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SHORT COMMUNICATION

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Genetic mixing facilitates adaptation to a novel environmental constraint

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Abstract

Climate change can affect the length and timing of seasons, which in turn can alter the time available for insects to complete their life cycles and successfully reproduce. Intraspecific hybridization between individuals from genetically distinct populations, or admixture, can boost fitness in populations experiencing environmental challenges. Admixture can particularly benefit small and isolated populations that may have high genetic load by masking deleterious alleles, thereby immediately increasing fitness, and also by increasing the genetic variation available for adaptive evolution. To evaluate the effects of admixture on populations exposed to a novel life cycle constraint, we used the red flour beetle, *Tribolium castaneum*, as a model system. Distinct laboratory lineages were kept isolated or mixed together to create populations containing 1–4 lineages. We then compared the fitness of admixed populations to 1-lineage populations while subjecting them to a shortened generation time for three generations. Admixture did not influence fitness after two generations. In contrast, in the third generation, admixed populations had significantly greater fitness compared with 1-lineage populations. The timing of the increase in fitness for the admixed populations suggests that adaptation to the novel environmental constraint occurred in the experimental populations. Our study highlights the importance of admixture for facilitating rapid adaptation to changes in seasonality, and more broadly to environmental change.

KEYWORDS
admixture, generation time, hybridization, red flour beetle, *Tribolium castaneum*

INTRODUCTION

Rapid adaptive evolution can enable plant and animal populations to persist through environmental change (Bell, 2013; Carlson et al., 2014). Given the strong selection pressures imposed by human actions in natural environments, a practical understanding of the factors that facilitate adaptation can help natural resource managers maintain healthy populations (Gonzalez et al., 2013). One process that has been shown to promote an increase in fitness in novel environments is admixture, or intraspecific hybridization between individuals from genetically distinct populations (Verhoeven et al., 2011).

Admixture tends to be most beneficial to small and isolated populations (Hardie & Hutchings, 2010), in which genetic drift, mutation, and inbreeding can lead to the loss of genetic variation and can increase the frequency of deleterious alleles. Such populations are said to harbour high genetic load, meaning their fitness is reduced due to maladaptive or deleterious alleles relative to an ideal population without those alleles (Hedrick & Garcia-Dorado, 2016). Admixture can increase population fitness through two mechanisms: (1) it can directly and immediately increase fitness by reducing genetic load (Whiteley et al., 2015) and (2) it can increase genetic variation and contribute beneficial alleles to the population, which enhances the potential for adaptive evolution in response to natural selection over time (Agashe et al., 2011; Barrett & Schluter, 2008). However, admixture may also result in reduced fitness (i.e., outbreeding depression) if the admixed populations are adapted to different environments or genetic incompatibilities exist (Lenormand, 2002).

Here, we evaluated the influence of admixture on populations exposed to a shortened and constrained generation time. Climate change and urbanisation have altered the timing and length of seasons that have conditions suitable for the survival and reproduction of many organisms. These shifts in seasonality often require organisms to evolve life history traits that make use of favourable seasons, including shorter generation times (Bradshaw & Holzapfel, 2008, 2010). For our experiment, we used the red flour beetle (*Tribolium castaneum*) (Herbst) as a model system similarly to Hufbauer et al. (2015). We abruptly transitioned beetle populations from an unregulated life cycle with overlapping generations to non-overlapping, 5-week generations, with reproduction confined to a short period each generation. We created experimental populations with four levels of admixture, or combinations of one to four lineages. These populations evolved for three discrete generations to allow us to address the following questions: (1) How does the level of admixture affect mean population fitness in the novel environment? (2) What mechanisms (reduction of genetic load, addition of genetic variation available for adaptation, or some combination) might underlie fitness differences among populations? By examining the effects of the level of admixture on population fitness for multiple generations, we can improve our understanding of how increased or restricted admixture might influence wild insect populations exposed to changes in phenology.

MATERIALS AND METHODS

Colony maintenance

We began with five lineages of *T. castaneum* (‘Lab-S Rusty’, ‘Estill’, ‘Ga-1’, ‘Z-2’, and ‘Z-4’) from a USDA stock centre (Kansas, USA). These lineages were chosen because they were wild-type phenotypes originally collected from a relatively limited geographic range (Midwestern and Southeastern US) and were therefore unlikely to have reproductive incompatibilities that can occur over large geographic distances, for example, across continents (Drury et al., 2011; Thomson et al., 1995). At the stock centre, beetles were reared without gene flow and under continuous and overlapping generations, thus without constraints on the timing of reproduction. Upon arrival at our laboratory, the *T. castaneum* lineages were kept separately in small 4 × 4 × 6 cm boxes, hereafter patches, containing 15 g of standard medium (95% wheat flour, 5% brewer’s yeast) and reared for a single generation prior to the start of the experiment.

Experiment initiation and propagation

To initiate the founding generation (G0), new experimental populations were created by outcrossing among the original lineages (Figure 1b: G0; Table S1). Here, we define a population as a group of interbreeding individuals. Thus, 56 populations were initiated consisting of four different admixture levels (1, 2, 3, or 4 lineages; 14 populations each) across three temporal blocks. Across blocks, there was partial overlap of the genetic background of individuals used to create populations (Table S1).

Each experimental population was founded with 39 or 40 adult beetles: 1-lineage populations contained 40 adults from a single lineage, 2-lineage populations contained 20 adults each from 2 lineages, 3-lineage populations contained 13 individuals from 3 lineages, and 4-lineage populations contained 10 individuals from 4 lineages. The beetles used to initiate these populations were about 5 weeks old (adults had enclosed within the week prior) and individuals were chosen without regard to their sex. Leaving sex ratio to chance can lead to variation in offspring number and the amount of admixture between populations, which may reduce our ability to detect differences among admixture levels. However, a study by Wade (1984) found that the apparent population size in laboratory colonies of *T. castaneum* was representative of the effective number of breeding adults in small populations (10 or 20 individuals with varying sex ratios). This suggests that approximately equal numbers of each lineage reproduced in the initial generation in our experiment (Wade, 1984).

In the experimental populations made up of 1–4 lineages, the founding adults were allowed to mate with other individuals in their patch and oviposit for 24 h. Adults were then discarded to allow the offspring to develop for another 34 days (Figure 1a). Thus, the generation time was constrained to 35 days in total. The abrupt shift from continuous generations to this strict life cycle regime, with oviposition occurring at 5 weeks old, likely imposed strong selection on these populations. Five weeks was used as a life cycle length to be comparable to other eco-evolutionary studies that use *T. castaneum* as a model organism in laboratory and mathematical modelling studies, for example, Hufbauer et al. (2015) (lab) and Melbourne and Hastings (2008) (lab and modelling).

With this design, the founding individuals enclosed as adults within their own lineage groups, so they were able to mate with other individuals within their lineage prior to founding the experimental populations. Therefore, the amount of outcrossing within populations containing multiple lineages in G0 was not known. However, two factors suggest that at least some offspring would be the product of outcrossing: (1) inbreeding, such as was likely in long-term maintenance of lineages at the stock centre, promotes increased mating frequency in female *T. castaneum* (Michalczyk et al., 2011), and (2) multiple mating is common in this species both within (Pai et al., 2007) and between lineages (Nilsson et al., 2003; Pai & Yan, 2002), with females mating with up to 12 males per hour (Pai et al., 2007).

To increase the sizes of our experimental populations and provide additional opportunities for outcrossing after the G0 mixing event, adults were allowed 72 h for mating and oviposition at the start of generation 1 (G1). Adults were then discarded and eggs were given an

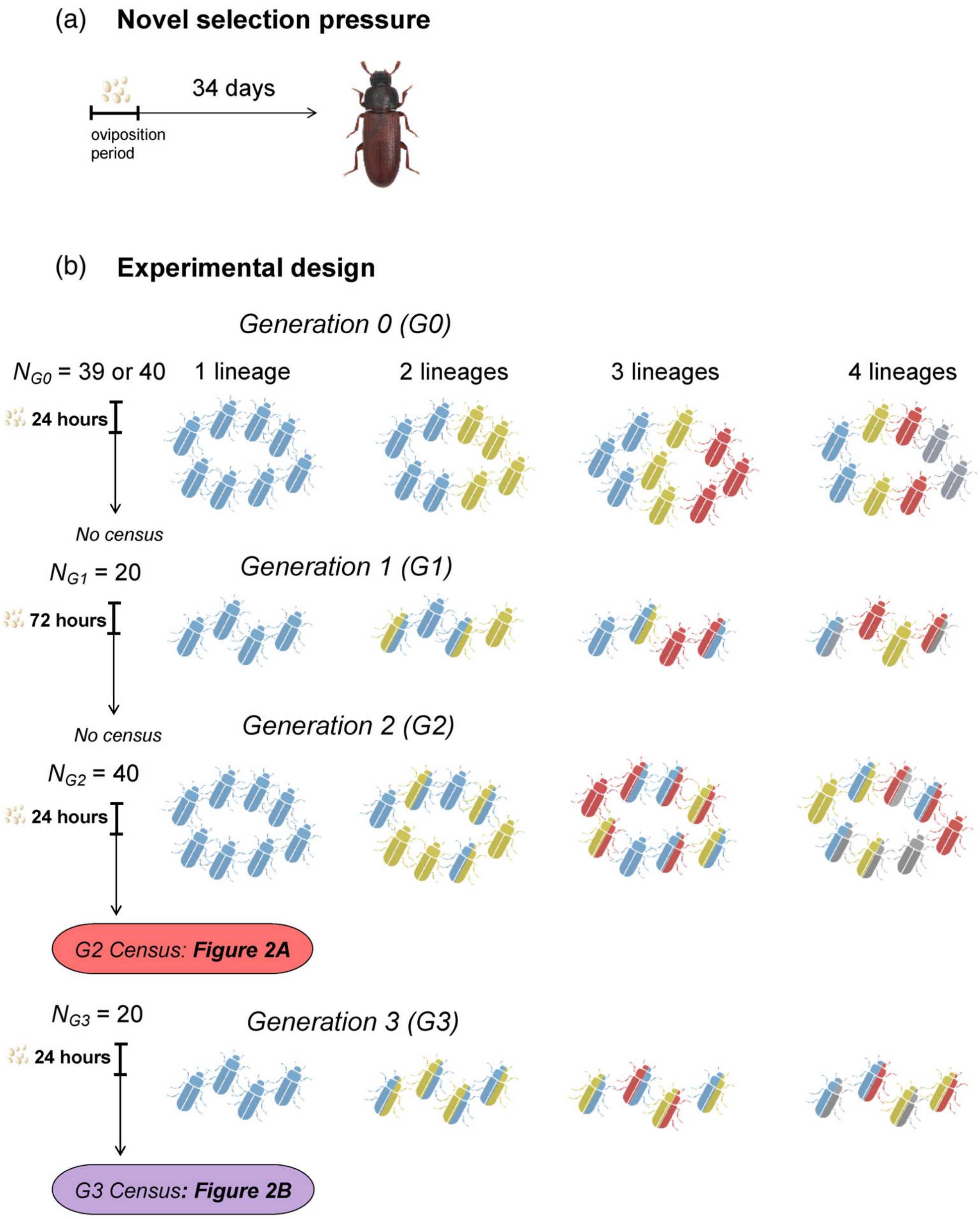

FIGURE 1 (a) The generation time and reproductive constraints imposed on the *Tribolium castaneum* populations in the experiment. (b) Experimental design showing how the four treatment groups were established through admixture over three generations. In generation 0 (G0), the 8 or 9 pictured beetles represent the 40 or 39 adults, respectively, that initiated each population. In G1, the 4 beetles represent 20 adults that initiated the generation, in G2 the 8 beetles represent 40 adults, and in G3 the 4 beetles again represent 20. Lower density was used in G1 and G3 to enable replication even when fewer adults were available. The colour changes represent admixture occurring, which likely increased over time. Photo by Tomasz Klejdysz/Shutterstock.com.

additional 34 days to develop (Figure 1b: G1). Thus, the generation time was constrained to 35 days in G0 and 37 days in G1. Generations 2 (G2) and 3 (G3) continued to constrain generation time, returning to a 24-h reproductive period and 34 days for development. Adult beetles were censused at the end of G2 and G3 (Figure 1b). Based on the number of adults available per population and to increase replication, G1 was initiated with 20 adults, G2 with 40, and G3 with 20 (Table S1). Individuals from each population were kept in 1–10 replicate patches in G2 and 1–20 patches in G3, depending on the size of the population.

Measuring population fitness and statistical analysis

We measured fitness as the mean number of surviving adult offspring per individual, that is, population size at census (N_t) divided by the initial population size (N_{t-1}) or $\frac{N_t}{N_{t-1}}$. Fitness data were analysed using two linear mixed effects models, one for each generation given the different values of N_{t-1}. Population fitness data were square root transformed for analysis. The models included the number of lineages per population (admixture level) as a fixed effect and temporal block and population identity (nested within block) as random effects. All

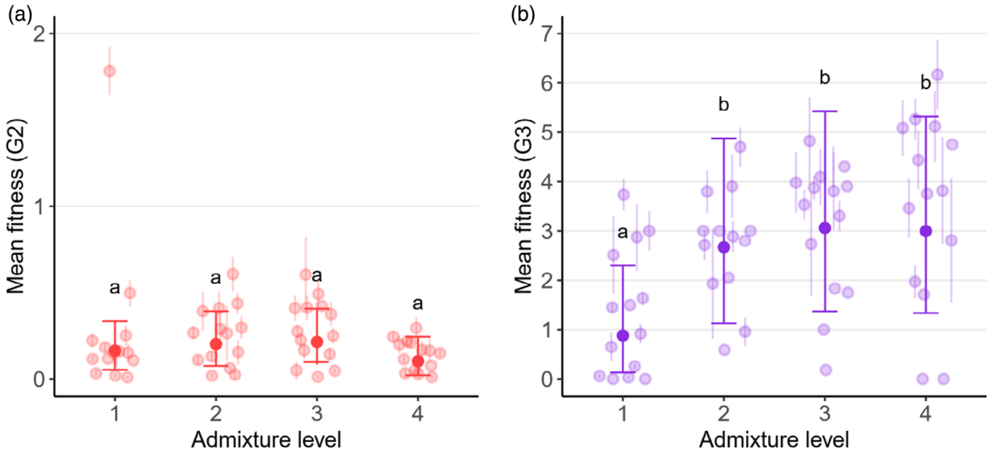

FIGURE 2 Mean fitness among populations with different admixture levels in (a) generation 2 (G2; red points), which was initiated with 40 individuals, and (b) generation 3 (G3; purple points), which was initiated with 20 individuals. Each point represents a population ±1 standard error generated from replicate patches (see Table S1). Points are jittered for clarity. Solid points are overall means from the fitted model for each admixture level with 95% model-generated confidence intervals. Letters represent significant differences at $p < 0.05$ after controlling for multiple comparisons (Tukey) within each generation. Note the different scales of *Y*-axes.

analyses were performed in R (R Core Team, 2021) using the *lme4* (Bates et al., 2015) and *emmeans* packages (Lenth, 2021). Residual versus fitted values and quantile–quantile plots of residuals were used to check that the assumptions of equal variance and normality were satisfied, respectively.

RESULTS

In generation 2 (Figure 2a), population fitness was not significantly different between admixture levels (Figure 2a; ANOVA: $F_{3,49.94} = 1.24$; $p = 0.305$).

In generation 3 (Figure 2b), the pattern was strikingly different, with admixture level significantly influencing fitness (Figure 2b; ANOVA: $F_{3,48.86} = 7.92$; $p < 0.001$). The 1-lineage populations had the lowest mean fitness of 0.88 (95% confidence interval [CI] [0.13, 2.30]) when compared to the 2–4 lineage populations (2.67, 95% CI [1.13, 4.88]; 3.06, [1.37, 5.42]; 3.00, [1.34, 5.32], respectively for admixture levels 2–4), which were comparable regardless of the number of lineages (Figure 2b).

The apparent overall difference in fitness between G2 and G3 should not be over interpreted given the difference in adult density (40 vs. 20) that initiated the populations in these generations.

DISCUSSION

This study evaluated the impacts of admixture on mean fitness under a shortened and constrained generation time, a life history shift that can be beneficial to insect populations experiencing changes in seasonality due to rapid climate change (Bradshaw & Holzapfel, 2010). Our results indicate that admixture (admixed vs. 1-lineage populations) increased fitness in populations exposed to this novel constraint in only three generations of selection. Furthermore, we found that there was not a clear effect of increased admixture (2- vs. 3- vs. 4-lineage populations) on fitness, as the 2-lineage populations performed similarly to the 3- and 4-lineage populations in G3. This suggests that even a low degree of outcrossing can facilitate population persistence in novel environments. These findings agree with other studies in model systems, including *Arabidopsis* (Crawford & Whitney, 2010), *Daphnia* (Loria et al., 2022), and other work with *Tribolium* (Agashe et al., 2011). Mean differences between G2 and G3 are likely due to negative density dependence primarily from egg cannibalism (Melbourne & Hastings, 2008), which had greater effects in G2 (initiated with 40 adult beetles) compared to G3 (initiated with half as many individuals).

We considered two mechanisms that can lead to increased fitness after admixture events: reduced genetic load and increased genetic variation for adaptation. In G2, all populations had statistically comparable fitness regardless of admixture level, suggesting that alleviation of genetic load did not occur in this generation. One possibility is that because we started with stock lineages that were likely highly inbred, deleterious alleles in those populations could have been purged prior to the start of the experiment. While purging of lethal alleles likely did occur, mildly deleterious mutations were likely to have been maintained. At the stock centre, the lineages were kept under benign conditions with adequate resources and little selection on development time, a setting that would allow mildly deleterious mutations to segregate, as well as alleles only deleterious in a more stressful environment (Fox & Reed, 2011). Results from a separate study (Olazcuaga et al., 2023) suggest that this is the case. Their experiment aimed to compare admixed populations, originally created by crossing the same *T. castaneum* lineages used in this experiment, that had experienced a bottleneck or not during their demographic history. After shifting them from a benign to a challenging environment, they found that growth rates were lower in the bottlenecked populations than in the admixed populations (Olazcuaga et al., 2023, summarised in Figure S1). This implies that there are segregated deleterious alleles in their admixed populations, and thus the lineages used in this experiment likely harboured some genetic load.

Here, we would expect fitness increases in admixed populations due to a reduction in genetic load to be apparent by G2, after two generations of outcrossing, which has been observed in animal populations in the field (Whiteley et al., 2015) and in *T. castaneum* populations in the lab (Stewart et al., 2017). However, fitness changes were not observed until G3, when we observed increased fitness in admixed populations relative to 1-lineage populations (Figure 2b). The three-generation time lag in the increase in fitness suggests that the dominant process was rapid adaptation to the shortened generation time, which occurred because selection for oviposition at 5 weeks of age acted on the increased genetic variation in the admixed populations. The relief of genetic load might have contributed to fitness increases as well, similar to the results of Hufbauer et al. (2015), who showed that adaptation and the reduction in inbreeding depression together increased fitness in declining populations.

Overall, this study provides experimental evidence for the benefits of admixture with as few as two lineages for promoting adaptation to a novel environmental constraint. More broadly, when thinking about biological invasions, our findings support limiting admixture via multiple introductions to mitigate the range expansion of invasive populations (Ellstrand & Schierenbeck, 2000). When applied to conservation, our findings support facilitating admixture for isolated populations to increase the genetic variation available for adaptation in novel environments imposed by climate change (Kardos et al., 2021; Whiteley et al., 2015).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

TABLE S1. The crossed lineages included in each experimental population and the replicate patches of each in generation 2 (G2) and generation 3 (G3). Populations are defined as individuals that interbreed each generation. Some crossed lineages (including single lineage groups with no admixture) were replicated temporally and thus had population representation in more than one block.

FIGURE S1. Comparison of growth rates between admixed and bottlenecked populations after experiencing a new challenging environment (modified from Olazcuaga et al., 2023). The admixed populations corresponded to replicated populations from a population created by crossing the five lineages used in this experiment, which was maintained for approximately 10 generations at a population size of ~10,000 individuals. The bottlenecked populations were created from this admixed population but had experienced a bottleneck event (reduction in population size to one pair of siblings for one generation) during their demographic history. These data represent the first generation of exposure to a challenging environment (corn flour) after several generations of rearing in a benign environment (wheat flour). Growth rate is measured as $\frac{N_t}{N_{t-1}}$ where $N_{t-1} = 100$. Olazcuaga et al. observed lower growth rates in the bottlenecked populations when compared to the admixed populations in this first generation in the challenging environment. Points represent population means and error bars represent 95% confidence intervals generated by a linear model (non-bottlenecked: 95% CI [3.02, 3.64], bottlenecked: 95% CI [2.34, 3.10]).

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