

FEATURE PAPER

Testing the demographic effects of divergent immigrants on small populations of Trinidadian guppies

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Abstract

Augmenting small and isolated populations with immigrants from elsewhere is a potentially powerful, yet controversial management tool. The goal of this approach was to increase population sizes via demographic and/or genetic rescue, but augmentation can also have the unintended consequence of breaking down local adaptation and reducing population fitness through outbreeding depression. In theory, outbreeding depression is more likely the more divergent immigrants are from the recipient population. Managers should therefore choose immigrant populations that are as adaptively and genetically similar as possible. However, for species of conservation concern, divergent source populations are often the only option. A crucial question that remains in applied conservation is whether the positive effects of augmentation with divergent immigrants will outweigh the potential risks of outbreeding depression. Here, we evaluate the demographic effects of augmenting small, inbred laboratory populations of Trinidadian guppies with two different types of immigrants: (1) adaptively divergent but genetically similar or (2) adaptively similar but genetically divergent, and compare them against the demography of control populations with no immigration. After 1–2 generations, we found that adult abundance remained constant or slightly declined over the duration of the experiment in the control populations. In contrast, adult recruitment and total abundance increased in augmented populations. Furthermore, treatments that received immigrants from the adaptively similar but genetically divergent population attained overall larger population sizes than those that received immigrants from the adaptively divergent but genetically similar population. Although our experimental design could not parse out the effects of demographic and genetic rescue, our results do suggest that augmentation can be better than no action, even in situations where only divergent immigrant sources are available.

Introduction

One of the most controversial issues in conservation biology is whether small populations should be supplemented with immigrants from larger populations as a means of increasing population fitness (Hedrick, 1995; Mills & Allendorf, 1996; Ingvarsson, 2001; Tallmon, Luikart & Waples, 2004; Frankham *et al.*, 2011; Weeks *et al.*, 2011; Whiteley *et al.*, 2015), a strategy known as augmentation. The controversy stems from uncertainty about whether this management practice will have the desired effect, as well as historical reluctance to actively move individuals from one population to another out of fear of 'diluting' local gene pools (Storfer, 1999; Tallmon *et al.*, 2004; Frankham, 2010). According to theory, augmenting populations could have both positive and negative effects on population fitness due to demographic and/or

genetic factors. From a demographic perspective, augmentation can increase population fitness by adding more individuals to the population (analogous to births), which decreases demographic stochasticity in a process termed 'demographic rescue' (Brown & Kodric-Brown, 1977). However, augmentation can also unintentionally introduce disease, potentially decreasing population fitness (Daszak, Cunningham & Hyatt, 2000). From a genetic perspective, augmentation often results in gene flow that can alleviate inbreeding depression and increase adaptive genetic variation in small populations, thereby increasing population fitness via 'genetic rescue' (Thrall *et al.*, 1998; Tallmon *et al.*, 2004). Alternatively, if groups of immigrant and recipient genes are no longer compatible with one another, augmentation can break apart coadapted gene complexes, reducing population fitness (Mayr, 1963; Edmands, 1999, 2007; Storfer, 1999; Lenormand,

2002; Edmands & Timmerman, 2003; Frankham *et al.*, 2011). This uncertainty about the potential outcome of augmenting small populations is a conservation conundrum and puts wildlife managers in a difficult situation. In many cases, managers choose not to augment out of concerns about the potential negative effects (Frankham, 2010; Frankham *et al.*, 2011).

If managers plan on augmenting a small population, they should ideally use individuals from a similar population to minimize the risk of outbreeding depression. However, for many species of conservation concern, a similar population may not exist because declining populations are often isolated from the rest of the species range and are locally adapted to different environments (e.g. Westemeier *et al.*, 1998; Hogg *et al.*, 2006; Johnson *et al.*, 2010). Theory predicts that outbreeding depression is more likely as adaptive divergence between immigrants and the recipient population increases (Storfer, 1999; Edmands & Timmerman, 2003; Frankham *et al.*, 2011) and as the length of time since last gene flow increases (Bateson, 1909; Dobzhansky, 1937; Muller, 1940; Lynch, 1991; Edmands, 1999, 2002; Edmands & Timmerman, 2003). Augmentation with adaptively and genetically similar immigrants is beneficial in the vast majority of cases (Frankham, 2015; Whiteley *et al.*, 2015). However, augmentation with divergent immigrants has received relatively little attention in the literature, and the handful of studies that have explored this issue have yielded mixed results. For example, some studies have found negative effects of augmentation with adaptively divergent (Lacy, 1998; Edmands, 1999, 2002; Fenster & Galloway, 2000; Lee, 2000) or genetically divergent individuals (Hwang *et al.*, 2011; Pekkala *et al.*, 2012). Other studies, however, have found positive effects of augmentation with phenotypically divergent individuals (Willi *et al.*, 2007), individuals from moderately different environments (Johnson *et al.*, 2010) or individuals from populations that are divergent at neutral loci (Hogg *et al.*, 2006; Bossuyt, 2007; Willi *et al.*, 2007; Tortajada, Carmona & Serra, 2010). Thus, two critically important and unresolved questions are as follows: (1) Does adding divergent immigrants increase or decrease the fitness of small populations? (2) If only divergent immigrant sources are available, is it better to choose populations that are adaptively divergent but genetically similar or adaptively similar but genetically divergent?

Trinidadian guppies *Poecilia reticulata* are small, live-bearing freshwater fish and they are ideal for addressing these questions for several reasons. First, adaptive divergence between guppy populations in 'low predation' (LP) and 'high predation' (HP) environments is well characterized from decades of evolutionary research in this system (Haskins *et al.*, 1961; Seghers, 1974; Reznick & Endler, 1982; Magurran, 2005), and can thus be treated as a predictor variable in factorial experiments. HP guppies are found in large, low-elevation streams with diverse communities of piscivorous fish, including a major guppy predator, the pike cichlid *Crenicichla* spp. (Reznick & Endler, 1982; Magurran, 2005). In contrast, LP guppies inhabit small, high-elevation headwater streams and coexist only with a killifish *Rivulus hartii*, a

gape-limited predator of juvenile and small-sized guppies. LP and HP guppies differ predictably across a suite of life history (Reznick, 1982; Reznick & Endler, 1982), morphological (Haskins *et al.*, 1961), color (Endler, 1983) and behavioral (Seghers, 1974; Houde & Endler, 1990) traits, which increase individual fitness in the respective environments and strongly suggest a repeated pattern of local adaptation. Second, LP populations are typically highly isolated (with low genetic variation and a high degree of inbreeding) and upstream immigration is rare due to the presence of downstream barrier waterfalls (Barson, Cable & Van Oosterhout, 2009; Suk & Neff, 2009; Willing *et al.*, 2010). This makes LP guppies an excellent model for small populations of conservation concern (van Oosterhout *et al.*, 2007). Lastly, guppies have short generation times (3–4 generations/year; Magurran, 2005) and can be reared in the laboratory (Reznick & Endler, 1982; Torres-Dowdall *et al.*, 2012), making it possible to rapidly test the effects of population augmentation across generations.

We used a replicated factorial experiment in the laboratory to test whether augmenting small, inbred guppy populations with immigrants from divergent sources had a positive or negative effect on population fitness relative to no augmentation. Specifically, if augmentation has a net positive effect, we would expect abundance, recruitment and survival to increase more in populations with immigrants than in those without immigrants. But, if augmentation has a net negative effect, abundance, recruitment and survival should decrease in populations that received immigrants relative to those that did not. To gain further insight into the effects of different *types* of divergent immigrants, we compared the demographic impacts of augmentation with immigrants from an adaptively divergent but genetically similar source to those of immigrants from an adaptively similar but genetically divergent source. Although our experiment was not designed to differentiate demographic from genetic rescue, our results shed light more generally on how divergent immigrant sources can differ in their ability to increase population fitness.

Materials and methods

Experimental mesocosms

To address our question about the effects of divergent immigrants on small populations, we used replicate 10-gallon glass aquaria, or mesocosms, in the laboratory to simulate small LP populations in upstream pools. Each mesocosm was seeded with 10 LP fish from the Aripo LP site: one wild-caught, three F₁ and one F₂ laboratory-bred individuals per sex. Generations were mixed in this way so that we could use all available LP fish and maximize the number of mesocosm replicates. Females were impregnated by randomly selected males from their population upon introduction. Although we were not able to estimate individual inbreeding coefficients or provide direct evidence for inbreeding depression in the recipient population, it did have low observed heterozygosity relative to HP streams ($H_o = 0.58$ vs. 0.77, respectively; Baillie, 2012), a trend

consistently observed in LP compared to HP guppy populations (Barson *et al.*, 2009; Suk & Neff, 2009; Willing *et al.*, 2010). Aripo LP mesocosm populations received one of the three treatments: (1) HP immigrants from a population in the same drainage, Aripo HP; (2) LP immigrants from a distant population on the opposite side of the Northern Range of Trinidad, Marianne LP; or (3) no immigration as a control. All immigrants were the F₁ offspring of wild-caught individuals, captured together at the same site per population. Importantly, the Aripo HP immigrants were adaptively divergent from the recipient Aripo LP populations (coming from a typical high-predation source) but were only moderately genetically divergent at 10 neutral microsatellite loci ($F_{ST} = 0.12$; Baillie, 2012), indicating relatively recent divergence and/or recent gene flow between LP and HP Aripo populations. Conversely, the Marianne LP immigrants were adaptively similar to the recipient Aripo LP populations, but considerably more genetically divergent at 10 microsatellite loci ($F_{ST} = 0.35$; Baillie, 2012), indicating long-standing isolation between these populations. We included three replicates per treatment according to a randomized controlled block design for a total of nine experimental mesocosm populations.

Full spectrum fluorescent bulbs were hung at a constant distance (30 cm) from each mesocosm. Three equally sized pieces of floating aquarium grass were kept in each tank to provide shelter for juvenile guppies. Fish in each tank were fed twice daily [AM: 165- μ L Tetramin™ tropical fish flake paste (Tetra, Melle, Germany); PM: 250- μ L hatched *Artemia* cysts]. Food quantity, temperature ($25 \pm 1^\circ\text{C}$) and light cycle (12 h light and 12 h dark) were held constant throughout the experiment. Sponge filter and partial water changes occurred once every 2 weeks, at which point tank surfaces were scrubbed of algae.

Population censuses

During the course of the study, we conducted monthly censuses for 6 months (seven total; one at the beginning of the study and each month thereafter). At each census, all adult fish (defined as having a standard length of 14 mm or longer) were anesthetized with a dilute solution of MS-222 and marked with visible implant elastomer (VIE; Northwest Marine Technology, Inc., Shaw Island, WA, USA) to label new recruits. Previous capture–mark–recapture studies on guppies demonstrate high mark retention and low marking mortality using these methods (Reznick *et al.*, 1996). Individuals smaller than 14 mm were not processed but were counted in the total number of sub-adult fish. After processing, individuals were placed in an aerated recovery tank and then returned to their experimental tank once they revived. We were able to conduct complete censuses of all individuals within each mesocosm.

Immediately after the third monthly census (2 months after the beginning of the study), we began adding one adult female and one adult male immigrant per month to the immigration treatment mesocosms from the appropriate source population. Guppies have a gestation of

approximately 1 month and reach sexual maturity at approximately 2 months of age. Thus, offspring resulting from matings between resident guppies and the first immigrants were most likely born by the fourth monthly census, sexually mature by the sixth monthly census and could have produced offspring by the final census. Immigrant females were sexually mature and pregnant upon addition to experimental mesocosms. Therefore, the demographic contribution of immigrant females included offspring *in utero* in addition to themselves. Despite this, we were still able to resolve treatment effects because our aim was to assess the overall effect of divergent immigrants on population fitness regardless of immigration rate.

Statistical analyses

We calculated total abundance, adult abundance, adult recruitment and adult survival for each mesocosm each month. Total abundance was measured as the total number of individuals (adults + sub-adults) in the tank at the time of each census, adult abundance was the number of adults at each census, adult recruitment was the number of new adults and adult survival was the proportion of adults that had survived since the previous census. To remove the direct demographic contribution of immigrants, we also calculated total and adult abundance for each mesocosm with immigrants excluded from the analysis.

For each of the above population metrics (total abundance, adult abundance, adult survival and adult recruitment), we fit Bayesian univariate generalized linear mixed models (GLMM) with the MCMCglmm function in the MCMCglmm R package (Hadfield, 2010) using R version 3.0.2 (R Development Core Team, 2011). These additive random effects models can be thought of as the Bayesian equivalent of repeated-measure models and therefore incorporate data from all months. Immigration treatment was modeled as a fixed effect and month nested within mesocosm and block were treated as random effects. Models were run for 300 000 iterations with a burn-in of 150 000 and a thinning interval of 200 with an inverse-Wishart proper prior (Hadfield, 2010). We estimated the means of controls, the effects of each immigration treatment and their 95% credible intervals (CI) using Markov chain Monte Carlo (MCMC) sampling of their posterior distributions, conditioned on the random effects. Effects were considered significant if the 95% CI did not overlap zero and the estimated pMCMC value was ≤ 0.05 (Longdon *et al.*, 2011). pMCMC values can be interpreted as the Bayesian equivalent of a *P*-value, and were calculated as two times the smaller MCMC estimates of: (1) the probability that the parameter estimate is greater than zero, or (2) the probability that the parameter estimate is less than zero.

Results

By the end of the study, total and adult abundance was generally higher in mesocosms that received immigrants than in control mesocosms with no immigration (Table 1). Mean total abundance was significantly higher in the adaptively

Table 1 Summary of the estimated means for the control mesocosms and the effects of the immigration treatments (relative to the mean values for the controls) on mesocosm abundances, number of adult recruits and survival rate, estimated from the posterior distributions of Bayesian univariate GLMMs

Response variable	Mean of control mesocosms with no immigrants (95% CI)	Adaptively divergent but genetically similar immigrant treatment (95% CI)	Adaptively similar but genetically divergent immigrant treatment (95% CI)
Total abundance including immigrants	12.09 (4.10, 20.24)	6.20 (−3.11, 14.75)	11.43 (3.82, 19.83)*
Total abundance excluding immigrants	12.17 (4.44, 20.05)	4.14 (−3.35, 13.08)	9.01 (0.10, 18.52)*
Adult abundance including immigrants	7.93 (5.77, 10.15)	3.62 (1.01, 6.01)**	4.22 (1.92, 6.63)***
Adult abundance excluding immigrants	8.01 (6.08, 9.86)	1.43 (−0.38, 3.25)	1.77 (−0.09, 3.61)†
Number of adult recruits	1.94 (−0.60, 4.63)	1.75 (0.29, 3.22)*	1.21 (−0.11, 2.58)†
Adult survival rate including immigrants (%) ^a	84.76 (70.17, 100.00)	0.10 (0.00, 3.39)	0.06 (0.00, 3.77)
Adult survival rate excluding immigrants (%) ^a	85.90 (71.58, 100.00)	0.003 (0.00, 5.77)	0.06 (0.00, 3.51)

The estimated level of significance (pMCMC) of effects is denoted by asterisks: † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

^aSurvival rates were arcsine square-root transformed prior to running the model. The means, effects and 95% CI presented here are back transformed. We corrected upper and lower bounds of back transformed 95% CIs using $B(X) = 100.00$ when $X > \pi/2$ and $B(X) = 0.00$ when $X < 0$.

similar but genetically divergent immigrant treatment mesocosms than in control mesocosms, regardless of whether or not the immigrants themselves were included in total abundance: 95% CI of the effects is (3.82, 19.83) individuals with immigrants and (0.10, 18.52) individuals without (Table 1). Moreover, mean total abundance started to decrease slightly in the control mesocosms after the third census, but continued increasing in both immigrant treatments for another 1–2 months before leveling off or starting to decrease (Fig. 1, Supporting Information Fig. S1).

Mean adult abundance was significantly higher in immigrant treatment mesocosms than in control mesocosms when immigrants were included: 95% CI (1.01, 6.01) for adaptively divergent but genetically similar treatments and (1.92, 6.63) for adaptively similar but genetically divergent treatments (Table 1). When immigrants were excluded, mean adult abundance was marginally significantly higher in the adaptively similar but genetically divergent treatment mesocosms than in control mesocosms: 95% CI (−0.09, 3.61) (Table 1). Mean adult abundance declined slightly in control mesocosms (Fig. 2), but consistently increased throughout the study in the immigrant treatment mesocosms when immigrants were included (Fig. 2a) and stayed constant when immigrants were excluded (Fig. 2b).

Mean adult survival rate was high (roughly 85%) and did not differ significantly among treatments (Table 1). However, mesocosms that received adaptively divergent but genetically similar immigrants had significantly higher mean adult recruitment than controls, and the adaptively similar but genetically divergent treatment had marginally significantly higher mean adult recruitment than controls: 95% CI (0.29, 3.22) and (−0.11, 2.58), respectively (Table 1). Mean recruitment leveled off in the controls after the fifth census, but continued increasing in immigrant treatment mesocosms (Fig. 3).

Discussion

Our results support the hypothesis that immigrants have a net positive demographic effect on the short-term fitness of

small populations, despite originating from divergent sources. Mean abundance and recruitment were higher in the immigrant mesocosms compared to the controls without immigrants. In the control mesocosms, population sizes declined slightly over the duration of the study. In the mesocosms that received immigrants from the adaptively divergent but genetically similar population, total population sizes either reflected the demographic contribution of immigrants and their babies or growth above this baseline (i.e. genetic rescue); our experiment was not designed to separate out the effects of demographic and genetic rescue. Our analyses excluding immigrants removed their direct demographic contribution, but not that of any offspring the female immigrants may have been carrying. It is therefore possible that the higher total abundance in these mesocosms after immigrant exclusion is the result of immigrant offspring rather than genetic rescue. Surprisingly, the mesocosms that received immigrants from the adaptively similar but genetically divergent (LP) population had a greater increase in total abundance than in adult abundance or recruitment, suggesting that population growth was driven by higher birth rates in these mesocosms. LP guppies typically have lower fecundity than HP guppies, with smaller brood sizes and longer inter-brood intervals, and take longer to reach sexual maturity (Reznick, 1982; Reznick & Endler, 1982). Our finding that LP guppies had higher fecundity suggests that the difference in ability of these divergent immigrant sources to increase population fitness was not solely an artifact of their fecundities in their respective native environments; we would have expected HP guppies to have higher reproductive output, but we found just the opposite. One potential explanation for this is that LP guppies, being better adapted than HP guppies to the competitive, predator-free mesocosm environment, were able to allocate resources toward reproduction more appropriately. We found that adaptively similar but genetically divergent immigrants were overall more beneficial to population fitness than adaptively divergent but genetically similar immigrants. This may come as a surprise because (coming from a LP environment) the adaptively similar but

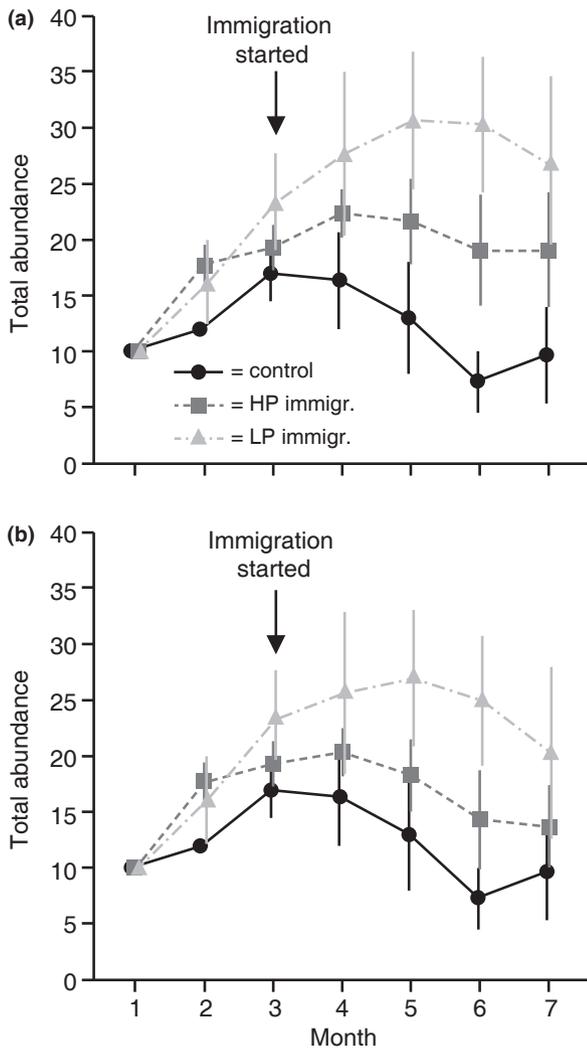


Figure 1 Mean monthly total abundance (adults + sub-adults) in control, adaptively divergent but genetically similar (HP), and adaptively similar but genetically divergent (LP) immigrant treatment mesocosms including (a) or excluding (b) immigrants. Error bars show standard errors. Monthly addition of immigrants (one female and one male per month) in immigrant treatment mesocosms started immediately after the third census.

genetically divergent immigrants likely had low genetic variation and therefore may contribute relatively little novel genetic information. But because recipients and immigrants came from population sources that have been long isolated from one another, and genetic drift is random, introgression between two inbred sources can still result in genetic rescue (Heber *et al.*, 2013). However, our results should be interpreted with some caution because we could only test one source population for each divergent immigrant treatment, and findings may differ depending on the genetic and demographic characteristics of divergent immigrants in other systems.

Outbreeding depression is expected to be the strongest in the F_2 generation, once recombination has shuffled alleles from

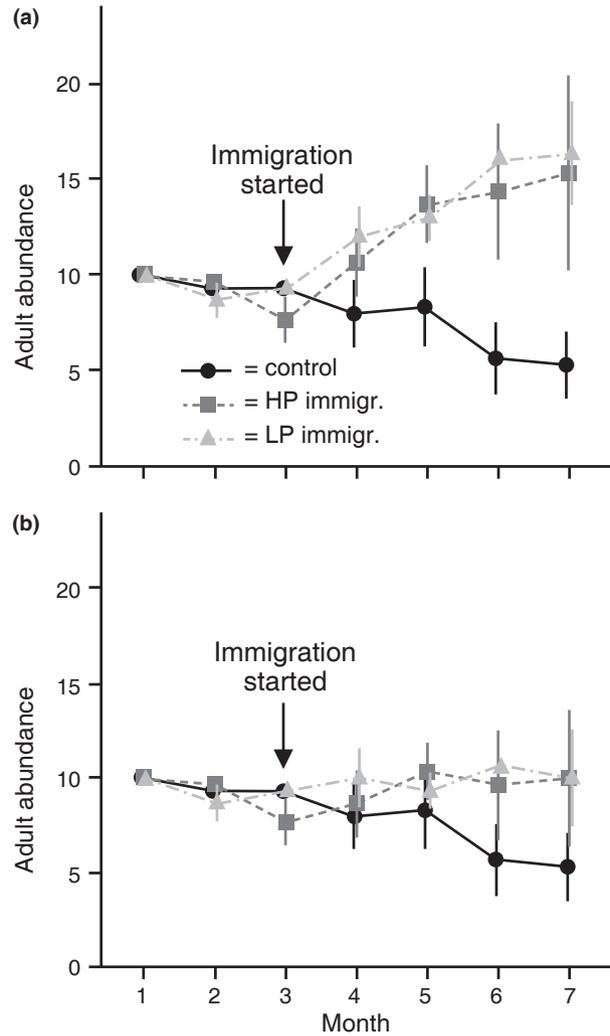


Figure 2 Mean monthly adult abundance in control, adaptively divergent but genetically similar (HP), and adaptively similar but genetically divergent (LP) immigrant treatment mesocosms including (a) or excluding (b) immigrants. Error bars show standard errors. Monthly addition of immigrants (one female and one male per month) in immigrant treatment mesocosms started immediately after the third census.

both populations (Bateson, 1909; Dobzhansky, 1937; Muller, 1940; Templeton, 1986; Lynch, 1991; Edmands, 2007). Our experiment ended shortly after the start of the F_2 generation and therefore only captured the effects of interpopulation recombination via some of the sub-adults; many negative outbreeding effects may not manifest until later in adulthood, and if our experiment had run for a longer period or our sample size were larger, we would have been better able to detect them. However, outbreeding depression is still possible in F_1 hybrids if immigrant genes are maladapted, there is underdominance, or detrimental epistatic interactions exist between heterozygotes or sex chromosomes (Edmands, 2007). Reduced fitness of F_1 hybrids has been demonstrated widely, including in plants (Quilichini, Debussche &

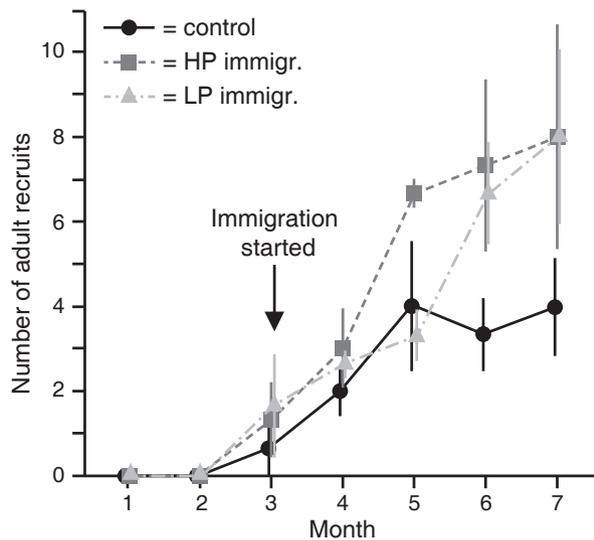


Figure 3 Mean monthly number of adult recruits in control, adaptively divergent but genetically similar (HP), and adaptively similar but genetically divergent (LP) immigrant treatment mesocosms. Error bars show standard errors. Monthly addition of immigrants (one female and one male per month) in immigrant treatment mesocosms started immediately after the third census.

Thompson, 2001; Galloway & Etterson, 2005; Heiser & Shaw, 2006), invertebrates (Lonsdale, Levinton & Rosen, 1988; Lee, 2000; Hwang *et al.*, 2012) and vertebrates (Sasa, Chippindale & Johnson, 1998; Gilk *et al.*, 2004; Neff, 2004). Therefore, while a thorough test for outbreeding depression requires monitoring over multiple generations, the lack of negative outbreeding effects in our experiment, though short term, is notable. There is also the question of whether the dynamics observed in our laboratory populations can be extrapolated to wild populations. We believe they generally can, in part because carrying capacity was not reached in these mesocosms (based on population sizes that reached ~50 individuals before declining in a subsequent experiment; J.A. Kronenberger, unpubl. data), and therefore density-dependent limits on population growth should not have influenced our results. Furthermore, our results are consistent with a similar increase in population fitness following immigration from a divergent population of guppies in the wild (Fitzpatrick *et al.*, 2016).

Previous studies testing the effects of non-divergent immigrants on population fitness have generally found positive results (reviewed by Frankham, 2015; Whiteley *et al.*, 2015). When immigrants are divergent, however, studies have been inconsistent, with some describing a positive effect (Hogg *et al.*, 2006; Bossuyt, 2007; Willi *et al.*, 2007; Johnson *et al.*, 2010; Tortajada *et al.*, 2010) and others a negative effect (Lacy, 1998; Edmands, 1999, 2002; Fenster & Galloway, 2000; Lee, 2000; Hwang *et al.*, 2011; Pekkala *et al.*, 2012). These inconsistencies may be due to a variety of factors, including the type and degree of divergence of immigrants (Frankham *et al.*, 2011), the severity of inbreeding

depression in recipient populations (Ingvarsson, 2001) and the duration of population monitoring (Tallmon *et al.*, 2004).

Future studies are needed to build upon our experiment and previous research. First, longer term studies will provide a more complete understanding of the ultimate effects of augmentation on population dynamics, as has been argued previously (Whitlock *et al.*, 1995; Fenster, Galloway & Chao, 1997; Edmands, 1999, 2007; Tallmon *et al.*, 2004; Willi *et al.*, 2007). Second, studies that manipulate the degree of divergence of immigrants and the level of inbreeding of recipient populations will identify key thresholds when the positive effects of gene flow become negative. Third, the goal of our study was to determine the overall effect of divergent immigrants on demography, rather than tease apart the contributions of demographic versus genetic rescue. Future studies should attempt to parse out the relative importance of these two mechanisms in rescuing populations (*sensu* Hufbauer *et al.*, 2015). To avoid unaccounted for variation in immigration rates, immigrants should not be pregnant upon introduction. We also suggest including a treatment with immigrants from the same population to control for the numerical input of the immigrants themselves; these immigrants would contribute little novel genetic information, and therefore their impact on population fitness would be almost entirely demographic. Contrasts between treatments with immigrants from the same population and those with immigrants from elsewhere would be able to differentiate demographic from genetic rescue. Finally, when managers decide to augment declining or small populations, they should utilize the opportunity to test the effects of immigrants on population dynamics, for example, using an adaptive management framework in which management actions are adjusted depending on the effectiveness of previous approaches (Walters, 1986; Williams, Nichols & Conroy, 2002).

Conservation implications

If managers decide to augment a population, they should ideally choose an immigrant source that is adaptively and genetically similar to the recipient population. However, our results suggest that if no similar source exists, augmenting with individuals from a divergent population may be better than doing nothing, particularly for very small populations that are highly vulnerable to extinction from demographic stochasticity. Individual mesocosms in our experiment showed widely varying population responses due to small initial population sizes (Fig. S1), but overall mesocosms that received divergent immigrants maintained higher abundances than controls that did not.

Our study is relevant to many species of conservation concern in which the only potential sources for augmenting small, isolated populations are adaptively and/or genetically divergent. For example, many small populations of Great Basin Columbia spotted frogs *Rana luteiventris* (a candidate for listing under the U.S. Endangered Species Act) are separated from each other by tens to hundreds of kilometers of inhospitable desert habitat and are also highly genetically differentiated from each other (Funk *et al.*, 2008). Similarly, island foxes *Urocyon littoralis* (listed as Endangered under

the U.S. Endangered Species Act) have been subdivided into six subspecies, each on a different island (Goldstein *et al.*, 1999; Aguilar *et al.*, 2004; Funk *et al.*, 2016). In both species, the potential sources for augmentation are highly divergent from recipient populations.

If deemed necessary for increasing population fitness and averting extinction, augmenting with individuals from divergent populations should at least be considered in these and other cases in which a similar source population is not available. We realize, however, that a single empirical study in guppies or any other experimental system does not necessarily inform all species and cases. Ultimately, the effects of immigration and gene flow will depend on many factors, particularly the degree of divergence of immigrants and the degree of inbreeding depression in recipient populations. More studies are needed to determine how these factors and others influence the outcome of gene flow if we are to come to a consensus about when demographic and/or genetic rescue via augmentation will work.

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References

- Aguilar, A., Roemer, G., Debenham, S., Binns, M., Garcelon, D. & Wayne, R.K. (2004). High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. *Proc. Natl Acad. Sci. USA* **101**, 3490–3494.
- Baillie, L. (2012). *Genetic population structure of the Trinidadian guppy (Poecilia reticulata) across Trinidad and Tobago*. MS Thesis, Dalhousie University.
- Barson, N.J., Cable, J. & Van Oosterhout, C. (2009). Population genetic analysis of microsatellite variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: evidence for a dynamic source-sink metapopulation structure, founder events and population bottlenecks. *J. Evol. Biol.* **22**, 485–497.
- Bateson, W. (1909). Heredity and variation in modern lights. In *Darwin and modern science*: 85–101. Seward, A.C. (Ed.). Cambridge: Cambridge University Press.
- Bossuyt, B. (2007). Genetic rescue in an isolated metapopulation of a naturally fragmented plant species, *Parnassia palustris*. *Conserv. Biol.* **21**, 832–841.
- Brown, J.H. & Kodric-Brown, A. (1977). Turnover rates in insular biogeography: effect of immigration on extinction. *Ecology* **58**, 445–449.
- Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2000). Emerging infectious diseases of wildlife - threats to biodiversity and human health. *Science* **287**, 443–449.
- Dobzhansky, T. (1937). *Genetics and the origin of species*. New York: Columbia University Press.
- Edmunds, S. (1999). Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* **53**, 1757–1768.
- Edmunds, S. (2002). Does parental divergence predict reproductive compatibility? *Trends Ecol. Evol.* **17**, 520–527.
- Edmunds, S. (2007). Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol. Ecol.* **16**, 463–475.
- Edmunds, S. & Timmerman, C.C. (2003). Modeling factors affecting the severity of outbreeding depression. *Conserv. Biol.* **17**, 883–892.
- Endler, J.A. (1983). Natural and sexual selection on color patterns in poeciliid fishes. *Environ. Biol. Fishes* **9**, 173–190.
- Fenster, C.B. & Galloway, L.F. (2000). Inbreeding and outbreeding depression in natural populations of *Chamaecrista fasciculata* (Fabaceae). *Conserv. Biol.* **14**, 1406–1412.
- Fenster, C.B., Galloway, L.F. & Chao, L. (1997). Epistasis and its consequences for the evolution of natural populations. *Trends Ecol. Evol.* **12**, 282–286.
- Fitzpatrick, S.W., Gerberich, J.C., Angeloni, L.M., Bailey, L.L., Broder, E.D., Torres-Dowdall, J., Handelsman, C.A., López-Sepulcre, A., Reznick, D.N., Ghalambor, C.K. & Funk, W.C. (2016). Gene flow from an adaptively divergent source causes rescue through genetic and demographic factors in two wild populations of Trinidadian guppies. *Evol. Appl.* (Online DOI: 10.1111/eva.12356)
- Frankham, R. (2010). Challenges and opportunities of genetic approaches to biological conservation. *Biol. Conserv.* **143**, 1919–1927.
- Frankham, R. (2015). Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. *Mol. Ecol.* **24**, 2610–2618.
- Frankham, R., Ballou, J.D., Eldridge, M.D.B., Lacy, R.C., Ralls, K., Dudash, M.R. & Fenster, C.B. (2011). Predicting the probability of outbreeding depression. *Conserv. Biol.* **25**, 465–475.
- Funk, W.C., Pearl, C.A., Draheim, H.M., Adams, M.J., Mullins, T.D. & Haig, S.M. (2008). Range-wide phylogeographic analysis of the spotted frog complex (*Rana luteiventris* and *Rana pretiosa*) in northwestern North America. *Mol. Phylogenet. Evol.* **49**, 198–210.
- Funk, W.C., Lovich, R.E., Hohenlohe, P.A., Hofman, C.A., Morrison, S.A., Sillett, T.S., Ghalambor, C.K., Maldonado, J.E., Rick, T.C., Day, M.D., Polato, N.R., Fitzpatrick, S.W.,

- Coonan, T.J., Crooks, K.R., Dillon, A., Garcelon, D.K., King, J.L., Boser, C.L., Gould, N. & Andelt, W.F. (2016). Adaptive divergence despite strong genetic drift: genomic analysis of the evolutionary mechanisms causing genetic differentiation in the island fox (*Urocyon littoralis*). *Mol. Ecol.* (Online DOI:10.1111/mec.13605)
- Galloway, L.F. & Etterson, J.R. (2005). Population differentiation and hybrid success in *Campanula americana*: geography and genome size. *J. Evol. Bio.* **18**, 81–89.
- Gilk, S.E., Wang, I.A., Hoover, C.L., Smoker, W.W., Taylor, S.G., Gray, A.K. & Gharrett, A.J. (2004). Outbreeding depression in hybrids between spatially separated pink salmon, *Onchorhynchus gorbuscha*, populations: marine survival, homing ability, and variability in family size. *Environ. Biol. Fishes* **69**, 287–297.
- Goldstein, D.B., Roemer, G.W., Smith, D.A., Reich, D.E., Bergman, A. & Wayne, R.K. (1999). The use of microsatellite variation to infer population structure and demographic history in a natural model system. *Genetics* **151**, 797–801.
- Hadfield, J.D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Softw.* **33**, 1–22.
- Haskins, C.P., Haskins, E.F., McLaughlin, J.J.A. & Hewitt, R.E. (1961). Polymorphism and population structure in *Lebistes reticulatus*, an ecological study. In *Vertebrate speciation*: 320–395. Blair, W.F. (Ed.). Austin: University of Texas Press.
- Heber, S., Varsani, A., Kuhn, S., Girg, A., Kempnaers, B. & Briskie, J. (2013). The genetic rescue of two bottlenecked South Island robin populations using translocations of inbred donors. *Proc. Roy. Soc. B* **280**, 20122228.
- Hedrick, P.W. (1995). Gene flow and genetic restoration—the Florida panther as a case-study. *Conserv. Biol.* **9**, 996–1007.
- Heiser, D.A. & Shaw, R.G. (2006). The fitness effects of outcrossing in *Calylophus serrulatus*, a permanent translocation heterozygote. *Evolution* **60**, 64–76.
- Hogg, J.T., Forbes, S.H., Steele, B.M. & Luikart, G. (2006). Genetic rescue of an insular population of large mammals. *Proc. Biol. Sci.* **273**, 1491–1499.
- Houde, A.E. & Endler, J.A. (1990). Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science* **248**, 1405–1408.
- Hufbauer, R.A., Szűcs, M., Kasyon, E., Youngberg, C., Koontza, M.J., Richards, C., Tuff, T. & Melbourne, B.A. (2015). Three types of rescue can avert extinction in a changing environment. *Proc. Natl Acad. Sci. USA* **112**, 10557–10562.
- Hwang, A.S., Northrup, S.L., Alexander, J.K., Vo, K.T. & Edmands, S. (2011). Long-term experimental hybrid swarms between moderately incompatible *Tigriopus californicus* populations: hybrid inferiority in early generations yields to hybrid superiority in later generations. *Conserv. Genet.* **12**, 895–909.
- Hwang, A.S., Northrup, S.L., Peterson, D.L., Kim, Y. & Edmands, S. (2012). Long-term experimental hybrid swarms between nearly incompatible *Tigriopus californicus* populations: persistent fitness problems and assimilation by the superior population. *Conserv. Genet.* **13**, 567–579.
- Ingvarsson, P.K. (2001). Restoration of genetic variation lost—the genetic rescue hypothesis. *Trends Ecol. Evol.* **16**, 62–63.
- Johnson, W.E., Onorato, D.P., Roelke, M.E., Land, E.D., Cunningham, M., Belden, R.C., McBride, R., Jansen, D., Lotz, M., Shindle, D., Howard, J., Wildt, D.E., Penfold, L.M., Hostetler, J.A., Oli, M.K. & O'Brien, S.J. (2010). Genetic restoration of the Florida panther. *Science* **329**, 1641–1645.
- Lacy, R.C. (1998). Partitioning additive, dominance, epistatic and maternal effects on reproductive performance in crosses between subspecies of *Peromyscus polionotes*. In *45th Annual Meeting of the Genetics Society of Australia*: 88. Sved, J. (Ed.). Sydney: Genetics Society of Australia.
- Lee, C.E. (2000). Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate “populations”. *Evolution* **54**, 2014–2027.
- Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **17**, 183–189.
- Longdon, B., Hadfield, J.D., Webster, C.L., Obbard, D.J. & Jiggins, F.M. (2011). Host phylogeny determines viral persistence and replication in novel hosts. *PLoS Pathog.* **7**, e1002260.
- Lonsdale, D.L., Levinton, J.S. & Rosen, S. (1988). Reproductive compatibility among latitudinally separated *Scotollana Canadensis* (Wiley). *Hydrobiologia* **167–168**, 469–476.
- Lynch, M. (1991). The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* **45**, 622–629.
- Magurran, A.E. (2005). *Evolutionary ecology: the Trinidadian guppy*. Oxford: Oxford University Press.
- Mayr, E. (1963). *Animal species and evolution*. Cambridge: Harvard University Press.
- Mills, L.S. & Allendorf, F.W. (1996). The one-migrant-per-generation rule in conservation and management. *Conserv. Biol.* **10**, 1509–1518.
- Muller, H.J. (1940). Bearing on the *Drosophila* work on systematics. In *The new systematics*: 185–268. Huxley, J.S. (Ed.). Oxford: Clarendon Press.
- Neff, B.D. (2004). Stabilizing selection on genomic divergence in a wild fish population. *Proc. Natl Acad. Sci. USA* **101**, 2381–2385.
- van Oosterhout, C., Smith, A.M., Hanfling, B., Ramnarine, I.W., Mohammed, R.S. & Cable, J. (2007). The guppy as a conservation model: implications of parasitism and inbreeding for reintroduction success. *Conserv. Biol.* **21**, 1573–1583.

- Pekkala, N., Knott, K.E., Kotiaho, J.S., Nissinen, K. & Puurtinen, M. (2012). The benefits of interpopulation hybridization diminish with increasing divergence of small populations. *J. Evol. Biol.* **25**, 2181–2193.
- Quilichini, A., Debussche, M. & Thompson, J.D. (2001). Evidence for local outbreeding depression in the Mediterranean island endemic *Anchusa crispata* Viv. (Boraginaceae). *Heredity* **87**, 190–197.
- R Development Core Team. (2011). *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Reznick, D. (1982). The impact of predation on life-history evolution in Trinidadian guppies: genetic basis of observed life-history patterns. *Evolution* **36**, 1236–1250.
- Reznick, D. & Endler, J.A. (1982). The impact of predation on life-history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**, 160–177.
- Reznick, D.N., Butler, M.J. IV, Rodd, F.H. & Ross, P. (1996). Life-history evolution in guppies (*Poecilia reticulata*). 6. Differential mortality as a mechanism for natural selection. *Evolution* **50**, 1651–1660.
- Sasa, M.M., Chippindale, P.T. & Johnson, N.A. (1998). Patterns of postzygotic isolation in frogs. *Evolution* **52**, 1811–1820.
- Seghers, B.H. (1974). Schooling behavior in guppy (*Poecilia reticulata*) - Evolutionary response to predation. *Evolution* **28**, 486–489.
- Storfer, A. (1999). Gene flow and endangered species translocations: a topic revisited. *Biol. Conserv.* **87**, 173–180.
- Suk, H.Y. & Neff, B.D. (2009). Microsatellite genetic differentiation among populations of the Trinidadian guppy. *Heredity* **102**, 425–434.
- Tallmon, D.A., Luikart, G. & Waples, R.S. (2004). The alluring simplicity and complex reality of genetic rescue. *Trends Ecol. Evol.* **19**, 489–496.
- Templeton, A.R. (1986). Coadaptation and outbreeding depression. In *Conservation biology: the science of scarcity and diversity*: 105–116. Soule, M.E. (Ed.). Sunderland: Sinauer Associates Inc.
- Thrall, P.H., Richards, C.M., McCauley, D.E. & Antonovics, J. (1998). Metapopulation collapse: the consequences of limited gene-flow in spatially structured populations. In *Modeling spatiotemporal dynamics in ecology*: 83–104. Bascompte, J. & Sole, R.V. (Eds). Berlin: Springer-Verlag.
- Torres-Dowdall, J., Handelsman, C.A., Reznick, D.N. & Ghalambor, C.K. (2012). Local adaptation and the evolution of phenotypic plasticity in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **66**, 3432–3443.
- Tortajada, A.M., Carmona, M.J. & Serra, M. (2010). Effects of population outcrossing on rotifer fitness. *BMC Evol. Biol.* **10**, 312.
- Walters, C. (1986). *Adaptive management of renewable resources*. New York: MacMillan.
- Weeks, A.R., Sgrò, C.M., Young, A.G., Frankham, R., Mitchell, N.J., Miller, K.A., Byrne, M., Coates, D.J., Eldridge, M.D.B., Sunnucks, P., Breed, M.F., James, E.A. & Hoffmann, A.A. (2011). Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evol. Appl.* **4**, 709–725.
- Westemeier, R.L., Brawn, J.D., Simpson, S.A., Esker, T.L., Jansen, R.W., Walk, J.W., Kershner, E.L., Bouzat, J.L. & Paige, K.N. (1998). Tracking and long-term decline and recovery of an isolated population. *Science* **282**, 1695–1698.
- Whiteley, A.R., Fitzpatrick, S.W., Funk, W.C. & Tallmon, D.A. (2015). Genetic rescue to the rescue. *Trends Ecol. Evol.* **30**, 42–49.
- Whitlock, M.C., Phillips, P.C., Moore, F.B.G. & Tonsor, S.J. (1995). Multiple fitness peaks and epistasis. *Annu. Rev. Ecol. Syst.* **26**, 601–629.
- Willi, Y., Van Kleunen, M., Dietrich, S. & Fischer, M. (2007). Genetic rescue persists beyond first-generation outbreeding in small populations of a rare plant. *Proc. Biol. Sci.* **274**, 2357–2364.
- Williams, B.K., Nichols, J.D. & Conroy, M.J. (2002). *Analysis and management of animal populations*. San Diego: Elsevier-Academic.
- Willing, E.-M., Bentzen, P., van Oosterhout, C., Hoffman, M., Cable, J., Breden, F., Weigel, D. & Dreyer, C. (2010). Genome-wide single nucleotide polymorphisms reveal population history and adaptive divergence in wild guppies. *Mol. Ecol.* **19**, 968–984.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Abundance of adult founders, adults recruited into the population and sub-adults (<14 mm) for each treatment and block over time.