Connectivity rescues genetic diversity after a demographic bottleneck in a butterfly population network

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Demographic bottlenecks that occur when populations fluctuate in size erode genetic diversity, but that diversity can be recovered through immigration. Connectivity among populations and habitat patches in the landscape enhances immigration and should in turn facilitate recovery of genetic diversity after a sudden reduction in population size. For the conservation of genetic diversity, it may therefore be particularly important to maintain connectivity in the face of factors that increase demographic instability, such as climate change. However, a direct link between connectivity and recovery of genetic diversity after a demographic bottleneck has not been clearly demonstrated in an empirical system. Here, we show that connectivity of habitat patches in the landscape contributes to the maintenance of genetic diversity after a demographic bottleneck. We were able to monitor genetic diversity in a network of populations of the alpine butterfly, *Parnassius smintheus*, before, during, and after a severe reduction in population size that lasted two generations. We found that allelic diversity in the network declined after the demographic bottleneck but that less allelic diversity was lost from populations occupying habitat patches with higher connectivity. Furthermore, the effect of connectivity on allelic diversity was important during the demographic recovery phase. Our results demonstrate directly the ability of connectivity to mediate the rescue of genetic diversity in a natural system.

alpine butterfly | genetic diversity | demographic bottleneck | recovery | connectivity

Genetic diversity represents the most fundamental level of biological diversity. Loss of genetic diversity is a central concern in conservation biology because populations with low genetic diversity may suffer from inbreeding and reduced fitness, lack the potential to adapt to future environmental change, and be more vulnerable to extinction (1, 2). Genetic diversity can be lost from populations through various mechanisms, with random drift in finite populations and demographic bottlenecks (temporary but severe reductions in population size), being of greatest relevance in conservation (3, 4).

Immigration into a genetically impoverished population can rescue genetic diversity and can be achieved artificially through translocations or through natural movement of individuals (5, 6). Natural immigration requires connectivity within the landscape, where connectivity measures the extent to which movement and gene flow can occur among populations (7). Connectivity can be defined at the level of the landscape or individual habitat patches and is a function of structural elements of the landscape in combination with the movement behavior of individual species (7, 8).

There is considerable interest in managing landscapes to improve connectivity among natural populations as this provides a variety of ecological and genetic benefits (9), including the potential for natural genetic rescue. Correlations between connectivity and genetic diversity shown in numerous systems suggest that connectivity contributes to maintenance of genetic diversity on some time scale (10, 11). However, the temporal scales involved in the establishment of such correlations are poorly understood. Although predicted in theory, the ability of connectivity to rescue genetic diversity rapidly after a demographic bottleneck has not, to our knowledge, been demonstrated in a natural system. Consequently, the extent to which connectivity may contribute directly to genetic diversity via immigration of novel alleles, vs. indirectly via effects on population size and stability, is also not well understood.

A network of populations of the Rocky Mountain Apollo butterfly, *Parnassius smintheus*, occupying patches of alpine meadow habitat in Alberta, Canada, has been monitored and studied continuously since 1995, and effects of both landscape structure and climate variation on population dynamics and dispersal have been described (12, 13) (Fig. 1). Population dynamics of *P. smintheus* are influenced by climate variation, with the Pacific Decadal Oscillation (PDO) index being a strong descriptor of annual population growth. More frequent extremely cold or warm winters, which can be expected as a result of climate change, are predicted to increase the occurrence of years with negative population growth for *P. smintheus* (12).

In this study site, two severe demographic bottlenecks have been documented and linked to poor overwintering weather conditions: in 2003 and 2010 (Fig. 2). The bottleneck that began in 2010 was more protracted, with population sizes remaining low in 2011 and recovering more slowly than after the 2003 bottleneck (Fig. 2). The reason for the differing nature of these two events may be more severe overwintering conditions in 2010, leading to higher egg mortality (12). A study comparing genetic

Significance

With climate change and associated climatic instability, populations are expected to experience more frequent and severe fluctuations in size. These population size fluctuations erode genetic diversity, which is a central concern for conservation. Connectivity among habitats should, in theory, protect against the erosion of genetic diversity because immigration of individuals into populations can counteract genetic diversity loss. Our study provides the first evidence in a natural system for a direct effect of connectivity in recovery of genetic diversity following a demographic bottleneck, highlighting the importance of maintaining connectivity in the face of climate change. Our results suggest an important potential interaction between the two global change factors of habitat fragmentation and climate change on genetic diversity of natural populations.

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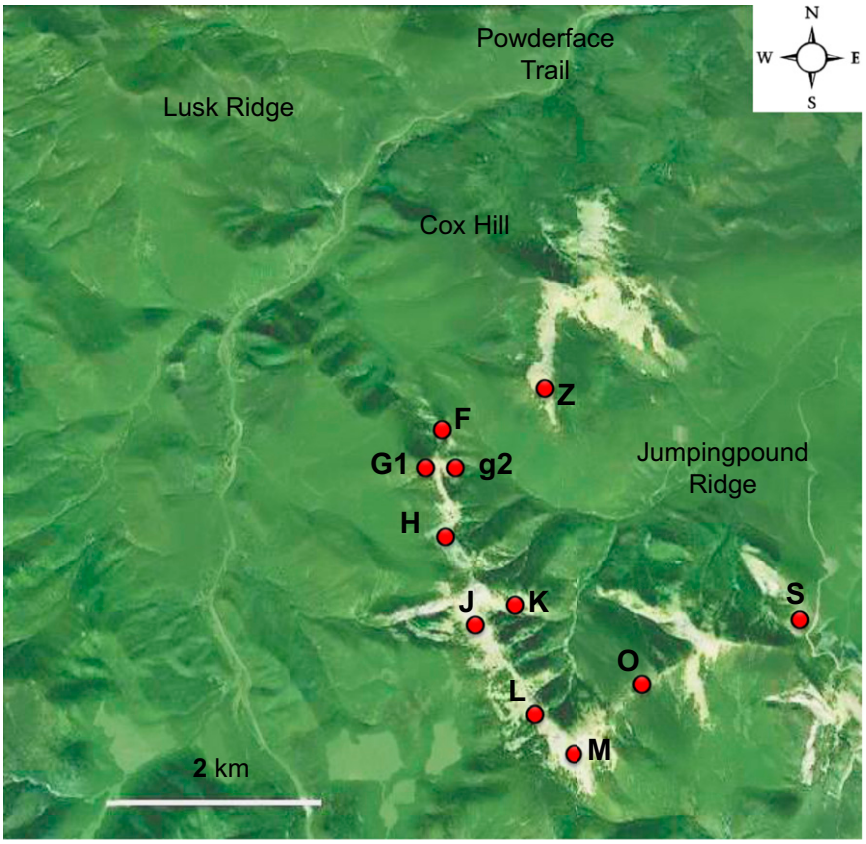

Fig. 1. Map of the study patches occupied by a network of *P. smintheus* populations in Kananaskis, Alberta, Canada. Ridge-top meadow habitat is in white. Locations of 11 patches included in this study are shown as red circles with black letter labels. Map data: Google, DigitalGlobe 2015.

diversity before and after the 2003 demographic bottleneck (14) yielded a key result: no overall loss of diversity across the network was detected, but an interaction between patch connectivity and severity of the demographic collapse affected loss of allelic richness within individual habitat patches. Although not definitive, this suggested some role of connectivity in maintaining genetic diversity in populations experiencing demographic bottlenecks.

Here, we assess effects of the more protracted 2010 demographic bottleneck on genetic diversity by comparing samples collected before (in 2008), during (in 2010 and 2011), and after

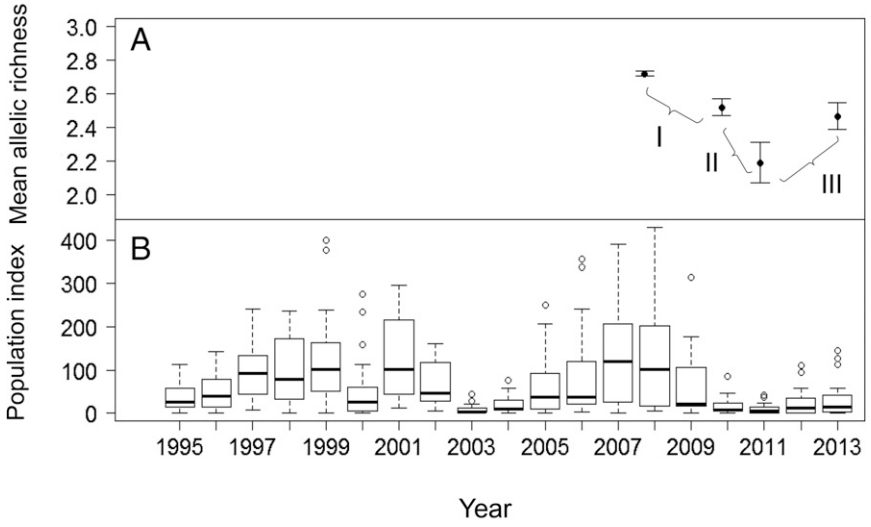

Fig. 2. Changes in *P. smintheus* population size and allelic richness (A_R) in the network over different years. (*A*) Mean A_R over seven microsatellite loci in 2008, 2010, 2011, and 2013 (with rarefaction to four genes). Significant predictors of A_R change were 2010 population size during phase *I*, none during phase II, and 2012 connectivity during phase III. (*B*) Boxplots of yearly *P. smintheus* abundance estimates for all populations, showing interquartile range (IQR; boxes), maximum and minimum estimates up to 1.5 × IQR (whiskers), and outliers beyond 1.5 × IQR (open circles).

(in 2013) the event. We show that across this bottleneck, connectivity plays a clear and significant role in recovery of genetic diversity, highlighting the importance of conserving connectivity in fluctuating populations.

Results and Discussion

We assessed genetic diversity using a panel of seven highly variable microsatellite loci (Dataset S1). Before the demographic bottleneck, we observed a total of 132 different alleles across all these loci and 123 cases of an allele within a patch having an observed frequency below 2%. Thus, with a large number of alleles per locus, these markers would have been particularly sensitive to changes in allelic diversity (15). We first established there were no effects of prebottleneck allelic richness (A_R) or expected heterozygosity (H_E) on 2010 or 2011 population size, indicating that levels of neutral genetic diversity did not predict or determine the severity of the demographic decline in each patch (2010: A_R, $P = 0.33$, H_E, $P = 0.86$; 2011: A_R, $P = 0.39$, H_E, $P = 0.95$).

Allelic diversity across the population network declined after the 2010 demographic bottleneck. Averaging over all patches and loci, A_R (rarefied to 10 genes) was reduced significantly from 4.76 (SE, ±0.026) in 2008 to 4.08 (SE, ±0.17) in 2013 (Wilcoxon–Mann–Whitney test, $W = 77.5$; $P = 0.001$). This change represents a mean loss of 14% (0.14 ± 0.039; Table S1) of the allelic richness present before the bottleneck. These results contrast with the 2003 demographic bottleneck where no overall loss of allelic diversity across the network occurred (14) and reflect differences in duration and recovery from the two events; a longer duration at low abundance has a stronger negative effect on genetic diversity (16). Expected heterozygosity also declined after the demographic bottleneck, from a mean of 0.71 (SE, ±0.005) across patches in 2008 to 0.67 (SE, ±0.016) in 2013, although the difference was marginally nonsignificant (Wilcoxon–Mann–Whitney test, $W = 62$; $P = 0.061$). The weaker response of heterozygosity is consistent with theoretical expectations that allelic diversity should respond more strongly and rapidly to a demographic bottleneck than heterozygosity (17).

Despite the clearly documented demographic bottlenecks in this system and the significant reduction in allelic diversity across the 2010 event, significant signatures of genetic bottlenecks using the program BOTTLENECK were not detected in samples collected before or after the 2010 event, using either the infinite allele model or two-phase model (all $P > 0.05$ for Wilcoxon tests). The low power of single sampling-period bottleneck detection methods has been previously noted (18, 19), and in our study system, the effectiveness of such methods may be particularly limited by the occurrence of immigration, which can erase a genetic bottleneck signature in two to three generations (20, 21).

We observed substantial variation among populations occupying different habitat patches in the amount of allelic diversity lost from 2008 to 2013, with the proportional loss of A_R (averaged across loci) ranging from −0.03 (A_R increased slightly in one site) to 0.31 (31% of A_R lost). We examined the ability of patch connectivity to explain these changes in A_R using a connectivity measure that accounts for both landscape structure (areas and distances between patches, and the nature of the intervening matrix) and movement parameters estimated from mark-recapture data. Severity of the demographic bottleneck, measured as population size during the lowest abundance years of 2010 or 2011, and the interaction between connectivity and bottleneck severity were included in the models analyzed. A measure of patch connectivity that was based on movement parameters from 2012 was the single best predictor of change in A_R from 2008 to 2013 ($r^2 = 0.81$, $F_{1,7} = 30.38$, $P = 0.0009$). Populations in patches with greater connectivity retained more allelic diversity through the demographic bottleneck (Fig. 3). There were no significant effects of bottleneck severity, measured as either 2010 or 2011 population size, on the loss of A_R. Compared with the 2003 demographic bottleneck, therefore, where the effect of connectivity on loss of A_R was complicated by an interaction with severity of local population size decline (14), across this more protracted event, we observed a very distinct and clear effect of connectivity on the loss of A_R.

Connectivity in 2012 was also a stronger predictor of A_R change from 2008 to 2013 than connectivity estimated using movement parameters from either 2010 ($r^2 = 0.62$, $F_{1,7} = 11.49$, $P = 0.011$) or 2011 ($r^2 = 0.71$, $F_{1,7} = 17.17$, $P = 0.004$). This result supports the hypothesis that the effect of connectivity on A_R change was via facilitation of immigration into patches, because this process should be acting most strongly during the demographic recovery phase (2011–2013) than during the initial decline (2008–2010). To examine this further and to characterize the behavior of A_R through the demographic bottleneck, we genotyped samples collected in 2010 and 2011; sample sizes in these years were necessarily small, and therefore a smaller number of patches and fewer individuals per patch could be analyzed. We then examined effects of patch connectivity and population size in various years, and their interactions, on changes in A_R (rarefied to four gene copies because of smaller sample sizes in 2010 and 2011) during the demographic decline phase and the recovery phase, separately.

Mean A_R declined from 2008 to 2010, accompanying the demographic bottleneck. Mean A_R dropped even further in 2011 with the continued time at low population size, and then increased from 2011 to 2013 as population sizes recovered, although not fully returning to prebottleneck (2008) levels (Fig. 2). Using the same number of populations as in our comparisons between 2008 and 2013, we found that loss of A_R from 2008 to 2010 was best explained by population size in 2010 ($r^2 = 0.57$, $F_{1,7} = 9.38$, $P = 0.018$; Fig. 2). This result indicates that severity of the demographic bottleneck, but not connectivity, determined the loss of genetic diversity during the demographic decline phase. In contrast, the increase in A_R from 2011 to 2013 was best explained by connectivity in 2012 ($r^2 = 0.91$, $F_{1,2} = 19.46$, $P = 0.047$; Fig. 2). Although this latter analysis of A_R gain was based on only four populations, in combination, our results do suggest that the effect of connectivity on A_R change observed across the entire demographic bottleneck (2008–2013) reflects the importance of connectivity during the recovery phase, rather than through any effect of connectivity on the loss of A_R during the initial population decline. The results are therefore consistent with the hypothesis that connectivity rescued genetic diversity via immigration and gene flow during the recovery phase. No predictors explained the additional losses of A_R between 2010 and 2011 (Fig. 2) that likely resulted from genetic drift. Compared with the contracted episode of population decline and recovery that occurred in 2003, it appears that across this more protracted, recent episode we were able tease apart the effects of the severity of demographic decline, which affects loss of A_R from populations, and connectivity, which affects recovery of A_R, that were previously found to interact.

Immigration accompanied by gene flow is a key process leading to recovery of genetic diversity after a demographic bottleneck (20, 22), allowing populations to maintain genetic diversity despite fluctuating dynamics (5). Although immigration is mediated by patch or landscape connectivity, empirical evidence for a direct effect of connectivity in rescuing genetic diversity has been lacking. Our study provides evidence in a natural system for a direct effect of connectivity in recovery of genetic diversity following a demographic bottleneck. Our study also underlines the importance of maintaining connectivity in the face of climate change, as natural populations are expected to experience more frequent and severe fluctuations in size as result of increasing climatic instability (12, 23). The two global change factors of loss of habitat connectivity and climate change may act together in this and other systems to reduce genetic diversity of populations.

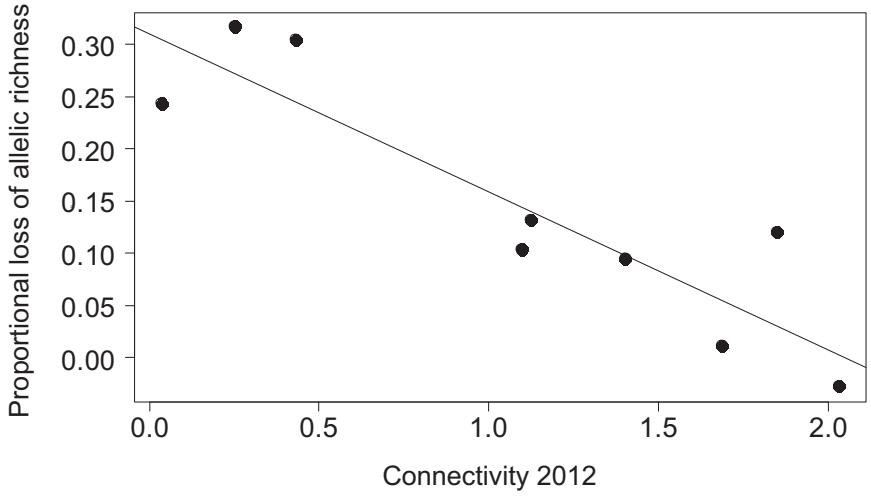

Fig. 3. Relationship between the proportional loss of allelic richness (A_R) across a demographic bottleneck and habitat patch connectivity in *P. smintheus*. Proportional loss of allelic richness was measured from 2008 and 2013 and was best explained by connectivity in 2012. Solid dots indicate individual patches. Least-square line of best fit is shown.

Materials and Methods

Sample Collection and Study Region. Wing-clips from adult *P. smintheus* have been collected since 1995, and yearly since 2005, from populations along Jumpingpound Ridge, in the Kananaskis region of Alberta, Canada (50°57′ N, 114°54′ W; Fig. 1). Here, *P. smintheus* occupies meadows above treeline (~2,100 m), and we delineated habitat patches that range in area from 0.2 to 22.7 ha and are separated from each other by either intervening forest or open meadow habitat (24). Butterflies were captured with hand-nets and individually marked, and ~0.2 cm^2 of wing tissue was removed using forceps or iris scissors and stored immediately in 95–100% (vol/vol) ethanol. To assess genetic diversity before and after the 2010 demographic bottleneck, we focused on individuals sampled from the same patches in 2008 (prebottleneck) and 2013 (postbottleneck). These years are those closest to the bottleneck in which larger samples from several patches were available (Fig. 2*A*). Supporting analyses also included individuals sampled during the low population size years of 2010 and 2011. The number of individuals sampled per population was by necessity small in the bottleneck years because of the low population sizes in those years. However, we sampled an equal or even larger proportion of the total population in these years, as well as in 2013, compared with 2008, as reflected in the ratios of sample size to index of population size for the different years (Table S2).

Mark-Recapture Study and Estimates of Patch Connectivity. Mark-recapture studies of *P. smintheus* have been conducted in the population network since 1995 and are described extensively elsewhere (12, 13). Adults are individually marked, and spatial locations of captures and recaptures are recorded. An index of population size in each habitat patch is determined using Craig's method, which provides an estimate of the number of adults in the population on a single day of sampling (25, 26). We used the maximum Craig's estimate from three to five different sampling days per year as an index of population size in each patch, each year. Rates of movement among patches were estimated with the virtual migration model (VMM) (27).

We defined the connectivity of patch k as its relative attractiveness and accessibility to emigrants from all other patches in the network. This value was estimated as the sum, over all other patches, of the probabilities of individuals leaving each other patch and reaching k (27)

$$\psi_k = \sum_{\substack{j=1 \\ j \neq k}}^{n} \frac{A_k^{\zeta} e^{\left(-\alpha_f d_{jkf} - \alpha_m d_{jkm}\right)}}{\frac{\lambda}{S_j} + S_j},$$

where

$$S_j = \sum_{\substack{k=1 \\ k \neq j}}^{n} A_k^{\zeta} e^{\left(-\alpha_f d_{jkf} - \alpha_m d_{jkm}\right)}.$$

A_k is the area of patch k; d_{jkf} and d_{jkm} are the distances through forest and meadow habitat between patches j and k, respectively; and λ is mortality during dispersal. Additional parameters describe the effect of forest and meadow on movement (α_f and α_m, respectively), and the scaling of immigration with patch area (ζ). Connectivity for each patch, each year, was calculated using parameters estimated from the mark-recapture data for that year. Because movements of *P. smintheus* are restricted along ridge tops (13), we calculated pairwise distances between patches along ridge tops between centroids of butterfly capture in each patch (Fig. 1). The total distance between any two patches was divided into two components, the distance comprised of forest and distance comprised of open meadow, which we estimated from digitized aerial photos.

DNA Extraction and Microsatellite Analysis. DNA was extracted from wing-clips using a DNeasy Blood and Tissue Kit (QIAgen), with a final elution volume of 200 μL. Each sample was genotyped at seven highly variable microsatellite loci (28, 29). PCR amplification of microsatellites occurred in two multiplex amplifications (multiplex 1: Ps 50, Ps 81, and Ps 85; multiplex 2: Ps 76 and Ps 163) and two individual locus amplifications (Ps 262 and Ps 165). PCR reactions occurred in a final solution of volume 10 μL; each amplification contained 1× AmpliTaq buffer (10 mM Tris, pH 8.8, 0.1% Triton ×100, 50 mM KCl), 3.125 mM $MgCl_2$, 0.075–0.275 μM of each primer, 0.25 mM dNTP, 0.0625 U of AmpliTaq DNA polymerase (Applied Biosystems), 0.15 μg BSA, and 3 μL genomic DNA. PCR amplifications were performed in a PTC 0200 DNA Engine Cycler (BioRad). One of the two primers (forward primer) was labeled with a fluorescent dye to allow visualization of PCR products.

Thermal cycling profiles followed one of two protocols as follows. (*i*) Multiplex amplification: denaturation for 60 s at 94 °C; followed by 3 cycles at of 30 s at 94 °C, 30 s at 56 °C annealing, and 30 s at 72 °C; 10 touchdown (TD) cycles when annealing temperature was reduced 0.5 °C per cycle, and all hold times were reduced to 15 s; 27 additional cycles of 15 s at 94 °C, 15 s at 51 °C, and 15 s at 72 °C; and final elongation at 72 °C for 180 s. (*ii*) Individual locus amplification: denaturation for 60 s at 94 °C; followed by 3 cycles of 30 s at 94 °C; 20 s at 54 °C, and 10 s at 72 °C; followed by 32 cycles of 15 s at 94 °C; 20 s at 54 °C; 5 s at 72 °C; and final elongation at 72 °C for 30s. Ramp speed was set to 1 °C/s for all thermal cycling.

PCR products were visualized and sized on an Applied Biosystems 3730S capillary DNA analyzer, using LIZ-500 size standard. All loci (the PCR products) for each individual were multiloaded in a single lane of the DNA analyzer. Electropherograms generated by the DNA analyzer were viewed and processed using GeneMapper software Ver. 4.0 (Applied Biosystems) to score microsatellite genotypes. All genotypes were checked manually and loci that failed to amplify were rerun up to two more times. If a locus failed to amplify in an individual after three attempts, the individual was considered null homozygous for that locus. However, any individuals with two or more failed loci were removed altogether from the dataset.

Linkage Disequilibrium and Hardy–Weinberg Tests. For each of the 4 y separately (2008, 2010, 2011, and 2013), linkage disequilibrium and conformity to Hardy–Weinberg proportions were tested for each locus in each population

using Genepop v.4.2 (30). For linkage tests, significance was assessed using a Markov chain method of 100 batches of 1,000 iterations per batch. Hardy–Weinberg tests used the Markov chain method and approximation of Fisher's exact test implemented in Genepop (31). Consistent with previous analyses of these loci (10, 14, 28, 29), there was no evidence for linkage disequilibrium but there were significant deviations from expected Hardy–Weinberg genotypic proportions. Of a total of 203 tests of conformity to Hardy–Weinberg proportions, 115 indicated significant homozygote excess, which was observed at all loci and in each time period (in 46 of 63 tests for 2008, 21 of 49 tests for 2010, 16 of 28 tests for 2011, and 32 of 63 tests for 2013). Homozygote excess at these loci is known to be a result of null alleles (28, 29), which are nonamplifying alleles that result from variation in microsatellite flanking regions. Null allele frequencies were estimated, and frequencies of other alleles were simultaneously reestimated, using the ENA method in the software FreeNA (32).

Changes in Genetic Diversity. For each year, within-population genetic diversity was quantified using two metrics: (*i*) unbiased expected heterozygosity (H_E) calculated using null-corrected allele frequencies for each locus (33) and then averaged over all loci in each population; and (*ii*) allelic richness (A_R) estimated in HP-Rare software (34, 35), also averaged over all loci in each population. Allelic richness represents a count of visible alleles at each locus, corrected for the number of sampled gene copies by rarefaction to the smallest sample size in the dataset (36), and is expected to show a stronger response to demographic bottlenecks than heterozygosity (17). Allelic richness also allows for robust comparisons of genetic diversity despite very unequal sample sizes (37). Because null allele frequency was consistent between sampling periods (mean of 10.8% in 2008 and 7.9% in 2013, with overlapping CIs; Table S1), the presence of null alleles should not affect temporal changes in allelic richness, which are estimated using the visible alleles (38). For comparisons between 2008 and 2013, we focused our analyses to the nine populations in which a minimum of five individuals were sampled in each year (Table S1), thus allowing us to estimate A_R with rarefaction to ten gene copies. For supplementary analyses involving samples from the bottleneck years of 2010 and 2011, the number of available samples was necessarily very small (Table S3). Inclusion of only those populations in which we could rarefy to 10, or even as few as 6, gene copies left us with very few populations (two or three for some pairs of years) to examine effects of connectivity and bottleneck severity on A_R change. Rarefaction to two gene copies allowed us to include more populations, but rarefaction to this small number of samples produced high variability in the A_R estimates and patterns of change that were not consistent with those detected when rarefaction was to four or more gene copies. Therefore, for the supplementary analyses involving 2010 and 2011 samples, we included populations with two or more individuals sampled in those years and estimated A_R for all years based on rarefaction to four gene copies. We note that in comparisons of A_R between 2008 and 2013, rarefaction to 4 or 10 gene copies gave highly consistent results, although linear models for A_R change had slightly less explanatory power with rarefaction to 4 gene copies. We used the nonparametric Wilcoxon–Mann–Whitney test to determine whether levels of genetic diversity differed between years.

We tested for evidence of genetic bottleneck signatures, separately for each population, using the software BOTTLENECK v.1.2.02 (39, 40). Wilcoxon signed-rank tests (WSRs) were used to compare the observed heterozygosity to that expected from the observed number of alleles, given the sample size, under the assumption of mutation-drift equilibrium, for each locus in each population. The infinite allele model (IAM) and the two-phase model (TPM) of mutation were used to simulate mutation-drift equilibrium. For TPM, two values (10, 30) were tested for the variance of the geometric distribution with a low probability of single-step mutations (70%). We did not include the strictly stepwise mutational model (SMM), because it is inappropriate here due to the occurrence of flanking-sequence insertions or deletions (28, 29).

Relationship to Patch Connectivity and Severity of the Demographic Bottleneck. We examined whether the level of genetic diversity within populations before the bottleneck (in 2008) affected bottleneck severity, by separately testing each of A_R and H_E as predictors of the 2010 and 2011 population size indices, using linear regression.

To examine factors affecting changes in allelic richness between years, we first quantified the proportional loss or gain of A_R between time periods. To improve interpretability of the results and minimize reference to negative changes in A_R, we quantified changes in A_R between years as either proportional loss or gain of A_R depending on whether allelic diversity, on average across all populations, decreased or increased between the time periods considered. For pairs of years where A_R had, on average, declined between the two time periods we estimated proportional loss of A_R as [(A_R in first period – A_R in second period)/A_R in first period]. Where A_R had, on average, increased between the two time periods, we estimated proportional gain of A_R as [(A_R in second period – A_R in first period)/A_R in first period].

We examined the effect of connectivity on A_R change using linear regression. We also included severity of the demographic bottleneck and the two-way interaction term as predictors in our models. Each population was considered a random effect. Proportional changes in A_R were arcsine transformed before being included as response variables in the regression analyses. For changes in A_R between 2008 and 2013, we tested separately the effects of connectivity in each of the years 2010, 2011, and 2012. Because we were interested in whether connectivity facilitated genetic rescue after the demographic bottleneck, it was most relevant to use connectivity estimated for these years, starting with the initial year in which population size crashed (2010) and up to the year before the 2013 samples were collected. The population size indices in the 2 y of lowest abundance (2010 and 2011) were used separately as measures of the severity of the demographic bottleneck in each population. We therefore tested models that included all possible combinations of connectivity for one of the 3 y, and bottleneck severity for one of the 2 y, and compared the performance of models based on the corrected Akaike Information Criterion (AICc) (Table S4). For supplementary analyses examining changes in A_R in the distinct phases of demographic decline (2008–2010) and recovery (2011–2013), the same sets of predictors were used. Although we would not expect causal relationships between some of the predictors and changes in A_R during these phases (e.g., we would not expect an effect of 2012 connectivity on A_R change between 2008 and 2010), we nonetheless examined all models so that we could have more confidence in the interpretation of factors affecting A_R change across the entire period of 2008–2013; for example, we wanted to confirm that the significant effect of 2012 connectivity on A_R change from 2008 to 2013 was indeed via immigration during the recovery stage and did not represent an artifact of processes operating during the decline phase (Table S5). The predictors used in each model were not collinear ($r^2 < 0.4$). All linear regressions were performed using the lm function of the Stats package in R v.3.1.2 (41). We confirmed that model residuals were not spatially autocorrelated using Moran's I (all $P > 0.05$) executed in the ape package (42) in R, based on the coordinates of the centroid of each sampled patch.

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