Parsing propagule pressure: Number, not size, of introductions drives colonization success in a novel environment

Michael J Koontz [a,b,c,1], Meagan F Oldfather [d,2], Brett A Melbourne[e,3], Ruth A Hufbauer[b,c,4]

a. Graduate Group in Ecology, University of California, Davis, CA 95616

b. Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO 80523

c. Department of Bioagricultural Science and Pest Management, Colorado State University, Fort Collins, CO 80523

d. Department of Integrative Biology, University of California, Berkeley, CA 94720

e. Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309

Running header: Parsing propagule pressure

[1] Email: mkoontz@ucdavis.edu; ORCID: orcid.org/0000-0002-8276-210X
[2] Email: meagan_oldfather@berkeley.edu; ORCID: orcid.org/0000-0003-3256-4786
[3] Email: brett.melbourne@colorado.edu
[4] Email: hufbauer@colostate.edu

ABSTRACT

Predicting whether individuals will colonize a novel habitat is of fundamental ecological interest and is crucial to both conservation efforts and invasive species management. The only consistent predictor of colonization success is the number of individuals introduced, also called propagule pressure. Propagule pressure increases with the number of introductions and the number of individuals per introduction (the size of the introduction), but it is unresolved which process is a stronger driver of colonization success. Furthermore their relative importance may depend upon the environment, with multiple introductions potentially enhancing colonization of fluctuating environments. To evaluate the relative importance of the number and size of introductions and its dependence upon environmental variability, we paired demographic simulations with a microcosm experiment. Using *Tribolium* flour beetles as a model system, we introduced a fixed number of individuals into replicated novel habitats of stable or fluctuating quality, varying the number of introductions through time and size of each introduction. We evaluated establishment probability and the size of extant populations after 7 generations. In the simulations and microcosms, we found that establishment probability increased with more, smaller introductions, but was not affected by biologically realistic fluctuations in environmental quality. Population size was not significantly affected by environmental variability in the simulations, but populations in the microcosms grew larger in a stable environment, especially with more introduction events. In general, the microcosm experiment yielded higher establishment probability and larger populations than the demographic simulations. We suggest that genetic mechanisms likely underlie these differences and thus deserve more attention in efforts to parse propagule pressure. Our results highlight the importance of preventing further introductions of undesirable species to invaded sites, and suggest conservation efforts should focus on increasing

the number of introductions or re-introductions of desirable species rather than increasing the size of those introduction events.

KEYWORDS: invasion, reintroduction, propagule pressure, biocontrol, conservation, microcosm, population dynamics, stochasticity, simulation

INTRODUCTION

Colonization is the ecologically fundamental process of population establishment in an unoccupied site, and it underlies the past, present, and future distributions of species. Colonization occurs naturally, but is increasingly prevalent due to anthropogenic influences (Sakai et al. 2001, Cassey et al. 2005, Ricciardi 2007). Incipient populations often face environments that are entirely novel, which is especially likely in the case of anthropogenic colonization (Cassey et al. 2005, Ricciardi 2007). Regardless of whether colonization events to novel habitats are natural (e.g., range expansion) or human-mediated (e.g., biological invasions, reintroductions of rare species, release of biological control agents), their successes or failures have significant implications for natural resource managers and society (Mack et al. 2000).

Most introductions to novel habitats fail, and colonization success can be difficult to predict (Lockwood et al. 2005, Zenni and Nuñez 2013). Incipient populations are commonly small, and face threats from environmental, demographic, and genetic stochasticity (Lande 1988, 1993, Fauvergue et al. 2012). Furthermore, the success of any given population can be idiosyncratic with respect to taxonomy and geography (Lodge 1993, Lockwood et al. 2005). Thus, it is crucial to understand more general features of the colonization process beyond the particular invading organism or the particular invaded environment (Lockwood et al. 2005). Propagule pressure is

64 one such general feature that is the only known consistent predictor of colonization success in
65 novel habitats (Lockwood et al. 2005, Colautti et al. 2006, Simberloff 2009).

66 Propagule pressure is the total number of potentially reproductive individuals (e.g., adults, eggs,
67 seeds, vegetative material) introduced to an area (Novak 2007). It is often described in this broad
68 sense, which belies its complexity. Two important components of propagule pressure are the
69 number of introduction events— sometimes termed propagule number, and the number of
70 individuals per introduction event— sometimes termed propagule size (*sensu* Fauvergue et al.
71 2012). Here, we use "introduction regime" to refer to different combinations of the number of
72 introduction events and the number of individuals introduced per event. The same total
73 propagule pressure, N, can be achieved by different introduction regimes depending on how
74 those N individuals are distributed in time or space (Haccou and Iwasa 1996). An introduction
75 regime of N individuals lies on a continuum bounded by maximizing the number of individuals
76 introduced per event (all N individuals introduced in one event to the same location) and
77 maximizing the number of introduction events (one individual introduced in each of N sequential
78 events through time or to each of N unique locations).

79 Colonization success increases with total propagule pressure, but it is unclear whether the
80 correlation is driven by the number of individuals introduced per event or the number of
81 introduction events (Lockwood et al. 2005, Colautti et al. 2006, Simberloff 2009). Historical data
82 suggest that multiple introductions can facilitate population establishment, but conclusions from
83 these studies are limited by the inability to control for the total number of individuals introduced
84 (Hopper and Roush 1993, Blackburn and Duncan 2001, Grevstad et al. 2011, Fauvergue et al.
85 2012). Models that hold the total propagule pressure constant agree that multiple, small
86 introductions distributed across space will lead to greater establishment probability compared to

a single, large introduction when Allee effects are weak (Haccou and Iwasa 1996, Grevstad 1999, Schreiber and Lloyd-Smith 2009). However, both modeling and empirical approaches have generated conflicting views on how an introduction regime affects colonization success when introductions are distributed through time and total propagule pressure is constant.

There is evidence from both models and experiments that colonization success can increase with more, smaller introduction events through time. Branching process models show that, in the long run, several, small introductions will always be more likely to successfully establish a population than a single, large introduction (Haccou and Iwasa 1996, Haccou and Vatutin 2003). This finding was corroborated by simulations with no Allee effects (Drolet and Locke 2016). In a *Daphnia* microcosm experiment, increasing introduction frequency (proportional to the number of introductions) positively affected population growth, but the number of individuals per introduction had no detectable effect. This pattern did not hold for establishment probability, which was only affected by total propagule pressure (Drake et al. 2005). However, Drake et al. (2005) did not continue scheduled introductions if a population went extinct, denying those populations one of the main benefits of repeated introductions and creating variability in the total propagule pressure. In a more recent experiment, several, small introductions led to a 65% increase in abundance of successfully colonizing invasive Pacific Oyster (*Crassostrea gigas*) compared to a single, large introduction (Hedge et al. 2012).

There is also evidence from both models and experiments that colonization success increases with fewer, larger introduction events through time. In simulations of bird invasions, a single, large introduction event always led to the greatest establishment probability (Cassey et al. 2014). When Drolet and Locke (2016) included Allee effects in their simulations, their previously observed pattern reversed and colonization success was instead more likely with fewer, larger

110 introductions than with more, smaller introductions. In a field experiment with the psyllid
111 biocontrol agent, *Arytainilla spartiophila*, the number of individuals per introduction event was a
112 better predictor of establishment success than the number of introduction events (Memmott et al.
113 2005). In this case however, the introduction regimes with the most individuals per event also
114 had the highest total propagule pressure (Memmott et al. 2005). In an experiment that controlled
115 total propagule pressure, a single, large introduction of the non-native mysid, *Hemimysis*
116 *anomala*, led to larger populations and greater survival probabilities compared to several, small
117 introductions through time (Sinclair and Arnott 2016).

118 Environmental stochasticity in the recipient environment may also affect which introduction
119 regime is optimal for colonization. Branching process models show that a more variable
120 environment can reduce the probability of population establishment for all introduction regimes
121 (Haccou and Iwasa 1996, Haccou and Vatutin 2003), and simulations of introductions distributed
122 in space suggest that greater environmental variability will magnify the benefit of multiple
123 introductions (Grevstad 1999). However, simulations of introductions through time did not
124 support either of these outcomes— a single, large introduction was most likely to establish a
125 population even with extreme levels of environmental stochasticity (Cassey et al. 2014).

126 Thus, modeling and empirical approaches have not resolved how different introduction regimes
127 with a fixed total propagule pressure will affect colonization success when introductions are
128 distributed through time. Further, there has been no experimental test of whether variability in
129 the recipient environment interacts with the introduction regime to affect colonization. We paired
130 demographic simulations with a laboratory microcosm experiment using the red flour beetle,
131 *Tribolium castaneum,* to reconcile conflicts in the literature and test how different introduction
132 regimes implemented through time affected colonization success in novel habitats. We explicitly

133 manipulated whether the novel habitat was stable or randomly fluctuating in quality to assess
134 how the success of different introduction regimes may depend upon variability in the recipient
135 environment. With the total number of individuals introduced held constant at 20, we varied the
136 size and number of introduction events used to distribute those individuals in four different
137 introduction regimes. We evaluated establishment probability and population size over 7 discrete
138 generations to ask: 1) does colonization success increase with more introduction events or with
139 more individuals per introduction event?, and 2) does the effect of the introduction regime on
140 colonization success depend on whether the recipient novel environment is stable or fluctuating
141 through time?

142 **METHODS**

143 *General Framework*

144 In simulations and a microcosm experiment, we evaluated the outcome of introducing 20 total
145 individuals to one of two environmental contexts (a stable or fluctuating novel environment),
146 varying the number of introduction events used to distribute those individuals through time. This
147 total was low enough to allow some population extinction within a reasonable timeframe, but
148 high enough to be representative of documented introductions in the literature (Simberloff 1989,
149 Simberloff 2009; Grevstad 1999; Berggren 2001; Taylor et al. 2005; Drake et al. 2005). The
150 introduction regimes were: 20 individuals introduced in the first generation, 10 individuals
151 introduced in each of the first 2 generations, 5 individuals introduced in each of the first 4
152 generations, and 4 individuals introduced in each of the first 5 generations. To create the
153 fluctuating environment, we imposed a magnitude of variability corresponding to environmental
154 stochasticity in nature, which leads to frequent, mild to moderate perturbations in population

growth rate due to external forces (Lande 1993). Populations were tracked for seven generations following the initial introduction.

Establishment probability and the size of established populations were used as measures of colonization success. Populations were deemed 'established' for any time step in which they were extant and population size was noted for all extant populations in every time step. For analysis, establishment probability and mean population size were assessed in generation 7.

Study System

Our simulations and microcosm experiment use *Tribolium castaneum* (red flour beetles) to model the life history of organisms with discrete, non-overlapping generations (e.g., annual insects and fishes) following Melbourne and Hastings (2008). Individual simulated colonists were randomly sourced from a randomly mating, infinitely large population. Individual experimental colonists came from a thoroughly mixed source population maintained at 800 individuals in each of the 4 temporal blocks over which the experiment was replicated. To obtain colonists, beetles from the source population were mixed freely on a plate, selected from many sections of the plate, and introduced to subsets of the experiment populations in a random order (about 24 subsets per block). All migrant females were assumed to arrive mated from the source population. The strong maternal effects exhibited by *Tribolium* flour beetles were reduced by rearing individuals from the source population on novel growth medium for 1 generation prior to using them as colonists (Van Allen and Rudolf 2013, Hufbauer et al. 2015).

Simulations

Population dynamics were simulated with a Negative Binomial-Binomial gamma (NBBg) Ricker model (Melbourne and Hastings 2008) with an additional mating function of the form:

$$
\begin{aligned}
&N_{t+1,i} \sim NegBinom\left(\mu_i, k_D F_{mated_{(t,i)}}\right)\\
&\mu_i = \frac{F_{mated_{(t,i)}}}{p} R_{E_i} e^{-\alpha N_{t,i}}\\
&R_{E_i} \sim Gamma\left(k_E, \frac{R_0}{k_E}\right)\\
&N_{t,i} = N_{migrants_{(t,i)}} + N_{residents_{(t,i)}}\\
&F_{mated_{(t,i)}} = \begin{cases} F_{migrants_{(t,i)}}, & F_{migrants_{(t,i)}} + F_{residents_{(t,i)}} = N_{t,i} \\ F_{migrants_{(t,i)}} + F_{residents_{(t,i)}}, & F_{migrants_{(t,i)}} + F_{residents_{(t,i)}} < N_{t,i} \end{cases}\\
&F_{migrants_{(t,i)}} \sim Binom\left(N_{migrants_{(t,i)}}, p\right)\\
&F_{residents_{(t,i)}} \sim Binom(N_{residents_{(t,i)}}, p)\\
&p = 0.5\\
&R_0 \sim Gamma(2.6, 1)\\
&\alpha \sim Gamma(0.0037, 1)\\
&k_E \sim Gamma(17.6, 1)\\
&k_D \sim Gamma(1.07, 1)
\end{aligned}
\qquad \text{(Eq. 1)}
$$

Where $N_{t+1,i}$ is the size of population i at time $t+1$, k_D represents stochasticity due to demographic heterogeneity, μ_i is the expectation of the size of population i in the next time step, $F_{mated(t,i)}$ is the latent number of mated females in population i at time t, p is the probability of an individual being female, R_{Ei} is the latent density-independent population growth rate for environment E, α is the egg cannibalism rate, $N_{t,i}$ is the total size of population i at time t, k_E represents environmental stochasticity, R_0 is the density-independent population growth rate for the average environment, $N_{migrants(t,i)}$ is the total number of migrants to population i at time t, and $N_{residents(t,i)}$ is the total number of residents in population i at time t.

186 We imposed a mating function such that a population would deterministically go extinct if it
187 comprised only non-migrant females. In cases with an all female population and a mixture of
188 residents and migrants, we only included the number of migrant females in the density-
189 dependent effect of the demographic heterogeneity term $k_D * F_{mated(t,i)}$ because this effect
190 manifests via the number of eggs laid by females and only migrant females, being pre-mated,
191 would lay eggs. Weakly regularizing gamma priors were taken from Melbourne and Hastings
192 (2008). The expected equilibrium population size for the model is:

193 $$K = \frac{\log(R_0)}{\alpha} \quad \text{(Eq. 2)}$$

194 For each combination of the 4 introduction regimes and 2 recipient environment types (described
195 above), we simulated 500,000 replicate populations for 7 generations. All simulations were
196 performed in R (R Core Team 2016). This model captures the stochastic and deterministic
197 ecological dynamics of the *Tribolium* system well, but does not include evolutionary processes
198 (Melbourne and Hastings 2008, Hufbauer et al. 2015).

199 To parameterize the model, we censused 125 *Tribolium* populations one generation after
200 establishing them at various, known densities (between 5 and 200 individuals) on the novel
201 growth medium following the rearing procedure described in "Microcosm Experiment" below.
202 We fit the hierarchical model (Eq. 1) to these data in a Bayesian framework to generate posterior
203 distributions for each of the parameters using the nimble package in R (de Valpine et al. 2016).
204 We used a Metropolis-Hastings random walk sampler with 3 chains having 50,000 samples each
205 (including 10,000 samples that were removed for burn in). The chains converged (multivariate $\hat{R}$

206 for the 4 key parameters =1.01) and produced a sufficient number of effective samples (R_0: 757,
207 α: 548, k_E: 4103, k_D: 1703).

208 A single novel growth medium mixture was used for these 125 populations (mixture 5,
209 Supplementary Table 1), so we treated the estimated environmental stochasticity parameter, k_E,
210 as the expectation for a stable environment. We parameterized a fluctuating environment model
211 by increasing the standard deviation of the density-independent per capita population growth rate
212 by approximately 10% for any given population at any given time step. This was accomplished
213 by multiplying all of the samples from the posterior distribution of the environmental
214 stochasticity parameter, k_E, by 100/121 (derived by moment matching for the gamma
215 distribution). By using random sets of parameters drawn from the samples of the posterior
216 distributions, we were able to propagate the model uncertainty through the simulations and
217 combine it with the deterministic and stochastic dynamics represented by the model itself.

218 *Microcosm Experiment*

219 We founded 842 *Tribolium* populations with different introduction regimes (20 individuals in the
220 first generation, 10 individuals in the first 2 generations, 5 individuals in the first 4 generations,
221 or 4 individuals in the first 5 generations) and environments (stable or fluctuating) with between
222 96 and 120 replicate populations per treatment combination (Supplemental Table 3). Each
223 population was reared in a 4cm x 4cm x 6cm plastic box (AMAC Plastic Products) with 2
224 tablespoons (approximately 15 g) of freshly prepared growth medium that had been humidified
225 for at least 24 hours. The growth medium used for the source population (the natal environment
226 of the colonists) comprised 95% wheat flour (Pillsbury Co. or Gold Medal Products Co.) and 5%
227 brewers' yeast (Sensient Flavors). Colonists were introduced into a novel growth medium

228 comprising a small percentage of natal medium mixed with corn flour (Bob's Red Mill). All
229 populations were reared in one of two dark incubators at 31° and approximately 70% relative
230 humidity (standard conditions) and were haphazardly rotated between incubators weekly.

231 For each population in each generation, a known number of adults laid eggs for 24 hours in fresh
232 medium, and were then removed. Offspring were given 35 days to develop, and adults were then
233 censused. Censused adults laid eggs on freshly prepared growth medium for 24 hours,
234 completing their laboratory life cycle. In censusing trials, we estimated the maximum
235 observation error to be 4.6% (median: 0%, mean: 0.26%) with no detectable difference across
236 observers or populations with different sizes.

237 Each replicate population experienced a novel environment that was either stable or randomly
238 fluctuating through time. The same novel growth medium mixture containing 99.05% novel corn
239 flour and 0.95% natal medium (mixture 5, Supplementary Table 1) was used for the stable
240 environment for the duration of the experiment, which preliminary results indicated would yield
241 a population growth rate of $\lambda = 1.2$ compared to a mean population growth rate of 3.36 on 100%
242 natal medium. To create the fluctuating environment, we randomly selected a novel growth
243 medium mixture from one of 9 possible media mixtures for each population in each generation.
244 Each population in the fluctuating environment therefore experienced a unique series of
245 environmental conditions. The 9 possible media mixtures represented a gradient of corn flour to
246 natal medium ratios, and were designed to yield expected population growth rates between 0.88
247 and 1.33 (Supplementary Table 1). We chose this range to mimic environmental stochasticity
248 measured in nature (Sæther and Engen 2002) while remaining within the bounds of biologically
249 realistic population growth (λ between 0.5 and 1.5) (Morris et al. 2008). The random choice was

250 constrained such that the expected geometric mean population growth rate for each population
251 resembled expected growth of populations in the stable environment ($\lambda_{expected}$ = 1.2±0.05).

252 We estimated the amount of environmental stochasticity that we achieved in the fluctuating
253 environment as the difference in mean total stochasticity between populations in the fluctuating
254 and stable environments (Sæther and Engen 2002). We assumed that total stochasticity was a
255 combination of demographic and environmental stochasticity for populations in the fluctuating
256 environment, and that demographic stochasticity was the sole contributor to total stochasticity
257 for populations in the stable environment. Total stochasticity (demographic plus environmental)
258 of each population that did not experience extinction (n=667) was calculated as the variance of
259 the natural logarithms of its population growth rates through 7 generations:

$$s_{total} = s_{demographic} + s_{environmental} = var(\log(\lambda_t)) \quad \text{(Eq. 3)}$$

260 where, for a particular population, s_{total} is its total stochasticity, $s_{demographic}$ is its demographic
261 stochasticity, $s_{environmental}$ is its environmental stochasticity (assumed to be 0 for populations
262 in the stable environment), and λ_t is its per capita population growth rate between generation *t-1*
263 and generation *t* (*t=1, 2, ... , 7*). We only calculated total stochasticity for populations that did
264 not experience any extinction in order to capture the full temporal extent of environmental
265 fluctuations and because extinctions would have an infinite effect on this measure of
266 stochasticity.

267 *Statistical analyses*

268 We evaluated how our environment treatment affected variability in population growth rate (total
269 stochasticity from Eq. 3) using a linear mixed effects model with environment (stable or
270 fluctuating) as a fixed effect and block as a random intercept effect.

271 We used a mixed effects logistic regression with a logit link to predict the binary response of
272 establishment, and a mixed effects Poisson regression with a log link to analyze population size.
273 In both models, introduction regime, environment treatment, and their interaction were treated as
274 fixed effects, and block was treated as a random intercept effect.

275 We assessed the effect of temporary extinctions on the establishment probability and mean
276 population size by fitting the generalized linear mixed effects models described above to data
277 from the multiple introduction regimes (i.e. not the 20x1 regime) and with additional predictor
278 variables. To assess the effect of the presence of a temporary extinction, we included an
279 additional Boolean predictor for whether a population went temporarily extinct or not. To assess
280 the role of the total propagule pressure, we included a numeric predictor representing the number
281 of beetles introduced after the latest temporary extinction.

282 Group-level significance of fixed effects was tested using likelihood ratio tests on nested models,
283 and least-squares contrasts were used to compare levels of the fixed effects. All statistical
284 analyses were performed in R, version 3.3.2 (R Core Team 2016). Generalized linear mixed
285 models were fit using the lme4 package, version 1.1-12 (Bates et al. 2015) and pairwise
286 comparisons were made using the lsmeans package, version 2.25 (Lenth 2016).

287 *Data availability*

288 The raw experiment data, simulated population trajectories, R code for the simulations, R code
289 fitting the NBBg model, samples from the posterior distributions of the NBBg parameters, and R
290 code fitting the mixed effects models are available as supplemental material, on Figshare
291 (https://doi.org/10.6084/m9.figshare.4648865.v2), and on GitHub
292 (https://github.com/mikoontz/ppp-establishment).

Note on egg contamination

Laboratory procedures after generation 3 resulted in occasional egg contamination between replicate populations of the same treatment. Some populations of 1 individual persisted when they should have deterministically gone extinct, so we manually edited those population trajectories such that they went extinct in the generation after having only 1 individual.

RESULTS

Simulations

Summary statistics for the posterior distributions of the four NBBg model parameters are given in Supplementary Table 2.

Our simulations showed introduction regimes with more introduction events were more likely establish a population by the seventh generation (Figure 1). Simulated introductions into a stochastically fluctuating environment resulted in slightly lower population establishment for all introduction regimes (difference of ~1%), but did not favor any particular regime (not shown). The mean population sizes for each introduction regime/environment combination were approximately equal in simulations. Mean population sizes only incorporated extant populations, so they were slightly larger than the expectation for the equilibrium population size (Figure 2).

Microcosm experiment

Mean environmental stochasticity of populations in the fluctuating environment was 0.052 (95% CI = 0.0073 to 0.0966; $p = 0.023$). This value is near the median value of 0.055 measured in

312 nature by Sæther and Engen (2002) in a metaanalysis of 35 avian populations, indicating that we
313 achieved biologically realistic fluctuations in population growth rate.

314 We found no evidence that the probability of establishment was affected by a main effect of
315 environment (χ^2=0.72, df=1, p=0.40), nor by an interaction between environment and
316 introduction regime (χ^2=3.49, df=3, p=0.32). However, there was strong support for an effect of
317 introduction regime on establishment probability (χ^2=59.76, df=3, p<0.0001). Pairwise
318 comparisons of the different introduction regimes averaged across the environment treatments
319 revealed that the 4x5 regime was the most likely to establish populations, with a probability of
320 about 0.98, whereas the 20x1 and 10x2 regimes were the least likely to establish populations,
321 with a probability reduced to about 0.8 (Figure 1, Figure 3).

322 The size of populations that persisted until generation 7 was shaped by significant effects of
323 introduction regime (χ^2=91.65, df=3, p<0.0001), environment treatment (χ^2=117.83, df=3,
324 p<0.0001), and their interaction (χ^2=44.62, df=3, p<0.0001). Populations established via more
325 introduction events were larger when averaged across the environment treatments. Extant
326 populations in the stable environment were larger than those in the fluctuating environment when
327 averaged across the introduction regimes. The interaction manifests as the benefit of a stable
328 environment increases with more, smaller introduction events (Figure 2, Figure 4).

329 Extinctions accumulated regularly throughout the experiment period, with 101 out of 842
330 populations (12.0%) going extinct by generation 7 (Figure 3). The additional introductions that
331 some populations received often restored a population that had temporarily gone extinct. Out of
332 602 populations that received more than 1 introduction (i.e. not the 20x1 introduction regime),
333 104 of them (17.3%) temporarily went extinct at least once before being replenished by

additional colonizing individuals. Twelve populations were rescued in this way at least twice, and one population was rescued in this way three times.

Temporary extinctions significantly affected colonization success. The presence of a temporary extinction significantly reduced average establishment probability from 92.4% to 82.1% (difference = -1.13 on logit scale, 95% CI = -0.22 to -2.04, χ^2 = 5.44, df = 1, p = 0.020) and mean population size from 47.8 to 45.4 (difference = -0.052 on log scale, 95% CI = -0.02 to -0.09, χ^2 = 9.42, df = 1, p=0.0021). Each additional colonist contributing to a population after the latest temporary extinction significantly increased the mean population size (estimate = 0.005 on the log scale, 95% CI = 6.4e-05 to 0.01, χ^2 = 3.94, df = 1, p=0.047).

Results from the simulations are expected to represent the dynamics in the microcosm experiment if the assumption of the model on which the simulations were built holds—that only demographic processes are responsible for the dynamics observed in the experiment. However, establishment probability at generation 7 was equal to or greater than that expected from the simulations (Figure 1) and mean population sizes were much larger in the experiment than in the simulations (Figure 2), together suggesting an influence of non-demographic mechanisms.

DISCUSSION

We assessed how the number and size of introduction events through time drive colonization success in a novel environment when the total number of individuals introduced to a site is fixed. We considered novel environments that were either stable or randomly fluctuating in quality through time, and evaluated populations through 7 discrete generations. We approached this question in two ways: 1) stochastic simulations of a demographic population dynamics model parameterized with empirical data, and 2) a highly replicated laboratory microcosm experiment.

By coupling these approaches, we were able to expand and test the theoretical understanding of how the introduction regime affects colonization in stable and fluctuating environments as well as to develop new avenues for research when observations did not align with predictions. We found that several, small introductions increase colonization success and that demographic processes alone are insufficient to explain the dynamics observed in the experiment.

We found minimal to no effect of a biologically realistic level of environmental stochasticity on establishment probability in demographic simulations. This finding is inconsistent with the prediction made by Grevstad (1999) who found with simulations that several, small introductions would produce an especially high establishment probability compared to a single, large introduction in a variable environment. The difference in findings is perhaps due to a difference in the kinds of introduction regimes modeled: Grevstad (1999) simulated multiple introductions distributed in space, while we focused on introductions separated in time. Additionally, we may not have detected the same effect of environmental stochasticity that Grevstad (1999) noted because of differences in the magnitude of environmental variation imposed. At the highest levels of simulated environmental variation imposed in Grevstad's study, over 65% of environments would result in population decline and over 40% of environments would result in population loss of greater than 90%. This kind of environmental variability might be characterized as random catastrophes—major events causing immediately disastrous effects to the population, rather than a level of environmental stochasticity leading to more moderate fluctuations in growth rates (Lande 1993). Our simulations and microcosm results do corroborate the results from simulations by Cassey et al. (2014) who also used introductions through time and found a minimal effect of environmental stochasticity on establishment probability. Cassey et al. (2014) further found a minimal effect of random, infrequent catastrophes and bonanzas,

379 suggesting that increasing environmental variability through time in the broadest sense (i.e.,
380 encompassing environmental stochasticity, random catastrophes, and bonanzas) does not
381 magnify the benefit of several, small introductions through time in the same way that Grevstad
382 (1999) found with several, small introductions across space.

383 There also did not appear to be a strong effect of environmental stochasticity on population size
384 in the demographic simulations. All treatments in the simulations reached similar mean
385 population size by generation 7, which was slightly higher than the equilibrium size due to our
386 measure only including extant populations. This suggests that strong density dependence in the
387 demographic simulations was the primary influence on population size.

388 We observed striking differences in the measures of colonization success between the
389 demographic simulations and the microcosm experiment. We found that establishment
390 probability increased with the number of introduction events (as in the simulations), but that all
391 microcosm establishment probabilities equaled or exceeded expectations from simulations. In the
392 microcosm, mean population size in stable environments was greater than in fluctuating
393 environments. There was also an interaction between environment and introduction regime,
394 whereby the mean population size was increasingly greater in stable compared to fluctuating
395 environments as the number of introductions increased. Furthermore, populations grew larger by
396 generation 7 in the microcosm experiment than in the simulations.

397 Differences between the results of the simulations and of the microcosm experiment suggest that
398 the demographic processes captured by the model do not account for all of the biological
399 processes that occurred in the microcosm. Recent work shows that adaptation to the novel, harsh
400 environment from standing variation is possible in this species (Hufbauer et al. 2015), and likely

401 explains the greater establishment probability and population sizes in the microcosm compared
402 to expectations derived from demographic simulations which do not include adaptation.

403 The rescue effect of immigration can act demographically by increasing the size of populations
404 (Hufbauer et al. 2015). Certainly, demographic rescue played a critical role for the 104
405 populations that went extinct temporarily until another introduction event revived them. Those
406 temporary extinctions had lasting effects on colonization success. Colonization success declined
407 for populations that experienced a temporary extinction, and the mean population size
408 significantly increased if more colonists contributed to the population after a temporary
409 extinction. These results reflect the overarching importance of total propagule pressure
410 regardless of introduction regime.

411 The rescue effect can also act genetically by increasing the fitness of populations (Frankham
412 2015, Hufbauer et al. 2015, Whiteley et al. 2015). The immigrants all came from the same large
413 external source population, so it is unlikely that gene flow from migrants produced novel genetic
414 combinations on which selection could act (Novak 2007). Thus, a more likely mechanism by
415 which immigration increased mean population size was by relieving inbreeding depression or
416 counteracting drift-induced allele loss. Small populations are more prone to experiencing
417 increased homozygosity and inbreeding depression, which can reduce population growth rates
418 and increase extinction risk (McCauley and Wade 1981, O'Grady et al. 2006, Szűcs et al. 2017).
419 Even small amounts of gene flow can alleviate these effects, so the additional small introductions
420 of mated individuals from the external source population were well-suited to bring about longer-
421 term relief (Slatkin 1985, Hufbauer et al. 2015). However, Cassey et al. (2014) found that
422 simulated inbreeding depression was especially detrimental for several, small introductions
423 through time, so other mechanisms might be at play.

Sustained immigration may affect adaptation of the incipient population. Introductions to a harsh, novel habitat can result in adaptive evolution with the right amount of gene flow if additional immigrants to a declining population prevent extinction long enough to allow for adaptation to occur (Holt and Gomulkiewicz 1997). However, too much gene flow can lead to genetic swamping whereby the homogenizing effects of gene flow overpowers ongoing local adaptation (Lenormand 2002). This may have been the case for populations in the 10x2 introduction regime, which had the highest average migration rate, lowest establishment probability, and lowest mean population size. 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). Though not significant, the lower establishment probability and mean population size in the 10x2 introduction regime warrants further work to assess whether they exemplify the yet-unseen scenario described by Blackburn et al. (2015) in which maladaptive gene flow from multiple introductions hampers population establishment in a novel range. More broadly, further study is necessary to evaluate how immigration affected adaptation in this system, if at all (Gomulkiewicz and Holt 1995, Boulding and Hay 2001).

CONCLUSION

Our experimental results suggest that several, small introductions through time lead to greater colonization success in a novel habitat, and that introductions into a stable recipient environment lead to larger population sizes, but not greater establishment probability. Further, introductions to a stable recipient environment are especially beneficial to populations established with more introduction events. These results defied our expectations derived from parallel simulations of a model that included demographic processes but not evolutionary ones, so we suspect a genetic mechanism might be at work. Genetic mechanisms are rarely incorporated when simulating the

447 effect of introduction regime on colonization (but see Cassey et al. 2014), and our multi-
448 generation microcosm is unique in bringing evolutionary processes to bear on parsing two key
449 components of propagule pressure in an experimental setting.

450 For invasions, our results highlight the importance of preventing further introductions to the
451 same location, even for established species. For conservation and biological control, our results
452 suggest that emphasis should be placed on increasing the number of introductions or re-
453 introductions to a location, rather than increasing the size of those events if Allee effects are
454 weak. Sustained introduction efforts should also bring about concomitant benefits in the form of
455 longer-term monitoring, increased data collection, and more opportunities for experimentation
456 and adaptive management (Lockwood et al. 2005, Godefroid et al. 2011).

457 **ACKNOWLEDGMENTS**

458 We thank Charlotte Hoover, Kaitlyn Adkins, Mary Catherine Cochran, Don Waters, Stacy
459 Endriss, Marianna Szűcs, and Jenny Rickford for help with data collection. Funding was
460 provided by NSF Graduate Research Fellowship Grants #DGE-1321845 Amend. 3 (to MJK) and
461 #DGE-1106400 (to MFO), NSF Grant DEB-0949619 (to RAH), and NSF Grant DEB-0949595
462 (to BAM). RAH also acknowledges the support of the USDA via the Colorado Experiment
463 Station. We thank the lab groups of Ruth Hufbauer, Paul Ode, Andrew Norton, Andrew Latimer,
464 Truman Young, and Jenny Gremer for comments on the project and manuscript.

465 **REFERENCES**

466 Bates, D. et al. 2015. Fitting linear mixed-effects models using lme4. - J. Stat. Softw. 67: 1–48.

467 Berggren, A. 2001. Colonization success in Roesel's bush-cricket *Metrioptera roeseli*: The

effects of propagule size. - Ecology 82: 274–280.

Blackburn, T. M. and Duncan, R. P. 2001. Establishment patterns of exotic birds are constrained by non-random patterns in introduction. - J. Biogeogr. 28: 927–939.

Blackburn, T. M. et al. 2015. The influence of numbers on invasion success. - Mol. Ecol.: 1–12.

Boulding, E. G. and Hay, T. 2001. Genetic and demographic parameters determining population persistence after a discrete change in the environment. - Heredity (Edinb). 86: 313–24.

Cassey, P. et al. 2005. Concerning invasive species: Reply to Brown and Sax. - Austral Ecol. 30: 475–480.

Cassey, P. et al. 2014. A population model for predicting the successful establishment of introduced bird species. - Oecologia 175: 417–428.

Colautti, R. I. et al. 2006. Propagule pressure: A null model for biological invasions. - Biol. Invasions 8: 1023–1037.

de Valpine, P. et al. 2016. Programming with models: writing statistical algorithms for general model structures with NIMBLE. - J. Comput. Graph. Stat. 8600: 1–20.

Drake, J. M. et al. 2005. Propagule pressure and persistence in experimental populations. - Biol. Lett. 1: 480–3.

Drolet, D. and Locke, A. 2016. Relative importance of propagule size and propagule number for establishment of non-indigenous species: A stochastic simulation study. - Aquat. Invasions 11: 101–110.

Fauvergue, X. et al. 2012. The biology of small, introduced populations, with special reference to

488 biological control. - Evol. Appl. 5: 424–43.

489 Frankham, R. 2015. Genetic rescue of small inbred populations: Meta-analysis reveals large and
490 consistent benefits of gene flow. - Mol. Ecol. 24: 2610–2618.

491 Godefroid, S. et al. 2011. How successful are plant species reintroductions? - Biol. Conserv. 144:
492 672–682.

493 Gomulkiewicz, R. and Holt, R. D. 1995. When does evolution by natural selection prevent
494 extinction? - Evolution (N. Y). 49: 201–207.

495 Grevstad, F. S. 1999. Factors influencing the chance of population establishment: Implications
496 for release strategies in biocontrol. - Ecol. Appl. 9: 1439–1447.

497 Grevstad, F. S. et al. 2011. Revisiting release strategies in biological control of weeds: Are we
498 using enough releases? - XIII Int. Symp. Biol. Control Weeds: 368–376.

499 Haccou, P. and Iwasa, Y. 1996. Establishment probability in fluctuating environments: A
500 branching process model. - Theor. Popul. Biol. 50: 254–280.

501 Haccou, P. and Vatutin, V. 2003. Establishment success and extinction risk in autocorrelated
502 environments. - Theor. Popul. Biol. 64: 303–314.

503 Hedge, L. H. et al. 2012. Manipulating the intrinsic parameters of propagule pressure:
504 Implications for bio-invasion. - Ecosphere 3: 1–13.

505 Holt, R. D. and Gomulkiewicz, R. 1997. How does immigration influence local adaptation? A
506 reexamination of a familiar paradigm. - Am. Nat. 149: 563–572.

507 Hopper, K. R. and Roush, R. T. 1993. Mate finding, dispersal, number released, and the success

of biological control introductions. - Ecol. Entomol. 18: 321–331.

Hufbauer, R. A. et al. 2015. Three types of rescue can avert extinction in a changing environment. - Proc. Natl. Acad. Sci. 112: 10557–10562.

Lande, R. 1988. Genetics and demography in biological conservation. - Science 241: 1455–1460.

Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. - Am. Nat. 142: 911–927.

Lenormand, T. 2002. Gene flow and the limits to natural selection. - Trends Ecol. Evol. 17: 183–189.

Lenth, R. V 2016. Least-squares means: The R package lsmeans. - J. Stat. Softw. 69: 1–33.

Lockwood, J. L. et al. 2005. The role of propagule pressure in explaining species invasions. - Trends Ecol. Evol. 20: 223–8.

Lodge, D. M. 1993. Biological invasions: Lessons for ecology. - Trends Ecol. Evol. 8: 133–137.

Mack, R. N. et al. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. - Ecol. Appl. 10: 689–710.

McCauley, D. E. and Wade, M. J. 1981. The populational effects of inbreeding in *Tribolium*. - Heredity (Edinb). 46: 59–67.

Melbourne, B. A. and Hastings, A. 2008. Extinction risk depends strongly on factors contributing to stochasticity. - Nature 454: 100–3.

Memmott, J. et al. 2005. The effect of propagule size on the invasion of an alien insect. - J. Anim.

Ecol. 74: 50–62.

Morris, W. F. et al. 2008. Longevity can buffer plant and animal populations against changing climatic variability. - Ecology 89: 19–25.

Novak, S. J. 2007. The role of evolution in the invasion process. - Proc. Natl. Acad. Sci. 104: 3671–2.

O'Grady, J. J. et al. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. - Biol. Conserv. 133: 42–51.

R Core Team 2016. R: A language and environment for statistical computing. http://www.r-project.org/. in press.

Ricciardi, A. 2007. Are modern biological invasions an unprecedented form of global change? - Conserv. Biol. 21: 329–36.

Sæther, B.-E. and Engen, S. 2002. Pattern of variation in avian population growth rates. - Philos. Trans. R. Soc. Lond. B. Biol. Sci. 357: 1185–95.

Sakai, A. K. et al. 2001. The population biology of invasive species. - Annu. Rev. Ecol. Syst. 32: 305–332.

Schreiber, S. J. and Lloyd-Smith, J. O. 2009. Invasion dynamics in spatially heterogeneous environments. - Am. Nat. 174: 490–505.

Simberloff, D. 1989. Which insect introductions succeed and which fail? - In: Drake et al., J. A. (ed), Biological Invasions: a Global Perspective. John Wiley & Sons Ltd, pp. 61–75.

Simberloff, D. 2009. The role of propagule pressure in biological invasions. - Annu. Rev. Ecol.

547 Evol. Syst. 40: 81–102.

548 Sinclair, J. S. and Arnott, S. E. 2016. Strength in size not numbers: Propagule size more
549 important than number in sexually reproducing populations. - Biol. Invasions 18: 497–505.

550 Slatkin, M. 1985. Gene flow in natural populations. - Annu. Rev. Ecol. Syst. 16: 393–430.

551 Szűcs, M. et al. 2017. Genetic and demographic founder effects have long-term fitness
552 consequences for colonising populations. - Ecol. Lett.: 1–7.

553 Taylor, S. S. et al. 2005. Successful island reintroductions of New Zealand robins and
554 saddlebacks with small numbers of founders. - Anim. Conserv. 8: 415–420.

555 Van Allen, B. G. and Rudolf, V. H. W. 2013. Ghosts of habitats past: Environmental carry-over
556 effects drive population dynamics in novel habitat. - Am. Nat. 181: 596–608.

557 Whiteley, A. R. et al. 2015. Genetic rescue to the rescue. - Trends Ecol. Evol. 30: 42–49.

558 Zenni, R. D. and Nuñez, M. A. 2013. The elephant in the room: The role of failed invasions in
559 understanding invasion biology. - Oikos 122: 801–815.

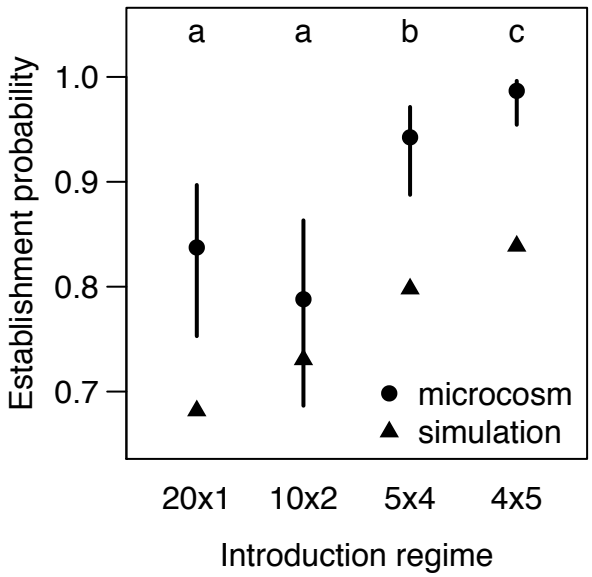

Figure 1. Establishment probabilities for each introduction regime assessed at generation 7. Triangles represent results from simulations. Dot-whiskers represent estimates and 95% confidence intervals from the mixed effects logistic regression model fit to data from the microcosm experiment. Different letters over dot-whiskers represent introduction regimes with significantly different establishment probabilities in the microcosm experiment.

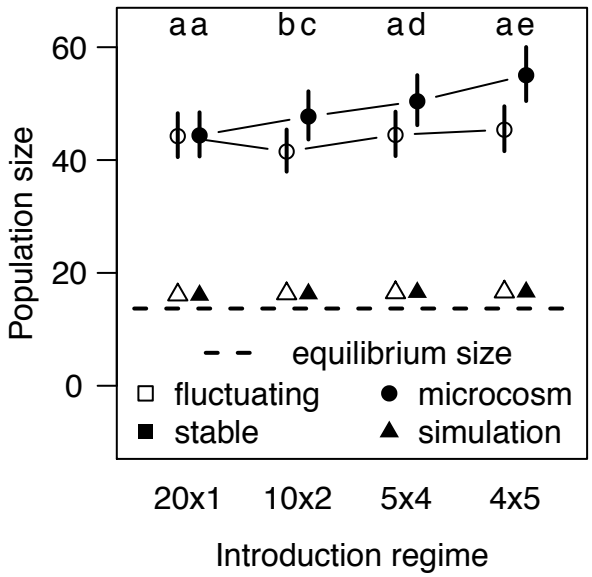

Figure 2. Mean sizes of extant populations for each introduction regime/environment combination in generation 7. Triangles represent results from simulations. Dot-whiskers represent estimates and 95% confidence intervals from the mixed effects Poisson regression model fit to data from the microcosm experiment. Different letters over dot-whiskers represent introduction regimes with significantly different mean population sizes. The dashed line represents the theoretical equilibrium population size derived from the NBBg model, which includes extinction and is therefore lower than the mean population size from the simulations.

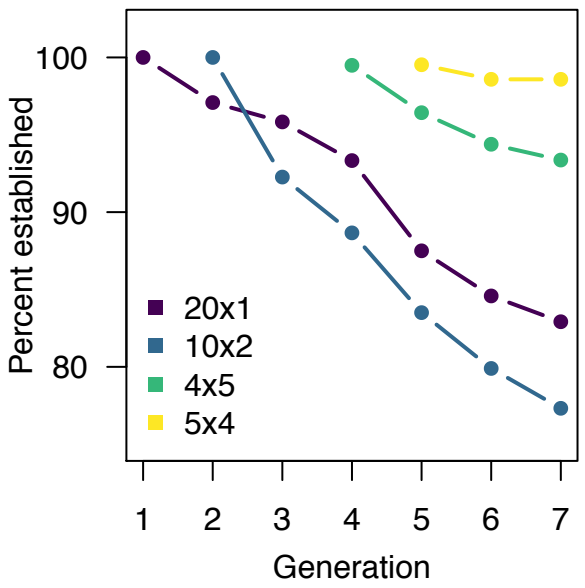

Figure 3. Percent of microcosm populations that were established in each generation for the 4 different introduction regimes. Data are pooled across the two environmental variability treatments. The introduction regimes are as follows: 20x1 = 20 individuals in the first generation, 10x2 = 10 individuals in each of the first two generations, 5x4 = 5 individuals in each of the first 4 generations, and 4x5 = 4 individuals in each of the first 5 generations.

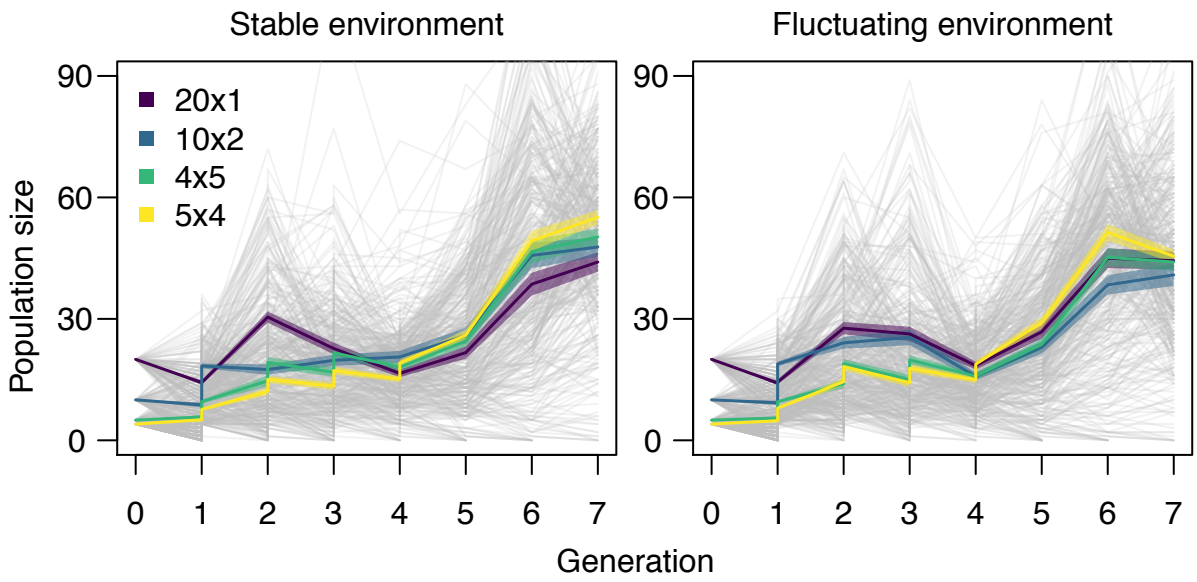

Figure 4. Population trajectories for 842 populations of *Tribolium* in the microcosm experiment. The first panel represents all populations in stable environments (n = 418) and the second panel represents all populations in fluctuating environments (n = 424). Vertical steps in the population size represent additional migrant individuals in introduction regimes with more than 1 introduction. Light grey lines represent individual population trajectories, and colored lines represent the mean size of extant populations for each of the 4 introduction regimes. The introduction regimes are as follows: 20x1 = 20 individuals in the first generation, 10x2 = 10 individuals in each of the first two generations, 5x4 = 5 individuals in each of the first 4 generations, and 4x5 = 4 individuals in each of the first 5 generations. Shading around the colored lines represents the mean size of extant populations plus and minus one standard error.