Minimizing extinction risk through genetic rescue

T. A. Waite, J. Vucetich, T. Saurer, M. Kroninger, E. Vaughn, K. Field & S. Ibargüen

Abstract

Minimizing extinction risk through genetic rescue.— According to the genetic rescue hypothesis, immigrants can improve population persistence through their genetic contribution alone. We investigate the potential for such rescue using small, inbred laboratory populations of the bean beetle (*Callosobruchus maculatus*). We ask how many migrants per generation (MPG) are needed to minimize the genetic component of extinction risk. During Phase 1, population size was made to fluctuate between 6 and 60 (for 10 generations). During this phase, we manipulated the number of MPG, replacing 0, 1, 3, or 5 females every generation with immigrant females. During Phase 2, we simply set an upper limit on population size ($\approx$10). Compared with the 0–MPG treatment, the other treatments were equivalently effective at improving reproductive success and reducing extinction risk. A single MPG was sufficient for genetic rescue, apparently because effective migration rate was inflated dramatically during generations when population size was small. An analysis of quasi–extinction suggests that replicate populations in the 1–MPG treatment benefited from initial purging of inbreeding depression. Populations in this treatment performed so well apparently because they received the dual benefit of purging followed by genetic infusion. Our results suggest the need for further evaluation of alternative schemes for genetic rescue.

Key words: Extinction risk, Founder events, Genetic rescue, Inbreeding.

Resumen

Minimización del riesgo de extinción mediante el rescate genético.— Según la hipótesis del rescate genético, los inmigrantes pueden mejorar la persistencia de una población mediante su contribución genética. Hemos investigado el potencial de un rescate de este tipo, utilizando pequeñas poblaciones endogámicas de laboratorio del gorgojo del haba *Callosobruchus maculatus*. Nos preguntamos cuántos migrantes por generación (MPG) son necesarios para minimizar el componente genético del riesgo de extinción. Durante la Fase 1, se hizo fluctuar el tamaño de la población entre 6 y 60 (durante 10 generaciones). En dicha fase manipulamos el número de MPGs, reemplazando 0, 1, 3, o 5 hembras nativas por hembras inmigrantes en cada generación. Durante la Fase 2, nos limitamos a poner un límite superior al tamaño de la población ($\approx$10). Comparados con el tratamiento de 0–MPG, los otros tratamientos resultaron ser igualmente efectivos en la mejora del éxito reproductivo y la reducción del riesgo de extinción. Un único MPG era suficiente para el rescate genético, aparentemente debido a que la tasa de migración efectiva quedaba espectacularmente sobredimensionada durante generaciones, cuando el tamaño de la población era pequeño. Un análisis de cuasi–extinción sugiere que las poblaciones replicadas durante el tratamiento 1–MPG se beneficiaron de un saneamiento inicial por la disminución de la endogamia. Aparentemente, las poblaciones de este tratamiento se comportaron tan bien debido a que recibieron el doble beneficio del saneamiento seguido de la inyección genética. Nuestros resultados sugieren la necesidad de posteriores evaluaciones del rescate genético mediante esquemas alternativos.

Palabras clave: Riesgo de extinción, Acontecimientos de hundimiento, Rescate genético, Endogamia.

T. A. Waite, T. Saurer, M. Kroninger, E. Vaughn, K. Field & S. Ibargüen, Dept. of Evolution, Ecology, and Organismal Biology, Ohio State Univ., Columbus, Ohio 43210–1293, U.S.A.– J. Vucetich, School of Forest Resources and Environmental Science, Michigan Technological Univ., Houghton, Michigan 49931, U.S.A.

Corresponding author: T. A. Waite. E–mail: waite.1@osu.edu

Introduction

Small isolated populations are subject to loss of genetic diversity through drift and inbreeding. Despite a large body of findings implicating inbreeding as a contributor to extinction risk (reviewed by Hedrick & Kalinowski, 2000), the strength of any causal linkage between inbreeding and extinction remains a point of contention. Until recently, there was no direct evidence that genetic deterioration contributes to extinction of wild populations (Frankham & Ralls, 1998). Lacking such evidence, some workers have argued that stochastic demographic and environmental events may typically drive small populations to the brink of extinction before genetic deterioration poses a serious threat (Lande, 1988; Pimm et al., 1988; Caro & Laurenson, 1994). Even so, there is widespread agreement that loss of genetic diversity can lead to extinction. Support for this perspective comes from theoretical studies (Mills & Smouse, 1994; Lande, 1998; Tanaka, 2000; Finke & Jetschke, 1999; Fowler & Whitlock, 1999), laboratory experiments (Frankham, 1995a; Bryant et al., 1999; Bijlsma et al., 2000; Reed & Bryant, 2000; Nieminen et al., 2001), field experiments (e.g., Newman & Pilson, 1997), a landmark study of a metapopulation in nature (Saccheri et al., 1998), and meta–analyses (Frankham, 1999). A recent review summarizes evidence, based on new pedigree data and new data made possible by molecular and analytical tools for estimating inbreeding, that inbreeding can adversely affect population performance (Keller & Waller 2002; see also Goudet & Keller 2002). Meanwhile, a flurry of recent experimental (e.g., Bryant et al., 1999; Reed & Bryant, 2000, 2001; Newman & Tallmon, 2001) and theoretical studies (e.g., Fu et al., 1998; Bataillon & Kirkpatrick, 2000; Kirkpatrick & Jarne, 2000; Wang, 2000; Whitlock, 2000; Linklater, 2003) have explored ways to minimize the genetic component of extinction risk.

What kind of genetic intervention, if any, is needed? Ideally, genetic risks could be minimized without intervention, simply by maintaining populations above minimum viable size (reviewed by Reed & Bryant, 2000; see also Lande, 1995; Lynch et al., 1995; Gilligan et al., 1997). However, when this approach is not feasible or has already failed, genetic diversity can be maintained or restored by facilitating gene flow via translocation of individuals or propagules (e.g., Madsen et al., 1999). Because the mere arrival of immigrants could forestall local extinction, to demonstrate unequivocally that gene flow *per se* is beneficial, one must perform experiments in which genetic diversity is introduced *without* a simultaneous increase in population size. Recent studies have sought to provide evidence for such genetic rescue (i.e., increase in fitness due to gene flow) of recently fragmented or newly colonized populations (reviewed by Ingvarsson, 2001; see also Vila et al., 2003). For example, pollen–mediated gene flow improved fitness in small populations of a dioecious weedy plant (*Silene alba*) (Richards, 2000). Likewise, gene flow via immigration improved various fitness components in the self–incompatible mustard (*Brassica campestris*) (Newman & Tallmon, 2001), and improved fitness and reduced extinction risk in the house fly (*Musca domestica*) (Bryant et al., 1999). Lastly, gene flow facilitated by an alien pollinator (African honeybee, *Apis mellifera scutellata*) is apparently responsible for improved reproductive output in an Amazonian tree (*Dinizia excelsa* [Fabaceae]) in pastures and forest remnants, where native pollinators are absent (Dick, 2001). Although some earlier studies provided contradictory findings (references in Newman & Tallmon, 2001), these recent studies indicate that pollen– or immigrant–mediated gene flow can dramatically improve fitness in small inbred populations.

Here, we describe an experiment that extends these recent findings. Using inbred laboratory populations of the bean beetle (*Callosobruchus maculatus*), we manipulated the number of MPG by replacing 0, 1, 3, or 5 females with immigrant females each generation. The experiment allowed us to evaluate: (1) whether even a single MPG could lead to genetic rescue, and (2) how many migrants are needed to minimize the genetic component of extinction risk.

Methods

Subjects

C. maculatus is an important pest species. The beetles used in our experiment were derived from a genetic strain from southern India and reared at Ohio State University. Several features make this species a suitable model organism (e.g., Vucetich et al., 2000): (1) it has a short generation time (4–6 weeks); (2) females oviposit on beans and offspring emerge synchronously, with the adults typically dying before the next generation emerges; and (3) because only one beetle typically emerges from each mung bean (*Vigna radiata*), carrying capacity can be controlled simply by limiting the number of beans available.

Overview and rationale

The experiment was designed to quantify the requisite number of MPG to minimize extinction risk in small inbred populations. It comprised two phases. During Phase 1, population size was made to fluctuate between 6 and 60 individuals across 10 generations. In each generation, the ♂♂:♀♂ sex ratio was 5:1. During this phase, we manipulated the number of MPG by replacing 0, 1, 3, or 5 females every generation with immigrant females from a large outbred population. At the end of this phase, we measured the reproductive fitness and founding success of each replicate population. During Phase 2, we limited N by simply providing 10 mung beans to each replicate population for 10 generations. During

this phase, we documented extinctions. The rationale for each of these procedures is described below.

Most tests of the genetic rescue hypothesis have treated the infusion of new genetic material dichotomously, testing the effect of a single level of infusion versus no infusion. In an attempt to titrate the level of genetic infusion minimizing extinction risk, we assessed the effects of several levels on reproductive fitness and extinction risk. To avoid confounding the results with effects attributable to demographic rescue, these migrants were replacements, not additions.

Due to fluctuations in population size (FPS) (Vucetich et al., 1997; Vucetich & Waite, 1999), skewed sex ratio, and extra–Poisson variation in fecundity, most real populations exhibit N_e/N ratios that are less than unity (Frankham, 1995b). Therefore, during Phase 1, we manipulated the FPS and sex ratio to achieve N_e/N ratios typical of real populations. This approach resulted in an N_e/N ratio of ~0.2, which is close to the median of surveyed populations (Frankham, 1995b).

The most straightforward way to perform genetic rescue is to infuse a population with genetic material for a pulse (i.e., one, two, or a few generations). In Phase 2 of the experiment, we assess the residual impact of genetic infusion. That is, we assess the effects of prior genetic management (imposed during Phase 1) on extinction risk.

Detailed protocol

Preliminary steps
To establish replicate inbred populations, we began by orchestrating two successive full–sibling matings. Eighty female–male pairs, representing 80 unique pairs of founders, were used. Offspring of these pairs comprised the parental generation. The inbreeding coefficient, F, in these progeny was 0.375. This procedure served several purposes. First, we were interested in investigating the effectiveness of genetic rescue of already–inbred populations. Second, we intended to purge the genetic load such that further purging would not confound our results. Finally, our prior work (unpubl. results) showed that additional full–sibling matings would push F beyond the extinction quasi–threshold (Frankham, 1995a).

To establish large outbred source populations of potential immigrants, we created five populations each comprising ~5,000 individuals. Through Phase 1(described below), we housed these five populations separately. Because the timing of emergence in the five source populations diverged over time, this procedure was used to ensure a continuous supply of immigrants.

Phase 1
We placed a female and a male with the same full–sibling parents into each of 80 petri dishes, each containing 40 pristine (eggless) mung beans. Following oviposition, we placed each of egg–laden bean in a separate Eppendorf tube. As adults

emerged, we placed one male and a specified number of females (5, 4, 2, or 0 in the 0–, 1–, 3–, and 5–MPG treatments, respectively) from the same replicate population along with a complimentary number of immigrant females (0, 1, 3, or 5) into petri dishes containing 250 pristine beans. For example, in each replicate in the 1–MPG treatment, one male was placed together with four females from the same replicate population along with one immigrant female. Thus, each of 80 dishes (20 replicates in each treatment) contained five females and one male in all odd–numbered generations. On the 21st day after the adults had been put together, any surviving adults were removed and each egg–laden bean was placed in a separate tube.

Upon emergence, we repeated the protocol, except that 10 males were put together with 50, 49, 47, or 45 females (in the 0–, 1–, 3–, and 5–MPG treatments, respectively) from the same replicate population and with 0, 1, 3, or 5 immigrant females. Each replicate population thus comprised 60 adults (50 females and 10 males) in generation 2 (and all even–numbered generations in Phase 1). We then repeated the above alternation between $N = 6$ in odd generations and $N = 60$ in even generations through the 10th generation.

Throughout this phase, a pool of immigrant females was kept available by placing egg–laden beans from the source population singly into 200 tubes every generation. By matching female immigrants by date of 4th emergence in candidate recipient populations, we ensured that female immigrants were approximately the same age (i.e., within 7 days) as most members of the recipient population. Any potential female immigrant not assigned within two weeks following her emergence was excluded. Females satisfying the criteria for inclusion were transferred to appropriate populations according to the following rules. Egg–laden beans (one in each of ≤ 250 tubes in each replicate) were monitored daily for onset of emergence. We designated the day of 4th emergence as Day 0. On Day 7, we determined whether at least one male had emerged. If so and if the criterion numbers of females and males had emerged, they were combined in a petri dish with the specified number of female immigrants. Mating was allowed to proceed. On Day 21, we transferred each egg–laden bean to a tube. If the criterion numbers of females and males had not been reached by Day 7, we placed the male(s) together with females (including immigrants) and added newly emerging individuals daily. This process continued until the criterion was met or until 3 consecutive days passed with no emergence. Then, 7 days after the last individual was added or 2 weeks after initially putting beetles together (whichever was longer), we transferred each egg–laden bean to a tube. Finally, if no males had emerged by Day 7, we waited until the first male emerged and then followed the just–described procedure. Some populations failed to reach the criterion numbers of adults, particularly in even–numbered genera-
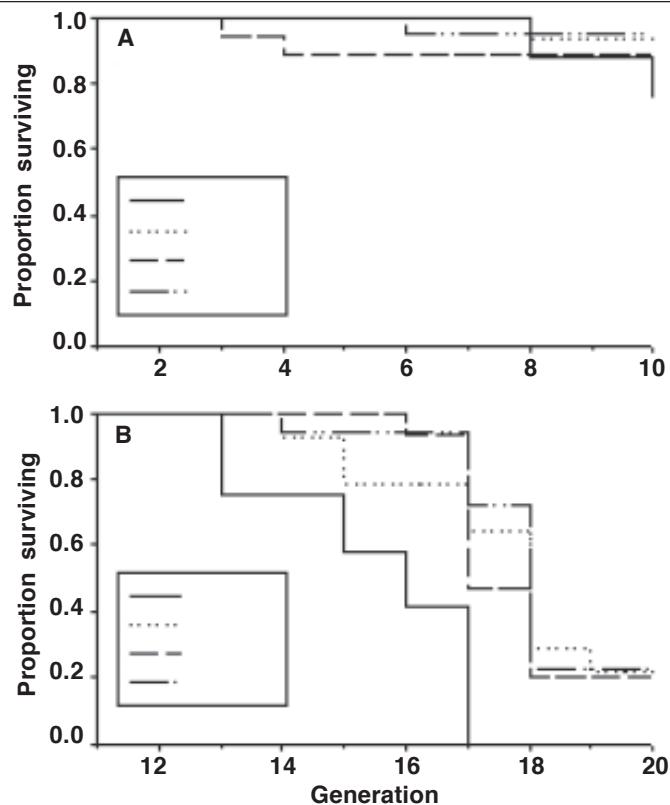
Fig. 1. Survivorship curves for small experimental populations of bean beetles (*Calosobruchus maculatus*) in four migrant–per–generation (MPG) treatments, with 20 replicate populations per treatment at the start of the experiment: A. Survivorship curves during Phase 1 of the experiment, when each population fluctuated between 6 individuals (1 male and 5 females) in odd generations and 60 individuals (10 males and 50 females) in even generations; B. Survivorship curves during Phase 2 of the experiment, when each population was subjected to an approximate carrying capacity of 10 individuals during every generation.

Fig. 1. Curvas de supervivencia para pequeñas poblaciones experimentales del gorgojo del haba Calosobruchus maculatus*, en tratamientos de cuatro migrantes por generación (MPG), con 20 poblaciones replicadas en cada tratamiento al inicio del experimento: A. Curvas de supervivencia durante la Fase 1 del experimento, cuando cada población fluctuaba entre 6 individuos (1 macho y 5 hembras) en las generaciones impares y 60 individuos (10 machos y 50 hembras) en las generaciones pares; B. Curvas de supervivencia durante la Fase 2 del experimento, cuando cada una de las poblaciones estaba sujeta a una capacidad de carga aproximada de 10 individuos por generación.*

tions, when numerous populations failed to produce 45 females. We refer to these failures as quasi–extinctions. We compare the incidence of quasi–extinction between treatments and across generations during Phase 1, when very few true extinctions took place.

Phase 2
After generation 10, replicate populations across all treatments were treated uniformly. Every population was subjected to a constant carrying capacity. No further immigration was orchestrated and no population variability was imposed. This phase lasted 10 generations. For each extant

population at the end of Phase 1, we placed all egg–laden beans (up to 210) in a large petri dish and then added 10 pristine beans. (Several replicates [10 in 0–MPG: 4 in 1–MPG , 2 in 3–MPG, 1 in 5–MPG] had gone extinct during Phase 1; others were lost to human error [2 in 0–MPG, 1 in 3–MPG].) Following oviposition, we discarded the original beans and placed 10 pristine beans in the dish with the 10 egg–laden beans. Following the next emergence and oviposition, we replaced the 10 old beans with 10 pristine beans. We repeated this process until extinction occurred or until the 20th generation (10th in Phase 2). For each population, time to extinction (in genera-

tions) was recorded. A population was judged to have gone extinct if: (1) no oviposition took place, (2) no beetles emerged, or (3) beetles of only one sex emerged.

Fitness measurement
To estimate individual reproductive success at the end of Phase 1, we began by randomly selecting 40 (of a possible 250) egg–laden beans from each population and placing two such beans in each of 20 tubes. Next, we monitored emergence and transferred five (whenever possible) female–male pairs to five petri dishes, each containing 100 pristine beans. We then allowed mating and oviposition to occur. Following emergence, we tallied the offspring produced by each pair. We used this quantity as our primary measure of fitness, but we also took advantage of the fact that some pairs failed to produce at least one adult offspring of each sex. We considered any such case to be a failed founding event. We thus compare both reproductive success and founding success across MPG treatments.

Data analysis

Survival analysis was performed using S–PLUS 2000 (1999). Kaplan–Meier nonparametric survival models were used to estimate mean time to extinction in each MPG treatment. Cox proportional hazards models were used to evaluate the effect of MPG treatment on risk of extinction. All pairwise comparisons were performed. Nominal P–values are reported, with an indication of whether each test is significant at the experimentwise ±–level of 0.05 (following Bonferroni correction). Other analyses were performed using SPSS (1999). Fisher's exact tests were used to perform pairwise comparisons of incidence of extinction and quasi–extinction between MPG levels and between generations during Phase 1. One–way ANOVA was used to compare fitness (number of offspring produced per female–male pair) across MPG levels. Pairwise comparisons were made using Tukey's HSD method. Finally, we compared the incidence (arcsin square root transformed proportion) of successful founding (production of at least one offspring of each sex) across MPG levels. Because the normality test failed (P < 0.001), we used Kruskal–Wallis nonparametric one–way "analysis of variance" on ranks. Pairwise comparisons were made using Dunn's method, with the critical ±–level set at 0.05.

Results

Survival analysis

Figure 1 shows the survival of replicate populations. No significant differences in incidence of extinction emerged among treatments by the end of Phase 1 (all Ps > 0.15, Fisher's exact test), when few extinctions occurred (i.e., 8 of 67 populations). In

Table 1. Results of pairwise Cox proportional hazards comparisons (test of effect of manipulating number of migrants per generation). For each comparison, β (= slope), exp(β), and P are shown. Each of the first three comparisons is significant following Bonferroni adjustment for the number of pairwise tests performed (i.e., the nominal P–value is less than 0.05/6). Any negative value of β corresponds with a value of exp(β) < 1, which indicates the decrease in relative risk of extinction associated with a unit increase in the experimental factor MPG.

Tabla 1. Resultados de las comparaciones al azar de Cox de los riesgos proporcionales por parejas (test del efecto de la manipulación del número de inmigrantes por generación). Para cada comparación se muestran β (= pendiente), exp(β), y P. *Cada una de las tres primeras comparaciones es significativa según el ajuste de Bonferroni para el número de test por parejas llevados a cabo (es decir, el valor nominal de* P *es menor de 0.05/6). Cualquier valor negativo de β se corresponde con un valor de exp(β) < 1, lo que indica un descenso del riesgo relativo de extinción asociado con un incremento unitario del factor experimental MPG.*

Between–treatment comparison	β	exp(β)	P
0– vs 1–MPG	−2.48	0.08	1.7×10^{-5}
0– vs 3–MPG	−0.76	0.47	3.6×10^{-6}
0– vs 5–MPG	−0.61	0.54	1.7×10^{-7}
1– vs 3–MPG	0.10	1.10	0.65
1– vs 5–MPG	−0.002	0.99	0.98
3– vs 5–MPG	−0.12	0.88	0.53

Phase 2, mean persistence of populations in the 0–MPG treatment (6.4 generations) was substantially shorter than in every other treatment (1–MPG: 8.9, 3–MPG: 8.7, and 5–MPG: 8.9), based on Kaplan–Meier survival analysis. Cox proportional hazards analysis revealed that extinction risk declined significantly with increasing MPG ($\beta = -0.23$, exp[β] = 0.80 [95% CI: 0.68–0.94], P = 0.007), where exp(β) quantifies the proportional effect of a unit increase in the experimental factor (MPG). Pairwise comparisons revealed significant effects of increasing MPG from 0 to any other level (i.e., 1, 3, or 5) (table 1). No other pairwise comparison was significant. Number of MPG was a significant predictor of extinction risk only when the 0–MPG treatment was included. The 1–, 3–, and 5–MPG treatments appeared to reduce extinction risk with equivalent effectiveness.
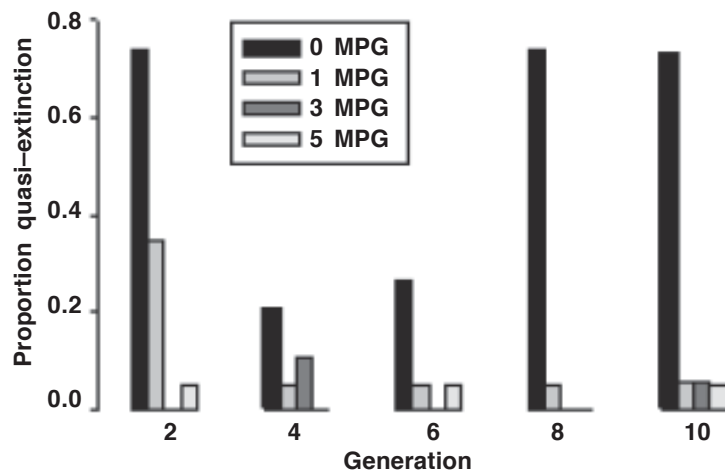
Fig. 2. The incidence of quasi–extinction (proportion of populations failing to produce at least 45 female offspring during even–numbered generations) in four migrant–per–generation treatments (MPG). Any extant population could experience quasi–extinction repeatedly.

Fig. 2. Incidencia de la cuasi–extinción (proporción de poblaciones que no consiguen producir al menos 45 descendientes hembra durante las generaciones pares) en tratamientos de cuatro migrantes por generación (MPG). Cualquier población existente podría experimentar la cuasi–extinción repetidas veces.

Quasi–extinction analysis

Figure 2 shows the incidence of quasi–extinction (defined as failure to produce at least 45 females in even–numbered generations during Phase 1). The incidence of quasi–extinction showed a pronounced temporal pattern in the 0–MPG treatment, decreasing from generation 2 to 4 ($P = 0.003$; Fisher's exact test) and then increasing (generation 6 vs. 8: $P = 0.009$; generation 4 *vs* 8: $P = 0.003$) to the initial level (generation 2 *vs* 10: $P = 1.0$). This temporal pattern of quasi–extinction in the 0–MPG treatment suggests an initial purging of inbreeding depression followed by onset of inbreeding depression. An initial decline, from generation 2 to 4, was detectable in the 1–MPG treatment (Ps = 0.044 for comparisons between generation 2 *vs* 4, 6, 8, and 10), suggesting an initial purging of inbreeding depression with no subsequent onset of inbreeding depression by the end of Phase 1. Other pairwise comparisons were nonsignificant (Ps = 1.0). No significant between–generation differences in incidence of quasi–extinction emerged for either the 3– or 5–MPG treatment (Ps > 0.48), suggesting neither an initial purging of inbreeding depression nor a subsequent onset of inbreeding depression in these treatments.

Within generations, several between–treatment differences emerged. In generation 2, quasi–extinction risk was reduced by the one–time immigration of a single female (i.e., incidence of quasi–extinction was lower in 1– than 0–MPG; $P = 0.025$).

Quasi–extinction risk was further reduced by the immigration of additional females (i.e., 1– *vs* 3– MPG: $P = 0.008$; 1– *vs* 5–MPG: $P = 0.02$). Incidence of quasi–extinction was minimized equivalently in the 3– and 5–MPG treatments. In generation 4, only one comparison (0– *vs* 5–MPG) was nominally significant. Within subsequent generations, incidence of quasi–extinction was higher in the 0–MPG treatment than in any other treatment (Ps < 0.001). All other pairwise comparisons were nonsignificant.

Fitness analysis

Figure 3A summarizes the results of the fitness test conducted at the end of Phase 1. Significant heterogeneity emerged across treatments ($F_{3,43.7} = 11.86$, $P < 0.001$), but not among replicates ($F_{19,37.9} = 1.33$, $P = 0.22$). Tukey's HSD tests revealed that the number of offspring produced per female–male pair was significantly lower in the 0–MPG treatment than in any other treatment (all Ps < 0.001). Although a visual inspection of Figure 3A suggests a weak tendency for female–male pairs in the 5–MPG treatment to produce more offspring (mean = 55.0) than pairs in the 1–MPG (49.3) and 3–MPG treatments (48.6), neither of these comparisons was significant (1– *vs* 5–MPG: $P = 0.20$; 3– *vs* 5–MPG: $P = 0.15$) (nor was comparison between 1– and 3–MPG: $P = 1.0$).

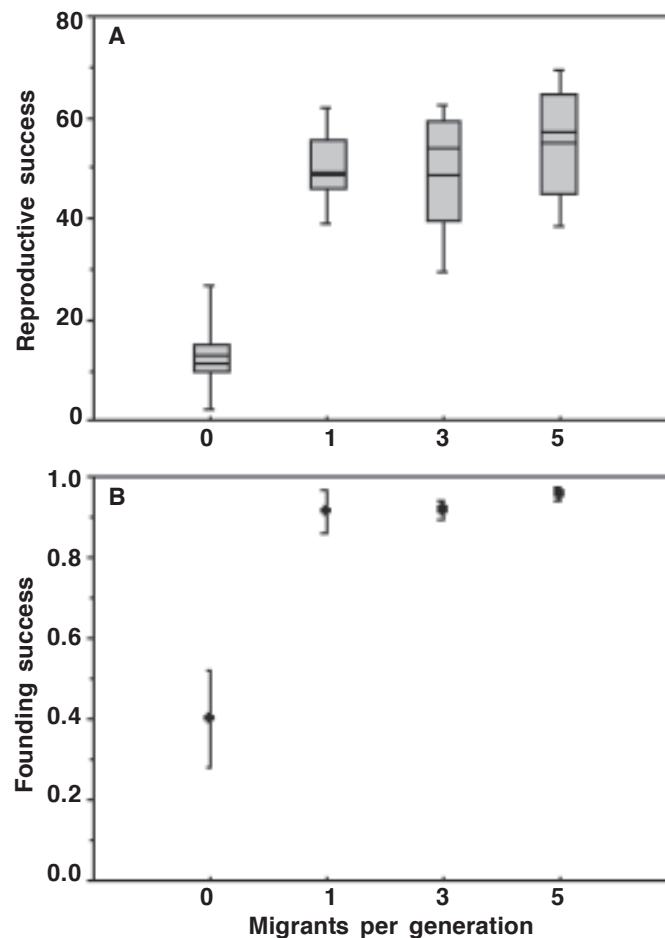Figure 3B shows the founding success (i.e., proportion of pairs that produced at least one adult

Fig. 3. A. Fitness measurements (mean number of offspring produced per female–male pair) in four migrant–per–generation (MPG) treatments at the end of Phase 1 of the experiment; the thin line within each box indicates the median, the thick line within each box indicates the mean, the box represents the interquartile interval (25th to 75th percentile), and the whiskers show the 10th and 90th percentiles. B. The founding success (defined as proportion of female–male pairs that produced at least one adult offspring of each sex) in four migrant–per–generation (MPG) treatments at the end of Phase 1 of the experiment. Symbols indicate means and the error bars represent standard errors. For both analyses, the numbers of replicate populations were as follows: 0–MPG, 8; 1–MPG, 16; 3–MPG, 17; and 5–MPG, 19 replicates.

Fig. 3. A. Mediciones de aptitud (número promedio de descendientes producidos por cada par macho–hembra) en los tratamientos de cuatro migrantes por generación (MPG), al final de la Fase 1 del experimento; la fina línea del interior de cada rectángulo indica la mediana, y la línea gruesa la media, mientras que los rectángulos representan los intervalos intercuartiles (los percentiles 25 a 75); los extremos de las barras verticales muestran los percentiles 10 y 90. B. Éxito de fundación (definido como la proporción de parejas macho–hembra que produjeron al menos un descendiente adulto de cada sexo) en tratamientos de cuatro migrantes por generación (MPG) al final de la Fase 1 del experimento. Los símbolos indican las medias y las barras de error los errores estándar. Para ambos análisis, los números de poblaciones replicadas fueron los siguientes: 0–MPG, 8; 1–MPG, 16; 3–MPG, 17; y 5–MPG, 19.

offspring of each sex) of female–male pairs. Significant heterogeneity emerged among treatments (Kruskal–Wallis test: $H_3 = 24.75$, $P < 0.001$). Dunn's multiple–comparison procedure revealed that founding success was significantly lower in the 0–MPG treatment than in any other treatment (qs > 3.54, Ps < 0.05). Other pairwise comparisons were non-significant.

Discussion

Our survival analysis suggests that the introduction of a small number of migrants per generation (MPG) was sufficient for genetic rescue of small, inbred populations of the bean beetle (fig. 1, table 1). Compared with the control (0–MPG treatment), all of the other treatments (1–, 3–, and 5–MPG) improved population persistence. Because immigrant females were replacements rather than extras, this finding may be attributable to beneficial effects of gene flow *per se*, perhaps including the masking of fixed deleterious mutations. That is, our manipulation apparently led to genetic rescue, as distinguished from demographic rescue.

In agreement with other studies, our findings suggest that even a single MPG can lead to improved fitness (e.g., Newman & Tallmon, 2001; Vila et al., 2003) and reduced extinction risk (e.g., Bryant et al., 1999). Moreover, our results suggest that the extent of genetic rescue was independent of number of MPG, provided there was at least one MPG. Compared with the 0–MPG treatment, the other treatments were equivalently effective both at improving fitness (fig. 3) and reducing extinction risk (table 1). This finding is superficially puzzling in light of recent theoretical arguments that one actual migrant per generation will often be inadequate (Mills & Allendorf, 1996), particularly when the recipient population fluctuates (Vucetich & Waite, 2000). However, this theory does not apply here (Kalinowsky & Waples, 2002) because the populations did not merely fluctuate; they also fell to a very small size (in alternate generations). When this happens, the migration rate associated with a fixed number of migrants can be dramatically inflated (Vucetich & Waite, 2001). Because we did not determine parentage, we cannot use genealogical data to calculate the realized genetically effective migration rate each generation. However, as a first approximation, we estimate that the average migration rate, $_m$ in the 1–, 3– and 5–MPG treatments was 4.5, 14, and 23 in odd generations (and 0.5, 1.7, and 2.8 in even generations), where   is mean population size and m is actual number of immigrants divided by current size of the recipient population. Thus, it appears that the 1–MPG treatment performed so well because the rate of genetic infusion was adequate after all.

Our analysis of quasi–extinction (fig. 2) suggests that populations in the 1–MPG treatment might have benefited also from initial purging of the genetic load (e.g., Fu et al., 1998; Fu, 1999; Wang, 2000; Reed & Bryant, 2001). Incidence of quasi–extinction in the 1–MPG treatment was high initially, but then decreased dramatically and remained low, suggesting initial purging followed by fitness–enhancing gene flow. By contrast, incidence of quasi–extinction in the 0–MPG treatment decreased initially but then increased, suggesting purging followed by onset of inbreeding depression in the absence of gene flow. Further evidence that 1–MPG populations benefited, in part, from gene flow

is provided by the observation that quasi–extinction risk was reduced by the first introduction of a single female (i.e., incidence of quasi–extinction was lower in 1–MPG than 0–MPG treatment in generation 2; fig. 2) (see also Spielman & Frankham, 1992). This result suggests that even a one–time immigration by a single individual can make a sufficient genetic contribution to provide a rescue effect (Ball et al., 2000; Vila et al., 2003). Our results also indicate, though, that quasi–extinction risk was further reduced by additional immigrants (fig. 2). Thus, the 1–MPG treatment did not perform as well as the 3– and 5–MPG treatments at this stage. Taken together, these results suggest a duel benefit for populations in the 1–MPG treatment: initial purging of inbreeding depression combined with subsequent fitness–enhancing gene flow.

In summary, our results suggest that even a single MPG may sometimes be useful for genetic management of small, inbred populations. A single actual MPG may sometimes be sufficient, particularly if the recipient population is small (Vucetich & Waite, 2001; see also Kalinowsky & Waples, 2002) and if inbreeding depression is purged initially (e.g., Backus et al., 1995). The adequacy of one MPG could be further enhanced if offspring of immigrants exhibit heterosis (Ingvarsson & Whitlock, 2000) or if immigrants are characterized by outbred vigor (Ball et al., 2000) and/or a mating advantage. Yet, it would be premature to promote the introduction of just one MPG as a general practice. Additional work should build upon experimental and theoretical studies (cited in Introduction) that have attempted to identify strategies for minimizing extinction risk.

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