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Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers

Lukas F. Keller[1*], Kathryn J. Jeffery[2†] Peter Arcese[3], Mark A. Beaumont[2‡], Wesley M. Hochachka[4], James N. M. Smith[5] and Michael W. Bruford[2†]

[1]*Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA*
[2]*Conservation Genetics Group, Institute of Zoology, Regent's Park, London NW1 4RY, UK*
[3]*Centre for Applied Conservation Biology, Forest Sciences, 2424 Main Mall, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z4*
[4]*Cornell Laboratory of Ornithology, 159 Sapsucker Woods Road, Ithaca, NY 14850, USA*
[5]*Centre for Biodiversity Research and Department of Zoology, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z4*

Population bottlenecks are often invoked to explain low levels of genetic variation in natural populations, yet few studies have documented the direct genetic consequences of known bottlenecks in the wild. Empirical studies of natural population bottlenecks are therefore needed, because key assumptions of theoretical and laboratory studies of bottlenecks may not hold in the wild. Here we present microsatellite data from a severe bottleneck (95% mortality) in an insular population of song sparrows (*Melospiza melodia*). The major findings of our study are as follows: (i) The bottleneck reduced heterozygosity and allelic diversity nearly to neutral expectations, despite non-random survival of birds with respect to inbreeding and wing length. (ii) All measures of genetic diversity regained pre-bottleneck levels within two to three years of the crash. This rapid recovery was due to low levels of immigration. (iii) The rapid recovery occurred despite a coincident, strong increase in average inbreeding. These results show that immigration at levels that are hard to measure in most field studies can lead to qualitatively very different genetic outcomes from those expected from mutations only. We suggest that future theoretical and empirical work on bottlenecks and metapopulations should address the impact of immigration.

Keywords: bottleneck; genetic variation; immigration; inbreeding; microsatellites

1. INTRODUCTION

Genetic diversity in populations and the evolutionary forces that affect it are central to both evolutionary (e.g. Wright 1931; Haldane 1932; Fisher 1958) and conservation biology (e.g. O'Brien *et al*. 1985; Lande 1988; Frankham 1995). When populations undergo temporary, large reductions in size—so-called population bottlenecks (Wright 1931; Nei *et al*. 1975)—they lose genetic diversity through random drift. Bottlenecks are therefore thought to be involved in speciation events (e.g. Mayr 1963; Carson 1990; Slatkin 1996), heterozygote deficiency in natural populations (Nei & Graur 1984), low levels of genetic variation (Bonnell & Selander 1974; O'Brien *et al*. 1983; Ellegren *et al*. 1996; Groombridge *et al*. 2000), and reduced reproductive function (Wildt *et al*. 1987; Madsen *et al*. 1999).

Following Nei *et al*. (1975) a number of theoretical (e.g. Maruyama & Fuerst 1984; Watterson 1984; Maruyama & Fuerst 1985*a*,*b*; Lacy 1987) and laboratory studies (e.g. Bryant *et al*. 1981; McCommas & Bryant 1990; Leberg 1992; Richards & Leberg 1996; Saccheri *et al*. 1999) have addressed the genetic effects of bottlenecks. However, while genetic data have often been used to infer the occurrence of bottlenecks in natural populations (e.g. Menotti-Raymond & O'Brien 1993; Hoelzel *et al*. 1993; Ross *et al*. 1993; Taylor *et al*. 1994; Dorit *et al*. 1995; Cornuet & Luikart 1996; Houlden *et al*. 1996; Vincek *et al*. 1997), few studies have actually documented the genetic consequences of known bottlenecks in natural populations (Gallardo *et al*. 1995; Brookes *et al*. 1997; Bouzat *et al*. 1998; Glenn *et al*. 1999). The genetic outcome of bottlenecks in nature may differ from those in experimental settings because some key assumptions made in those studies may not hold in the wild (Carson 1990) and because the ecological context of bottlenecks is different in the wild. For example, in laboratory studies individuals are removed at random to simulate a bottleneck. In the wild, however, individuals may not die at random and selection may be strong (Bancroft *et al*. 1995*a*,*b*). Moreover, most theoretical and laboratory studies of bottlenecks have assumed a single, completely isolated population. Many natural populations, however, are part of a set of connected populations and exchange genes with other populations. Thus, data from natural populations with known bottleneck histories, including data on the individuals that survived the bottleneck, are needed to establish the degree to which laboratory and theoretical results reflect bottleneck effects in nature (Ardern *et al*. 1997; Amos & Harwood 1998).

*Author and address for correspondence: Division of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, UK (l.keller@bio.gla.ac.uk).
†Present address: Cardiff School of Biosciences, Cardiff University, Cardiff CF1 3TL, UK.
‡Present address: School of Animal and Microbial Sciences, University of Reading, Whiteknights, PO Box 228, Reading RG6 6AJ, UK.

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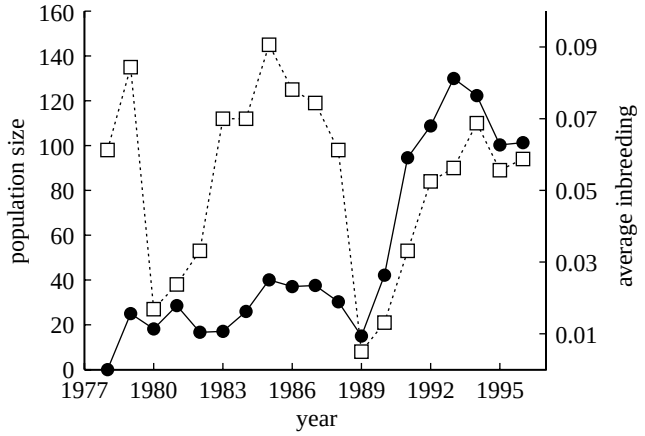

Figure 1. Population size (number of breeding birds, open squares) and average inbreeding among the breeding birds (filled circles) in each year of our study of the Mandarte Island song sparrow population.

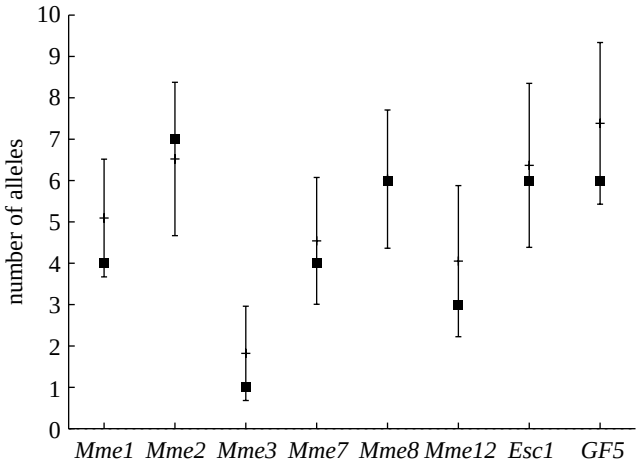

Figure 2. Observed number of alleles surviving the bottleneck at eight microsatellite loci (filled squares) and the expectation (with the 95% confidence interval) under a neutral model (solid lines).

Here we present the first study (to the best of our knowledge) of a bottleneck in a natural population, with genetic and pedigree data gathered before, during and after a severe population bottleneck. The data stem from a population of song sparrows (*Melospiza melodia*) living on Mandarte Island, Canada. In 1989, 94.8% of the population died during a severe winter storm (Rogers *et al*. 1991), with inbred birds surviving less well than non-inbred birds (Keller *et al*. 1994), and longer-winged females surviving better than others (Rogers *et al*. 1991). While the previous studies investigated which ecological and genetic variables affected survival of individuals during the storm, this paper reports the consequences of the severe bottleneck on molecular genetic variation in the population. We present new data on the genetic trajectory of this natural bottleneck using data from eight microsatellite loci from two years before the bottleneck to seven years afterwards. First, we report the decline in allelic diversity and heterozygosity due to the sudden drop in numbers through the bottleneck (following Denniston (1978) we call this the reduction phase) and ask how closely it matched the neutral expectations underlying much of the theoretical work on bottlenecks. Next, we analysed the dynamics of genetic diversity in the recovery phase after the bottleneck, when the population grew back to its original size. Here we sought to establish whether known immigration in the years following the bottleneck, when the occurrence of inbreeding increased sharply, had an effect on the dynamics of genetic diversity in the population.

2. METHODS

We studied the entire, year-round resident population of song sparrows on Mandarte Island from 1974 until 1996 (Smith 1981, 1988; Arcese *et al*. 1992). Mandarte Island (48°38′ N, 123°17′W) is a small island in Haro Strait, British Columbia, Canada, and supported a median of 89 breeding song sparrows during the study period. The population size fluctuated 18-fold during the study due to two population crashes, one in 1979–1980, and the other in February 1989 (figure 1). Blood samples were first collected in 1987, so we only consider the second bottleneck here. Eight males and three females survived the storm in 1989, and a female born on a neighbouring island in 1988 immigrated to Mandarte nine weeks after the storm. Thus, four pairs bred on Mandarte in 1989 and four males were territorial but unmated. One of the four unmated males bred from 1990 until 1992; the other three never bred on the island. The population grew rapidly after the crash (figure 1) and reached the long-term average size again by 1992.

We studied the genetic trajectory of the 1989 bottleneck using eight microsatellite markers. Six markers were developed for the purpose of this study (Jeffery *et al*. 2001) and two more were obtained from the literature (*Escμl*: Hanotte *et al*. 1994; *GF5*: Petren 1998). Two song sparrow microsatellite loci (*Mme3* and *Mme7*) were inherited in a Z-linked fashion. Blood sampling techniques, microsatellite primer development, primer sequences, and polymerase chain reaction (PCR) conditions are described in detail in Jeffery *et al*. (2001). Genotypes at each locus were represented by the length (in bp) of the two alleles found. 670 known mother–offspring pairs were used to exclude the possibility of the occurrence of null alleles (Pemberton *et al*. 1995) and allelic dropout (Jeffery *et al*. 2001).

Blood sampling effort varied over time (table 1), but in several years samples from the entire breeding population were available. Moreover, we had blood samples from all but one crash survivor (an unmated male that never bred after the crash). All alleles observed on Mandarte after the bottleneck could be traced either to one of the ten genotyped survivors or to an immigrant. This suggests that the one survivor who was not genotyped did not sire any offspring after the bottleneck. We obtained genotypes at all eight microsatellite loci from all breeding birds that were sampled between 1987 and 1996, totalling 324 individuals.

(a) *Data analysis*

We examined two different measures of genetic variability, expected heterozygosity (H_e) and the number of alleles at a locus (allelic diversity). We calculated unbiased average expected heterozygosities, following Nei (1987), as $H_e = 2n(1 - \Sigma_{i=1}^{k} x_i^2), (2n - 1)$, where n is the number of individuals sampled, k is the number of alleles at a locus, and x_i is the frequency of the ith allele. The two Z-linked loci were excluded from heterozygosity calculations. H_e combines information on both number of alleles and their relative frequencies, and its *average* behaviour following a bottleneck is

Table 1. *Population sizes and percentage sampled*

(The number of breeding birds and the percentage of breeding birds that were genotyped in each year of the study.)

year	number of breeding birds	% genotyped
1987	120	76
1988	99	88
survivors	11	91
1989	8	100
1990	21	67
1991	53	47
1992	85	72
1993	90	98
1994	110	100
1995	89	100
1996	94	100

well understood (Nei *et al*. 1975; Maruyama & Fuerst 1985*a*). Thus, H_e is commonly the measure of choice for overall genetic variation (Hedrick *et al*. 1986). However, because most empirical studies only have data for a relatively small number of loci, random changes in allele frequencies can lead to increased heterozygosity following a bottleneck (Leberg 1992; Brookes *et al*. 1997). Thus, additional measures of genetic variation are desirable.

Because selection can change gene frequencies after a bottleneck provided they do not reach zero, the number of alleles retained at a locus (allelic diversity) is another genetic measure of interest. Allelic diversity is more sensitive to bottlenecks than H_e (e.g. Denniston 1978; McCommas & Bryant 1990; Leberg 1992; Brookes *et al*. 1997) because alleles are lost more quickly than heterozygosity. A disadvantage of allelic diversity as a measure of genetic variation is that it is sensitive to sampling (e.g. Sjögren & Wyöni 1994). However, because we had blood samples from all or most individuals in many years of the study (table 1) this disadvantage is minimal in our case. The expected numbers of alleles surviving under a neutral model and the 95% confidence intervals were calculated following Denniston (1978), adjusting for the Z-linked loci.

Differences in allelic diversity are often analysed using *t*-tests or analyses of variance. However, because for most markers allelic diversity is a discrete variable, often with a skewed distribution, the assumptions of those tests are typically not met. Thus, we estimated, for the *j*th locus, the probability function of observing x alleles among the survivors out of a maximum of n_j as $p_j(x) = P_j(X = x)$, $x = 1, 2, \ldots, n_j$, and the cumulative distribution function, $F_j(x) = P_j(X \leqslant x)$, using a simulation procedure. We ran 5000 simulations where ten individuals, corresponding to our ten genotyped bottleneck survivors, were chosen at random without replacement from the 87 genotyped individuals breeding in 1988. From these simulation runs we estimated $p_j(x)$ and $F_j(x)$. Given the number of alleles actually observed at each locus after the bottleneck, k_j, we wished to test whether the joint set of values of $F_j(k_j)$ was an extreme result. Motivated by Fisher's method of combining *p*-values from different experiments (Fisher 1950), but noting that this method cannot be directly applied here because the probabilities are discrete, we tested for whether $\Pi^j F_j(k_j)$ is an extreme result. This assumes that the loci are independent. To do this we calculated the probability function $q(x) = P(X = \Pi^j F_j(\mathbf{v}_j) = x) = \Sigma^{\mathbf{l}} \Pi^j p_j(\mathbf{v}_{\mathbf{l}j})$, where $\mathbf{v}_{\mathbf{l}}$ is a vector giving the lth configuration of the number of alleles observed at each locus such that $X = x$. The reason for the summation is that different combinations of number of alleles, indexed by l, may give rise to the same X. From this distribution we can then estimate $P(X \leqslant \Pi^j F_j(k_j))$, the probability of obtaining as extreme a result as that observed.

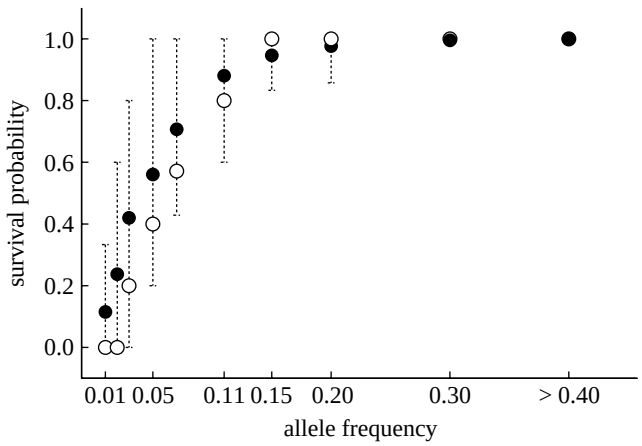

Figure 3. Survival probability of alleles over the bottleneck as a function of their frequency before the crash. The observed survival probability in each allele frequency class is given by the open circles. The mean (filled circles) and 95% confidence intervals of the neutral expectation derived by simulations are also given.

We also analysed the survival of alleles as a function of their frequency before the bottleneck. Two approaches were employed to overcome the problem that allele frequencies at a locus are not independent. First, we removed the most common allele at each locus from the data for the logistic regressions (Leberg 1992). Second, we used the simulation procedure described above to estimate the expected survival of alleles as a function of pre-bottleneck frequency, assuming random survival of individuals. For these analyses, we grouped allele frequencies (figure 3). Allele frequency classes were chosen such that at least five alleles fell in each class.

Pedigree data spanning up to 17 generations were utilized to estimate the incidence of inbreeding on Mandarte. PEDSYS (http://www.sfbr.org) was used to estimate inbreeding and kinship coefficients following Keller (1998).

3. RESULTS

(a) *Decline of genetic diversity during the reduction phase*

Allelic diversity declined through the bottleneck at all eight loci (sign test, $p = 0.008$) and the number of alleles was reduced by 22–67% (mean of 42.5%). When each locus was considered separately, this reduction did not differ significantly from that expected under a neutral model: at no locus did the observed number of surviving alleles lie outside the 95% confidence interval expected when neutrality is assumed (figure 2). However, the number of surviving alleles was lower than expected at seven out of eight loci. Over all loci, the probability of obtaining a result as or more extreme by chance was non-significant, at 0.089. The average degree of relatedness among bottleneck survivors was 0.0375 (s.d. = 0.0607) versus 0.0267 (s.d. = 0.0464) among all breeding birds in 1988 (sign test, $p = 0.009$). Thus, this higher degree of relatedness among the bottleneck survivors

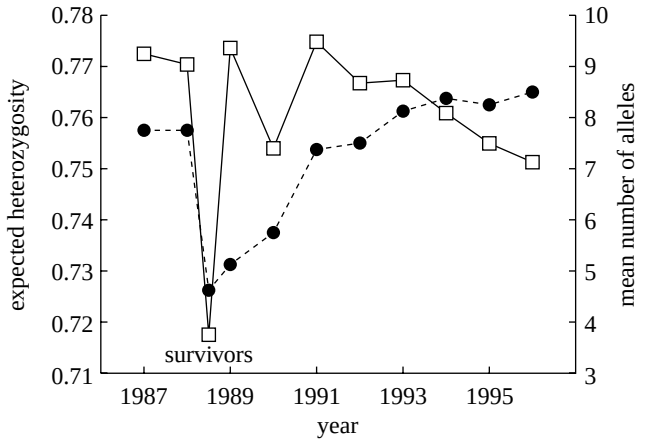

Figure 4. Average expected heterozygosity (open squares) at six microsatellite loci and average number of alleles (filled circles) at eight microsatellite loci over the study period.

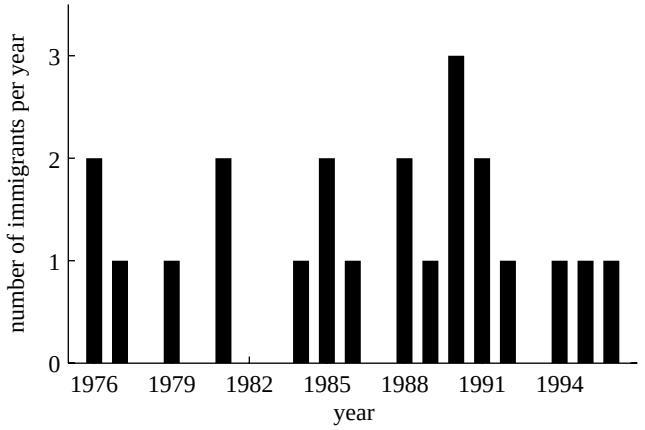

Figure 5. Number of immigrants to the Mandarte Island breeding population during the study period. No birds immigrated to Mandarte in years with no bar in the graph.

might have been responsible for the slightly lower number of alleles surviving.

As expected from neutral theory, the chances of a particular allele surviving the crash were highly correlated with its frequency before the bottleneck (logistic regression, $\beta = 82.48$, $\chi^2 = 6.57$, $p = 0.01$, $n = 54$). There was no evidence of differences between loci ($\chi^2 = 3.60$, $p = 0.82$). We used simulations to assess whether the relationship between allele frequency and bottleneck survival differed from the neutral expectation of random sampling (see § 2a, figure 3). For all allele frequency classes the observed average survival probability was within the 95% confidence interval established by simulation. Throughout, however, alleles that were rare before the crash (allele frequencies below 0.11) survived less often than expected (figure 3). A higher degree of relatedness among bottleneck survivors may again explain this deviation.

Average expected heterozygosity (H_e) was significantly reduced by the bottleneck. H_e among the surviving birds declined by 0.053 (s.d. = 0.033) from $H_e = 0.770$ immediately before the bottleneck to 0.717 among the bottleneck survivors (paired *t*-test (Nei 1987), $t_5 = 3.97$, $p = 0.011$). With ten genotyped bottleneck survivors the sampling effect of genetic drift would have been expected (Crow & Kimura 1970) to result in an H_e equal

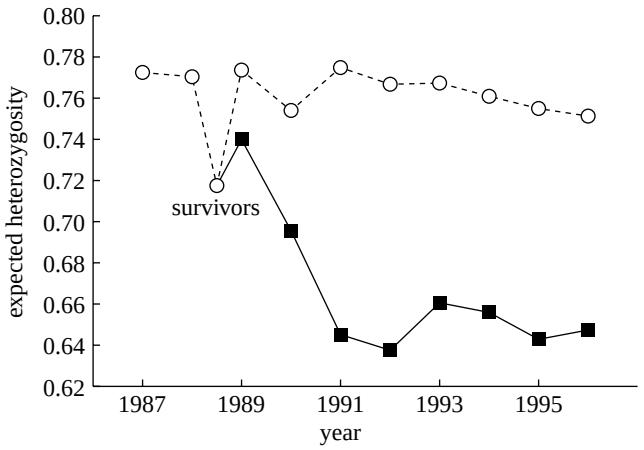

Figure 6. Average expected heterozygosity during the study period calculated for the entire data set (open circles) and the subset excluding all immigrant lineages (closed squares).

to $(1 - 1/20) = 95\%$ of the H_e before the crash, i.e. $H_e = 0.732$. The actual value of H_e among the crash survivors was 0.718, only slightly smaller than the expected value (paired *t*-test, $t_5 = -0.96$, $p \geqslant 0.38$). Changes in observed heterozygosity over the bottleneck showed a pattern similar to H_e but were less pronounced (data not shown).

(b) *Dynamics of genetic diversity in the recovery phase*

Both heterozygosity and allelic diversity returned to pre-bottleneck levels within only one to three years (figure 4). The cause of this rapid restoration of genetic variability to Mandarte Island was immigration. A mean of 1.05 song sparrows immigrated into Mandarte each year between 1976 and 1996 (figure 5), and 86% of the 22 immigrants were females. Immigrants regularly introduced new alleles to Mandarte, re-establishing allelic diversity and heterozygosity lost over the bottleneck. Indeed, H_e recovered to pre-bottleneck levels by April 1989 ($H_e = 0.774$), within two months of the crash and without intervening reproduction. About 40% of this increase in H_e was due to immigration of a single female from a neighbouring island immediately after the storm. The remaining recovery was due to random allele frequency fluctuations caused by the exclusion from the breeding population of three male survivors that did not breed in 1989, reducing the frequencies of the most common alleles and thus increasing H_e.

The effects of immigration versus selection or drift in the re-establishment of heterozygosity can be shown by removing all individuals from the data set that are descendants of immigrants after the bottleneck (figure 6). Removing the effects of immigration in this way shows that H_e would have declined substantially after the bottleneck (from *ca.* 0.72 to 0.64 by 1995) with no recovery.

4. DISCUSSION

A combination of detailed ecological, demographic and molecular analyses allowed us to test hypotheses concerning the processes affecting the molecular genetic consequences of a severe natural population bottleneck. Our major findings were as follows: (i) the bottleneck reduced

heterozygosity and allelic diversity close to neutral expectations, despite non-random survival of birds with respect to inbreeding and wing length. (ii) All measures of genetic diversity regained pre-bottleneck levels within two to three years of the crash. This rapid recovery was due to immigration. (iii) The rapid recovery occurred despite a coincident, strong increase in average inbreeding. We will discuss these findings separately for the reduction and the recovery phase of the bottleneck.

(a) *The reduction phase*

Despite non-random survival of birds with respect to wing length and inbreeding, the loss of genetic variation at eight microsatellite loci through the bottleneck was close to random: allelic diversity and expected heterozygosity were lost as predicted by neutral theory. The fact that drift rather than selection was mostly responsible for the changes in allele frequencies is further highlighted by the fact that the loss of alleles through the bottleneck was well predicted by their frequencies before the bottleneck (figure 3). Laboratory experiments and theoretical work where survivors are picked randomly (McCommas & Bryant 1990; Saccheri *et al*. 1999) thus yielded valid approximations of the molecular effects of the reduction phase of a bottleneck that we observed in nature. It is possible, however, that the ecological causes of a bottleneck may affect the assumption that survival of alleles is random. Unpredictable and short-lived catastrophic events such as winter storms may have different selective consequences from the starvation and parasite-mediated mortality associated with selection in, for example, Soay sheep (Bancroft *et al*. 1995*a*, Coltman *et al*. 1999).

Higher relatedness among the survivors than among individuals before the crash may have caused the survival of slightly fewer alleles (0.55 fewer per locus on average) than expected under neutrality. The differences were not statistically significant ($p = 0.089$), but with only eight loci, we had low statistical power. Increased relatedness of survivors might be a normal outcome of bottlenecks in nature if traits that promote survival under the extreme environmental conditions are heritable. For example, wing length was correlated positively with female survival in 1989 and is heritable on Mandarte ($h^2 = 0.31$ (Schluter & Smith (1986)). Thus, selection on wing length could lead to the survival of relatives.

Our results also provide further empirical corroboration of the fact that allelic diversity is a more sensitive measure of bottleneck effects than H_e (Denniston 1978; McCommas & Bryant 1990; Leberg 1992; Cornuet & Luikart 1996; Brookes *et al*. 1997). Between 22 and 67% of alleles were lost at each locus compared with a loss of 6.9% in H_e.

(b) *The recovery phase*

The most remarkable result of our study is the rapid recovery of genetic variation following the bottleneck. Pre-bottleneck levels of H_e and of allelic diversity were regained within only 1–3 years (figure 4). With an average generation time of about two years, this recovery was many orders of magnitude faster than expected in a closed population under a drift/mutation model (Nei *et al*. 1975). The cause of this rapid recovery was immigrants (figure 5). In 1990, three individuals immigrated into Mandarte, more than in any other year. Although this did not represent a statistically significant increase in the number of immigrants compared with all other years of the study (Mann–Whitney *U*-test, $n_1 = 20$, $n_2 = 1$, $p = 0.10$), when combined with the small population size immediately after the bottleneck it resulted in a breeding population that consisted of almost 50% immigrants and offspring of immigrants in 1991. This, in turn, caused a rapid increase in genetic variation.

It is important to note that the levels of immigration that led to this rapid recovery of genetic diversity would be undetectable in most field studies. Unless the entire population is individually marked, as it was in our study, it is impossible to detect low levels of immigration. Our study confirms a theoretical result first shown by Wright (1931) that low levels of immigration restore genetic variation rapidly. However, empirical support for this familiar result is rare (e.g. Spielman & Frankham 1992; Hansson *et al*. 2000).

Serially bottlenecked populations are expected to show reduced inbreeding depression due to selection against inbred individuals (purging, e.g. Frankham 1995). However, this expectation will only be met in the absence of immigration, because immigration can re-establish deleterious recessive alleles that were purged following a bottleneck. In our study, immigration restored genetic variation at microsatellite loci very rapidly following the bottleneck. Deleterious recessive alleles that cause inbreeding depression were probably also re-established quickly to the Mandarte population and contributed to the continued inbreeding depression in this serially bottlenecked population. Genetic load in a typical song sparrow egg on Mandarte is approximately five lethal equivalents, inbreeding depression in several fitness measures is considerable (Keller 1998), and there is no evidence that its effects were reduced following the 1989 bottleneck (L. F. Keller, unpublished data). Thus, genetic load due to immigration (immigration load; Wright 1977) is likely to have been responsible for the continued inbreeding depression on Mandarte Island.

Mandarte Island forms part of a metapopulation that shows extinction–recolonization dynamics in addition to within-island population dynamics. Rogers *et al*. (1991) and Smith *et al*. (1996) describe the metapopulation and its dynamics in detail. In brief, Mandarte Island, situated 1.3 km from the nearest other island and 8.2 km from the very large Vancouver Island (31 284 km^2), supports one of the largest populations in the metapopulation. The population dynamics on the different islands are largely uncorrelated. Mandarte was the only island in the metapopulation that experienced the dramatic decline in February 1989. Due to the prevalence of natural or anthropogenic fragmentation of habitat, the genetics of metapopulations are of great interest (e.g. Gilpin 1991; Hedrick & Gilpin 1997; Whitlock & Barton 1997; Nunney 1999). While most genetic and demographic metapopulation models only consider gene flow between patches during recolonization events, Hedrick & Gilpin (1997) showed that gene flow between occupied patches can greatly change the conclusions. Even low levels of gene flow that still allow for considerable genetic differentiation between patches can greatly increase the effective metapopulation size by restoring genetic variation to

patches. Our results confirm the critical role of immigration (gene flow) in empirical and theoretical studies of metapopulation biology (e.g. Stacey *et al*. 1997).

Theoretical studies of the genetic effects of bottlenecks that assume no immigration (e.g. Nei *et al*. 1975) predict that heterozygosity will recover more slowly than allelic diversity. On Mandarte, however, genetic variation was restored through immigration, and heterozygosity did not recover more slowly than allelic diversity, because immigrants brought in new and thus rare alleles at several loci simultaneously. Summed over loci, this caused a sudden and appreciable increase in the frequency of rare alleles, and a rapid recovery of H_e. Furthermore, the effects of immigration on population-level variation at a limited number of marker loci can hide dramatic increases in inbreeding. Average inbreeding first dropped over the bottleneck as a consequence of the selection against inbred birds (Keller *et al*. 1994) but then increased approximately eightfold from 1989 to 1991 (figure 1). This increase coincided with the recovery of genetic variation (figure 4). Among Mandarte song sparrows, measures of allelic diversity and H_e (together with observed heterozygosity), therefore, failed to detect not only a recent bottleneck after only a few years, but also a strong increase in inbreeding. More sensitive measures recently described to detect bottlenecks from allele frequency data (Cornuet & Luikart 1996) also failed to detect the recent bottleneck on Mandarte (data not shown).

Overall, our results show that immigration can lead to results that differ substantially from those expected in an isolated population. Yet, most theoretical work on bottlenecks is based on the assumption of no immigration. Completely isolated natural populations do exist (e.g. Komdeur 1996; Gilbert *et al*. 1990; Groombridge *et al*. 2000), but they are clearly the exception in nature. In fact, in many cases even seemingly isolated populations can exchange immigrants at rates that are high enough to maintain levels of genetic variation yet low enough to defy direct observation (Kiester *et al*. 1982; Edwards 1993). Thus, if we are to understand the dynamics of genetic diversity in natural populations, future theoretical and empirical work needs to incorporate the effects of immigration explicitly. Recent work on bottlenecks and metapopulations has started to do so (e.g. Halley & Hoelzel 1996; Hedrick & Gilpin 1997, Marr *et al*. 2001).

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