THESIS

THE ECO-EVOLUTIONARY CONSEQUENCES OF MULTIPLE INTRODUCTIONS FOR COLONIZING INDIVIDUALS

Submitted by

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ABSTRACT

THE ECO-EVOLUTIONARY CONSEQUENCES OF MULTIPLE INTRODUCTIONS FOR COLONIZING INDIVIDUALS

Predicting the fate of individuals colonizing novel habitats is an elusive but critical goal in fields as diverse as invasion biology, biological control, climate change-induced species range shifts, and reintroductions of rare species. Propagule pressure, which comprises the number of introduction events (propagule number) and the number of individuals per introduction event (propagule size), consistently correlates with a greater probability of population establishment. It is unclear which component, propagule number or propagule size, is more important for establishment, or under what environmental conditions their relative importance may shift. We used 917 independent *Tribolium* flour beetle populations in a microcosm experiment to disentangle the importance of the different components of propagule pressure. In a factorial design, we held the total number of introduced individuals constant (20) and varied the number of introductions used to distribute them (1, 2, 4, or 5 events) into stable or randomly fluctuating novel environments. Counter to expectations, we found no effect of environmental stability on extinction probability or time to extinction. We also found that several, small introduction events resulted in the lowest extinction probability and the longest time to extinction. We propose that continuing introductions provided low amounts of gene flow that were critical to alleviating inbreeding depression and/or reducing allelic loss by drift in the incipient populations. Our results speak to the importance of preventing future introductions of

invasive species (even those that are already established), and using sustained efforts to establish biological control agents or reintroduce desirable organisms to their former range.

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INTRODUCTION

For a population to exist and thrive, it must initially colonize a location in space. Thus, colonization is a fundamental process in ecology that underlies the past, present, and future distributions of species across the globe. Colonization can be a strictly natural process, but it is increasingly prevalent due to the influence of anthropogenic forces (Sakai et al. 2001, Cassey et al. 2005, Ricciardi 2007). The ubiquity and vast spatial extent of anthropogenic colonization often leads incipient populations to face environments that are entirely novel (Cassey et al. 2005, Ricciardi 2007). Whether colonization events to novel habitats are accidental (e.g. biological invasions) or deliberate (e.g. reintroductions of rare species, release of biological control agents), their success or failure has significant implications for natural resource managers and society (Mack et al. 2000).

Populations introduced to a novel environment can either diminish to extinction or successfully establish, gaining the potential to expand their geographic range and adapt to the new conditions (Lockwood et al. 2005, Colautti et al. 2006). Incipient populations are often small and face many threats to their existence from both external and internal forces

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[1] This is the working title for the manuscript that will be derived from this chapter and which will be submitted for publication in a peer-reviewed journal. These additional people will be coauthors on the manuscript submitted for publication: Meagan Oldfather, Brett Melbourne, Ruth Hufbauer.

(Fauvergue et al. 2012). External threats such as unsuitable novel environments (Gomulkiewicz and Holt 1995), random environmental catastrophes (Lande 1993), and environmental stochasticity (Lande 1993, Drake and Lodge 2004, Melbourne and Hastings 2008) act to reduce long-run population growth rates and increase extinction probability. Intrinsic threats to the population can be either demographic or genetic in origin, with some debate over which is more important for predicting extinction risk (Lande 1988, Boyce 1992, Spielman et al. 2004, Hufbauer et al. 2013, Szücs et al. 2014). Demographic threats include those of stochasticity (Lande 1993, Melbourne and Hastings 2008) and heterogeneity (Melbourne and Hastings 2008), Allee effects (Tobin et al. 2007, Fauvergue et al. 2012), as well as skewed sex ratios (a form of demographic heterogeneity) (Engen et al. 2003, Melbourne and Hastings 2008). Genetic drift can overpower selection in small populations, which can result in fixation of maladaptive alleles or the loss of adaptive alleles (Futuyama 1998). Drift and bottlenecks can reduce genetic diversity overall, representing a loss of evolutionary potential (Spielman et al. 2004). The reduction in diversity is likely to be especially important in the colonization of novel habitats (Crawford and Whitney 2010, Hufbauer et al. 2013). Prolonged periods of small population size will increase the probability that mating will occur between closely related individuals, at which point inbreeding depression may arise and further erode population fitness (Spielman et al. 2004). A population that is to be successful after colonization must mitigate these threats well enough to prevent or delay extinction.

A majority of the research on colonization success focuses on the invasiveness of organisms or the invasibility of the novel habitats. These species- and habitat-specific approaches may be limited in their generality about how and when a single population will establish (Lockwood et al. 2005). Instead, characteristics of the colonization event itself may form a stronger basis for predicting colonization success. Perspectives from studying the colonization event are more general because they transcend idiosyncrasies of taxa or geography, and can apply outside of invasion contexts. The same species can be introduced in different ways in different places, and the same place can experience different kinds of introductions by different species. By the same token, different species or places can experience similar kinds of introductions. Propagule pressure is one characteristic of a colonization event that is gaining increased attention as arguably the only consistent predictor of colonization success in novel habitats (Lockwood et al. 2005, Colautti et al. 2006, Simberloff 2009).

Propagule pressure has two components: the number of individuals per introduction event—termed propagule size, and the number of introduction events— termed propagule number. Greater propagule pressure, as the combined concept of these two components, correlates with greater colonization success across taxa (Lockwood et al. 2005, Colautti et al. 2006, Simberloff 2009), but the two components themselves have been historically challenging to parse (Lockwood et al. 2005, but see Drake et al. 2005). It is not clear which component of propagule pressure is more important for colonization success. Even if the total number of individuals introduced to a new area is fixed, theory suggests that the probability of population establishment will be affected by an interaction between the number of introduction events and the degree of environmental variability in the recipient environment. Several, smaller introductions should establish populations with a greater probability in variable environments due to the accumulating likelihood that one of those independent events will occur serendipitously in a particularly good habitat patch or time period. A single, larger event should be more likely to establish a population in stable environments because the greater extinction risks inherently faced by small populations can be avoided (Grevstad 1999).

Undoubtedly, a confluence of environmental, demographic, and genetic factors will determine how a single, large introduction event versus several, small introduction events will affect colonization success (Grevstad 1999, Dlugosch and Parker 2008, Szücs et al. 2014). Laboratory microcosm experiments are optimally suited to disentangle factors that confound each other in natural settings. Additionally, multiple generations can be observed in short time periods in lab studies, which is both convenient—as multiple introductions will necessarily take time to implement, and generalizable— as extinction risk scales better to generations than years (O'Grady et al. 2008). We used the red flour beetle, *Tribolium castaneum*, as a model system to assess how the number of introduction events affects colonization success in stable and fluctuating novel environments when the total number of individuals introduced is held constant. We hypothesized that populations established via a single, large introduction event would have the lowest extinction probability (and the longest time to extinction) in stable environments, but that several, small introductions would have the lowest extinction probability (and the longest time to extinction) in fluctuating environments.

METHODS

Study System

Our laboratory rearing of *Tribolium castaneum* flour beetles was modified from Melbourne and Hastings (2008, 2009). *Tribolium* individuals used in this experiment were all taken from the same meta-population source, representing thousands of randomly mating individuals. Each experimental population was reared in a 4cm x 4cm x 6cm plastic box (AMAC Plastic Products) with 2 tablespoons of freshly prepared growth medium that had been humidified for at least 24 hours. Standard growth medium, the natal environment, comprised 95% wheat flour (Pillsbury Co. or Gold Medal Products Co.) and 5% brewers' yeast (Sensient Flavors). The novel growth medium comprised a small percentage of standard growth medium mixed with corn flour (Bob's Red Mill). *Tribolium* flour beetles are known to exhibit strong maternal effects (Dawson 1964, Enfield et al. 1966), so individuals from the source population were reared on novel growth medium for 1 generation prior to using them in the experiment. All populations were reared in one of two dark incubators (Sanyo) at 31° and approximately 70% relative humidity (standard conditions) and were haphazardly rotated between incubators weekly.

In each box, a known number of adults laid eggs for 24 hours in fresh medium, and were then removed. The eggs remained and developed through larval and pupal stages into adults. A complete census of the resulting adults was conducted for each population after exactly 35 days, at which point those adults were allowed to lay eggs on freshly prepared growth medium for 24 hours, completing their laboratory life cycle. This manipulation modeled organisms with non-overlapping generations, as in the case of annual life history strategies. We estimated census error to be less than 2% per population in any given time-step, with approximately equal variance across observers (see Appendix D). Experimental populations were maintained in this way for 9 generations.

Treatments

Tribolium flour beetles were introduced with varying propagule size (number of individuals per introduction event) and propagule number (number of introduction events) to experimental patches whose environment was either stable or fluctuating through time. The total number of individuals introduced to each patch was held constant at 20. This total was low enough to allow some population extinction within the experiment timeframe, but high enough to be representative of documented introductions in the literature (Simberloff 1989, with other references in Simberloff 2009; Grevstad 1999; Berggren 2001; Taylor et al. 2005; Drake et al. 2005).

The 20 individuals were introduced into patches in four ways: all 20 at once in the first generation, 10 individuals in each of the first 2 generations, 5 individuals in each of the first 4 generations, and 4 individuals in each of the first 5 generations. These four introduction treatments were repeated for two types of novel environmental regimes: stable and randomly fluctuating. Patches with a stable environment were replenished with the same novel growth medium mixture for all 9 generations, while patches with a fluctuating environment were replenished with a random growth medium mixture in each generation.

The percentage of standard medium in the environment used to create the novel growth medium was chosen in light of the expected, long-term, geometric mean population growth rate and the expected degree of environmental stochasticity that might affect that growth rate (assuming no adaptation). Expected mean growth rates and environmental stochasticity values were selected to be biologically realistic and to produce manageable population growth without deterministic extinction (that is, expected λ slightly greater than 1). A pilot study corroborated findings from Szücs and Hufbauer (unpublished data) in suggesting that a novel growth medium containing 0.95% standard medium (99.05% corn flour) would result in a discrete, one-generation, mean population growth rate of 1.2 (compared to a mean population growth rate of 3.36 on 100% standard medium). This mixture was used through all generations for populations in the stable environment treatment. To implement the randomly-fluctuating treatments, a range of 9 possible environment mixtures from 0.58% standard medium (99.42% corn flour) to 1.32% standard medium (98.68% corn flour) was chosen to mimic environmental stochasticity measured in nature (Sæther and Engen 2002) while maintaining population growth rates that were between 0.5 and 1.5, the cutoff for biologically realistic population trajectories used by Morris et al. (2008). These mixtures were predicted to yield mean population growth rates between 0.88 to 1.33 (Table 1).

Because demographic stochasticity was likely important due to both the population sizes throughout the experiment (Lande 1993) and the study system itself (Melbourne and Hastings 2008), we derived environmental stochasticity as the difference in mean total stochasticity between populations in the fluctuating and stable environment treatments (Sæther and Engen 2002). We assumed that total stochasticity was a combination of demographic and environmental stochasticity for populations in the fluctuating environment, and that demographic stochasticity was the sole contributor to total stochasticity for populations in the stable environment treatment. Thus, the difference in mean total stochasticity between the two treatments represents the experimentally imposed environmental stochasticity.

We only calculated total stochasticity for populations that did not experience extinction throughout the experiment period. We used this subset to capture the full temporal extent of environmental fluctuations during the experiment, and because extinctions would have an infinite effect on this measure of stochasticity.

Table 1: Novel growth medium mixtures and $\lambda_{expected}$ for populations in the fluctuating treatment. The 0.95% standard medium mixture was used in each generation for populations in the stable treatment.

% Standard	% Corn	$\lambda_{expected}$
0.580	99.420	0.877
0.72	99.328	0.981
0.765	99.235	1.070
0.857	99.143	1.141
0.950	**99.050**	**1.20**
1.042	98.958	1.245
1.135	98.865	1.282
1.228	98.772	1.311
1.320	98.680	1.333

The growth medium for each patch was replenished just prior to oviposition for both environment treatments. For the stable treatment, the 0.95% standard medium mixture was used ($\lambda_{expected}$ = 1.2 across all 9 generations) while the patches in the fluctuating treatment were replenished with a growth medium that was randomly chosen from 9 possible mixtures (Table 1). Each population in the randomly fluctuating treatment experienced a different sequence of growth medium mixtures independent of the sequences of all other populations. Sequences were selected to ensure that the expected, long-term geometric mean population growth rate for each patch resembled expected growth of populations in the stable treatment ($\lambda_{expected}$=1.2±0.05 across all 9 generations).

Wheat flour contamination with pesticide

We discovered that the wheat flour (Pillsbury Co.) mixed into the growth medium was contaminated with an insect growth regulator pesticide during development of the F_5 generation. This did not appear to affect experimental population growth rates, likely because populations were exposed to such a low volume of the wheat-flour-containing standard medium (Table 1). For the F_6 through F_7 generations (all blocks) and the F_8 generation (blocks 1 and 2), a different wheat flour was used to create growth media (Gold Medal). Partially through development of the F_8 generation, we discovered a different kind of pesticide action when using this Gold Medal wheat flour. Again, it did not appear to affect experimental populations that were reared on growth media that were mostly corn flour. It was already too late to switch the type of wheat flour used to make media for the F_8 generation of blocks 1 and 2, but for the F_8 generation and onwards in blocks 3 and 4 (and the F_9 generation and onwards in blocks 1 and 2), all media was made using Gold Medal organic wheat flour. It cannot be ruled out that the higher population growth rate experienced by populations between the F_5 and F_6 generations isn't attributed to Gold Medal traditional flour being a superior food resource, but this seems unlikely since no population increase was observed in control populations reared on Gold Medal traditional flour in a parallel experiment.

Statistical analyses

Populations were deemed extinct if no adults were present upon census and no further introductions were forthcoming. After 9 generations, a binary response of extinct vs. extant was assessed for each population. For each extinct population, we noted the number of generations before the extinction occurred since the beginning of the experiment period as well as since the final introduction for its particular introduction scenario.

Total stochasticity (demographic plus environmental) of each population that didn't experience extinction (n=667) was calculated as the variance of the natural logarithms of its population growth rates through 9 generations:

$$s_{total} = s_{demographic} + s_{environmental} = var(\log(\lambda_t))$$

Where, for a particular population, s_{total} is its total stochasticity, $s_{demographic}$ is its demographic stochasticity, $s_{environmental}$ is its environmental stochasticity (assumed to be 0 for populations in the stable environment), and λ_t is its per capita population growth rate in the *t* generation (*t=1, 2, … , 9*).

Environmental stochasticity was calculated as the difference in mean total stochasticity between populations in the fluctuating treatment and in the stable treatment using the least squared means from a linear mixed effects model. We used environmental stability as the only fixed effect, and block as the only random effect with a Kenward-Roger approximation for the degrees of freedom.

To assess extinction probability in 9 generations, a generalized linear mixed model was used to predict the binary response of extinction (logistic regression) after checking for overdispersion. Linear mixed models were used to analyze both measures of time to extinction after checking for homoskedasticity and normality of residuals. In each of the three mixed models, the introduction scenario, the environmental stability, and their interaction were treated as fixed effects, while block and its interactions with all fixed effects were treated as random effects. We used likelihood ratio tests in a backward elimination procedure to test the significance of random effects by comparing saturated models to a model with a single term left out. Group-level significance of fixed effects was also tested using likelihood ratio tests, but only to focus the interpretation of contrasts; all fixed effects were left in the final model. Pairwise comparisons without adjustment were made between the levels of the significant fixed effects (Gotelli and Ellison 2004). All statistical analyses were performed in R, version 3.1.1 (R Core Team 2014). Generalized linear mixed models were constructed using the lme4 package (Bates et al. 2014). Likelihood ratio tests of random effects were performed using the anova() function in base R. To determine group-level significance of the fixed effects in the generalized linear mixed models, we used the likelihood ratio test method in the mixed() function from the afex package (Singmann 2014) as recommended by Bates et al. (2014). To assess group-level significance of fixed effects in our linear mixed models, we used a Kenward-Roger approximation for denominator degrees of freedom using the lmerTest package

(Kuznetsova et al. 2014). Pairwise comparisons were made using the lsmeans package (Lenth 2014). The R code fitting these models is included in Appendix A and the raw data that were analyzed are included in Appendix B.

Note on experimental conditions

During the experiment period, relative humidity dropped to ~20% for a period of 2 days for about half of the experimental populations approximately evenly across treatments and blocks. This "drought" was treated as a fixed effect in each mixed model, but was deemed not significant using a likelihood ratio test and was therefore dropped from future models. Also, 75 populations scheduled for an additional introduction during generation 2 received that input in generation 3 instead due to a lack of available *Tribolium* individuals from the source population at that time. This introduction gap was also treated as a fixed effect in all models but was dropped given that likelihood ratio test were not significant in each case.

RESULTS

Extinctions accumulated regularly throughout the experiment period (Figure 1), with 150 out of 917 populations (16.36%) going extinct within 9 generations. The additional introductions that some populations received were often immediately important for recolonizing a patch that had temporarily gone extinct. Out of 677 populations that received more than 1 introduction (i.e. not the 20x1 introduction scenario), 115 of them (16.99%) temporarily went extinct at least once before being replenished by additional colonizing individuals. Thirteen populations were rescued in this way at least twice, and one population was rescued in this way three times.

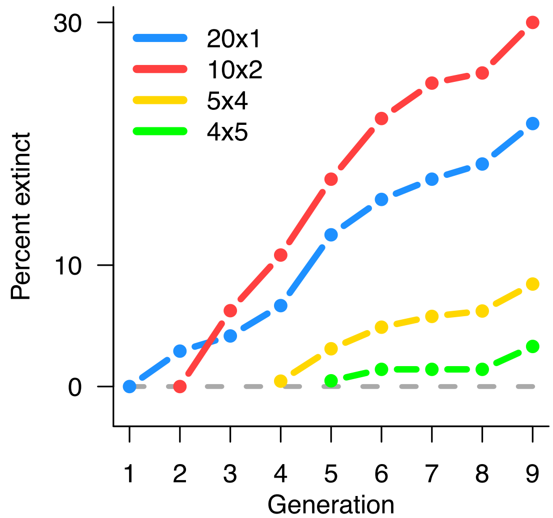
The variation of all block interactions were not significantly different from zero as determined by non-significant likelihood ratio tests, so the random effects structures for all models were simplified to a random intercept effect of temporal block.

Imposed Environmental Stochasticity

Environmental stochasticity imposed by the fluctuating treatment, calculated as the mean total stochasticity for the fluctuating environment treatment minus the mean total stochasticity for the stable environment treatment, amounted to 0.047 ($t=2.33$, $df=662.7$, $p=0.0201$). This value is near the median value of 0.055 measured in nature by Sæther and Engen (2002) in a metaanalysis of 35 avian populations.

Extinction Probability

The probability of extinction did not increase in the variable environment, contrary to our initial expectations ($\chi^2=1.001$, $df=1$, $p=0.317$), nor was it affected by an interaction between environmental stability and introduction scenario ($\chi2=2.922$, $df=3$, $p=0.404$). However, extinction probability was shaped strongly by introduction scenario ($\chi2=80.722$, $df=3$, $p<0.0001$). After pooling data by environmental stability, pairwise comparisons revealed that the extinction probabilities of all introduction scenarios were significantly different from each other. The 10x2 introduction scenario had the greatest extinction probability and the 4x5 introduction scenario had the lowest extinction probability (Figure 2).



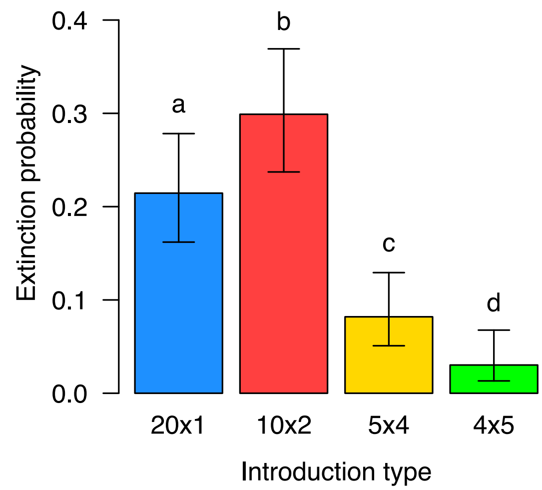


Figure 1: Percent of populations that went extinct for the 4 different introduction scenarios. Data are pooled across the two environmental stability treatments.

Figure 2: Modeled extinction probabilities and 95% confidence intervals for each introduction scenario from a logistic regression. Bars with different letters over them represent introduction scenarios with significantly different extinction probabilities at an α=0.05 level.

Time to Extinction

For populations destined for extinction, the environmental stability did not affect how long it took for them to go extinct. This was true regardless of the reference time for calculating this response (since the first introduction: $F_{1, 141.8}$<0.001, p=0.992; since the final introduction $F_{1, 141.9}$<0.001, p=0.986). There was also no significant interaction between environmental stability and introduction scenario in predicting time to extinction measured from either reference time (since first introduction: $F_{3, 141.3}$=2.606, p=0.0541; since final introduction: $F_{3, 139.2}$, p=0.0611). The introduction scenario did have a significant effect on the time to extinction for both reference times (since first introduction:

$F_{3, 140.9}$=3.771, p=0.012; since final introduction: $F_{3, 141.3}$=5.839, p<0.001). Restricting the post hoc pairwise comparisons to the levels of introduction scenario, we found that the fewer, larger introduction scenarios (20x1 and 10x2) led to a shorter time to extinction compared to the many, smaller introduction scenarios (5x4 and 4x5) when measuring since the first introduction (Figure 3a). However, when measuring time to extinction since the final introduction event for a given introduction scenario, populations established via a single large introduction persisted significantly longer than all other introduction scenarios (Figure 3b).

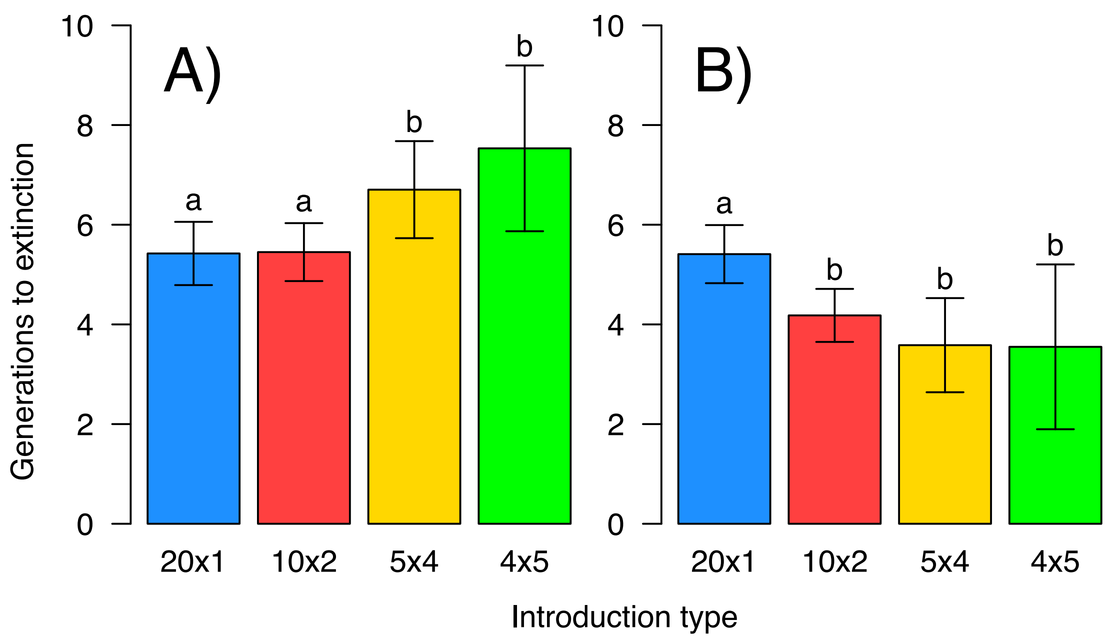


Figure 3: Time to extinction in generations for each introduction type measured since (A) the first introduction and (B) the final introduction for each introduction scenario. Within a panel, bars with different letters above them are significantly different from each other at an α=0.05 level.

DISCUSSION

In this experiment, we were able to parse the components of propagule pressure by controlling the total number of individuals introduced to a novel habitat while manipulating the number of introduction events used to distribute those individuals. Patches that were colonized via several, smaller introduction events (5 individuals in each of 4 events and 4 individuals in each of 5 events) were significantly less likely to go extinct compared to patches colonized by fewer, larger introduction events (20 individuals in 1 event and 10 individuals in each of 2 events). Surprisingly, this trend held in both stable and fluctuating environments. The number of introduction events also significantly affected the time to extinction for those populations that did go extinct. When time to extinction was measured from the first introduction, more introduction events forestalled extinction. However, when time to extinction was measured from the most recent introduction event, a single large introduction persisted the longest. Again, the environmental stability had no significant bearing on either measure of time to extinction.

Despite imposing a biologically realistic and statistically discernible environmental stochasticity treatment, we found no effect on extinction probability or time to extinction. This finding is inconsistent with the prediction made by (Grevstad 1999) who suggested that more, smaller introductions would lead to greater colonization success with greater environmental stochasticity. Environmental stochasticity is known to decrease a population's long-run growth rate, but it may not be a primary driver of extinction (Lande 1993, Melbourne and Hastings 2008). The implication that environmental stochasticity will be important in predicting colonization success may arise from how environmental stochasticity was imposed by Grevstad (1999) in her model, whereby survivorship from year to year was sampled from a beta distribution with a mean of 0.02 and a standard deviation between 0.01 and 0.04 depending on the magnitude of environmental variability. Especially with a greater environmental variability (above 0.02), a large proportion of time steps in this model will result in considerable population loss. This kind of environmental variability may be more akin to random catastrophes—major events causing immediately disastrous effects to the population, rather than environmental stochasticity—regular, mild to moderate perturbations in population growth rate due to external forces from year to year (*sensu* Lande 1993). If Grevstad's (1999) increasingly variable environment is interpreted in the context of random catastrophes instead, then her prediction that several, smaller introductions should reduce extinction probability coincides more closely with Lande's (1993) expectation of increased extinction risk with a greater frequency or intensity of catastrophes. Since we didn't impose environmental variability that could be considered random catastrophes, but still found that many, small introductions reduced extinction probability, some other mechanism must be at work.

The simplest explanation of this pattern is that the 9-generation experiment period did not provide for an equal number of population extinction opportunities for all introduction scenarios. For instance, the 5x4 (5 individuals in each of 4 generations) introduction scenario only had 6 generations to accumulate extinctions, while the 4x5 scenario could only accumulate extinctions for 5 generations, due to sustained inputs of beetles through the first half of the experiment. On the other hand, the 20x1 scenario had 9 generations and the 10x2 scenario had 8 generations to accumulate extinctions. This simple explanation seems unlikely, however. The longest time period after a final introduction event that was common to all introduction scenarios was 5 generations. Standardizing across the four different introduction scenarios by looking at extinction probability 5 generations after the final introduction event (at generation 5 for the 20x1 scenario, generation 6 for 10x2, generation 8 for 5x4, and generation 9 for 4x5; Fig. 1), we see that the fewer, larger scenarios still exhibit significantly more extinction.

We propose instead that the sustained immigration from the external source population was a sufficient mechanism by which extinction probability was reduced. We expected that the many, small introduction scenarios would be plagued by detrimental effects of demographic stochasticity compared to the scenarios with larger introduction sizes, but their relative success suggests that the benefits of immigration outweighed those detriments. The rescue effect of immigration can act demographically (e.g. increasing the size of populations) or genetically (e.g. increasing the fitness of populations), or by some combination (Brown and Kodric-Brown 1977). Certainly, demographic rescue played a critical role for the 115 populations that went extinct temporarily until another introduction event revived them. The demographic story can't be whole picture, however. A temporary extinction in a population's past did not significantly increase its future extinction probability, and the cumulative number of beetles added to the population from the external source prior to a temporary extinction (i.e. representing the total "loss" of potential inputs) also did not lead to a greater extinction probability.

Because all populations received the same total number of beetles (20) from the external source, and any "losses" of those inputs didn't affect extinction probability, we expect that a genetic component of immigration is important in this case. This genetic effect seems to be greatest at low migration rates, likely ruling out the mechanism of immigrants providing additional raw genetic material on which selection can act (Novak 2007), which should benefit from greater migration (Perron et al. 2007). Thus, a more likely mechanism by which immigration reduced extinction probability was by relieving inbreeding depression or counteracting drift-induced allele loss. Small populations are more prone to experiencing inbreeding depression and loss of allelic richness, which can reduce population growth rates and increase extinction risk (McCauley and Wade 1981, Saccheri et al. 1998, Spielman et al. 2004, Frankham 2005, O'Grady et al. 2006, Szücs et al. 2014). However, even small amounts of gene flow can alleviate these effects (Slatkin 1985, Mills and Allendorf 1996), so the several, smaller introductions with propagules taken from the large source population were well-suited to bring about longer-term relief. This is borne out in the time to extinction, as well. The several, smaller introduction scenarios resulted in a longer time to extinction when measuring from the first introduction event, but the single, large introduction scenario resulted in the longest time to extinction when measuring from the final introduction event for each scenario. This difference suggests that additional introductions are important for sustaining populations through the introduction period, thereby delaying extinction.

The importance of a genetic component to the sustained immigration will have long-term implications for population persistence and adaptation. First, adaptation is expected to occur in different ways depending upon immigration rate, relative population fitness, and the speed at which the environment changes (Perron et al. 2006, 2007). Introductions to a novel habitat, representing a discrete change in the environment, can result in adaptive evolution with the right amount of gene flow (Holt and Gomulkiewicz 1997) and so introductions that sustain a sink population may prevent extinction long enough to allow for adaptation. Further study is necessary to evaluate how immigration affected adaptation in this system, if at all (Gomulkiewicz and Holt 1995, Boulding and Hay 2001). Second, the populations in the 10x2 introduction scenario, which had the highest migration rate and the greatest extinction probability, may have suffered from genetic swamping whereby the homogenizing effects of gene flow overpowered ongoing local adaptation (Mills and Allendorf 1996, Rhymer and Simberloff 1996, Lenormand 2002). Alternatively, negative density dependence may have reduced population fitness when migration rates were high, reducing population growth rates and hampering the spread of adaptive alleles (Holt and Gomulkiewicz 1997).

Our work does not consider Allee effects explicitly. Negative density dependence can be observed in this system at low population densities (Szücs et al. 2014), which implies that Allee effects in the classic sense (Allee 1931) may play a limited role in population fitness. Limitations to mate-finding are most often implicated as Allee effects (Boukal and Berec 2002), but the small microcosm size in this experiment and high potential rate of mating in *Tribolium* (Arnaud and Haubruge 1999) may suggest that mate-finding isn't limiting in this system. A lack of Allee effects may still mimic natural colonization, however. Other detriments to small populations (e.g. demographic stochasticity, skewed sex ratio) were inherently present in our experiment and could be considered Allee effects since they produce the same patterns in natural populations (Lande 1998, Engen et al. 2003, but see

Dennis 2002). The fact that multiple mechanisms may give rise to a positive relationship between fitness and population size may be why experimental evidence of Allee effects in natural colonization may be weak (Fauvergue et al. 2012) or why it may not be found at all (Fauvergue et al. 2007). If present, Allee effects would increase the extinction probability of populations below a certain size, leading to an overall greater extinction probability for populations introduced by several, smaller introduction events (Hopper and Roush 1993, Grevstad 1999). This could dampen or reverse the results that we found in our experiment, where many, smaller introduction events decreased extinction probability and increased time to extinction. Despite this uncertainty about whether Allee effects are present in natural colonization, it is important to consider our results with them in mind.

CONCLUSION

The threat of extinction from environmental stochasticity in the case of many, small introduction events to a novel habitat may be overstated. Though random catastrophes may shift this expectation of extinction, our research suggests that the impact of environmental stochasticity per se may be minimal. The combination of immigration and environmental stochasticity may actually augment populations in some cases such as when the stochasticity is autocorrelated (Stacey and Taper 1992, Gonzalez and Holt 2002, Holt et al. 2004, Matthews and Gonzalez 2007).

When the total number of founding individuals is limited, a sustained introduction effort through time may be more important in promoting population establishment and persistence than the size of each of those introduction events so long as Allee effects are small or can be minimized. For invasions, this highlights the importance of preventing further introductions, even for established species. For conservation and biological control, this suggests emphasis should be placed on increasing the number of (re)introduction events, rather than increasing the size of those events. Sustained introduction efforts should also bring about concomitant benefits in the form of longer-term monitoring, increased data collection, and more opportunities for experimentation (Lockwood et al. 2005, Godefroid et al. 2011).

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APPENDIX A: R CODE

EXTINCTION PROBABILITY ANALYSIS

```
### Title: Extinction probability analysis script
###
### Author: Michael Koontz
###
### Date Created: 20140609
### Last Updated: 20141004
###
### Purpose: Executes a generalized linear mixed model (logistic regression) to assess
 whether treatments have different probabilities of populations going extinct after 9
 generations. First determines if it is appropriate to drop a few factors that arose as
 logistical complications throughout the course of the experiment (gap in introduction
 history when founding the 2nd generation and the drought in one incubator during
 development of F3 offspring). I then assess whether the interaction between environment
 type (stable vs. fluctating) and introduction scenario (20x1, 10x2, 5x4, 4x5) is significant,
 followed by the factors individually. The final model includes only the introduction type
 plus a random block intercept.

# Set working directory and source in data modification functions
setwd("/Users/mikoontz/Documents/Research/Tribolium/Demography")

# Load required packages; uncomment the next line if they need to be installed first
library(lme4)
library(lsmeans) # For using anova() and then
library(afex)

# Read data and coerce Block and intro columns to be factors
beetles <- read.csv("Analysis code/Extinction
 analysis/20140723_parsing_propagule_pressure_logistic_regression_extinction_data.csv"
 )
beetles$Block <- as.factor(beetles$Block)
beetles$intro <- as.factor(beetles$intro)

### A different approach to testing the effect of an introduction gap on extinction
 probability. First subset the data such that only the introduction treatments that may
 have had a gap are being analyzed (10x2 and 5x4)
### Then build a full model with all possible predictor factors and a reduced model that
 doesn't include introduction gap

### First subset to only have 10x2 and 5x4 treatments
beetles.gap.test <- beetles[(beetles$intro %in% c(10,5) & beetles$Block %in% c(1,2,4)) , ]

### Full model
intro.gap.full.model <- glmer(extinct ~ intro*env + gap + drought + (1 + env*intro | Block),
```

```
 family="binomial", data=beetles.gap.test, control=glmerControl(optimizer="bobyqa"))
### Reduced model with introduction gap removed
intro.gap.model <- glmer(extinct ~ intro*env + drought + (1 + env*intro | Block),
 family="binomial", data=beetles.gap.test, control=glmerControl(optimizer="bobyqa"))
summary(intro.gap.model)

# Use the anova function to perform a likelihood ratio test
anova(intro.gap.full.model, intro.gap.model)
# Conclusion: Model with introduction gap is not more likely than the model without
 (when analyzed with just the treatments that could have had the gap), so this factor will
 be dropped from even the full model in future analyses

beetles.binomial <- beetles

### First test for 3 way interaction with block
full.model <- glmer(extinct ~ intro * env + drought + (1 + env*intro | Block),
 family="binomial", data=beetles, control=glmerControl(optimizer="bobyqa",
 optCtrl=list(maxfun=1e6)))
summary(full.model)

three.way.interaction.model <- glmer(extinct ~ intro * env + drought + (1 + env+intro |
 Block), family="binomial", data=beetles, control=glmerControl(optimizer="bobyqa",
 optCtrl=list(maxfun=1e6)))

anova(full.model, three.way.interaction.model)

### Conclusion: Model with 3 way interaction is not more likely than model without, so
 we drop it.
full.model <- three.way.interaction.model

env.block.model <- glmer(extinct ~ intro * env + drought + (1 + intro | Block),
 family="binomial", data=beetles, control=glmerControl(optimizer="bobyqa",
 optCtrl=list(maxfun=1e6)))

anova(full.model, env.block.model)
### Conclusion: Model with random slope for environment not more likely than model
 without. Drop it from future models.

full.model <- env.block.model

intro.block.model <- glmer(extinct ~ intro * env + drought + (1 | Block), family="binomial",
 data=beetles, control=glmerControl(optimizer="bobyqa", optCtrl=list(maxfun=1e6)))

anova(full.model, intro.block.model)
full.model <- intro.block.model
# Conclusion: Model with random slope for introduction type is not more likley than
```

model without

```
### We have simplified our random effects structure. Now let's check drought...

drought.model <- glmer(extinct ~ intro * env + (1 | Block), family="binomial",
 data=beetles, control=glmerControl(optimizer="bobyqa", optCtrl=list(maxfun=1e6)))

anova(full.model, drought.model)
### Conclusion: Model with drought is not more likely than model without, so drop it.

final.model.binomial <- drought.model

# Check for group-level significance of each covariate
mixed(extinct ~ intro*env + (1 | Block), data=beetles.binomial, family="binomial",
 method="LRT")

# Check for overdispersion by dividing deviance by the residual degrees of freedom (732.2
 / 908). This is less than 1, so the model is not overdispersed

results <- summary(final.model.binomial)
posthoc.prob <- lsmeans::lsmeans(final.model.binomial, pairwise ~ intro, adjust="none")

# least square means (averaged across environment)
# 95% confidence intervals
# Back transforming using inverse logit to get proportion
mean.prop <- rev(plogis(summary(posthoc.prob)$lsmean$lsmean))
lower.CI <- rev(plogis(summary(posthoc.prob)$lsmean$asymp.LCL))
upper.CI <- rev(plogis(summary(posthoc.prob)$lsmean$asymp.UCL))

### Bar plot showing results of pairwise comparisons across introduction scenario
par(mar=c(4,2,2,2), oma=c(0,2,0.5,0))
figure2 <- barplot(mean.prop, ylim=c(0, 0.45), col=c("dodgerblue", "brown1", "gold",
 "green"), ylab=NA, xlab=NA, las=2)
mtext(text=c("20x1", "10x2", "5x4", "4x5"), side=1, at=figure2, line=0.5)

mtext(text="Extinction probability", side=2, line=2.5)
mtext(text="Introduction type", side=1, line=2)

arrows(x0=figure2, y0=lower.CI, x1=figure2, y1=upper.CI, angle=90, code=3, length=0.1)
text(x=figure2, y=upper.CI+0.03, label=c("a", "b", "c", "d"))
```

TIME TO EXTINCTION ANALYSIS

```
### Title: Time to extinction analysis script
###
### Author: Michael Koontz
###
### Date Created: 20140609
### Last Updated: 20141020
###
### Purpose: Executes a linear mixed models to assess whether treatments have different
 times to extinction. First determines if it is appropriate to pool the two environment
 treatments (stable vs. fluctuating) and if we can ignore the drought and the introduction
 gap.

setwd("/Users/mikoontz/Documents/Research/Tribolium/Demography")

# Load libraries
library(lme4)
library(afex)
library(lsmeans)

# Read data
beetles <- read.csv("Analysis code/Extinction
 analysis/20140723_parsing_propagule_pressure_logistic_regression_extinction_data.csv")

beetles$Block <- as.factor(beetles$Block)
beetles$intro <- as.factor(beetles$intro)
beetles$gap <- as.factor(beetles$gap)
beetles$drought <- as.factor(beetles$drought)

# Subset data such that we are only using populations that did go extinct
beetles.time <- subset(beetles, subset=!is.na(time.to.extinct))

# Testing for importance of introduction gap first. Subset by those treatments that had the
 potential for a gap.
beetles.gap.test <- beetles.time[beetles.time$intro %in% c(10,5) &
 beetles.time$Block %in% c(1,2,4) , ]

# Residuals look normal, with equal variance so we can use a linear mixed effects model
 instead of a generalized linear mixed effects model.
# Use REML=FALSE when using likelihood ratio tests to compare models
full.intro.gap.lmer <- lmer(time.to.extinct ~ intro*env + gap + drought + (1 + intro*env |
 Block), data=beetles.gap.test, REML=FALSE)
plot(full.intro.gap.lmer)
qqnorm(resid(full.intro.gap.lmer))
```

```
intro.gap.lmer <- lmer(time.to.extinct ~ intro*env + drought +  (1 + intro*env | Block),
 data=beetles.gap.test, REML=FALSE)

anova(full.intro.gap.lmer, intro.gap.lmer)
# Conclusion: Model with introduction gap is not more likely. Drop it.
### Test random effects first
# Start with 3 way interaction

full.model <- lmer(time.to.extinct ~ intro*env + drought +  (1 + intro*env | Block),
 data=beetles.time, REML=FALSE, control=lmerControl(optCtrl=list(maxfun=1e6)))

# Check assumptions
plot(full.model) # Residuals look normal and with equal variance
three.way.lmer <- lmer(time.to.extinct ~ intro*env + drought +  (1 + intro+env | Block),
 data=beetles.time, REML=FALSE, control=lmerControl(optCtrl=list(maxfun=1e6)))

anova(full.model, three.way.lmer)
# Conclusion: Model with 3 way interaction is not more likely than model without. Drop it.

full.model <- three.way.lmer
env.block.lmer <- lmer(time.to.extinct ~ intro*env + drought +  (1 + intro | Block),
 data=beetles.time, REML=FALSE, control=lmerControl(optCtrl=list(maxfun=1e6)))

anova(full.model, env.block.lmer)
# Conclusion: Random slope for environment doesn't make the model more likely so drop
 it.

full.model <- env.block.lmer

intro.block.lmer <- lmer(time.to.extinct ~ intro*env + drought +  (1 | Block),
 data=beetles.time, REML=FALSE, control=lmerControl(optCtrl=list(maxfun=1e6)))

anova(full.model, intro.block.lmer)
# Conclusion: Model with introduction scenario random slope not more likely than model
 without. Drop it.

###
### Now test fixed effect of drought
###

full.model <- intro.block.lmer

drought.model <- lmer(time.to.extinct ~ intro*env +  (1 | Block), data=beetles.time,
 REML=FALSE, control=lmerControl(optCtrl=list(maxfun=1e6)))

anova(full.model, drought.model)
```

```
# Conclusion: Model with drought is not more likely than model without. Drop it.

final.model <- drought.model

final.model <- lmer(time.to.extinct ~ intro*env + (1 | Block), data=beetles.time,
 REML=FALSE, control=lmerControl(optCtrl=list(maxfun=1e6)))

# Check assumptions of final model
plot(final.model)
qqnorm(resid(final.model)) # Residuals look normally distributed and have equal variance

anova.table <- summary(mixed(time.to.extinct ~ intro*env +  (1 | Block), data=beetles.time,
 method="KR"))
posthoc.time <- lsmeans::lsmeans(final.model, pairwise ~ intro, adjust="none")

### Adjust time to extinction so that it represents the number of generations to go extinct
 AFTER the last introduction
time.adj <- c(0, 1, 3, 4)
intro <- c("20", "10", "5", "4")
beetles.time$time.adj <- beetles.time$time.to.extinct - time.adj[match(beetles.time$intro,
 intro)] - as.logical(beetles.time$gap)


time.adj.model <- lmer(time.adj ~ intro*env + (1 | Block), data=beetles.time, REML=FALSE)
adj.anova.table <- summary(mixed(time.adj ~ intro*env + (1 | Block), data=beetles.time,
 method="KR"))
posthoc.time.adj <- lsmeans::lsmeans(time.adj.model, pairwise ~ intro, adjust="none")

mean.time.adj <- rev(summary(posthoc.time.adj)$lsmean$lsmean)
lower.CI.time.adj <- rev(summary(posthoc.time.adj)$lsmean$lower.CL)
upper.CI.time.adj <- rev(summary(posthoc.time.adj)$lsmean$upper.CL)

# Two-panel bar plot showing time to extiction for the 4 different introduction scenarios.
 First panel uses reference of time since the start of the experiment and second panel uses
 reference of time since most recent introduction for a given introduction scenario.
par(oma=c(0,2,0.5,1))
layout(mat=matrix(1:2, nrow=1, ncol=2))
par(mar=c(4,2,2,1))
figure3 <- barplot(mean.time, ylim=c(0, 10), col=c("dodgerblue", "brown1", "gold",
 "green"), ylab=NA, xlab=NA, las=2)
mtext(text=c("20x1", "10x2", "5x4", "4x5"), side=1, at=figure3, line=0.5)
arrows(x0=figure3, y0=lower.CI.time, x1=figure3, y1=upper.CI.time, angle=90, code=3,
 length=0.1)
text(x=figure3, y=upper.CI.time+0.4, label=c("a", "a", "b", "b"))
text(figure3[1], y=9, label="A)", cex=2)
```

```
par(mar=c(4,1,2,2))
figure4 <- barplot(mean.time.adj, ylim=c(0, 10), col=c("dodgerblue", "brown1", "gold",
 "green"), ylab=NA, xlab=NA, las=2)
mtext(text=c("20x1", "10x2", "5x4", "4x5"), side=1, at=figure4, line=0.5)
arrows(x0=figure4, y0=lower.CI.time.adj, x1=figure4, y1=upper.CI.time.adj, angle=90,
 code=3, length=0.1)
text(x=figure4, y=upper.CI.time.adj+0.4, label=c("a", "b", "b", "b"))
mtext(text="Generations to extinction", side=2, padj=-0.75, adj=0.5, outer=TRUE, line=0)
mtext(text="Introduction type", side=1, outer=TRUE, line=-2)
text(figure4[1], y=9, label="B)", cex=2)
```

APPENDIX B: DATA

COLUMN DESCRIPTIONS OF DATA

ID: The unique identifying number for each replicate population.

Block: One of 4 temporal blocks (1=first week, Mon-Tues; 2=first week, Wed-Thurs; 3=second week, Mon-Tues; 4=second week, Wed-Thurs).

intro: The introduction scenario (20=20 individuals introduced in the first generation; 10=10 individuals introduced in each of the first 2 generations; 5=5 individuals introduced in each of the first 4 generations; 4=4 individuals introduced in each of the first 5 generations).

env: The environmental stability (stable or fluctuating).

drought: Whether the population experienced the drop in relative humidity during development of the F_3 generation.

gap: Whether there was a gap in the introduction period due to a shortage of adult beetles to add to the populations.

extinct: A binary response telling whether the population went extinct (1) or not (0).

time.to.extinct: The generation that the population became extinct if it did go extinct and NA if the population was extant by the end of 9 generations.

RAW DATA

ID	Block	intro	env	drought	gap	extinct	time.to.extinct
1	1	20	fluctuating	FALSE	FALSE	0	NA
2	1	20	fluctuating	FALSE	FALSE	0	NA
3	1	20	fluctuating	FALSE	FALSE	1	6
4	1	20	fluctuating	TRUE	FALSE	0	NA
5	1	20	fluctuating	FALSE	FALSE	0	NA
6	1	20	fluctuating	TRUE	FALSE	0	NA
7	1	20	fluctuating	FALSE	FALSE	0	NA
8	1	20	fluctuating	FALSE	FALSE	0	NA
9	1	20	fluctuating	FALSE	FALSE	0	NA
10	1	20	fluctuating	FALSE	FALSE	0	NA
11	1	20	fluctuating	TRUE	FALSE	0	NA
12	1	20	fluctuating	FALSE	FALSE	0	NA
13	1	20	fluctuating	TRUE	FALSE	1	4
14	1	20	fluctuating	FALSE	FALSE	1	2
15	1	20	fluctuating	TRUE	FALSE	1	2
16	1	20	fluctuating	FALSE	FALSE	0	NA
17	1	20	fluctuating	FALSE	FALSE	0	NA
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30	1	20	fluctuating	FALSE	FALSE	1	9
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944	4	4	stable	FALSE	FALSE	0	NA
945	4	4	stable	TRUE	FALSE	0	NA

APPENDIX C: ADAPTATION AND GENETIC LOAD

INTRODUCTION

In a factorial microcosm experiment, we parsed the components of propagule pressure by fixing the total number of individuals introduced (20) and varying the number of introduction events (1, 2, 4, or 5 events) into a novel environment that was either stable or fluctuating through time. We found that several, small introductions lead to greater colonization success compared to fewer, larger introductions by reducing extinction probability and delaying time to extinction. We proposed that this was likely due to sustained immigration alleviating genetic load. We also suggested that sustained immigration might have affected how adaptation occurred, if at all. If both adaptation and genetic load affected populations, their influences on population fitness may cancel out and be undetectable without further experimentation. We set out to assess whether adaptation to the novel growth medium occurred, and whether it occurred differentially depending on introduction scenario or environmental stability.

METHODS

Experimental populations from blocks 3 and 4 of the experiment described in the main text of this thesis (hereafter referred to as the "main experiment") were reared for 2 additional generations on a novel corn medium with 0.95% standard medium (Ch. 1, Table 1). A large, external meta-population of *Tribolium* was maintained in parallel to the main experiment on the natal growth medium (100% standard medium). Subpopulations were thoroughly mixed prior to the oviposition period and 20 new subpopulations per block were each founded with 40 adults every generation. Thus, the large, external meta-population represents thousands of randomly mating individuals but with reduced negative density dependence, and which are purportedly non-adapted to a novel corn growth medium.

Calculating Expected Loss of Heterozygosity

Populations that remain small for prolonged periods lose heterozygosity at selectively neutral loci in a predictable way. We can calculate the expected degree of inbreeding for each population based on its census history and known immigration rate (from additional introductions) using the following series of equations from McCauley and Wade (1981):

$$\frac{H_t}{H_0} = 1 - f_t$$

Where $\frac{H_t}{H_0}$ represents the expected amount of heterozygosity at neutral loci at time *t* relative to initial conditions, and f_t is the inbreeding coefficient for the population at time *t*, representing the probability that an individual is homozygous at a neutral locus with both alleles descending from a common ancestor. The inbreeding coefficient is calculated as:

$$f_t = [\left(\frac{1}{2N_e}\right) + f_{t-1}\left(1 - \left(\frac{1}{2N_e}\right)\right)](1 - m)^2$$

Where N_e is the effective population size, f_{t-1} is the inbreeding coefficient at time *t-1*, and *m* is the migration rate calculated as:

$$m = \frac{migrants}{migrants + residents}$$

Where *migrants* are the number of individuals being introduced, and *residents* are the number of individuals already present.

Effective population size, N_e, is reduced with non-random mating or non-even sex ratios, but we assumed that N_e was equivalent to population size for the purposes of this experiment. Thus, our estimates of expected loss of heterozygosity underestimate the actual loss. Using the metric of $\frac{H_t}{H_0}$, a population with a low probability of autozygosity would have a high $\frac{H_t}{H_0}$, indicating not much heterozygosity was lost compared to initial conditions. A population with a high probability of autozygosity would have an $\frac{H_t}{H_0}$ value that was very low, indicating considerable loss in heterozygosity at neutral loci. Both inbreeding and drift-induced allelic loss can increase the inbreeding coefficient and lead to fitness declines, which have been observed in other *Tribolium* research (McCauley and Wade 1981, Szücs et al. 2014), but need to be verified for this experiment.

Treatment Groups

To explicitly test how adaptation and increasing probability of autozygosity may be affecting experimental populations, we compared population fitness across the following groups:

1. Experimental populations that we expect to be more autozygous (based on low $\frac{H_t}{H_0}$ values), and which could also be adapted to the novel medium
2. Experimental populations that we expect to be less autozygous (based on high $\frac{H_t}{H_0}$ values), and which could also be adapted to the novel medium
3. Several experimental populations mixed together, which would alleviate inbreeding depression and drift-induced allelic loss but retain adaptation

4. Base populations from large, external source population reared on the natal environment which would be neither adapted or autozygous

Creating Treatment Groups in the F_{10} Generation

For each of 8 treatments in each of 2 blocks, 5-10 populations (about a third of populations in the treatment) were randomly selected from the half of populations with the lowest $\frac{H_t}{H_0}$ values, representing Group 1—the high inbreeding group. Similarly, 5-10 populations (also a third of populations in the treatment) were randomly selected from the half of populations with the highest $\frac{H_t}{H_0}$ values, representing Group 2—the low inbreeding group. The remaining 5-10 populations from each treatment (a third of populations in the treatment) were mixed together, representing Group 3—the mixed group. Any population that had 40 or more individuals was split evenly into subpopulations such that no subpopulation had greater than 40 individuals. Offspring from these subpopulations were recombined as adults at the next generation's census such that there was still full admixture at the whole-population level. For the F_{10} generation, populations in Groups 1 and 2 were founded with all F_9 adults (unless there were more than 40). The mixed F_9 adults in Group 3 were used to found as many populations as possible with 20 individuals per population.

Control Group in the F_{10} Generation

Thirty populations per block were founded using 20 individuals each from the large, external source meta-population. This represented the control group, Group 4, and rearing these populations on novel growth medium for 1 generation standardized maternal effects.

The F_{11} Generation

After census of the F_{10} generation, all populations in Group 3 and Group 4 within each block were thoroughly mixed. This maintained non-inbred individuals for Group 4, and allowed further genetic admixture for Group 3. The F_{11} generation was founded using 16 adult beetles per population, with beetles in excess of 16 discarded. For Group 3, the mixed group, as many populations of 16 as possible were founded using the mixture of all F_{10} adults from each treatment. For Group 4, the control group, 30 populations with 16 individuals each were founded for each block. For Group 1 and 2, populations with multiples of 16 individuals were split into subpopulations, which were treated as replicates of that population (e.g. a population with 34 individuals could be used to found 2 replicate populations, with 2 individuals discarded). Any populations that did not have 16 or more adult individuals at the F_{10} census were discarded. We chose 16 individuals to found the F_{11} generation to ensure that a sufficient number of populations would be large enough to be included in the study and because there was a reasonably high probability that the sex ratio of such populations would be nearly even. The one-generation population growth rate, λ, was calculated and used as a metric of population fitness.

DATA

Column Descriptions of Data

ID: The unique identifier for each replicate. Three-digit IDs correspond to the original main experiment IDs. Four-digit IDs are control groups or mixed groups and the digits are "block-treatment-ID" so populations in the 4th treatment and 3rd block will be 3401 to

34XX. The control group is considered treatment 0. Any decimal after the ID indicates the subpopulation replicate number.

Group: One of 4 treatment groups (high inbreeding, low inbreeding, mixed, or control).

Blk: Temporal block (3 or 4).

Trt: Treatment number from main experiment (1-8).

Intro: Introduction scenario (20=20x1, 10=10x2, 5=5x4, and 4=4x5).

Env: Environmental stability (stab=stable, fluc=fluctuating).

H8/H0: Relative amount of heterozygosity remaining at neutral loci in generation 8.

H9/H0: Relative amount of heterozygosity remaining at neutral loci in generation 9.

F9: Average population size (across subreplicates) of the F_9 generation adults. Decimals here mean that the F_9 population was 40 or greater and was split. The value here then represents the mean population size of those split populations. This gives a sense of any potential grandmaternal effects.

F10: Average population size (across subreplicates) of the F_{10} generation adults. Gives a sense of how F_{10} generation performed.

N0: The number of adults (16) used to found all populations for the F_{11} generation.

F11: The number of F_{11} adults upon census.

Raw Data

ID	Group	Blk	Trt	intro	env	H8 H0	H9 H0	F9	F10	N0	F11
3001	control	3	0	0		NA	NA	20	39.5	16	21
3002	control	3	0	0		NA	NA	20	39.5	16	19
3003	control	3	0	0		NA	NA	20	39.5	16	29
3004	control	3	0	0		NA	NA	20	39.5	16	16
3005	control	3	0	0		NA	NA	20	39.5	16	21
3006	control	3	0	0		NA	NA	20	39.5	16	18
3007	control	3	0	0		NA	NA	20	39.5	16	22
3008	control	3	0	0		NA	NA	20	39.5	16	15
3009	control	3	0	0		NA	NA	20	39.5	16	23
3010	control	3	0	0		NA	NA	20	39.5	16	23
3011	control	3	0	0		NA	NA	20	39.5	16	28
3012	control	3	0	0		NA	NA	20	39.5	16	7
3013	control	3	0	0		NA	NA	20	39.5	16	17
3014	control	3	0	0		NA	NA	20	39.5	16	17
3015	control	3	0	0		NA	NA	20	39.5	16	11
3016	control	3	0	0		NA	NA	20	39.5	16	24
3017	control	3	0	0		NA	NA	20	39.5	16	2
3018	control	3	0	0		NA	NA	20	39.5	16	9
3019	control	3	0	0		NA	NA	20	39.5	16	24
3020	control	3	0	0		NA	NA	20	39.5	16	15
3021	control	3	0	0		NA	NA	20	39.5	16	16
3022	control	3	0	0		NA	NA	20	39.5	16	31
3023	control	3	0	0		NA	NA	20	39.5	16	14
3024	control	3	0	0		NA	NA	20	39.5	16	18
3025	control	3	0	0		NA	NA	20	39.5	16	11
3026	control	3	0	0		NA	NA	20	39.5	16	13
3027	control	3	0	0		NA	NA	20	39.5	16	14
3028	control	3	0	0		NA	NA	20	39.5	16	18
3029	control	3	0	0		NA	NA	20	39.5	16	18
3030	control	3	0	0		NA	NA	20	39.5	16	17
481.1	low	3	1	20	fluc	0.884	0.869	29	29	16	48
484.1	low	3	1	20	fluc	0.881	0.871	22	17	16	30
485.1	high	3	1	20	fluc	0.781	0.771	20.5	40	16	24
485.2	high	3	1	20	fluc	0.781	0.771	20.5	40	16	39
487.1	high	3	1	20	fluc	0.853	0.829	18	16	16	39
489.1	low	3	1	20	fluc	0.883	0.872	38	30	16	34
492.1	high	3	1	20	fluc	0.826	0.804	19	26	16	21
493.1	high	3	1	20	fluc	0.85	0.835	30	19	16	11
501.1	low	3	1	20	fluc	0.897	0.883	31	36	16	30
501.2	low	3	1	20	fluc	0.897	0.883	31	36	16	27
502.1	low	3	1	20	fluc	0.865	0.841	18	17	16	32

508.1	high	3	1	20	fluc	0.846	0.83	26	29	16	13
3101.1	mixed	3	1	20	fluc	NA	NA	20	10.88	16	50
3102.1	mixed	3	1	20	fluc	NA	NA	20	10.88	16	29
3103.1	mixed	3	1	20	fluc	NA	NA	20	10.88	16	23
3104.1	mixed	3	1	20	fluc	NA	NA	20	10.88	16	41
3105.1	mixed	3	1	20	fluc	NA	NA	20	10.88	16	39
512.1	low	3	2	20	stab	0.823	0.811	35	17	16	6
513.1	high	3	2	20	stab	0.739	0.73	20	54	16	20
513.2	high	3	2	20	stab	0.739	0.73	20	54	16	15
513.3	high	3	2	20	stab	0.739	0.73	20	54	16	34
515.1	high	3	2	20	stab	0.743	0.729	25	27	16	6
516.1	low	3	2	20	stab	0.839	0.809	14	19	16	35
522.1	low	3	2	20	stab	0.84	0.827	31	20	16	22
525.1	low	3	2	20	stab	0.822	0.809	30	16	16	14
528.1	low	3	2	20	stab	0.847	0.834	34	20	16	16
530.1	high	3	2	20	stab	0.749	0.732	22	29	16	7
531.1	low	3	2	20	stab	0.864	0.855	25	32	16	27
531.2	low	3	2	20	stab	0.864	0.855	25	32	16	18
532.1	low	3	2	20	stab	0.766	0.757	20.5	48	16	38
532.2	low	3	2	20	stab	0.766	0.757	20.5	48	16	20
532.3	low	3	2	20	stab	0.766	0.757	20.5	48	16	34
536.1	high	3	2	20	stab	0.73	0.719	31	38	16	8
536.2	high	3	2	20	stab	0.73	0.719	31	38	16	4
3201.1	mixed	3	2	20	stab	NA	NA	20	15.8	16	35
3202.1	mixed	3	2	20	stab	NA	NA	20	15.8	16	49
3203.1	mixed	3	2	20	stab	NA	NA	20	15.8	16	33
3204.1	mixed	3	2	20	stab	NA	NA	20	15.8	16	33
545.1	low	3	3	10	fluc	0.886	0.86	17	23	16	14
546.1	high	3	3	10	fluc	0.744	0.726	21	23	16	24
552.1	low	3	3	10	fluc	0.868	0.858	22	40	16	25
552.2	low	3	3	10	fluc	0.868	0.858	22	40	16	30
560.1	high	3	3	10	fluc	0.85	0.831	23	24	16	34
563.1	high	3	3	10	fluc	0.792	0.756	11	16	16	21
3301.1	mixed	3	3	10	fluc	NA	NA	20	21.33	16	19
3302.1	mixed	3	3	10	fluc	NA	NA	20	21.33	16	13
3303.1	mixed	3	3	10	fluc	NA	NA	20	21.33	16	34
3304.1	mixed	3	3	10	fluc	NA	NA	20	21.33	16	30
3305.1	mixed	3	3	10	fluc	NA	NA	20	21.33	16	27
3306.1	mixed	3	3	10	fluc	NA	NA	20	21.33	16	28
3307.1	mixed	3	3	10	fluc	NA	NA	20	21.33	16	33
3308.1	mixed	3	3	10	fluc	NA	NA	20	21.33	16	28
571.1	low	3	4	10	stab	0.841	0.832	22.5	32	16	18
571.2	low	3	4	10	stab	0.841	0.832	22.5	32	16	15
575.1	high	3	4	10	stab	0.84	0.828	36	39	16	47
575.2	high	3	4	10	stab	0.84	0.828	36	39	16	19
577.1	high	3	4	10	stab	0.804	0.783	19	18	16	31

595.1	low	3	4	10	stab	0.889	0.875	32	21	16	38
599.1	low	3	4	10	stab	0.891	0.873	24	23	16	22
600.1	low	3	4	10	stab	0.839	0.831	29	34	16	17
600.2	low	3	4	10	stab	0.839	0.831	29	34	16	18
3401.1	mixed	3	4	10	stab	NA	NA	20	22.25	16	25
3402.1	mixed	3	4	10	stab	NA	NA	20	22.25	16	32
3403.1	mixed	3	4	10	stab	NA	NA	20	22.25	16	10
3404.1	mixed	3	4	10	stab	NA	NA	20	22.25	16	21
3405.1	mixed	3	4	10	stab	NA	NA	20	22.25	16	16
602.1	low	3	5	5	fluc	0.866	0.855	20.5	46	16	33
602.2	low	3	5	5	fluc	0.866	0.855	20.5	46	16	31
606.1	high	3	5	5	fluc	0.831	0.814	25	40	16	7
606.2	high	3	5	5	fluc	0.831	0.814	25	40	16	9
607.1	high	3	5	5	fluc	0.811	0.777	12	16	16	17
611.1	low	3	5	5	fluc	0.883	0.865	24	20	16	24
614.1	low	3	5	5	fluc	0.863	0.838	17	31	16	6
617.1	high	3	5	5	fluc	0.812	0.794	22	40	16	6
617.2	high	3	5	5	fluc	0.812	0.794	22	40	16	22
621.1	low	3	5	5	fluc	0.874	0.86	31	26	16	41
622.1	high	3	5	5	fluc	0.818	0.809	23	40	16	23
622.2	high	3	5	5	fluc	0.818	0.809	23	40	16	13
624.1	low	3	5	5	fluc	0.876	0.864	37	29	16	17
625.1	low	3	5	5	fluc	0.877	0.861	28	19	16	11
626.1	low	3	5	5	fluc	0.869	0.852	26	39	16	2
626.2	low	3	5	5	fluc	0.869	0.852	26	39	16	5
627.1	high	3	5	5	fluc	0.787	0.765	18	16	16	7
628.1	high	3	5	5	fluc	0.83	0.815	27	17	16	5
3501.1	mixed	3	5	5	fluc	NA	NA	20	20.27	16	28
3502.1	mixed	3	5	5	fluc	NA	NA	20	20.27	16	43
3503.1	mixed	3	5	5	fluc	NA	NA	20	20.27	16	34
3504.1	mixed	3	5	5	fluc	NA	NA	20	20.27	16	30
3505.1	mixed	3	5	5	fluc	NA	NA	20	20.27	16	23
3506.1	mixed	3	5	5	fluc	NA	NA	20	20.27	16	28
3507.1	mixed	3	5	5	fluc	NA	NA	20	20.27	16	30
3508.1	mixed	3	5	5	fluc	NA	NA	20	20.27	16	16
3509.1	mixed	3	5	5	fluc	NA	NA	20	20.27	16	20
3510.1	mixed	3	5	5	fluc	NA	NA	20	20.27	16	20
631.1	high	3	6	5	stab	0.862	0.823	11	25	16	17
633.1	high	3	6	5	stab	0.806	0.789	23	22	16	11
636.1	low	3	6	5	stab	0.868	0.851	25	24	16	2
637.1	high	3	6	5	stab	0.841	0.789	8	17	16	12
639.1	high	3	6	5	stab	0.825	0.809	27	30	16	5
653.1	low	3	6	5	stab	0.868	0.85	24	26	16	32
654.1	high	3	6	5	stab	0.859	0.811	9	24	16	28
655.1	low	3	6	5	stab	0.899	0.886	35	21	16	8
657.1	low	3	6	5	stab	0.842	0.824	24	18	16	21

658.1	low	3	6	5	stab	0.869	0.849	22	17	16	19
659.1	low	3	6	5	stab	0.857	0.841	26	19	16	13
3601.1	mixed	3	6	5	stab	NA	NA	20	18.88	16	18
3602.1	mixed	3	6	5	stab	NA	NA	20	18.88	16	23
3603.1	mixed	3	6	5	stab	NA	NA	20	18.88	16	33
3604.1	mixed	3	6	5	stab	NA	NA	20	18.88	16	23
3605.1	mixed	3	6	5	stab	NA	NA	20	18.88	16	23
3606.1	mixed	3	6	5	stab	NA	NA	20	18.88	16	14
3607.1	mixed	3	6	5	stab	NA	NA	20	18.88	16	33
3608.1	mixed	3	6	5	stab	NA	NA	20	18.88	16	24
3609.1	mixed	3	6	5	stab	NA	NA	20	18.88	16	12
664.1	high	3	7	4	fluc	0.875	0.862	35	26	16	14
667.1	high	3	7	4	fluc	0.888	0.877	21	22	16	33
668.1	low	3	7	4	fluc	0.893	0.885	29	29	16	29
670.1	low	3	7	4	fluc	0.892	0.882	20.5	49	16	16
670.2	low	3	7	4	fluc	0.892	0.882	20.5	49	16	35
670.3	low	3	7	4	fluc	0.892	0.882	20.5	49	16	21
673.1	low	3	7	4	fluc	0.902	0.886	29	18	16	21
678.1	high	3	7	4	fluc	0.885	0.872	33	38	16	19
678.2	high	3	7	4	fluc	0.885	0.872	33	38	16	20
681.1	high	3	7	4	fluc	0.886	0.867	23	19	16	47
3701.1	mixed	3	7	4	fluc	NA	NA	20	21.3	16	20
3702.1	mixed	3	7	4	fluc	NA	NA	20	21.3	16	16
3703.1	mixed	3	7	4	fluc	NA	NA	20	21.3	16	32
3704.1	mixed	3	7	4	fluc	NA	NA	20	21.3	16	24
3705.1	mixed	3	7	4	fluc	NA	NA	20	21.3	16	21
3706.1	mixed	3	7	4	fluc	NA	NA	20	21.3	16	18
3707.1	mixed	3	7	4	fluc	NA	NA	20	21.3	16	13
3708.1	mixed	3	7	4	fluc	NA	NA	20	21.3	16	20
3709.1	mixed	3	7	4	fluc	NA	NA	20	21.3	16	26
3710.1	mixed	3	7	4	fluc	NA	NA	20	21.3	16	27
696.1	low	3	8	4	stab	0.895	0.88	29	20	16	4
698.1	high	3	8	4	stab	0.865	0.845	22	25	16	18
701.1	high	3	8	4	stab	0.876	0.821	8	17	16	17
702.1	low	3	8	4	stab	0.886	0.872	31	21	16	22
705.1	high	3	8	4	stab	0.832	0.809	18	42	16	23
705.2	high	3	8	4	stab	0.832	0.809	18	42	16	20
706.1	high	3	8	4	stab	0.89	0.867	20	22	16	29
707.1	low	3	8	4	stab	0.885	0.871	31	27	16	14
708.1	low	3	8	4	stab	0.902	0.886	28	25	16	27
709.1	high	3	8	4	stab	0.856	0.838	24	21	16	28
712.1	high	3	8	4	stab	0.881	0.867	31	19	16	37
713.1	low	3	8	4	stab	0.89	0.878	36	47	16	24
713.2	low	3	8	4	stab	0.89	0.878	36	47	16	22
720.1	low	3	8	4	stab	0.893	0.88	33	27	16	4
3801.1	mixed	3	8	4	stab	NA	NA	20	26.55	16	28

3802.1	mixed	3	8	4	stab	NA	NA	20	26.55	16	31
3803.1	mixed	3	8	4	stab	NA	NA	20	26.55	16	35
3804.1	mixed	3	8	4	stab	NA	NA	20	26.55	16	20
3805.1	mixed	3	8	4	stab	NA	NA	20	26.55	16	32
3806.1	mixed	3	8	4	stab	NA	NA	20	26.55	16	33
3807.1	mixed	3	8	4	stab	NA	NA	20	26.55	16	26
3808.1	mixed	3	8	4	stab	NA	NA	20	26.55	16	49
3809.1	mixed	3	8	4	stab	NA	NA	20	26.55	16	30
3810.1	mixed	3	8	4	stab	NA	NA	20	26.55	16	45
4001	control	4	0	0		NA	NA	20	47.1	16	25
4002	control	4	0	0		NA	NA	20	47.1	16	25
4003	control	4	0	0		NA	NA	20	47.1	16	21
4004	control	4	0	0		NA	NA	20	47.1	16	31
4005	control	4	0	0		NA	NA	20	47.1	16	11
4006	control	4	0	0		NA	NA	20	47.1	16	15
4007	control	4	0	0		NA	NA	20	47.1	16	7
4008	control	4	0	0		NA	NA	20	47.1	16	15
4009	control	4	0	0		NA	NA	20	47.1	16	15
4010	control	4	0	0		NA	NA	20	47.1	16	23
4011	control	4	0	0		NA	NA	20	47.1	16	34
4012	control	4	0	0		NA	NA	20	47.1	16	11
4013	control	4	0	0		NA	NA	20	47.1	16	14
4014	control	4	0	0		NA	NA	20	47.1	16	36
4015	control	4	0	0		NA	NA	20	47.1	16	21
4016	control	4	0	0		NA	NA	20	47.1	16	25
4017	control	4	0	0		NA	NA	20	47.1	16	19
4018	control	4	0	0		NA	NA	20	47.1	16	33
4019	control	4	0	0		NA	NA	20	47.1	16	16
4020	control	4	0	0		NA	NA	20	47.1	16	9
4021	control	4	0	0		NA	NA	20	47.1	16	13
4022	control	4	0	0		NA	NA	20	47.1	16	18
4023	control	4	0	0		NA	NA	20	47.1	16	35
4024	control	4	0	0		NA	NA	20	47.1	16	10
4025	control	4	0	0		NA	NA	20	47.1	16	14
4026	control	4	0	0		NA	NA	20	47.1	16	29
4027	control	4	0	0		NA	NA	20	47.1	16	9
4028	control	4	0	0		NA	NA	20	47.1	16	19
4029	control	4	0	0		NA	NA	20	47.1	16	22
4030	control	4	0	0		NA	NA	20	47.1	16	16
725.1	high	4	1	20	fluc	0.828	0.811	24	28	16	27
726.1	high	4	1	20	fluc	0.792	0.771	19	27	16	20
728.1	high	4	1	20	fluc	0.743	0.725	21	27	16	16
729.1	low	4	1	20	fluc	0.88	0.872	30	60	16	27
729.2	low	4	1	20	fluc	0.88	0.872	30	60	16	47
729.3	low	4	1	20	fluc	0.88	0.872	30	60	16	28
730.1	high	4	1	20	fluc	0.822	0.792	14	19	16	37

734.1	high	4	1	20	fluc	0.786	0.742	9	19	16	8
737.1	high	4	1	20	fluc	0.854	0.834	22	19	16	19
741.1	low	4	1	20	fluc	0.848	0.84	27.5	39	16	36
741.2	low	4	1	20	fluc	0.848	0.84	27.5	39	16	37
742.1	high	4	1	20	fluc	0.811	0.798	30	23	16	16
745.1	low	4	1	20	fluc	0.879	0.869	22.5	72	16	29
745.2	low	4	1	20	fluc	0.879	0.869	22.5	72	16	39
745.3	low	4	1	20	fluc	0.879	0.869	22.5	72	16	28
745.4	low	4	1	20	fluc	0.879	0.869	22.5	72	16	34
749.1	high	4	1	20	fluc	0.756	0.735	18	24	16	28
4101.1	mixed	4	1	20	fluc	NA	NA	20	23	16	32
4102.1	mixed	4	1	20	fluc	NA	NA	20	23	16	18
4103.1	mixed	4	1	20	fluc	NA	NA	20	23	16	27
4104.1	mixed	4	1	20	fluc	NA	NA	20	23	16	26
4105.1	mixed	4	1	20	fluc	NA	NA	20	23	16	26
4106.1	mixed	4	1	20	fluc	NA	NA	20	23	16	50
4107.1	mixed	4	1	20	fluc	NA	NA	20	23	16	32
4108.1	mixed	4	1	20	fluc	NA	NA	20	23	16	20
4109.1	mixed	4	1	20	fluc	NA	NA	20	23	16	27
4110.1	mixed	4	1	20	fluc	NA	NA	20	23	16	23
753.1	high	4	2	20	stab	0.839	0.828	36	37	16	26
753.2	high	4	2	20	stab	0.839	0.828	36	37	16	32
754.1	low	4	2	20	stab	0.871	0.858	33	23	16	28
755.1	high	4	2	20	stab	0.703	0.696	23	44	16	19
755.2	high	4	2	20	stab	0.703	0.696	23	44	16	9
757.1	high	4	2	20	stab	0.837	0.823	29	21	16	18
763.1	low	4	2	20	stab	0.889	0.874	31	34	16	11
763.2	low	4	2	20	stab	0.889	0.874	31	34	16	20
764.1	high	4	2	20	stab	0.666	0.658	20.5	31	16	33
768.1	low	4	2	20	stab	0.871	0.862	24.5	16	16	31
769.1	low	4	2	20	stab	0.845	0.829	27	18	16	32
771.1	low	4	2	20	stab	0.855	0.841	29	23	16	18
772.1	high	4	2	20	stab	0.829	0.819	21.5	25	16	34
776.1	low	4	2	20	stab	0.84	0.831	23.5	37	16	25
776.2	low	4	2	20	stab	0.84	0.831	23.5	37	16	18
779.1	high	4	2	20	stab	0.754	0.743	34	20	16	32
4201.1	mixed	4	2	20	stab	NA	NA	20	20	16	23
4202.1	mixed	4	2	20	stab	NA	NA	20	20	16	16
4203.1	mixed	4	2	20	stab	NA	NA	20	20	16	15
4204.1	mixed	4	2	20	stab	NA	NA	20	20	16	17
4205.1	mixed	4	2	20	stab	NA	NA	20	20	16	13
4206.1	mixed	4	2	20	stab	NA	NA	20	20	16	25
4207.1	mixed	4	2	20	stab	NA	NA	20	20	16	28
4208.1	mixed	4	2	20	stab	NA	NA	20	20	16	39
4209.1	mixed	4	2	20	stab	NA	NA	20	20	16	26
4210.1	mixed	4	2	20	stab	NA	NA	20	20	16	34

781.1	low	4	3	10	fluc	0.864	0.852	37	21	16	25
782.1	high	4	3	10	fluc	0.803	0.797	32	51	16	32
782.2	high	4	3	10	fluc	0.803	0.797	32	51	16	14
782.3	high	4	3	10	fluc	0.803	0.797	32	51	16	15
787.1	high	4	3	10	fluc	0.598	0.573	12	18	16	20
791.1	low	4	3	10	fluc	0.867	0.844	19	38	16	11
791.2	low	4	3	10	fluc	0.867	0.844	19	38	16	15
792.1	high	4	3	10	fluc	0.771	0.758	28	16	16	18
800.1	high	4	3	10	fluc	0.806	0.781	16	28	16	4
801.1	low	4	3	10	fluc	0.881	0.856	18	27	16	36
805.1	low	4	3	10	fluc	0.855	0.843	34	26	16	10
806.1	low	4	3	10	fluc	0.884	0.863	21	18	16	31
4301.1	mixed	4	3	10	fluc	NA	NA	20	18.4	16	32
4302.1	mixed	4	3	10	fluc	NA	NA	20	18.4	16	33
4303.1	mixed	4	3	10	fluc	NA	NA	20	18.4	16	32
4304.1	mixed	4	3	10	fluc	NA	NA	20	18.4	16	10
4305.1	mixed	4	3	10	fluc	NA	NA	20	18.4	16	8
811.1	low	4	4	10	stab	0.899	0.892	32	27	16	13
812.1	low	4	4	10	stab	0.818	0.808	20.5	22	16	11
835.1	high	4	4	10	stab	0.772	0.756	24	17	16	11
4401.1	mixed	4	4	10	stab	NA	NA	20	22.71	16	29
4402.1	mixed	4	4	10	stab	NA	NA	20	22.71	16	36
4403.1	mixed	4	4	10	stab	NA	NA	20	22.71	16	35
4404.1	mixed	4	4	10	stab	NA	NA	20	22.71	16	51
4405.1	mixed	4	4	10	stab	NA	NA	20	22.71	16	46
4406.1	mixed	4	4	10	stab	NA	NA	20	22.71	16	1
4407.1	mixed	4	4	10	stab	NA	NA	20	22.71	16	33
4408.1	mixed	4	4	10	stab	NA	NA	20	22.71	16	27
4409.1	mixed	4	4	10	stab	NA	NA	20	22.71	16	36
844.1	high	4	5	5	fluc	0.847	0.839	26.5	33	16	25
844.2	high	4	5	5	fluc	0.847	0.839	26.5	33	16	25
846.1	high	4	5	5	fluc	0.843	0.833	23	28	16	21
847.1	high	4	5	5	fluc	0.728	0.707	18	20	16	19
851.1	low	4	5	5	fluc	0.894	0.884	21	29	16	19
853.1	high	4	5	5	fluc	0.778	0.755	17	20	16	22
856.1	low	4	5	5	fluc	0.861	0.85	39	27	16	32
857.1	low	4	5	5	fluc	0.868	0.855	34	20	16	31
858.1	high	4	5	5	fluc	0.839	0.821	23	20	16	27
859.1	low	4	5	5	fluc	0.867	0.855	34	17	16	17
861.1	low	4	5	5	fluc	0.85	0.839	20	60	16	21
861.2	low	4	5	5	fluc	0.85	0.839	20	60	16	26
861.3	low	4	5	5	fluc	0.85	0.839	20	60	16	24
862.1	low	4	5	5	fluc	0.907	0.891	27	28	16	24
864.1	high	4	5	5	fluc	0.754	0.736	20	20	16	13
866.1	low	4	5	5	fluc	0.864	0.848	27	17	16	43
867.1	low	4	5	5	fluc	0.871	0.859	37	27	16	18

868.1	low	4	5	5	fluc	0.857	0.846	38	16	16	4
869.1	high	4	5	5	fluc	0.793	0.782	37	22	16	40
870.1	low	4	5	5	fluc	0.881	0.868	35	21	16	26
4501.1	mixed	4	5	5	fluc	NA	NA	20	21.67	16	32
4502.1	mixed	4	5	5	fluc	NA	NA	20	21.67	16	26
4503.1	mixed	4	5	5	fluc	NA	NA	20	21.67	16	38
4504.1	mixed	4	5	5	fluc	NA	NA	20	21.67	16	37
4505.1	mixed	4	5	5	fluc	NA	NA	20	21.67	16	26
4506.1	mixed	4	5	5	fluc	NA	NA	20	21.67	16	41
4507.1	mixed	4	5	5	fluc	NA	NA	20	21.67	16	36
4508.1	mixed	4	5	5	fluc	NA	NA	20	21.67	16	33
4509.1	mixed	4	5	5	fluc	NA	NA	20	21.67	16	29
4510.1	mixed	4	5	5	fluc	NA	NA	20	21.67	16	11
872.1	low	4	6	5	stab	0.877	0.862	29	27	16	18
874.1	low	4	6	5	stab	0.868	0.848	21	19	16	33
877.1	high	4	6	5	stab	0.832	0.819	31	24	16	12
883.1	high	4	6	5	stab	0.791	0.775	24	19	16	20
4601.1	mixed	4	6	5	stab	NA	NA	20	15.1	16	31
4602.1	mixed	4	6	5	stab	NA	NA	20	15.1	16	11
4603.1	mixed	4	6	5	stab	NA	NA	20	15.1	16	38
4604.1	mixed	4	6	5	stab	NA	NA	20	15.1	16	49
4605.1	mixed	4	6	5	stab	NA	NA	20	15.1	16	39
4606.1	mixed	4	6	5	stab	NA	NA	20	15.1	16	31
4607.1	mixed	4	6	5	stab	NA	NA	20	15.1	16	41
4608.1	mixed	4	6	5	stab	NA	NA	20	15.1	16	13
4609.1	mixed	4	6	5	stab	NA	NA	20	15.1	16	33
901.1	high	4	7	4	fluc	0.882	0.865	26	25	16	23
903.1	low	4	7	4	fluc	0.894	0.884	22	46	16	42
903.2	low	4	7	4	fluc	0.894	0.884	22	46	16	33
904.1	high	4	7	4	fluc	0.867	0.857	21.5	45	16	20
904.2	high	4	7	4	fluc	0.867	0.857	21.5	45	16	29
909.1	low	4	7	4	fluc	0.908	0.897	21.5	20	16	20
916.1	low	4	7	4	fluc	0.889	0.882	33	48	16	27
916.2	low	4	7	4	fluc	0.889	0.882	33	48	16	34
916.3	low	4	7	4	fluc	0.889	0.882	33	48	16	34
917.1	high	4	7	4	fluc	0.888	0.872	28	21	16	34
4701.1	mixed	4	7	4	fluc	NA	NA	20	18.17	16	49
4702.1	mixed	4	7	4	fluc	NA	NA	20	18.17	16	26
4703.1	mixed	4	7	4	fluc	NA	NA	20	18.17	16	51
4704.1	mixed	4	7	4	fluc	NA	NA	20	18.17	16	34
4705.1	mixed	4	7	4	fluc	NA	NA	20	18.17	16	19
4706.1	mixed	4	7	4	fluc	NA	NA	20	18.17	16	36
934.1	low	4	8	4	stab	0.896	0.887	23.5	22	16	17
935.1	high	4	8	4	stab	0.871	0.857	31	19	16	25
940.1	high	4	8	4	stab	0.87	0.854	28	19	16	41
942.1	low	4	8	4	stab	0.899	0.883	28	22	16	25

945.1	low	4	8	4	stab	0.883	0.872	20.5	45	16	0
945.2	low	4	8	4	stab	0.883	0.872	20.5	45	16	15
4801.1	mixed	4	8	4	stab	NA	NA	20	13.33	16	23
4802.1	mixed	4	8	4	stab	NA	NA	20	13.33	16	37
4803.1	mixed	4	8	4	stab	NA	NA	20	13.33	16	22
4804.1	mixed	4	8	4	stab	NA	NA	20	13.33	16	7
4805.1	mixed	4	8	4	stab	NA	NA	20	13.33	16	33
4806.1	mixed	4	8	4	stab	NA	NA	20	13.33	16	14
4807.1	mixed	4	8	4	stab	NA	NA	20	13.33	16	25

APPENDIX D: MEASUREMENT ERROR

INTRODUCTION

To collect census data for this experiment, adult beetles and growth medium from each microcosm box were separated using a soil sieve. Adults were dumped onto a plate and counted as they were brushed back into the emptied microcosm box. Because all individuals are counted, no extrapolation is needed from the observed population size to an estimate of the true population size. Thus this census procedure yields very good estimates of the true population size. However, observational measurement error can still create disparity between the true population size and the census estimate. This measurement error is historically low using this procedure (1% per population; Melbourne and Hastings 2008), but needs to be quantified given that different observers participate in each experiment.

METHODS

Setup

An unknown number of adult beetles were added to 15 boxes with 2 tablespoons of standard medium by pouring them from a box containing many hundreds of individuals. Three relative densities were represented within these 15 boxes with 5 populations each: low, medium, and high. This range of densities was estimated visually and approximately spanned the range observed during the main experiment.

Each observer (n=12) censused each population, recorded their population size data, and stored their datasheet in a closed envelope. Observers neither looked at previous data nor shared their data with new observers.

Analysis

The residual for observer *i* and population *k* was calculated as the squared difference between the mean population size across all observers for population *k* and the population size reported by observer *i* for population *k*. The mean of those residuals across all *i* observers was calculated for each population, which was then square-root transformed and divided by the group mean to calculate a coefficient of variation for each population. The mean of these coefficients of variation across observers was 0.0037 with a range of 0 to 0.017, suggesting that on average there is less than half a percentage point of spread (and a maximum of about 1.7%) around estimates of population size for all 12 observers. This increased slightly as the relative densities of the population increased, and with minimal change across observers (ranging from 0.00046 to 0.0121).

Column Descriptions of Data

Person: The initials of the observer for the entry

Date: The date that the population census was taken. Some variability was likely added as individuals were accidentally lost from populations during repeated censusing. Therefore, the analysis presented here probably overestimates the measurement error in the protocol.

Box: The replicate id number for the 15 different populations that were censused. Boxes 1-5 represented the low density treatment, boxes 6-10 represented the medium density treatment, and boxes 11-15 represented the high density treatment.

Census: The amount of adult beetles counted for each box by each observer.

Notes: One population was not censused by one observer and this is noted in this column.

Raw Data

Person	Date	Box	Census	Notes
JAR	20140423	1	6	
JAR	20140423	2	12	
JAR	20140423	3	13	
JAR	20140423	4	6	
JAR	20140423	5	19	
JAR	20140423	6	39	
JAR	20140423	7	36	
JAR	20140423	8	33	
JAR	20140423	9	38	
JAR	20140423	10	30	
JAR	20140423	11	144	
JAR	20140423	12	200	
JAR	20140423	13	175	
JAR	20140423	14	165	
JAR	20140423	15	180	
SBE	20140423	1	6	
SBE	20140423	2	12	
SBE	20140423	3	13	
SBE	20140423	4	6	
SBE	20140423	5	19	
SBE	20140423	6	39	
SBE	20140423	7	36	
SBE	20140423	8	33	
SBE	20140423	9	38	
SBE	20140423	10	30	
SBE	20140423	11	144	
SBE	20140423	12	204	
SBE	20140423	13	175	
SBE	20140423	14	165	
SBE	20140423	15	178	
CAH	20140424	1	6	
CAH	20140424	2	12	
CAH	20140424	3	13	
CAH	20140424	4	6	
CAH	20140424	5	18	
CAH	20140424	6	39	
CAH	20140424	7	36	
CAH	20140424	8	33	
CAH	20140424	9	38	

CAH	20140424	10	30
CAH	20140424	11	144
CAH	20140424	12	203
CAH	20140424	13	175
CAH	20140424	14	165
CAH	20140424	15	180
KRA	20140424	1	6
KRA	20140424	2	12
KRA	20140424	3	13
KRA	20140424	4	6
KRA	20140424	5	19
KRA	20140424	6	39
KRA	20140424	7	36
KRA	20140424	8	33
KRA	20140424	9	38
KRA	20140424	10	30
KRA	20140424	11	144
KRA	20140424	12	203
KRA	20140424	13	175
KRA	20140424	14	165
KRA	20140424	15	180
MFO	20140428	1	6
MFO	20140428	2	12
MFO	20140428	3	13
MFO	20140428	4	6
MFO	20140428	5	19
MFO	20140428	6	39
MFO	20140428	7	36
MFO	20140428	8	33
MFO	20140428	9	38
MFO	20140428	10	30
MFO	20140428	11	144
MFO	20140428	12	203
MFO	20140428	13	174
MFO	20140428	14	165
MFO	20140428	15	180
RAH	20140429	1	6
RAH	20140429	2	12
RAH	20140429	3	13
RAH	20140429	4	6
RAH	20140429	5	19
RAH	20140429	6	39
RAH	20140429	7	36
RAH	20140429	8	33
RAH	20140429	9	38
RAH	20140429	10	30

RAH	20140429	11	144	
RAH	20140429	12	203	
RAH	20140429	13	176	
RAH	20140429	14		No data taken for this population
RAH	20140429	15	180	
MS	20140501	1	6	
MS	20140501	2	12	
MS	20140501	3	13	
MS	20140501	4	6	
MS	20140501	5	19	
MS	20140501	6	39	
MS	20140501	7	36	
MS	20140501	8	33	
MS	20140501	9	37	
MS	20140501	10	30	
MS	20140501	11	144	
MS	20140501	12	203	
MS	20140501	13	175	
MS	20140501	14	164	
MS	20140501	15	183	
MJK	20140501	1	6	
MJK	20140501	2	12	
MJK	20140501	3	13	
MJK	20140501	4	6	
MJK	20140501	5	19	
MJK	20140501	6	39	
MJK	20140501	7	35	
MJK	20140501	8	33	
MJK	20140501	9	37	
MJK	20140501	10	30	
MJK	20140501	11	144	
MJK	20140501	12	203	
MJK	20140501	13	175	
MJK	20140501	14	164	
MJK	20140501	15	180	