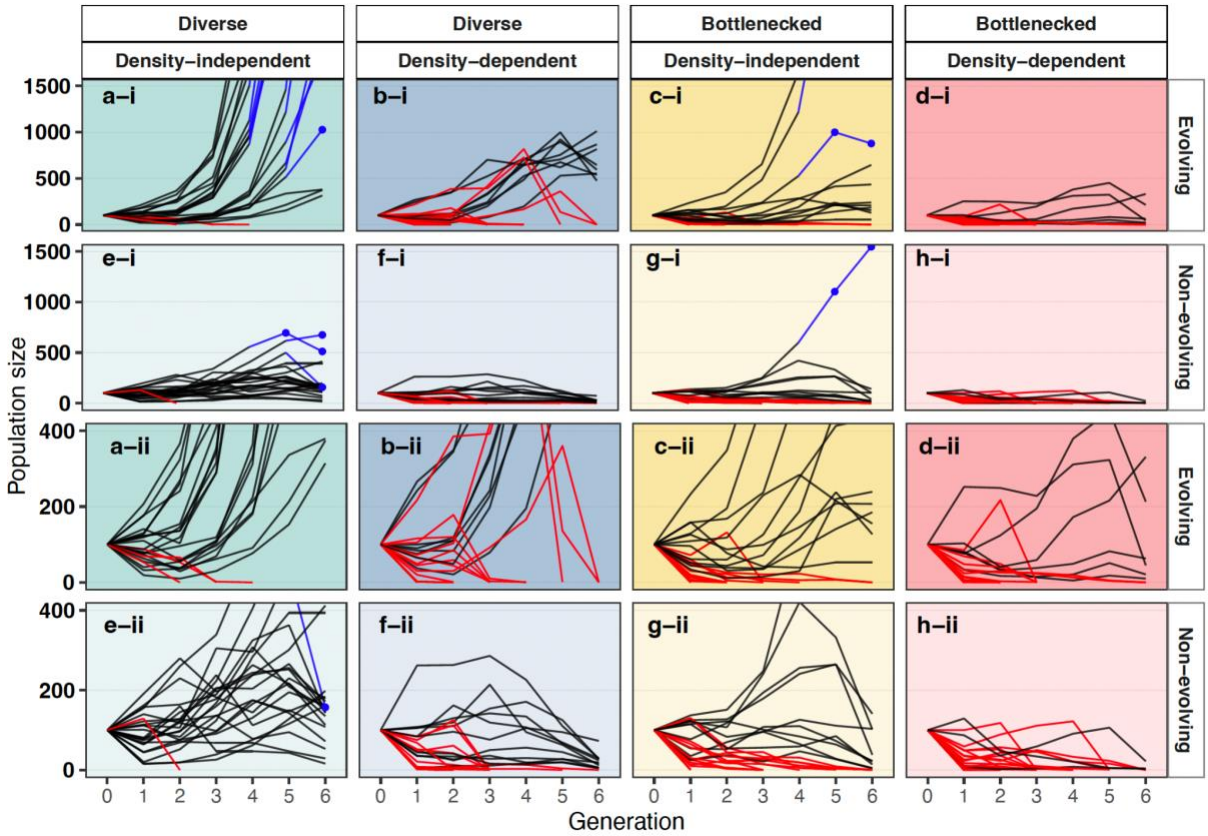

Figure 3. Size of experimental populations through time, showing that a population bottleneck and density dependence constrained population size and increased extinction risk, while density independence and evolution facilitated population growth. *For easier comparison, the first two rows are on a scale of up to 1,500 individuals (a-i:h-i), while the last two rows repeat the same data but on a scale of up to 400 individuals (a-ii:h-ii). Red lines indicate populations that went extinct. Some density-independent populations grew too large to maintain in the incubators; in these cases, from generation 4 only 500 individuals were used to produce the next generation and their population size (shown in blue) was projected from their growth rate at constant low density. See methods for details.*

We used a generalized additive mixed model (GAMM) to estimate the mean dynamics of surviving populations through time. Density dependence, bottlenecks and evolution each shaped these dynamics ($LRT_{\text{triple interaction}} = 10.36$, *P-value* < 0.001; Fig. 4). Diverse, evolving populations grew the most quickly, but density dependence limited the final size so that it was comparable to bottlenecked, density-independent populations. Non-evolving populations were substantially smaller than

their evolving counterparts with density independence increasing final population size relative to density dependence (Fig. 4b, Fig. S3).

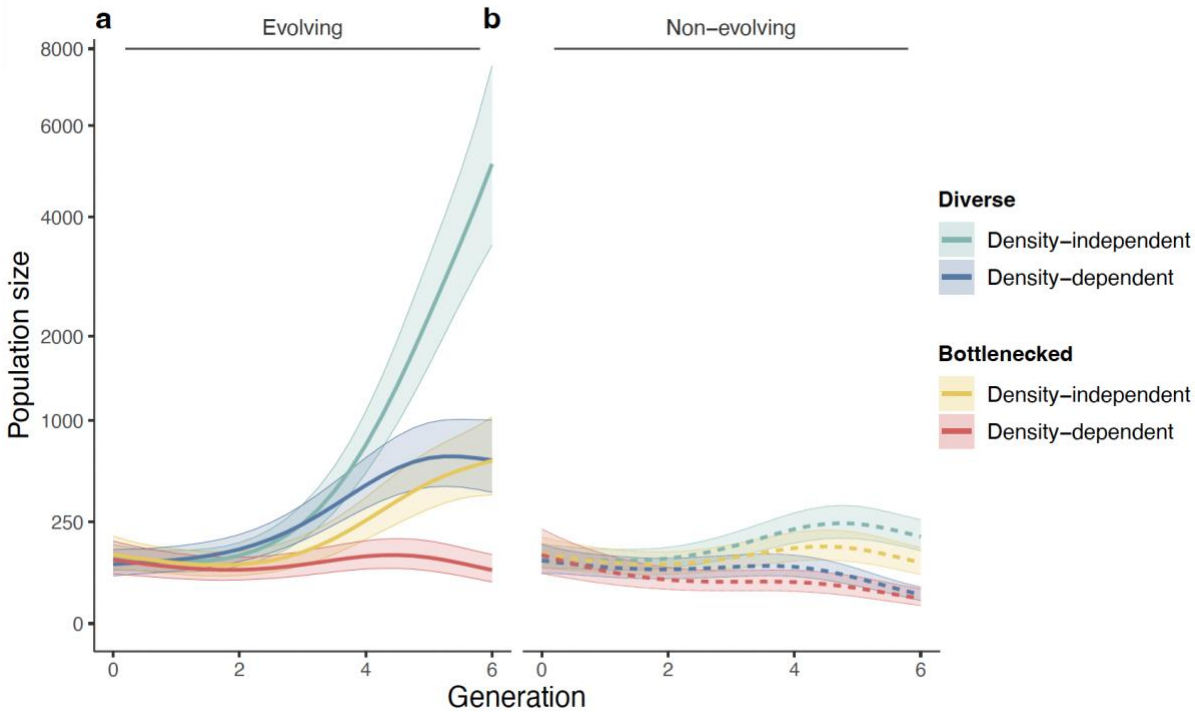

Figure 4. Trajectories of surviving populations through time, estimated by the generalized additive mixed model, showing that population sizes increased rapidly when diverse populations could adapt under density independence, and remained small when evolution was prevented, particularly under density dependence. *The y-axis is on a square root scale to increase the visibility of small population sizes. The lines are estimates of the mean population size for each treatment from the model, averaged over the four temporal blocks and the 47 source populations (included in the model as random effects). Shaded areas are 95% confidence intervals.*

Intrinsic growth rates increased for evolving populations early on, demonstrating adaptation

To quantify evolution of the intrinsic growth rate, and when that adaptation occurred, we fitted population models independently to each of the four types of evolving populations (Fig. 5). For diverse populations, density dependence led to a higher maximum intrinsic growth rate (r_{max} = 1.53, Fig. 5a) than density independence

(r_{max} = 0.95, Fig. 5a). Bottlenecked populations achieved much lower intrinsic growth rates (r_{max} = 0.19 under density dependence and 0.46 under density independence), with confidence intervals overlapping the predicted value (Fig. 5b). Adaptation is evident in the positive slope of intrinsic growth rate over time, and occurred mainly in the early generations of the experiment, with an adaptation period ranging from 2.44 to 3.00 generations for all treatments.

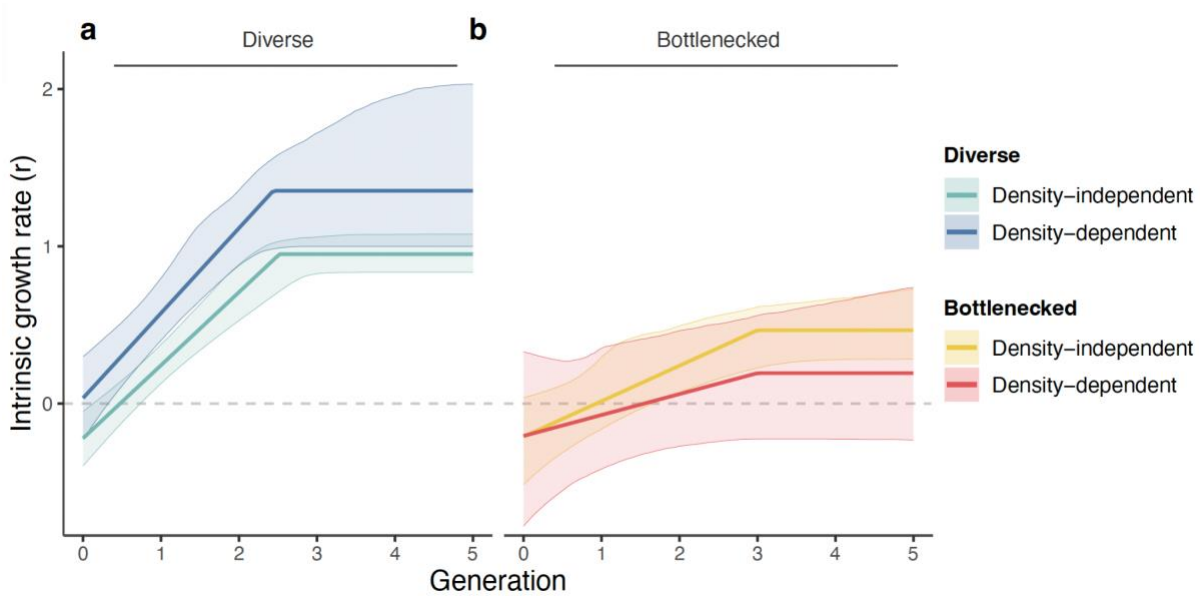

Figure 5. Evolution of the intrinsic growth rate (r) through time for surviving populations, showing that populations adapted mainly during the first few generations and that the rate of adaptation was greater for diverse populations than bottlenecked ones. Among surviving diverse populations, density dependence led to higher intrinsic growth rate than density independence. *The lines are estimates of the evolving mean intrinsic growth rate (from population models fitted to the data for each treatment separately) averaged over 20, 11, 8 and 6 source populations (included in the model as a random effect) in the four independent models. Models included a Ricker form for the effect of density for the populations evolving under density dependence. We used a piecewise linear model for the evolution of the intrinsic growth rate up to a maximum. Shaded areas are 95% confidence intervals (parametric bootstrap). The grey dashed line, where the intrinsic growth rate is equal to 0, corresponds to the value above which the population could grow from low density.*

Realized fitness at the end of the experiment confirmed adaptation in evolving populations

After six generations, we evaluated the realized fitness of the surviving populations at a range of controlled densities to quantify adaptation experimentally. Populations that were allowed to evolve during the experiment had higher realized fitness in the challenging environment than the non-evolving populations, thus showing clear adaptation (Fig. 6). Adaptation was particularly strong in the diverse populations (Fig. 6). Realized fitness was reduced at higher densities, particularly for bottlenecked populations, showing their greater sensitivity to negative density dependence ($LRT_{density\ x\ evolution\ x\ bottleneck\ interaction}$: $\chi^2 = 6.36$, *P-value* = 0.01). Populations that evolved under density dependence and density independence showed similar patterns (Fig. 6, $LRT_{intercept}$: $\chi^2 = 0.12$, *P-value* = 0.73, LRT_{slope}: $\chi^2 = 0.39$, *P-value* = 0.53 and always P-values > 0.33 when considered in interaction with other variables). Nevertheless, at high density, realized fitness of density-dependent populations appeared slightly higher than density-independent populations (Fig. 6), a pattern consistent with adaptation to higher density, more efficient selection, or both in density-dependent populations.

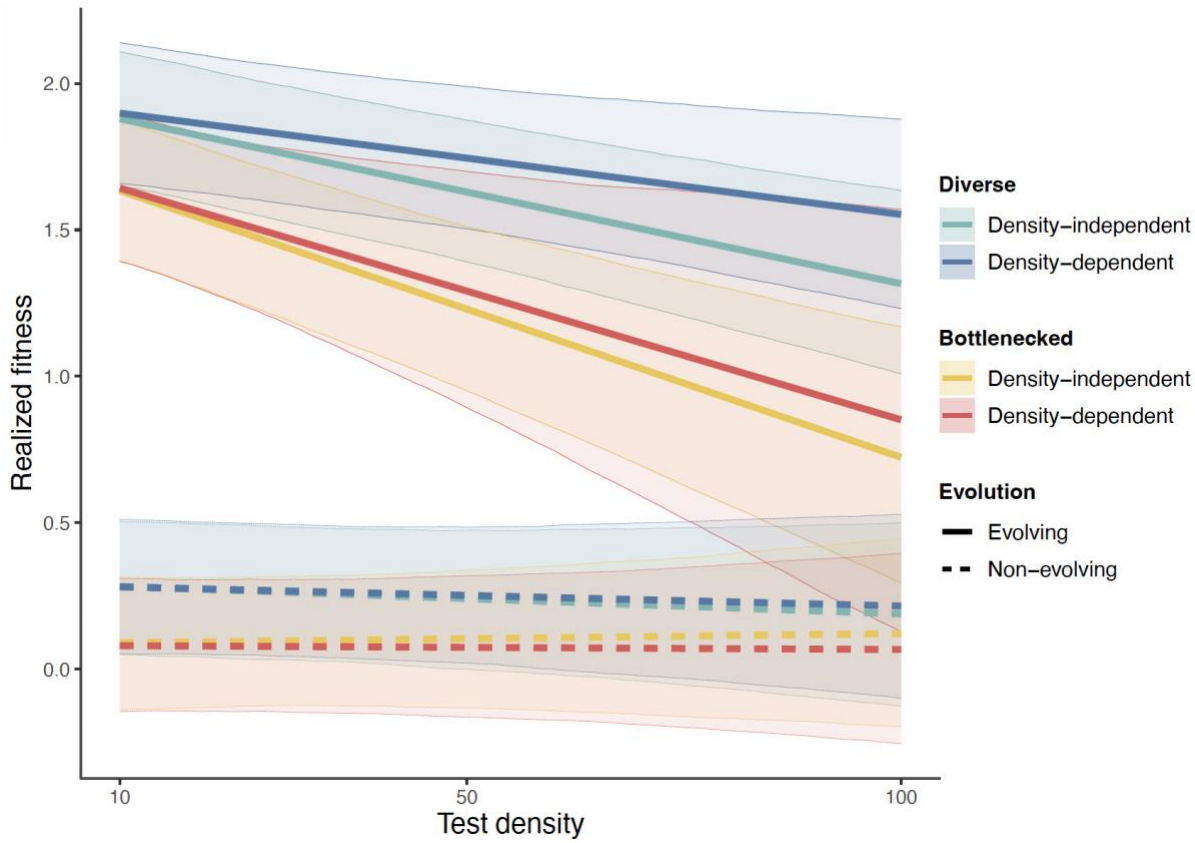

Figure 6. Mean realized fitness of surviving populations after the rescue experiment, as a function of test density. *The lines are estimates from the linear mixed model of the mean realized fitness for each treatment, averaged over the four temporal blocks and the 47 source populations (included in the model as random effects). The model included all aspects of the experimental design, including all interactions of the treatment factors. Shaded areas are 95% confidence intervals (parametric bootstrap).*

Discussion

Here, we show experimentally for the first time that negative density dependence dramatically increases the extinction risk of populations in challenging environments, but nonetheless does not prevent adaptive evolution in populations able to persist. Furthermore, the effects of density dependence are influenced by the genetic diversity or demographic history of the population. Populations that have experienced bottlenecks in size, which can both reduce genetic diversity and increase genetic load, have a yet higher risk of extinction under density dependence, and a much lower ability to adapt to environmental change. Finally, our results

support the main theoretical results[23,24] as they are consistent with selection under density dependence being more efficient, despite a higher extinction rate. Overall, this study highlights the crucial importance of both preserving genetic diversity and increasing habitat size and resource density to relieve density dependence for populations of conservation concern.

Density dependence increases extinction without necessarily limiting adaptation. In our experiment, density dependence had a strong impact on the trajectory of populations and their ability to persist over time. Density dependence increased the probability of extinction and reduced population realized fitness throughout the experiment. These negative effects of density dependence were even more visible as populations increased in size, i.e., for diverse populations than for bottleneck populations. These findings disagree with theory that includes ecological release associated with population decreases under density dependence[19,20] and instead align with theoretical frameworks that start with equivalent intrinsic fitness and thus density dependence reduces vital rates[21,23]. Fundamentally, what occurs in nature may thus depend upon the degree to which, and the mechanisms by which, populations adapt to density dependence prior to detrimental environmental change. If density dependence generally reduces vital rates, natural populations adapting to drastic environmental change while experiencing negative density dependence may suffer the demographic consequences, increasing their risk of extinction. Our experimental set-up considers fitness directly (combining reproduction and survival), but the nature of density dependence may be more complicated in other natural contexts (for instance, see[20,27] where the effect of density dependence differs for reproduction and survival). Given that density-dependent dynamics are widespread in natural populations[28], it is crucial to consider these effects in the management of populations that may be at risk of extinction when environments change.

The adaptive potential of populations that experienced a bottleneck in their demographic history was substantially reduced, particularly in the context of density dependence. The combination of bottlenecks and density dependence was nearly as

detrimental to the ability of populations to adapt and grow over time as being unable to evolve at all. Experiencing a bottleneck essentially erased the advantages of density independence. The bottlenecked populations evolving under density independence reached sizes similar to those of the diverse populations evolving under density dependence. Furthermore, even though the bottlenecked populations that persisted showed signs of adaptation at the end of the experiment, they never reached the fitness of the diverse populations. This is because bottlenecked populations have a combination of both lower diversity and higher genetic load[29,30], which makes them less able to adapt and therefore less able to take advantage of the density-independent conditions. These findings are of particular significance in the context of species conservation, as populations facing extinction frequently experience both bottlenecks and restricted habitat sizes[31]. For instance, Stoffel et al. examined 30 species of pinnipeds and demonstrated that the three species most significantly impacted by a bottleneck, resulting from hunting or habitat loss, had been designated as endangered[32]. Our findings emphasize that bottlenecked populations are particularly vulnerable to extinction following a severe environmental change when density-dependent effects are considered. In addition, density dependence and bottlenecks can be associated in nature, particularly when restricted habitat size leads to both a bottleneck and density dependence. As bottlenecks and density dependence are common in natural populations, our findings could help explain why adaptation to sudden environmental change is not commonly observed in nature[33].

Our experimental design may have reduced extinction in non-evolving populations, leading to extinction probabilities similar to evolving populations. Individuals introduced each generation originated from the ancestral environment (without the toxin) and thus were not adapted to the stressful environment. The benign maternal environment may have enhanced reproduction and larval performance in the stressful environment, as in other studies[12,34]. This would potentially prevent populations from declining to sizes where they are at risk of extinction due to demographic stochasticity. However, without the ability to adapt,

the non-evolving populations remain small, while evolving populations become considerably larger. Consequently, it is likely that if natural populations are unable to evolve at all, they may have a higher probability of extinction than demonstrated by the non-evolving populations in this experiment.

The long-term performance of populations after evolutionary rescue remains unclear. Our experiment demonstrates that if populations are able to persist and adapt to severe environmental change, density dependence may improve their fitness by increasing the strength of selection, particularly if they have adequate genetic diversity. Whether such a fitness boost lasts is unknown, as our experiment was limited to six generations. In the future, it would be interesting to re-examine these dynamics over an extended period for two key reasons. First, having passed through low population sizes, populations may have accumulated genetic load[35], which would reduce their persistence over the long term[36]. Second, the suppressing effects of high density increase in strength again as population size increases with adaptation. Consequently, because diverse populations are better able to adapt, they will experience stronger effects of density dependence in the long term. Few studies have examined the long-term persistence of populations following evolutionary rescue, which would be particularly relevant in the context of density dependence.

These findings have clear broader implications for managers of natural populations, highlighting the importance of both genetic diversity and habitat size. While even the bottlenecked populations were able to adapt to the stressful environment, the diverse populations attained much higher fitness. Thus, in natural populations known to or likely to have low diversity, genetic diversity should be enhanced by active management[37,38]. This should both reduce extinction risk and improve the ability to adapt to changing environments. This recommendation is in alignment with the substantial literature on genetic rescue (the assisted movement of individuals among populations to enhance diversity and increase fitness). Genetic rescue can have large and beneficial effects on populations[37,39], and we agree with

others[40,41] that masking genetic load and enhancing diversity is of paramount importance.

Along with genetic diversity, habitat size and quality should remain a focus in management and conservation efforts. Alleviating negative density dependence by enhancing either habitat size or resource availability should substantially aid populations, even bottlenecked or inbred ones. Evidence from natural populations shows that challenging environmental conditions are all the more detrimental to populations when they are close to their carrying capacity and density dependence is high (e.g., in feral sheep[42] and Alpine ibex[43]). However, as challenging as it is to move organisms to enhance diversity, increasing habitat size and quality is likely to be more challenging. Nonetheless, our findings illustrate that reducing density dependence can be extremely powerful in alleviating extinction risk, and we hope they can help support the political and social decisions needed to provide resources for habitat improvement.

To conclude, negative density dependence is common in natural plant and animal populations, often in the form of intraspecific competition[44–46]. Its role in shaping communities and increasing community diversity is well documented [e.g.,47]. Additionally, density dependence is known to evolve, exemplified by the evolution of carrying capacities[48,49]. Here, we show experimentally for the first time that density dependence dramatically influences the ability of populations to persist in challenging environments, reducing the likelihood of evolutionary rescue.

Our results provide both challenges and hopes for the management of natural populations. The smaller size of populations experiencing density dependence clearly places them at risk of extinction, and alleviating density dependence through habitat improvements is extremely challenging. On the more hopeful side, even populations in strongly density-dependent conditions may adapt to rapid environmental change, particularly if they harbor adequate genetic diversity. Adaptive evolution thus can counter some of the effects of density dependence, buying time for habitat restoration.

Methods

Model system and lab conditions

Our experiment used *Tribolium castaneum*, the red flour beetle, to test the effects of negative density dependence, a bottleneck event, and evolutionary processes on evolutionary rescue. The same experimental conditions and life cycle were used during laboratory maintenance, the evolutionary rescue experiment, and the phenotyping. *Tribolium castaneum* populations were kept in 4 cm by 4 cm by 6 cm acrylic containers with 20 g of medium, which served as both a 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 and experimental population of *T. castaneum*, and single patches were used in phenotyping. The life cycle of *T. castaneum* was constrained to mimic that of a seasonally breeding organism with non-overlapping generations. Adult beetles were placed in groups of adults in fresh 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. Beetles were kept in incubators at 31°C with 54 ± 16% relative humidity. Several incubators were used, and patches were randomized among and within incubators once each week to prevent any systematic effects of incubation conditions.

Laboratory populations

To consider the effect of bottleneck events during the demographic history of the populations, we used 23 diverse and 24 bottlenecked populations of *T. castaneum* for our experiment. These 47 populations were initiated from a large stock population created from multiple crosses of different stock strains (“GA1”, “Lab-S”, “Z2”, “Z4” and “Estill” from USDA stock maintenance) maintained at a population size of 10,000 individuals for multiple generations. Then, each of the 24 bottlenecked populations was initiated from an independent sibling pair from the large source population, corresponding to a bottleneck event of 2 individuals for 2 generations (see “strong bottleneck populations” in[12]). Each of the 23 diverse populations was initiated with

500 individuals from the large source population. These 47 populations were maintained at a population size of 400-500 individuals for about ten generations under optimal development conditions on standard medium (95% wheat flour and 5% brewer's yeast), distributed over four temporal blocks, one week apart.

Evolutionary rescue experiment

To test the effects of negative density dependence as well as a bottleneck event and evolutionary processes on evolutionary rescue, we performed an experiment where populations experienced a new challenging environment for six generations (Fig. 1). All the experimental populations (described below) were initiated with 100 individuals. For each generation, the adults in each population were censused completely, then the individuals were mixed and split evenly in the newly connected patches with fresh challenging media for the 5-week life cycle as described above. A population was considered extinct when the population size was 0 or 1 at the census.

A challenging medium was created by adding a pyrethroid insecticide, deltamethrin (DeltaDust, Bayer), to the standard medium at a concentration of 11.30 ppm (92.9% wheat flour, 4.9% brewer's yeast and 2.2% DeltaDust). A concentrated deltamethrin medium at 2.97ppm is known to strongly reduce the growth rate that can cause unadapted populations to go extinct of *T. castaneum*[26]. We therefore increased the concentration to observe extinctions in all treatments, even those evolving under the most favorable conditions (i.e., diverse populations evolving in density independence treatment).

We imposed a multi-factorial experiment crossing two levels of density dependence with two levels of bottleneck and two levels of evolution as follows:

To test the influence of density dependence on extinction and adaptation, we compared experimental populations with naturally varying densities to populations with low and constant density intended to approximate density independence. Populations in the density dependence treatment spent the whole experiment in a two-patch connected environment, regardless of population size. Populations in the density independence treatment were maintained at 10 individuals/patch, and as populations grew or declined, the number of patches increased or decreased accordingly. In summary, while habitat size was constant for density-dependent

populations, habitat size was adjusted each generation to maintain constant low density for density-independent populations. The density chosen for the density independence treatment of 10 individuals/patch did not represent full density independence since competition and cannibalism could potentially still occur. However, this choice of 10 individuals/patch represented significantly less competition compared to the density dependence treatment while avoiding possible Allee effects (e.g., difficulty in finding a sexual partner) if the density had been lower. The size of the populations evolving under density independence increased rapidly during the experiment. For feasibility and logistical reasons, we constrained the size of these populations for the penultimate and final generations. While all adults were counted, only a portion (500 randomly selected individuals) were used to initiate the last two generations (generations 5 and 6). These procedures were necessary for 24 populations among the 63 surviving density-independent populations. Even though the population size could be constrained for the last two generations, the density of individuals (10 individuals/patch) was kept constant for the whole experiment.

To test the influence of a bottleneck event during demographic history, individuals used for this experiment came from bottlenecked populations or diverse populations. We used the 23 diverse and 24 bottlenecked independent lab populations described above. Due to the multifactorial design, each independent lab population was used to initiate four experimental populations (density-dependent—evolving, density-independent—evolving, density-dependent—non-evolving, and density-independent—non-evolving).

To test the influence of adaptation, experimental populations evolved under conditions where adaptation was possible (non-constrained) or where adaptation was not possible (non-allowed). In evolving populations, individuals emerging from the challenging environment each generation were used to create the next generation. The dynamics of evolving populations were due to demographic and adaptive effects. In non-evolving populations, the individuals emerging from the challenging environment each generation were replaced by the same number of individuals of the same age from the population used to initiate the experimental population. These replacement individuals came from lab populations that were maintained in parallel in the laboratory on standard medium with a population size of 600 individuals, under the conditions described in laboratory maintenance. This treatment allowed the genetic composition of the non-evolving experimental

populations to remain the same. The dynamics of the non-evolving populations were therefore due to demographic effects only. These non-evolving populations were a control treatment allowing to refer to adaptation and thus to observe evolutionary rescue. As an illustration, it is possible to discern an increase in population size due to demographic effects from demographic effects plus adaptive effects by comparing density independence-evolving treatments with density independence-non-evolving treatments.

The multi-factorial experiment of these three variables corresponded to eight treatment combinations (2 x 2 x 2). We initiated 23 to 24 populations of each of the treatment combinations, for a total of 188 experimental populations distributed in four temporal blocks.

At the last generation of the evolutionary rescue experiment, the weight of individuals emerging was measured. Weight is a trait known to evolve in correlation with the response to the selection of a focal trait[25]. The measurement of body mass was performed in groups of 10 individuals at 35-day old after 6 generations in a challenging environment during the experiment. We replicated to the maximum extent following the number of individuals emerging from the reciprocal transplant experiment. We measured 5 groups of 10 individuals for each population, or less if the population size was less than 50 individuals (average 4.41 replicates per population).

Phenotyping

After evolving for five generations under these experimental conditions, the realized fitness of the experimental populations that did not go extinct (hereafter surviving populations) was measured in a reciprocal transplant experiment. Specifically, directly after laying the eggs of the 6th generation, the 5th generation adults of 36 days old were used to measure the growth under different conditions. The realized fitness of each experimental population was measured as the number of offspring produced per 10 individuals, 50 individuals, or 100 individuals in a patch of challenging medium. For evolving populations, the number of replicates depended on population size. For the non-evolving populations, we completed the number of parents by individuals from the lab populations that we maintained for 24 hours in the corresponding experimental conditions (35 days on standard medium at 50

individuals/patch then 24 hours in a challenging medium at 10 individuals/patch for density-independent populations and 420 individuals in 2-connected patches for density-dependent populations) to have enough individuals. *A posteriori*, we confirmed that the non-evolving individuals coming directly from the experiment (those that laid the 6th generation) or from the laboratory populations (those that waited 1 day in the same experimental conditions) had the same pattern of realized fitness across the three densities. In total, we were able to measure for each surviving population on average 6.4 replicates at a density of 10 individuals (total: 874 measurements), 4.5 replicates at 50 individuals (total: 599 measurements), and 1.4 replicates at 100 individuals (total: 186 measurements).

Statistical analysis

All statistical models were fitted in R version 3.4.2[50]. 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 the challenging environment was influenced by density dependence, bottlenecking, and evolution, we used survival analysis where the probability of extinction was defined as 1 minus the probability of survival. We described survival over time using a log-rank statistic to compare the survival dynamics across treatments using the package survival[51]. We used a Cox proportional-hazards model[52] to simultaneously assess the effect of explanatory variables on survival time and probabilities. The explanatory variables in the model were density dependence (a categorical variable with two levels: “density dependence” and “density independence”), bottleneck (a categorical variable with two levels: "diverse" and "bottleneck"), evolution (a categorical variable with two levels: "evolving" and "non-evolving") and their double interaction and the triple interaction as fixed effects, with source population identity used to initiate the experimental populations and temporal block as random effects. This mixed model was implemented with the package *coxme*[53].

To understand how the dynamics of the surviving populations was influenced by density dependence, bottleneck status, and evolution, we analyzed population size through time using a Generalized Additive Mixed Model (GAMM[54]) with a

negative binomial distribution to account for overdispersion using the package *mgcv*[55]. We focused on surviving populations at the end of the experiment and used projected population size (see above) when population size was experimentally truncated, in generations 5 and 6 for density-independent populations. To allow for the nonlinear relationship, we modeled population size as a smooth function of generation (a continuous variable from 1 to 6) using a thin plate regression spline[54]. Explanatory variables included categorical density dependence, bottleneck, evolution, and the interaction between each of these variables and generation (thus allowing the shape of the curve to vary by treatment) as fixed effects and source population identity and temporal block as random effects. This model was compared with a reduced model using a likelihood ratio test with the *compareML* function of the package *itsadug*[56].

To determine how the intrinsic growth rate of the populations evolved over time, we fitted a generalized linear model with a density effect (Ricker model) or without a density effect (exponential model). The Ricker model provides an accurate description of the biological processes occurring in this *Tribolium castaneum* model system[57]. We fitted the linearized model as a linear mixed model, with the response ln(Nt+1/Nt) as a function of time, and the trend in intrinsic growth rate described by a piecewise linear model with a breakpoint beyond which the intrinsic growth rate no longer increases. The breakpoint was estimated by profiling the likelihood function. In addition, the linear model for populations evolving under density dependence included an effect of population density in the previous generation as a fixed effect. Source population identity was also included as a random effect. The intrinsic growth rate over time, as well as the breakpoint in evolution, were estimated independently for each treatment with four independent models (for diverse populations and bottlenecks each evolving in density-dependent and density-independent conditions). Confidence intervals for intrinsic growth rates were estimated by parametric bootstrapping.

In addition, to determine how the final population size was influenced by density dependence, bottleneck, and evolution, we analyzed the estimated population size at the last generation (generation 6) with a generalized linear mixed model, using the package *lme4*[58]. The projected population size of the surviving population was rounded to integer values and analyzed with a Poisson distribution. The explanatory variables in these models were density dependence, bottleneck,

evolution, and their double interaction and triple interaction as fixed effects, and source population identity and temporal block as random effects. To account for overdispersion, we added an observation-level random effect.

To understand how the populations adapted to the challenging environment and how this depended on the density in which they were measured, we studied the realized fitness of each surviving population at different densities with a linear mixed model using the package *lme4*[58]. Realized fitness (i.e., number of offspring divided by the number of parents) was analyzed with a normal distribution and identity link, after confirming that not transforming the data provided the best approximation of normally distributed residuals compared with the log-normal transformation or square root transformation. In addition to the previously studied model structure (fixed effects on the mean/intercept: density dependence, bottleneck, evolution, and their interactions, and random effects on the variance: initial lab population identity and temporal block), we studied how these factors could influence the slope of the realized fitness as a function of the density of the individuals (continuous variable ranging from 0 to 100). By adding interactions between the discrete variables (density dependence, bottleneck, evolution) and the continuous variable (test density), we were able to test whether the slopes were significantly different between treatments.

To estimate how weight evolved during the experiment, we modeled this trait measured at the last generation of the experiment (generation 6) with a linear mixed model using the package *lme4*[58]. The weight (i.e., body mass of ten adult beetles) was analyzed with a normal distribution and identity link, after confirming that not transforming the data provided the best approximation of normally distributed residuals compared with the log-normal transformation or square root transformation. The explanatory variables in these models were density dependence, bottleneck, evolution, and their double interaction and triple interaction as fixed effects, and source population identity and temporal block as random effects (Fig. S4).

Data availability

The data and R scripts for our analyses are available at:

https://anonymous.4open.science/r/AnalysisNDDDelta-86C3/

They will be archived on Dryad upon acceptance of the manuscript.

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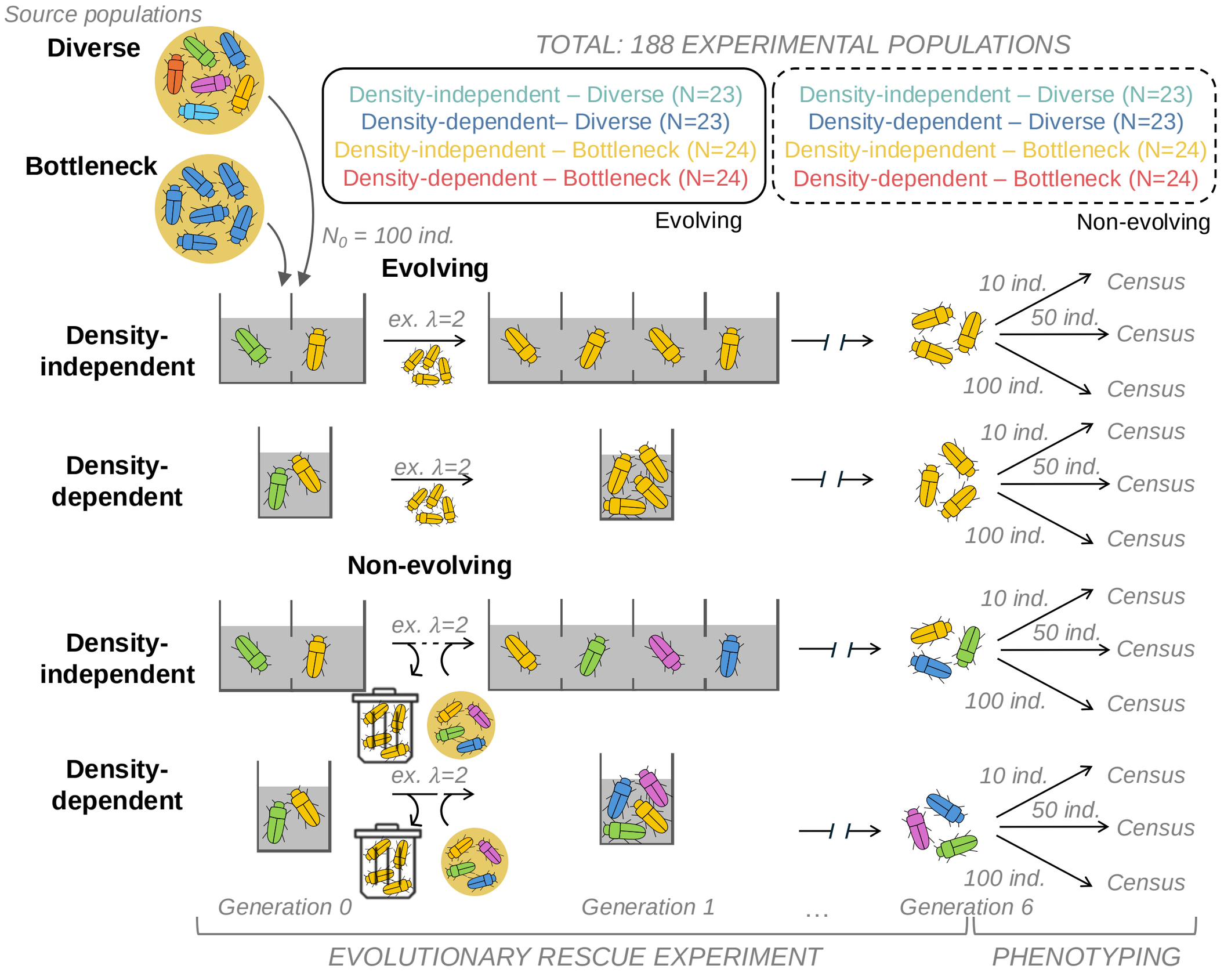
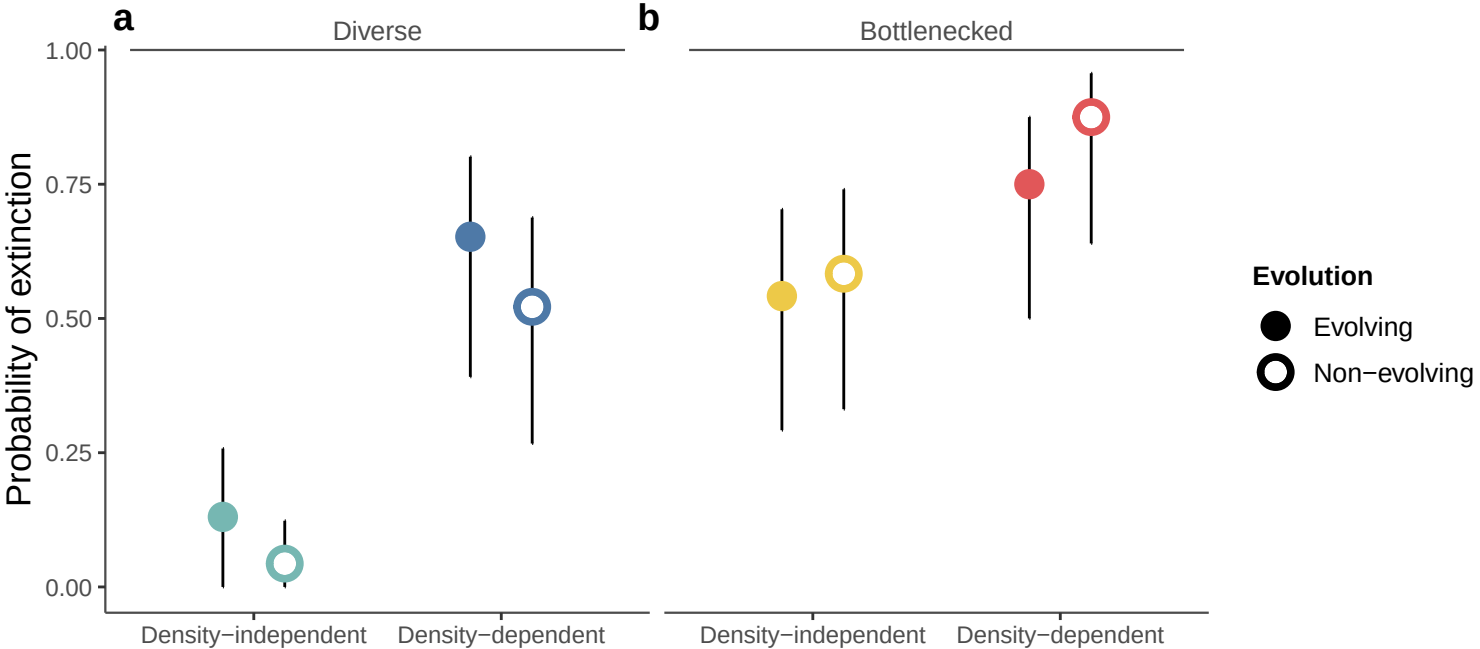
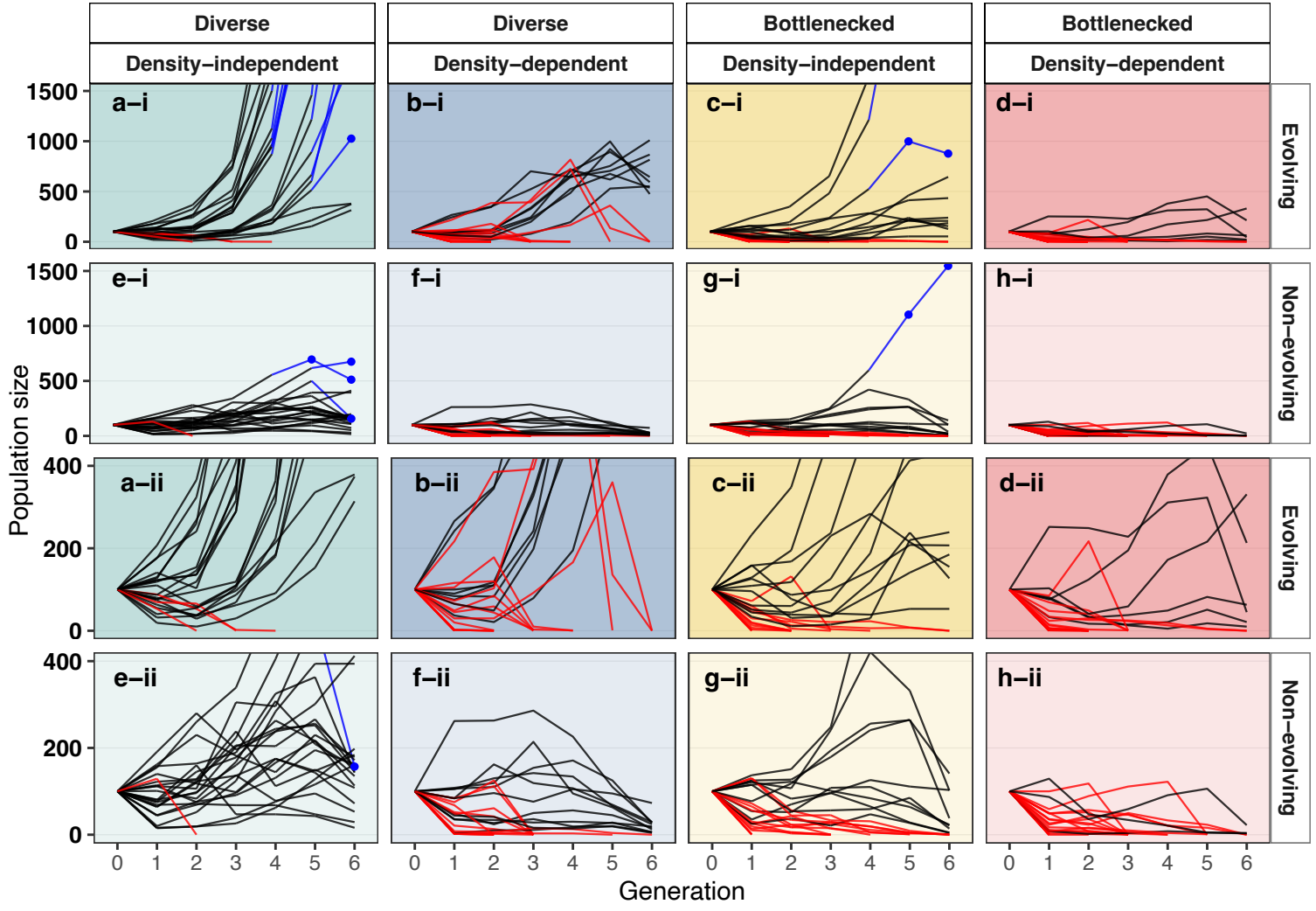
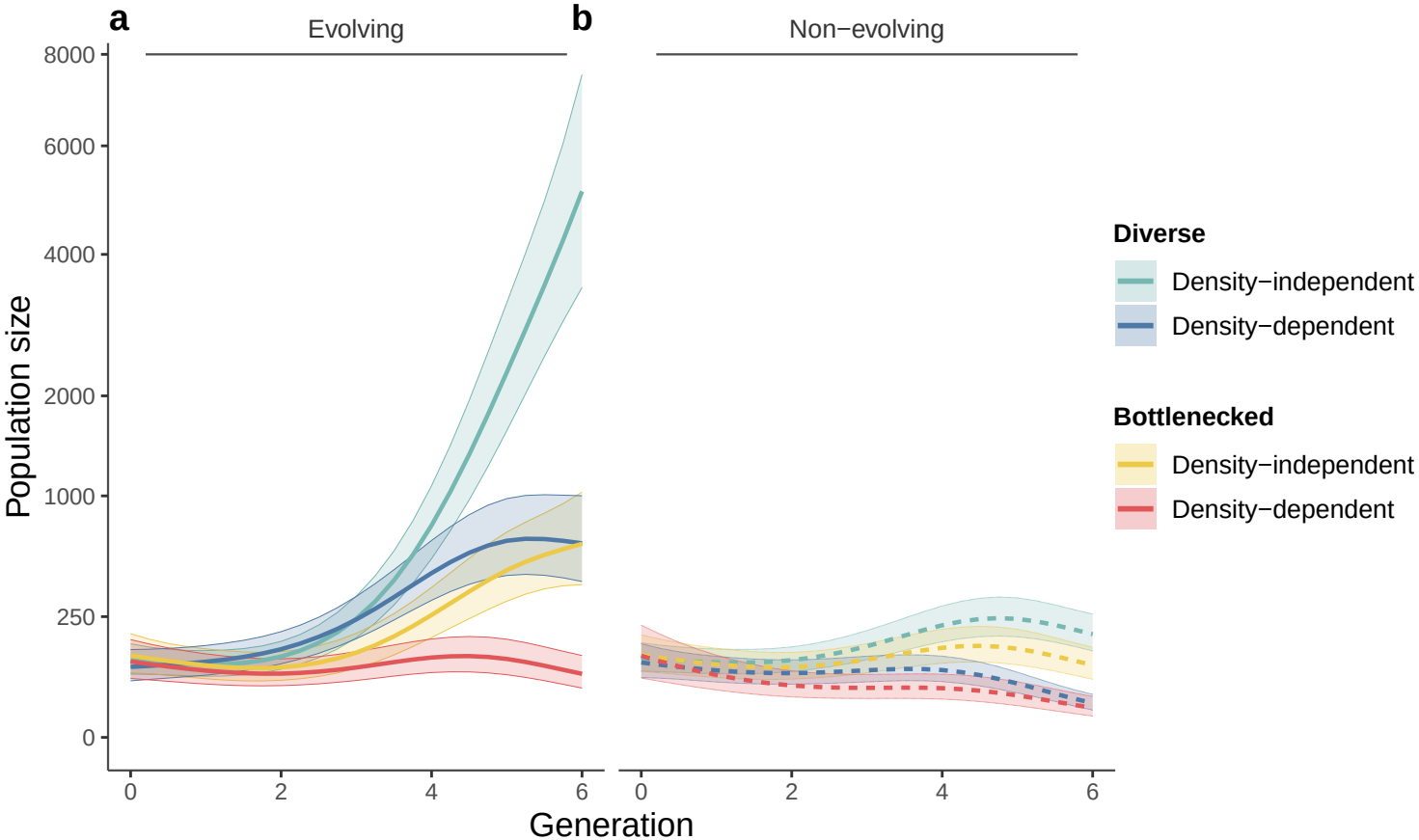
Ethics declarations

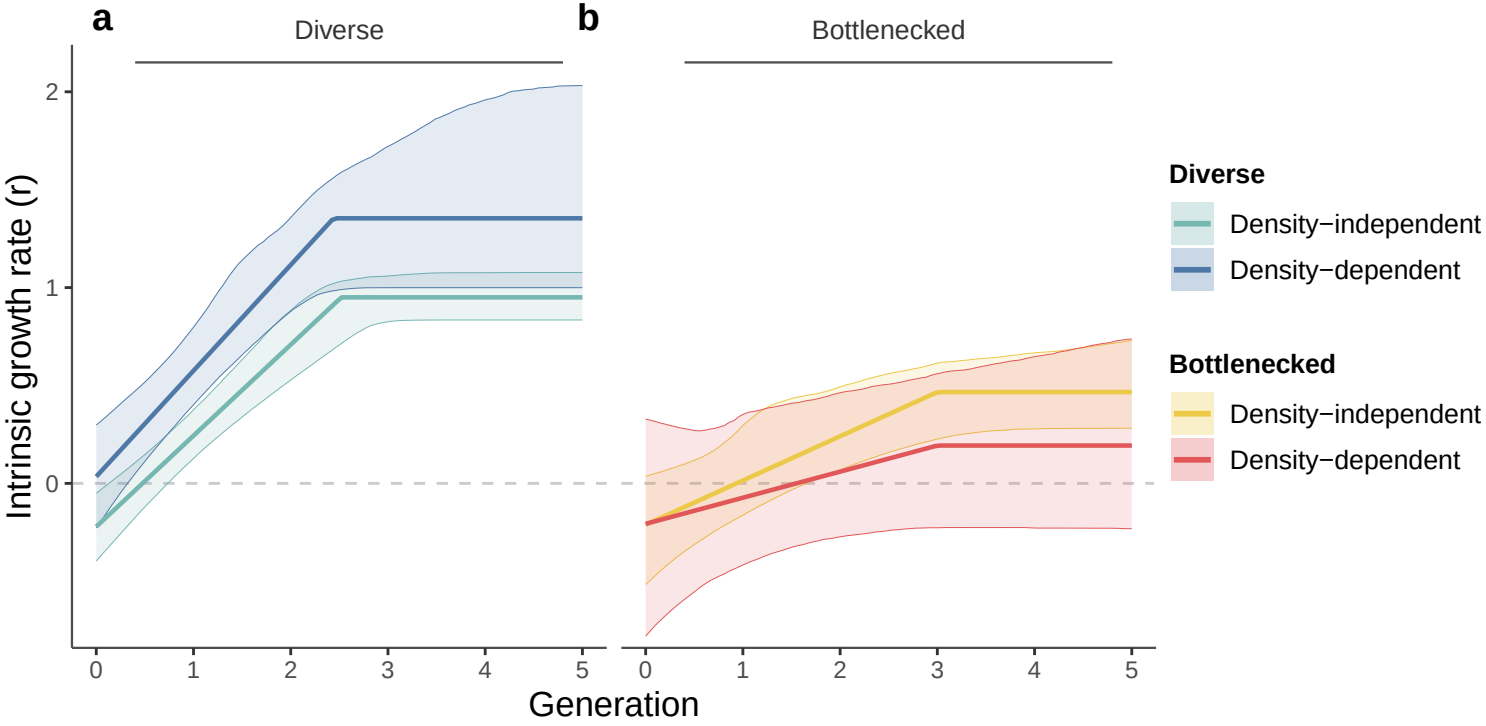
Competing interests: The authors declare no competing interests.

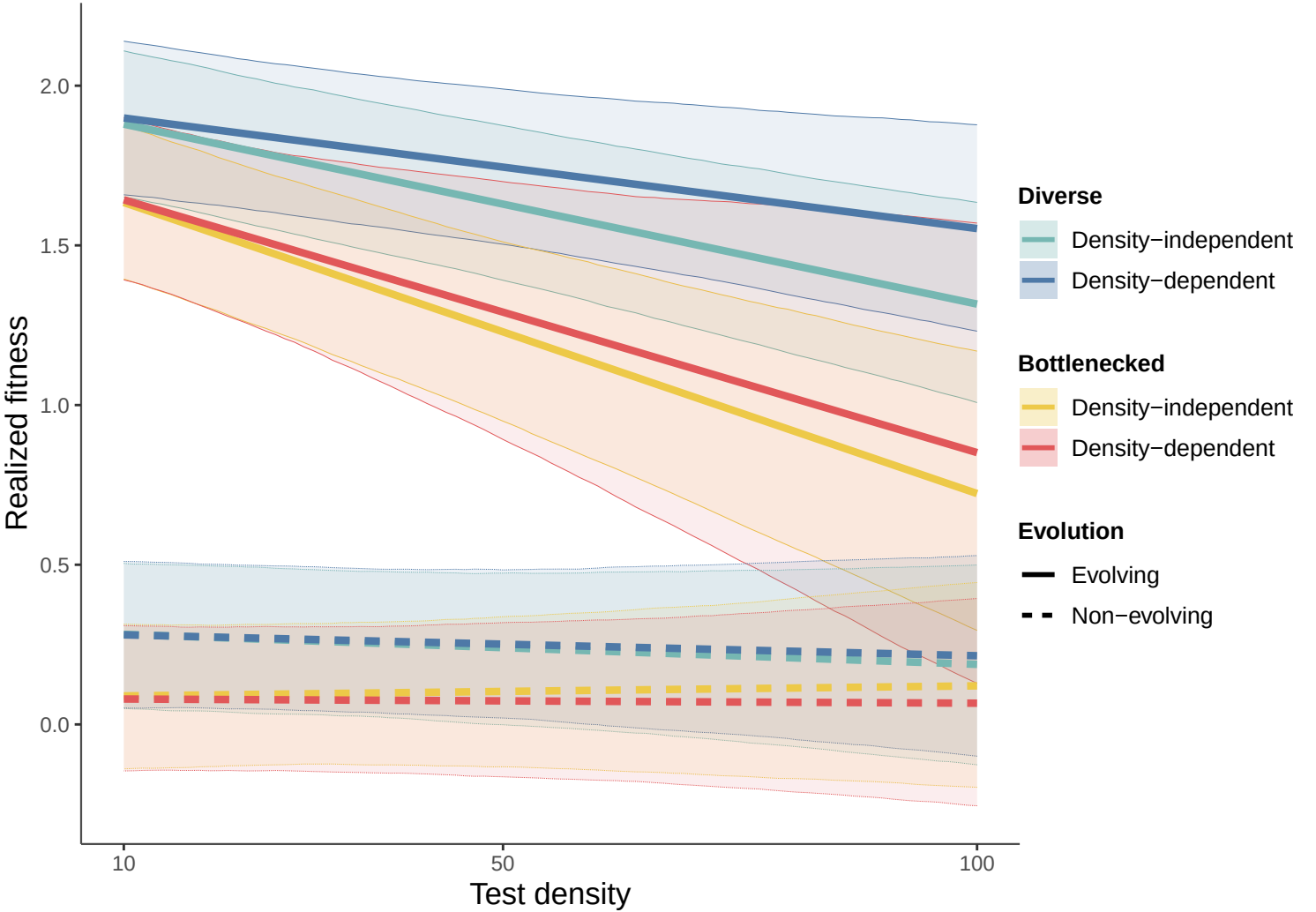

Source populations
Diverse
Bottleneck
TOTAL: 188 EXPERIMENTAL POPULATIONS
Density-independent – Diverse (N=23)
Density-dependent– Diverse (N=23)
Density-independent – Bottleneck (N=24)
Density-dependent – Bottleneck (N=24)
Evolving
Density-independent – Diverse (N=23)
Density-dependent – Diverse (N=23)
Density-independent – Bottleneck (N=24)
Density-dependent – Bottleneck (N=24)
Non-evolving
N_0 = 100 ind.
Evolving
Density-independent
ex. λ=2
10 ind.
50 ind.
100 ind.
Census
Density-dependent
Non-evolving
Density-independent
Density-dependent
Generation 0
Generation 1
…
Generation 6
EVOLUTIONARY RESCUE EXPERIMENT
PHENOTYPING


a
Diverse
b
Bottlenecked
Probability of extinction
1.00
0.75
0.50
0.25
0.00
Density−independent
Density−dependent
Density−independent
Density−dependent
Evolution
Evolving
Non−evolving


Diverse
Density−independent
Diverse
Density−dependent
Bottlenecked
Density−independent
Bottlenecked
Density−dependent
Evolving
Non−evolving
Evolving
Non−evolving
a−i
b−i
c−i
d−i
e−i
f−i
g−i
h−i
a−ii
b−ii
c−ii
d−ii
e−ii
f−ii
g−ii
h−ii
Population size
1500
1000
500
0
400
200
0
0 1 2 3 4 5 6
Generation


a
Evolving
b
Non−evolving
Population size
8000
6000
4000
2000
1000
250
0
0
2
4
6
Generation
Diverse
Density−independent
Density−dependent
Bottlenecked
Density−independent
Density−dependent


a
Diverse
b
Bottlenecked
Intrinsic growth rate (r)
2
1
0
0
1
2
3
4
5
Generation
Diverse
Density−independent
Density−dependent
Bottlenecked
Density−independent
Density−dependent


Realized fitness
2.0
1.5
1.0
0.5
0.0
10
50
100
Test density
Diverse
Density−independent
Density−dependent
Bottlenecked
Density−independent
Density−dependent
Evolution
Evolving
Non−evolving