Landscape structure and the genetic effects of a population collapse

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Both landscape structure and population size fluctuations influence population genetics. While independent effects of these factors on genetic patterns and processes are well studied, a key challenge is to understand their interaction, as populations are simultaneously exposed to habitat fragmentation and climatic changes that increase variability in population size. In a population network of an alpine butterfly, abundance declined 60–100% in 2003 because of low over-winter survival. Across the network, mean microsatellite genetic diversity did not change. However, patch connectivity and local severity of the collapse interacted to determine allelic richness change within populations, indicating that patch connectivity can mediate genetic response to a demographic collapse. The collapse strongly affected spatial genetic structure, leading to a breakdown of isolation-by-distance and loss of landscape genetic pattern. Our study reveals important interactions between landscape structure and temporal demographic variability on the genetic diversity and genetic differentiation of populations. Projected future changes to both landscape and climate may lead to loss of genetic variability from the studied populations, and selection acting on adaptive variation will likely occur within the context of an increasing influence of genetic drift.

1. Introduction

Genetic diversity and spatial genetic structure are important population traits relevant to persistence and evolutionary potential and are influenced by both landscape characteristics and demographic fluctuations [1–3]. While the independent effects of landscape variables and population declines on population genetics have been studied extensively in recent years [4–6], relatively few studies have assessed the potentially complex interactions of these factors on genetic patterns and processes [2,7]. Understanding such interactions is important as natural populations are exposed simultaneously to landscape changes such as habitat fragmentation, and to other environmental changes, notably climate change, that lead to greater temporal variability in population numbers [8].

A population collapse, or severe and sudden reduction in population size, is predicted to lead to a loss of genetic diversity, particularly allelic diversity, producing what is referred to as a genetic bottleneck [9]. However, genetic diversity lost as a result of a population collapse can be rapidly recovered through immigration [10]. Therefore, connectivity of a population (the extent to which it exchanges individuals with other populations) should be important in mitigating any loss of genetic diversity that might result from a collapse. Connectivity among populations, in turn, is strongly influenced by landscape characteristics [11–13]. Conversely, landscape composition and configuration can influence background levels of genetic drift and gene flow, resulting in associations between landscape variables and patterns of genetic diversity or spatial genetic structure [2,14]. Within a network of populations, severe reductions in local or regional population size would lead to greater genetic differentiation, essentially as a result of
increased genetic drift [1,15]. At the same time, a rapid reduction in population size could disrupt the underlying balance between genetic drift and gene flow [16], thereby altering or removing any association between contemporary landscape structure and genetic patterns. To date, the ability of population connectivity within the landscape to mitigate loss of genetic diversity due to a collapse has not been directly investigated in empirical systems, nor have the effects of a collapse on the association between contemporary landscape and genetic patterns. Here, we take advantage of a natural, severe collapse that affected a network of interconnected populations to directly address both of these questions.

A network of populations of the alpine butterfly *Parnassius smintheus* has been studied continuously since 1995 with the aim of examining effects of landscape structure and patch connectivity on population dynamics [4,12,13,17,18]. In 2003, a severe population collapse was documented wherein a 60–100% decline in adult abundance was observed across all populations within the network (electronic supplementary material, figure S1). This event was attributed to low-overwinter survival of larvae as a result of reduced snow cover [8,18]. Population abundances rebounded quickly, reaching pre-collapse levels within two years.

Our study describes the population genetic response to the demographic collapse by using samples collected before and after the event to assess the effect on genetic diversity and landscape genetic patterns. We examine whether patch connectivity mediated the loss of diversity during the collapse, and how spatial genetic structure and landscape genetic patterns changed as a result of the collapse. With both climate change and anthropogenic habitat fragmentation increasing globally, the question of how demographic and landscape factors interact to shape genetic diversity and spatial genetic structure is important for predicting the persistence and evolutionary trajectories of natural populations.

2. Material and methods

(a) Study system

*Parnassius smintheus* occurs in the North American Rocky Mountains, primarily in high-altitude meadows above the treeline. Our study focused on a network of populations occupying patches of meadow habitat arranged in a linear fashion along three ridge-tops in the Kananaskis Country region of Alberta, Canada (figure 1). These meadow patches occur above 2100 m and are separated primarily by intervening forest habitat.

(b) Mark–release–recapture studies, population size and dispersal

Mark–release–recapture studies of *P. smintheus* have been conducted in the population network since 1995 [8]. Adult butterflies are individually marked, and daily indices of population size are estimated using Craig’s method [19–21]. The maximum estimate from three to five different sampling days per year was used as a single index of population size in each meadow, each year. Maximum-likelihood estimates of movement between patches in each year were obtained from the individual capture histories using the Virtual Migration Model (VMM) [22].

(c) Microsatellite genotyping

Wing tissue samples from adult *P. smintheus* were collected prior to the 2003 collapse (in 1995 and 1996, referred to as the ‘pre-collapse’ period) and two years after (in 2005, referred to as the ‘post-collapse’ period) from 17 different sampling locations or meadow ‘patches’ within the study system (electronic supplementary material, figure S1 and figure 1). After the collapse, 2005 was the first year in which sufficient numbers of butterflies could be sampled for a robust population genetic analysis. The same tissue collection protocol was used in both periods [4], and each year sampling was carried out throughout the flight season. Samples from two patches (P and Q, figure 1) were not included in the current study as a separate study on the effects of experimental population removal was ongoing from 2001 to 2007, where all observed adults of *P. smintheus* in these two sites were removed [18]. Also, data from sites D and Y were excluded from further analyses because of small sample sizes (less than or equal to two individuals per site) in one of the two sampling periods. Thus, subsequent population genetic analyses focus on data from the remaining 13 habitat patches.

DNA was extracted from wing samples using a DNeasy Blood and Tissue Kit (QIAGen, Germantown, MD, USA). Individuals were genotyped at seven microsatellite markers (Ps50, Ps76, Ps81, Ps85, Ps163, Ps165 and Ps262) [4,23]. As the pre-collapse samples had been analysed previously [4,13], a subset of twenty were re-genotyped alongside the post-collapse samples to confirm consistent genotype calling between the two time periods. Null alleles are common in Lepidopteran microsatellites [24] and are documented in *P. smintheus* [4,23]. Thus, if one or two loci failed to amplify for any individual, with a minimum of three attempts, the individual was scored as homozygous for a null allele at those loci. If three or more loci failed to amplify, the individual was removed from the dataset (three and five such individuals were removed from the pre- and post-collapse datasets, respectively).

(d) Hardy–Weinberg and linkage tests, and estimation of null allele frequencies

Results of Hardy–Weinberg and linkage equilibrium tests for the pre-collapse dataset were reported previously [4,13]. For the...
post-collapse dataset, these tests were performed for each locus in each population using Genepop v. 4.0.10 [25]. For both time periods, null allele frequencies were estimated, and the frequencies of all other alleles simultaneously re-estimated, based on the expectation–maximization (EM) algorithm [26] using the software FREENA [27].

(e) Genetic diversity, genetic bottlenecks and patch connectivity

For each patch in each time period (pre- or post-collapse), we calculated unbiased heterozygosity (\(H_0\)) according to Nei & Roychoudhury [28] using null-corrected allele frequencies, and allelic richness (\(A_R\)) with rarefaction to 10 genes [29–31] using HP-RARE software [32]. We tested for genetic evidence of a bottleneck (hereafter referred to as a ‘genetic bottleneck’) in each patch, and in each period, using the approach of Cornuet & Luikart [33], as implemented in the software BOTTLENECK. We used Wilcoxon signed-rank (WSR) tests to determine whether observed heterozygosity was greater than expected for a population with the same number of alleles and under mutation-drift equilibrium. The infinite allele model (IAM) and the two-phase model (TPM) of mutation were used to simulate mutation-drift equilibrium; a strictly stepwise mutational model is inappropriate here because of the occurrence of indels in flanking regions [4,23]. For the same reason, for the TPM we used a relatively low probability of single-step mutations (70%) and tested two values (10 and 30) for the variance of the number of repeat units per multi-step mutation.

We examined the effect of patch connectivity (described below) on how much \(A_R\) changed within sites between our two sampling periods. We focused on \(A_R\), which is expected to show a stronger response to a short-duration bottleneck than would heterozygosity [34], and used the proportional loss of \(A_R\) between time periods (i.e. (pre-collapse \(A_R\) – post-collapse \(A_R\))/pre-collapse \(A_R\)) as the response variable (arsine transformed), to account for variation among patches in initial levels of allelic diversity. Because the severity of the population collapse is expected to influence \(A_R\) loss, we also included the 2003 population size index as an explanatory variable. We performed mixed stepwise model selection by Akaike information criterion (AIC), including the two-way interaction term, using the stepAIC function of the MASS package in R v. 3.0.2 [35,36]. The two predictors were not collinear (\(r^2 = 0.32\)). Population \(E\) was removed from the analysis due to missing abundance data.

We calculated the connectivity of each patch in the network using a measure that reflects rates of immigration into the focal patch, and accounts for the area and population size of the surrounding source patches and for differential rates of movement through forest and meadow [18]:

\[
S_j = \sum_{i \neq j} A_{ij}^{\text{f}} \sum_{k=1}^n \frac{d_{ik}^{-\text{f}}(1-\eta)}{A_{ik}^{\text{f}}} \eta A_{ik}^{\text{m}} N_i
\]

where for patches \(i\) and \(j\), \(S_j\) is the connectivity of patch \(j\); \(A_i\) and \(A_j\) are the areas; \(d_{ik}^{\text{f}}\) and \(d_{ik}^{\text{m}}\) are the distances through forest and meadow, respectively; and \(N_i\) is the population size estimate. Meadow areas and distances were estimated from 1:40 000 digitized aerial photos [93]. Since the movement and distribution of \(P.\) smitheus in the study area are constrained to higher elevations, pairwise distances were calculated along ridge-tops between the centroids of butterfly capture within each patch [17]. These distances were divided into two components: distance over meadow and distance over forest (i.e. for each pair of patches, these two distances sum to the total geographical distance between patch centroids). We accounted for the adult removal experiment in meadows P and Q by setting their population sizes to zero. Parameters in the connectivity equation represent effects of intervening forest and meadow distance on movement (\(a_f\) and \(a_m\), respectively), and effects of patch size on rates of immigration (\(\mu_m\) and emigration (\(\eta\) and \(\mu_m\)), and were estimated using the YMM [22] approach to 2004 mark–recapture data. Parameter values were \(a_f = 2.76, a_m = 3.87, \mu_m = 1.06, \eta = 0.11\) and \(\mu_m = 0.32\). Since we were interested in whether patches with higher connectivity experienced genetic ‘rescue’ after the population collapse, it was most relevant to use connectivity estimated for 2004, the year after the collapse but immediately before the genetic samples were collected. Note however that in 2004 there was no difference in emigration rate among populations in different sized patches (i.e. \(\mu_m = 0\)) and that, unlike most other years, movement distance through meadow was less than through forest. To test the sensitivity of our results to the use of these particular 2004 parameter estimates, we also evaluated a regression model using the mean value of patch connectivity for all available years previous to the collapse (1995–2002), 2003 population size index and their interaction, as predictors.

(f) Change in genetic structure in response to demographic collapse

We estimated global and pairwise \(F_{ST}\) among patches, within sampling periods, using the ‘ENA’ method of Chapuis & Estoup [27], as implemented in the software FinzNA. This method is unbiased in the presence of null alleles. For comparison, we also estimated pairwise \(F_{ST}\) between time periods, for each patch. Separately for pre- and post-collapse, we tested for a correlation between pairwise genetic distance (\(D_{ST}\)) and total geographical distance (along ridge-tops), indicating isolation-by-distance (IBD), using Mantel tests. In initial analyses, we found that pairwise \(F_{ST}\) showed the strongest pattern of IBD (highest \(r^2\)) in the pre-collapse period compared to Nei’s genetic distance [36] and chord distance [37] (also corrected for null alleles). To determine potential effects of intervening land cover on genetic differentiation, we performed partial Mantel tests to determine the effect of intervening forest distance on pairwise \(F_{ST}\) while controlling for the distance through meadow, and vice versa [4]. All Mantel and partial Mantel tests were performed with the ecodist [38] and vegan [39] packages in R with 1000 permutations.

We tested for IBD and effects of intervening landscape using the pre- and post-collapse datasets containing our 13 patches, as well as in a dataset where patches M, O, R and S were removed. We conducted analyses without patches M, O, R and S to ensure that any observed changes in spatial genetic structure were not influenced by the experimental removal in patches P and Q. Based on our connectivity metric, the effect of population size changes in patches P and Q would extend only to M, O, R and S (figure 1). For example, using the connectivity metrics parametrized with 1995–1996 mark–recapture data, and artificially setting population sizes for patches P and Q to zero, only the connectivity metrics for sites M, O, R and S are changed by more than 1%. Connectivity metrics of all other sites are virtually unchanged; the largest effect is on patch L, with reduction of only 0.4% in connectivity. Estimates of genetic differentiation, the power to detect IBD, as well as effects of intervening landscape on genetic differentiation, may be affected by the number of individuals sampled from each site because smaller samples lead to greater variance in \(F_{ST}\) estimates. Sample sizes for most patches were smaller in our post-collapse dataset (table 1). To examine how similar pre-collapse sample sizes might have influenced our conclusions, in the \(R\) environment we generated 25 subsampled datasets for the pre-collapse period, randomly sampling without replacement an equivalent number of individuals as were sampled post-collapse, from those patches that had smaller post-collapse samples. Global and pairwise \(F_{ST}\) values were estimated for each subsampled dataset. All Mantel and partial Mantel tests were also conducted for each subsampled dataset, and for each
expected heterozygosity ($H$)
data. Patch connectivity is estimated from aerial photos and 2004 mark–recapture data. ‘Post’ from 2005. ‘No. Genotyped Individuals’ is the number of individuals that amplified at a minimum of five loci. Allelic richness ($A$) was measured by rarefaction to 10 genes, expected heterozygosity ($H_e$), and frequency of null allele (Null Freq.) were averaged across loci. $N_{2003}$ is 2003 adult abundance estimated using mark–recapture data. Patch connectivity is estimated from aerial photos and 2004 mark–recapture data.

Table 1. Genetic diversity and patch characteristics for populations of *Parnassius smintheus*. ‘Pre’ indicates samples from 1995 and 1996 (before collapse) and ‘Post’ from 2003. ‘No. Genotyped Individuals’ is the number of individuals that amplified at a minimum of five loci. Allelic richness ($A$), expected heterozygosity ($H$), and frequency of null allele (Null Freq.) are averaged across loci. $N_{2003}$ is 2003 adult abundance estimated using mark–recapture data. Patch connectivity is estimated from aerial photos and 2004 mark–recapture data.

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<th>$H_E$</th>
<th>null freq.</th>
<th>$N_{2003}$</th>
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test the median correlation coefficient across subsamples was tested against a null hypothesis of zero using WSR tests in R.

(g) Relationship between genetic structure and dispersal patterns

For each period, we determined the extent to which genetic differentiation between patches related to rates of movement as determined by mark–release–recapture. We summed the number of migrants moving between each pair of patches, as estimated by the VMM, to obtain an index of total flow of individuals between those patches. For the pre-collapse period, estimates of total flow were obtained using capture histories for 1995 and 1996, and summed across the two years. For the post-collapse period, estimates were obtained using 2004 and 2005 capture histories. The relationship between genetic differentiation ($F_{ST}$) and total flow of individuals (log transformed) was assessed using Mantel tests, and as for the IBD analyses, the 25 random subsamples of the pre-collapse data were used to assess the effect of sample size difference between the two time periods on our conclusions. Population E was not included in these analyses due to missing mark–recapture data.

3. Results

(a) Hardy–Weinberg and linkage tests, and estimation of null allele frequencies

Results of linkage and Hardy–Weinberg tests for the post-collapse data were similar to those previously reported for the pre-collapse data [4,13,23,40]. No significant linkage disequilibrium was detected after correction for multiple tests, but many instances of significant heterozygote deficiency were detected (in 43 of 91 tests for 2005, and 57 of 91 comparable tests for 1995–1996). Heterozygote deficiencies occurred at all loci and are most probably due to null alleles [4,24]. Estimated null allele frequencies were similar in the two periods (table 1), and consistent with the observed number of null homozygote individuals.

(b) Genetic diversity, bottleneck effects and patch connectivity

Averaging across patches, mean expected heterozygosity did not change after the population collapse (mean $H_e$ ± s.e.: 0.71 ± 0.01 pre-collapse and 0.72 ± 0.008 post-collapse; table 1), nor did mean allelic richness (mean $A_R$ ± s.e.: 4.55 ± 0.04 pre-collapse and 4.56 ± 0.07 post-collapse; table 1). The proportional loss of $A_R$ averaged across patches, was very close to zero (−0.004 ± 0.02). There was considerable variation among populations in how $A_R$ changed however, with seven experiencing losses of $A_R$ after the collapse (G1, g2, I, J, K, O, R) and six patches actually experiencing increases in $A_R$ (i.e. negative ‘loss’; E, F, L, M, S, Z). Patches that lost $A_R$ tended to be those with smaller 2003 populations (mean population size index ± s.e.: 6.8 ± 3.6), while those that gained $A_R$ had larger 2003 populations (mean index ± s.e.: 18.3 ± 7.4), although the difference was not statistically significant (Wilcoxon rank sum test, $W = 27.5$, $p = 0.12$). No significant genetic bottleneck was detected in samples collected either before or after the population collapse using either the IAM or TPM models (all $p > 0.05$ for Wilcoxon tests). There was also no relationship between either $A_R$ or $H_e$ in the pre-collapse period and the intensity of the population collapse, as measured by 2003 population size index ($A_R$: Spearman’s $\rho = −0.12$, $p = 0.72$; $H_e$: Spearman’s $\rho = −0.40$, $p = 0.20$).

A model including both predictors (patch connectivity (2004) and population size (2003)), as well as their interaction
best explained proportional changes in $A_R$ associated with the population collapse ($r^2 = 0.79$, $F_{3,8} = 9.77$, $p = 0.005$). All three terms were significant (estimate $ \pm $ s.e.: connectivity $ -0.08 \pm 0.03$, $p = 0.04$; population size $ -0.01 \pm 0.003$, $p = 0.0007$; interaction $ 0.007 \pm 0.002$, $p = 0.002$). Using mean connectivity values for the years 1995–2002, instead of 2004 connectivity, did not qualitatively change the results although the model fit was reduced ($r^2 = 0.65$, $F_{3,8} = 4.97$, $p = 0.03$).

The effects of connectivity and severity of population collapse on allelic diversity must therefore be interpreted in light of their interaction. We examined this interaction by plotting proportional loss of $A_R$ versus 2003 population size separately for patches of low and high connectivity (low 2004 connectivity = 0.10–0.95; high = 1.1–2.8; figure 2). Overall, among low connectivity patches, there was a strong effect of 2003 population size on $A_R$ loss, wherein the most severely collapsed populations (smallest size) lost the most diversity (highest loss). Among high connectivity patches, however, there was no relationship between 2003 population size and $A_R$ change. If populations were grouped into three categories based on 2004 connectivity (low = 0.10–0.45, medium = 0.76–1.1, high = 1.4–2.8), then a similar pattern of interaction emerged where there was a strong negative relationship between loss of $A_R$ and 2003 population size for low connectivity patches, a weaker negative relationship for medium connectivity patches and no relationship for high connectivity patches (electronic supplementary material, figure S2).

One population (patch S) appeared influential in determining the negative relationship between $A_R$ change and 2003 population size for low connectivity patches. This population experienced the largest observed increase in $A_R$ and maintained moderately large population size in 2003. However, our results were robust to the removal of this patch from analyses; the overall model was still significant ($r^2 = 0.77$, $F_{3,7} = 7.87$, $p = 0.01$), as was the interaction between 2004 connectivity and 2003 population size (estimate $ \pm $ s.e.: 0.006 $ \pm $ 0.001, $p = 0.003$), and there was still a negative relationship between $A_R$ change and 2003 population size for low connectivity populations ($r = -0.68$).

We note that the greatest loss of $A_R$ occurred in two populations (patches I and R) that are both isolated and had very small 2003 population sizes (figure 2). However, there was also one population (patch J) that both maintained a reasonably large population size in 2003 and is of high connectivity, yet nonetheless lost a relatively high level of $A_R$. There is still therefore unexplained variation in allelic diversity change, particularly among the higher connectivity populations.

(c) Change in genetic structure in response to demographic collapse

Global $F_{ST}$ among all 13 sampled patches was higher after the collapse ($F_{ST} = 0.018$, 95% confidence interval (CI): 0.013–0.024) than before ($F_{ST} = 0.015$, 95% CI: 0.011–0.022), but not significantly so as indicated by overlap of 95% CIs. Pairwise $F_{ST}$ values were significantly higher after the collapse (WSR test: $V = 988$, $p = 0.006$; pre-collapse mean $ \pm $ s.e. = 0.015 $ \pm $ 0.001, post-collapse mean $ \pm $ s.e. = 0.024 $ \pm $ 0.002), and when subsamples were taken from the pre-collapse dataset, the median pairwise $F_{ST}$ of 0.019 across those subsamples was significantly less than the median post-collapse pairwise $F_{ST}$ value of 0.024 (WSR test: $V = 85$, $p = 0.04$). Pairwise $F_{ST}$ between time periods for individual patches ranged from 0.005 to 0.078, and with a mean of 0.025 was comparable to mean pairwise $F_{ST}$ among patches in the post-collapse period.

For the pre-collapse data, there was a significant positive correlation between pairwise genetic differentiation ($F_{ST}$) and total distance; by contrast, the post-collapse data showed no correlation between pairwise $F_{ST}$ and total distance (table 2 and figure 3). When individuals were subsampled from the pre-collapse dataset to generate sample sizes equivalent to those in the post-collapse data, there was still strong evidence for a significant correlation between pairwise $F_{ST}$ and total distance. Across the 25 subsamples, the median correlation coefficient ($r$) was 0.44 and was significantly greater than zero (WSR test: $V = 325$, $p < 0.001$).

For the pre-collapse data, there was a significant effect of intervening forest distance on genetic differentiation after controlling for meadow distance, but not vice versa (table 2). When individuals were subsampled, these patterns still held. Across the 25 subsamples, the median partial correlation coefficient ($r$) between forest distance and $F_{ST}$, controlling for meadow distance, was 0.34 and was significantly greater than zero (WSR test: $V = 325$, $p < 0.001$). For the partial correlation between meadow distance and $F_{ST}$, controlling for forest distance, the median correlation coefficient ($r$) across subsamples was 0.05 and was not significantly greater than zero (WSR test: $V = 234$, $p = 0.06$). For the post-collapse data there was no effect of intervening forest or meadow on genetic differentiation, and both partial correlations were not significant (table 2).

The removal of patches M, O, R and S from the IBD and landscape genetic analyses did not alter our results. The pre-collapse data still displayed significant IBD (table 2), including when individuals were subsampled (across 25 subsamples, the median correlation coefficient ($r$) of 0.47 was significantly greater than zero (WSR test: $V = 325$, $p < 0.001$)). The post-collapse data excluding M, O, R and S did not display IBD (table 2). For the pre-collapse data excluding M, O, R and S,
Table 2. Correlations between $F_{ST}$ and (i) geographical/landscape distances and (ii) adult movement. Mantel tests were performed for pairwise $F_{ST}$ against the total distance between patch centroids, and the estimated number of individuals moving between a pair of patches. Partial Mantel tests were performed for pairwise $F_{ST}$ against forest distance, controlling for meadow distance (Forest effect) and vice versa (Meadow effect). For the pre-collapse data, 25 random subsamples equivalent in size to the post-collapse dataset were generated; for each analysis, median $r$ across subsamples is reported along with results of a WSR test for $H_{c}$ median $= 0$. Bold values indicate statistical significance.

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<tr>
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<table>
<thead>
<tr>
<th>pairwise $F_{ST}$ and movement</th>
<th>pre-collapse</th>
<th>post-collapse</th>
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<tr>
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<td>$p$-value</td>
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<td>removal of M, O, R, S</td>
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There was a significant effect of intervening forest distance on genetic differentiation after controlling for meadow distance, but not vice versa (table 2). This pattern held with subsampling (across 25 subsamples, the median partial correlation coefficient between forest distance and $F_{ST}$, controlling for meadow distance, was 0.36 and significantly greater than zero (WSR test: $V = 325, p < 0.001$); median partial correlation coefficient between meadow distance and $F_{ST}$ controlling for forest distance, was 0.006 and not significantly greater than zero (WSR test: $V = 172, p = 0.81$)). For the post-collapse data excluding M, O, R and S, there was no significant effect of intervening forest habitat on genetic differentiation, and both partial correlations were not significant (table 2).

### 4. Discussion

We assessed the population genetic consequences of a demographic collapse, within a network of interconnected populations, taking into account the effects of landscape structure. We found no overall loss of genetic diversity nor evidence for a genetic bottleneck, but did find an interaction between patch connectivity and severity of the collapse in determining how allelic diversity changed. We also found a marked change in spatial genetic structure, and an uncoupling of genetic patterns from both landscape structure and patterns of individual movement, after the collapse.

Although the 2003 *P. smintheus* demographic collapse was severe, after only two generations there was no detectable loss of genetic diversity ($H_{E}$ or $A_{E}$) across the network of populations, nor any evidence of genetic bottlenecks (i.e. no excess heterozygosity relative to values expected based on observed allelic diversity) [34]. Maintenance or recovery of allelic diversity after a severe population collapse has been observed in several studies and can be attributed to various factors [5]. One possibility for *P. smintheus* is that previous cycles of population collapse have already removed rare alleles. However, microsatellite diversity in the pre-collapse period was high with 128 alleles observed across seven loci in the 15 patches, and with over 133 cases of an allele within a patch having an observed frequency between 1 and 2%. Furthermore, existence of IBD and spatial genetic patterns in the pre-collapse samples, in sharp contrast to the post-collapse samples, suggests that in 1995–1996 we were not sampling immediately after a collapse. Therefore, in *P. smintheus*, the two most probable factors contributing to recovery of genetic diversity are short duration at low abundance followed by rapid population rebound, and the occurrence of immigration and gene flow within a network of connected populations [5,10]. We predicted that if immigration was important in countering loss of genetic diversity (i.e. through a ‘genetic rescue effect’), then patch connectivity should mediate the genetic response of individual populations to the demographic collapse. We did find that patch connectivity affected the observed change in allelic diversity within individual patches, however, within the context of a significant interaction with severity of local population size decline. Specifically, more allelic diversity was lost in populations that were reduced to lower abundance during the collapse, but only in isolated habitat patches (figure 2). Thus, potentially complex interactions between demographic and landscape factors can shape the response of genetic diversity to a population collapse.
Identification of previous demographic collapses based on a single temporal sample is a frequent goal of conservation genetic studies. However, as with our study, analyses can frequently fail to detect a genetic bottleneck, even when a population collapse is known to have occurred [41]. This result reflects potential limitations of genetic bottleneck detection methods in cases with rapid demographic recovery [42], complex dispersal patterns [5] and variable patterns of microsatellite evolution [33]. Peery et al. [41] found that only 47–56% of studies detected a genetic bottleneck when a demographic bottleneck was known to have occurred, and falsely identified a bottleneck in stable populations 13–20% of the time. Our results support their recommendation to apply caution when using such methods as the sole means of inferring previous demographic collapses.

While the *P. smintheus* demographic collapse had little effect on mean genetic diversity measures across the sampled patches, we observed notable changes in spatial genetic patterns. Specifically, we observed a loss of IBD, and an uncoupling of pairwise genetic distances from both landscape structure and movement. Removing patches M, O, R and S from analyses did not affect the presence of IBD or landscape genetic patterns (i.e. significant effect of intervening forest on genetic differentiation) prior to the collapse, nor did it change the loss of IBD and landscape genetic patterns seen post-collapse. These results indicate that the experimental removals of butterflies from patches P and Q in 2001–2008 [18], and their impact on adjacent patches, did not underlie the observed break-down of both IBD and landscape effects in our post-collapse data.

Interestingly, in 2004 intervening forest did not have as strong an isolating effect as is normally observed in this system, as evident in the parameter estimates from the VMM: the estimated resistivity of forest to movement was 2.76 (0.61–5.95; 95% CI), while that of meadow habitat was atypically higher at 3.97 (2.37–6.32; table 2). Preliminary analyses suggest this may have been due to a combination of low density and relatively warm, sunny conditions during the flight season, promoting among-population dispersal, perhaps due to conspecific attraction or mate finding behaviour (S. F. Matter & J. Roland 2014, unpublished data). However, this cannot be the reason for the observed loss of landscape genetic pattern in the post-collapse sample, given the lack of association between genetic differentiation (pairwise $F_{ST}$) and movement between patches for this period (figure 2). Indeed, pairwise $F_{ST}$ for the 2005 samples does not correlate with movement in any previous year (data not shown). Instead, increased pairwise genetic differentiation among populations, along with the uncoupling of genetic and movement patterns, support random drift (occurring at a high level in a sudden and temporary episode) as the primary process leading to the loss of genetic spatial structure and landscape genetic relationships post-collapse.

Through the action of gene flow, IBD and landscape genetic patterns should eventually re-establish. Given that we were previously able to detect significant IBD and landscape genetic patterns in this system, despite the fact that demographic fluctuations are predicted to occur regularly, perhaps as often as every decade [8], we predict that this pattern also re-establishes frequently and rapidly, probably within a few years. This prediction is supported by theoretical and empirical studies that indicate patterns of genetic differentiation among populations can reach equilibrium quickly [13,43,44]. For example, populations of the Glanville fritillary butterfly (*Melitaea cinxia*) display a time-lag of only 6–7 years between genetic patterns and demographic processes [45].

Importantly, had we conducted our initial landscape genetic study in 2005 without knowledge of the recent collapse, we would have erroneously concluded that populations are highly isolated and the intervening landscape (i.e. the forest) has no effect on spatial genetic structure. Clearly, landscape genetic inference can be strongly influenced by the recent demographic history of populations. Our study underlines the importance of considering recent demographic history in landscape genetic studies. When demographic variability is expected to be high, sampling at more than one time point is valuable for making more complete inferences about landscape effects on genetic patterns and processes.

We recognize there is a seven year gap between collection of our first set of samples (1995–1996) and the 2003 population collapse. While some factor occurring in this period, other than the collapse, could have influenced the genetic patterns...
observed in the post-collapse samples, we note that the change in spatial genetic structure observed between the pre- and post-collapse samples is consistent with the effects of a sudden and severe population decline leading to increased drift among populations. Furthermore, in the period leading up to the 2003 collapse (1997–2002), population sizes were never lower than they were in 1995–1996 when the pre-collapse samples were taken (electronic supplementary material, figure S1). Thus, we are confident that the changes in genetic structure observed between our temporal samples can be attributed to the 2003 demographic collapse.

With climate change, conditions leading to low-overwinter survival in *P. smintheus* and the incidence of demographic collapses are predicted to increase in frequency. *Parnassius smintheus*’ population growth appears to be linked to variations in the Pacific Decadal Oscillation (PDO) index, with declines in population size coinciding with extreme (either warm dry, or cold snowy) winter PDO values [8]. Climate change may also indirectly lead to habitat fragmentation via tree-line encroachment with severity of population collapse, can affect the loss of genetic diversity. Overall, increasing climatic and demographic variability will interact with rising tree-line and fragmentation of high-altitude meadow habitat to produce *P. smintheus* populations that are more strongly dominated by genetic drift and characterized by lower genetic diversity. While the effects on adaptive genetic diversity will ultimately also depend on the strength and direction of selection, any selection acting on these populations in the future will occur within the context of an increasing influence of random genetic drift and less gene flow.

Data accessibility. Microsatellite genotypes, pairwise movement indices, and forest and meadow distances for this study are available at doi:10.5061/dryad.d715g.

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12. Matter SF, Roland J, Keyghobadi N, Sabourin K. 2003 The effects of isolation, habitat area and population size coinciding with extreme (either warm dry, or cold snowy) winter PDO values [8]. Climate change may also indirectly lead to habitat fragmentation via tree-line encroachment into alpine meadow habitats [17]. Therefore, in the future there will be more frequent severe demographic fluctuations, and these will occur within smaller and more isolated habitat patches. We have shown that patch connectivity, via an interaction with severity of population collapse, can affect the loss of genetic diversity. Overall, increasing climatic and demographic variability will interact with rising tree-line and


