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Genomic sweep and potential genetic rescue during limiting environmental conditions in an isolated wolf population

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Genetic rescue, in which the introduction of one or more unrelated individuals into an inbred population results in the reduction of detrimental genetic effects and an increase in one or more vital rates, is a potentially important management tool for mitigating adverse effects of inbreeding. We used molecular techniques to document the consequences of a male wolf (*Canis lupus*) that immigrated, on its own, across Lake Superior ice to the small, inbred wolf population in Isle Royale National Park. The immigrant's fitness so exceeded that of native wolves that within 2.5 generations, he was related to every individual in the population and his ancestry constituted 56 per cent of the population, resulting in a selective sweep of the total genome. In other words, all the male ancestry (50% of the total ancestry) descended from this immigrant, plus 6 per cent owing to the success of some of his inbred offspring. The immigration event occurred in an environment where space was limiting (i.e. packs occupied all available territories) and during a time when environmental conditions had deteriorated (i.e. wolves' prey declined). These conditions probably explain why the immigration event did not obviously improve the population's demography (e.g. increased population numbers or growth rate). Our results show that the beneficial effects of gene flow may be substantial and quickly manifest, short-lived under some circumstances, and how the demographic benefits of genetic rescue might be masked by environmental conditions.

Keywords: *Canis lupus*; fitness; genetic rescue; genomic sweep; inbreeding; Isle Royale

1. INTRODUCTION

Inbreeding is an important threat to the viability of many populations [1,2]. Genetic rescue, in which the introduction of one or more unrelated individuals into an inbred population results in an increase of vital rates, is expected to mitigate inbreeding depression (genetic load, see [3]) and other detrimental effects of inbreeding. The effects of genetic rescue have been observed in the wild on several occasions [3–9]. In four cases, genetic rescue was associated with human-mediated translocations of relatively large numbers of individuals. Populations of adders (*Vipera berus*) and prairie chickens (*Tympanuchus cupido*) experienced an increase in population growth, a bighorn sheep (*Ovis canadensis*) population exhibited increases in reproduction, survival and five fitness traits [6,8,9] and the fitness of Florida panthers (*Puma concolor*) has benefited from genetic rescue [10].

In two additional cases, the natural immigration of a few individuals into inbred populations of the Scandinavian wolf (*Canis lupus*) and Mandarte Island song sparrow (*Melospiza melodia*) resulted in genetic rescue [5,7]. The arrival of a single immigrant was associated

with increased population growth for Scandinavian wolves, and the Mandarte Island song sparrow population exhibited higher fitness after immigration in the F₁ generation. However, in later generations in both examples, the populations experienced lowered fitness [5,7]. Apart from these cases, the effects of genetic rescue in wild populations are essentially unknown. An obstacle for better understanding genetic rescue is the difficulty of adequately monitoring a population before and after a rescue event so that its effects can be understood [11].

Wolves colonized Isle Royale (ISRO; 48°00' N, 89°00' W), a wilderness island (544 km²) in Lake Superior, North America, in 1949 or 1950. The population is isolated from mainland wolves by a channel of frigid water, 24 km wide. In many, but not all years, this channel freezes for several days or weeks. Although an occasional ice bridge makes immigration possible, the analysis of mitochondrial DNA and the Y chromosome suggests that the population was originally founded by only one female and two males ([12], electronic supplementary material). The population is typically comprised of three or four wolf packs (figure 1), average census number is 24 (interquartile range = (18,26)), long-term effective population size is approximately 3.8 individuals and generation time is 4.2 years [13].

By the late 1990s, the population's estimated inbreeding coefficient (f) had risen to 0.81 (figure 2a). Fifty-eight per cent of ISRO wolves showed congenital bone deformities

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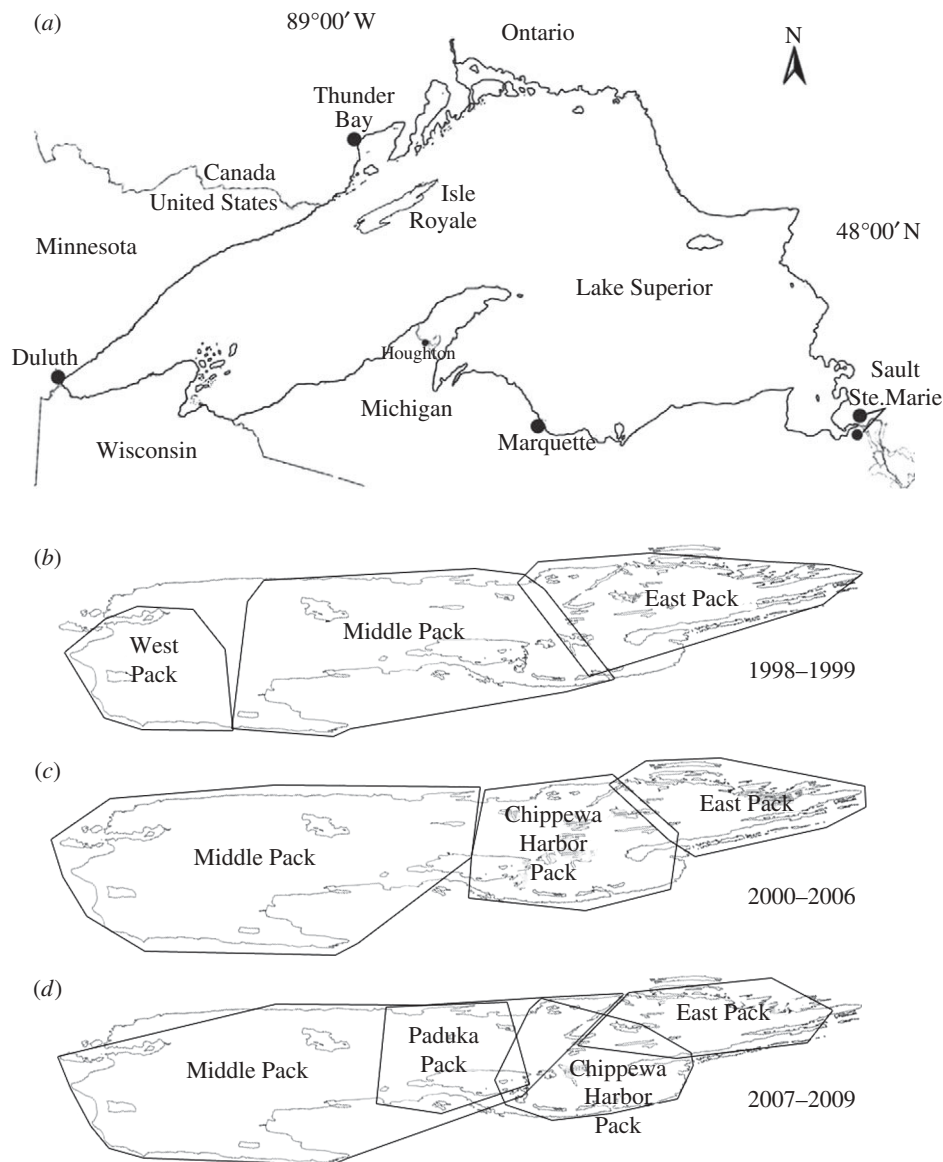


Figure 1. Location of ISRO within (a) Lake Superior and maps of ISRO (b)–(d) showing approximate territorial boundaries of wolf packs for different time periods. During 2007–2009, Paduka Pack and Middle Pack territories overlapped greatly.

compared with only 1 per cent in two outbred wolf populations [14]. Some of these deformities could reduce individual fitness, particularly components of fitness associated with predation and reproduction. An effect of inbreeding has never been detected in the population's basic vital rates. That is, the population's growth rate, recruitment rate and survival rate appear comparable with those of outbred wolf populations [13]. However, because the ecological conditions for ISRO wolves are complex and dynamic, detecting an effect would be difficult. Specifically, vital rates depend on kill rates and prey availability (indexed by moose:wolf ratio), which are extremely variable ($CV_{\text{kill rate}} = 0.36$; $CV_{\text{moose:wolf}} = 0.65$), and a large portion of the variability in vital rates is owing to demographic stochasticity [15].

Here, we document the genetic and demographic impact of a male wolf (*C. lupus*) that immigrated across Lake Superior ice from mainland Ontario in 1997 to the small, inbred wolf population in Isle Royale National Park. This migration event was discovered in 2009 from detailed molecular genetic analysis of samples (mainly scat) from specific individuals and the

subsequent construction of a pedigree. We monitored this population for 40 years (10 generations) prior to the immigration event and 10 years (2.5 generations) after the event [13,15].

2. MATERIAL AND METHODS

(a) *Field methods*

We collected samples of DNA from skeletal remains of 67 wolves discovered between 1966 and 2007, blood from 29 wolves that were live-captured and radio-collared between 1988 and 2007, and 1738 faecal samples collected between 1999 and 2009 at sites where wolves had fed on moose carcasses. The genotype from each faecal sample was assigned to one of the population's five packs, based upon the pack territory where the kill site was located and the genotypes of other wolves (i.e. pack mates) detected at the same kill site (figure 1).

We collected faecal samples systematically and intensively from 1999 to 2009; thus we genotyped at least 90 per cent of the individuals living during this period (see §2e below). Behavioural observations indicate that we collected DNA from all of the breeding individuals from 1999 to 2009.

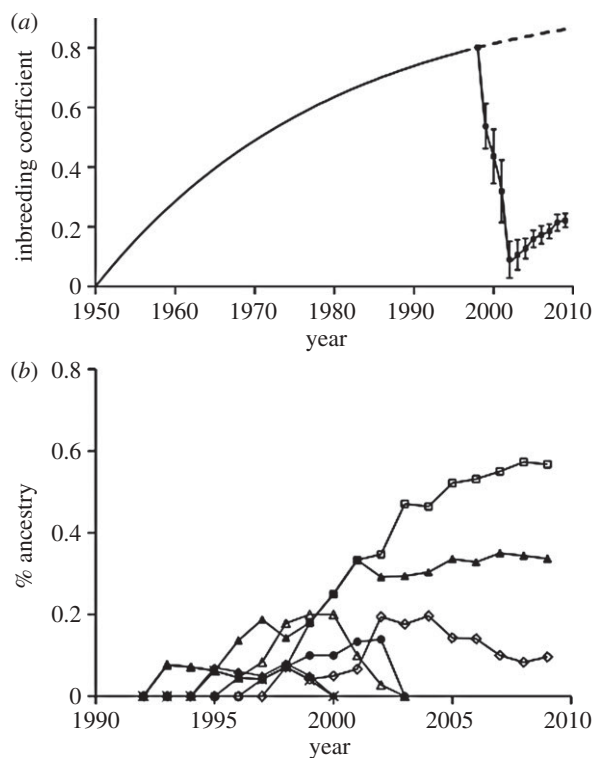


Figure 2. (a) The population inbreeding coefficient (f) averaged over each individual present in the population from 1950 to 2009. For the period 1950–1998 (before rescue, continuous line), f was calculated from estimates of effective population size (3.8) and generation time (4.2 years) derived from the population's demographic properties [12]. For the period 1999–2009 (after rescue, continuous line), mean f and its standard error was calculated from the pedigree in figure 3. (a) Also projects what expected f would have been for the period 1999–2009 had the immigrant not arrived (dashed line). All individuals not descended from or related to the immigrant male were assigned the average f for their year of birth. (b) The proportion of ancestry of immigrant wolf 93 and six native breeding wolves (i.e. wolves 99, 67, 55, 61, 91 and 92) for each year since the immigrant's arrival. Wolf 99 is the first mate of the immigrant, wolf 93. Offspring of wolf 93 who became breeders are excluded in order to compare the ancestry of wolf 93 with those of wolves not influenced by wolf 93's genes. Wolf identification numbers (ID) in the legend correspond to the pedigree (figure 3). Open squares, wolf 93; filled triangles, wolf 99; open diamonds, wolf 67; open triangles, wolf 55; filled circles, wolf 61; asterisks, wolf 91; filled diamonds, wolf 92.

Of the individuals we genotyped, 28 lived between 1960 and 1998, and 112 lived between 1999 and 2009.

To assess whether the migration event had any impact on the population's demography, we compared estimates of wolf abundance, survival and recruitment before and after the migrant's arrival. The methods for estimating these vital rates and their values have been previously reported [15,16].

(b) Microsatellite analysis

We extracted DNA from bone samples using a silica-based method [17,18], and from blood and faecal samples using DNeasy Blood and QIAamp DNA Stool protocols (Qiagen, Valencia, CA, USA). We performed bone and faecal sample extractions in a laboratory dedicated to low quality and quantity DNA sources. One negative extraction was included in each DNA extraction to monitor contamination.

We generated genotypes at eight autosomal microsatellite loci, selected on the basis of their relative size and probability of identity (PID). Specifically, the loci that we used were AHT125, PEZ19 and FH2137 [19], FH2054 and FH2226 [20], AHT121 [21], C05.377 [22] and CXX.20 [23]. Polymerase chain reactions (PCRs) for blood and faecal samples were carried out in 10 μ l reactions containing 5 μ l of Qiagen Multiplex PCR Master Mix, 1 μ l Q-Solution and 0.06 μ M of FH2054, 0.08 μ M of AHT125, 0.1 μ M of FH2137, 0.15 μ M of MS34A and AHT121, 0.2 μ M of FH2226, PEZ19 and C05.377, 0.25 μ M of CXX.20, and 1 μ l of DNA extract. The thermocycler profile was 13 cycles of 94°C for 30 s, 63°C–0.6°C/cycle for 1.5 min and 72°C for 1 min, 28 cycles of 94°C for 30 s, 55°C for 1.5 min and 72°C for 1 min, and 60°C for 30 min following a hot start of 94°C for 15 min. PCRs for bone samples were carried out in two 15 μ l multiplex reactions. Reaction one contained 1.5 mM MgCl₂, 0.4 mM dNTPs, 1X PCR Gold Buffer, 1 U AmpliTaq Gold DNA Polymerase (Applied Biosystems Inc., Foster City, CA, USA) and the same concentrations of primers FH2054, FH2137, FH2226 and MS34A as listed above. Reaction two was the same as reaction one, but with primers AHT121, AHT125, PEZ19, C05.377 and CXX.20 in the same concentrations as above. The thermocycler profile was 13 cycles of 94°C for 30 s, 63°C–0.6°C/cycle for 1.5 min and 72°C for 1 min, and 42 cycles of 94°C for 30 s, 55°C for 1.5 min and 72°C for 1 min following a hot start of 94°C for 10 min.

(c) Probability of identity and heterozygosity

PID values were calculated for these loci from 126 individuals who were born into the population between 1984 (this is the estimated year of birth for the first wolf radio-collared on ISRO) and 2009 using program Gimlet [24]. Observed and expected heterozygosities and number of alleles were also calculated for each locus using Gimlet [24]. To assess what impact the male immigrant had on the heterozygosity of the ISRO population, observed heterozygosities were calculated for 18 wolves present on the island prior to the immigration event and 81 wolves present on the island after the immigration event. In addition, observed and expected heterozygosities were calculated for a population of mainland wolves (see STRUCTURE analysis) from Ontario, Canada ($n = 11$) and MN, USA ($n = 24$).

(d) Data screening method

Blood genotypes were accepted for further analysis after one positive amplification at all loci. Bone and faecal genotypes were screened for accuracy using the comparative reference genotype data filtering method [25]. Briefly, a heterozygous result was accepted after observing each allele twice across multiple PCR replicates. Homozygous results were accepted after three positive PCR results. Once consensus genotypes were obtained for all sample types, genotype matches were identified. Faecal samples with seven locus consensus genotypes were included in the matching analysis. All identical genotypes were scored as originating from the same individual. If two genotypes differed by one or two alleles and that difference could have been owing to allelic dropout, further PCR replicates were performed until the genotype differences were resolved. If after seven PCR replicates, a homozygous result caused a one- or two-allele difference between two genotypes, those genotypes were scored as separate individuals [26]. Sex was assigned to a sample after three identical positive

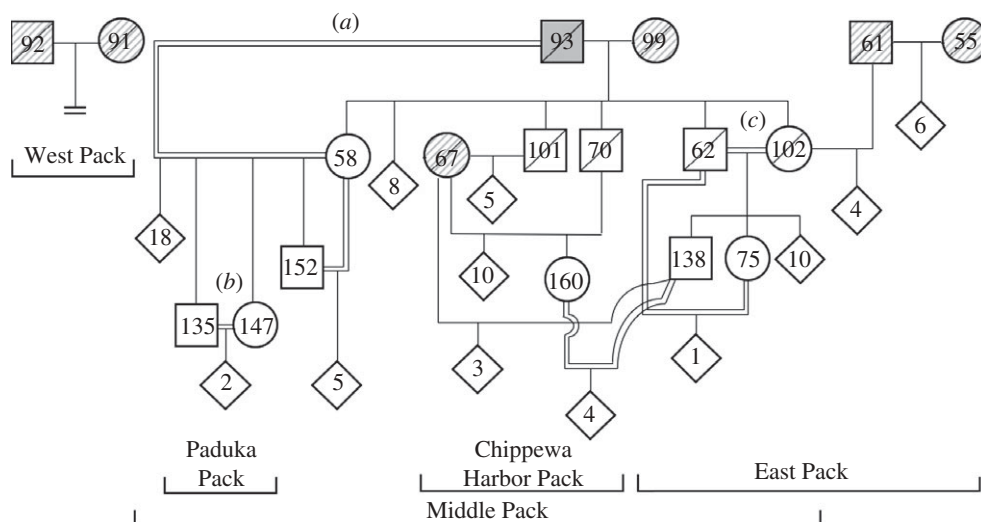


Figure 3. Pedigree of ISRO wolves from 1999 to 2009. Circles are females, squares are males and numbers represent wolf IDs. Diamonds represent several individuals of both sexes who never reproduced. The numbers in the diamonds are the number of individuals represented by each diamond. Double lines represent matings between related individuals (i.e. full-sibs, parent–offspring or cousins). Symbols with a diagonal line were known to be deceased by 2008. The double line below wolves 91 and 92 indicates that this pair produced no litters. The letters (a), (b) and (c) denote specific breeding events that are described in the text. Since 1999, all ISRO wolves are descended from wolves represented by shaded or hatched symbols. Shaded square, immigrant male; crossed squares, breeders with no. 93 ancestry.

amplifications at Y-linked microsatellite locus MS34A for males and three negative amplifications for females [27].

(e) *Pedigree analysis*

We used genotypes and field observations to construct a pedigree of individuals living between 1999 and 2009. Field observations include knowing that wolves live in social groups called packs, which are family units, comprised of a socially dominant, breeding pair (i.e. alpha male and alpha female) and their subordinate offspring. Packs are territorial, and spend much time at sites where they have killed prey within their territories. While feeding at these sites, they defaecate frequently. We collected faecal samples from kill sites that had been detected from light aircraft flown throughout a 44-day field study conducted each winter [13,28]. We also determined, by direct observation of movement and behaviour, territorial boundaries during these aerial surveys (figure 1).

We determined the genetic identity of alpha wolves from direct observations and genetic exclusion. All family relationships assigned from field observations were tested genetically using exclusion. Alphas can be identified in the field by their behavioural interactions with subordinate wolves. Several of the breeding wolves in figure 3 (i.e. wolves 55, 58, 61, 62, and 67) were radio-collared and identified as alphas by social behaviour we observed. The genetic identity of other alpha wolves was determined when a sample of their faeces was collected immediately after observing them defaecate (i.e. wolves 93 and 75). The genetic identity of one alpha female (wolf 102) was identified from oestrus blood in urine deposited in the snow. One male alpha (wolf 70) was genetically identified through tissue collected after his death.

The genetic identity of most subordinate, non-breeding wolves was known only through faecal samples collected from kill sites. Pups were identified through the appearance of genotypes detected from faecal samples that had not been detected in previous years. The accuracy of observed numbers of genotypes representing offspring in each pack, each year, was checked by comparing those numbers with the number of offspring observed during winter field season [28].

All pedigree relationships assigned from field observations were confirmed using genetic exclusion. For the 2002 and 2009 Chippewa Harbor Pack and 2008 and 2009 Middle Pack, litters parentage was assigned using the program CERVUS v. 3.0 [29]. The identity of wolf 58's last mate cannot be determined with certainty using eight microsatellite loci. Wolf 152 and his brother 156 are equally likely to have fathered Middle Pack's litters in 2008 and 2009 (figure 3). Because wolves 152 and 156 are both sons of wolf 58 and her previous mate wolf 93 and therefore would not affect the calculations of inbreeding coefficients, it was decided to arbitrarily designate 152 as alpha male until further data can resolve the issue (figure 3). Once the pedigree relationships were assigned, the per cent ancestry values in the population in 2009 were calculated for the immigrant (93, figure 3) and breeding wolves born on ISRO prior to the immigrants arrival (figure 2b).

(f) *Genetic structure analysis*

Multi-locus genotypes were generated for wolves from Ontario (ON, $n = 15$), Minnesota (MN, $n = 38$) and individuals born on ISRO between 1984 and 1999 ($n = 18$) at 17 microsatellite loci. The microsatellite loci used in addition to the eight listed above were PEZ5, PEZ11 [19], FH2001, FH2062, FH2140 [20] AHT103 [21], C09.173 [23], FH2422 [30] and FH2869 [31]. PCRs for these nine loci were carried out in 10 μ l reactions containing 5 μ l of Qiagen Multiplex PCR Master Mix, 1 μ l Q-Solution and 0.08 μ M of FH2140, 0.1 μ M of FH2001, FH2062, FH2422 and FH2869, 0.15 μ M of AHT103 and C09.173, and 0.2 μ M of PEZ5 and PEZ11 and 1 μ l of DNA extract. The thermocycler profile was the same as listed for blood and faecal samples under microsatellite analysis above.

Related individuals (full siblings or parent offspring) from ON and MN were identified using the program MLRelate [32] and removed from further analysis (ON, $n = 4$; MN, $n = 14$). Genotype data were analysed using STRUCTURE 2.3 [33,34] to assess whether wolf 93 was an immigrant from the mainland. We ran STRUCTURE with the admixture

Table 1. Heterozygosity and demographic parameters before and after genetic rescue. The period before genetic rescue is 1959–1998 for population growth rate (i.e. r), and 1971–1998 for recruitment and survival. Prior to 1971, recruitment and survival data are not available. The period after genetic rescue is 1998–2009. The p -values are for t -tests comparing log-transformed population growth rates before and after genetic rescue, and logit-transformed recruitment and survival rates before and after genetic rescue. Because the effect of the rescue might not be expected to be manifest immediately, we also repeated these tests when before and after periods are 1959–2000 and 2001–2009. The results of these tests were similar to those shown above (i.e. no significant difference in survival, recruitment or growth). The methods for collecting these data, and much of the data represented here are presented in Peterson *et al.* [13] and Vucetich & Peterson [15].

parameter	mean values (and standard errors) before and after genetic rescue		p
	before	after	
average heterozygosity	0.49 (0.014)	0.59 (0.010)	0.04
annual survival rate	0.758 (0.032)	0.741 (0.050)	0.94
annual recruitment rate	0.247 (0.023)	0.316 (0.047)	0.11
annual population growth rate	9.1×10^{-3} (0.040)	0.049 (0.088)	0.28
moose-to-wolf ratio	57.9 (5.8)	33.0 (4.5)	7.8×10^{-4}

model of ancestry, the correlated allele frequencies model and a burn-in period of 200 000 repetitions followed by 800 000 Markov chain Monte Carlo (MCMC) iterations. The number of groups (K) was varied between 1 and 4 and five replicates were performed for each K with the results being averaged across the replicates. Ancestry (q) values for the possible immigrant were averaged across replicate runs for $K = 2$. A histogram of the results was created using program Distruct ([35], the electronic supplementary material, figure S1).

3. RESULTS

(a) Probability of identity and heterozygosity

The probabilities that two individuals share the same genotype by chance (PID_{OBS}) and the probability of siblings sharing the same genotype (PID_{SIBS}) were 4.3×10^{-6} and 4.2×10^{-3} for faecal genotypes based on eight microsatellite loci ($n = 510$). Because some genotypes were based on seven loci ($n = 154$), we also calculated PID_{OBS} and PID_{SIBS} for the six combinations of seven loci used. PID_{OBS} for seven loci ranged from 1.5×10^{-5} to 3.0×10^{-5} , and PID_{SIBS} ranged from 7.4×10^{-3} to 9.4×10^{-3} . The average observed and expected heterozygosities per locus for wolves born between 1984 and 2009 ($n = 126$) were 0.60 and 0.63, respectively (see the electronic supplementary material, table S1). The average number of alleles per locus was 3.8 (electronic supplementary material, table S1). The average observed heterozygosity was significantly different between the pre-immigrant (average $H_O = 0.49 \pm 0.014$, SE) and post-immigrant (average $H_O = 0.59 \pm 0.010$, SE) population ($p = 0.036$, d.f. = 14, two sample t -test, unequal variances; table 1 and the electronic supplementary material, table S2). The average observed heterozygosity was not significantly different between the post-immigrant ISRO population and the mainland (average $H_O = 0.66 \pm 0.023$, SE) wolf population ($p = 0.102$, d.f. = 14, two sample t -test, unequal variances; the electronic supplementary material, table S2).

(b) Microsatellite analysis

Analysing nuclear DNA of blood ($n = 29$), bone ($n = 57$) and faecal ($n = 1738$) samples collected between 1966 and 2009, we identified 141 individuals and relationships

among ISRO wolves. Thirty-five of these individuals lived between 1966 and 1998, 79 lived between 1999 and 2009 and 27 spanned both time periods. Faecal genotyping identified a male wolf (wolf 93) in 1999, who possessed alleles at three different loci not previously observed in the ISRO wolf population among 35 genotyped individuals living from 1966 to 1998. One new allele was at locus MS34A on the Y chromosome, and two other alleles were at unlinked loci FH2226 and C05.377. The discovery of these alleles in the ISRO wolf population and the scat collected from the alpha male in the Middle Pack is most probably owing to immigration rather than mutation.

(c) Pedigree analysis and inbreeding coefficient

Pedigree assignments were determined for 94 individuals. The immigrant produced 34 pups during his 8-year tenure as a breeder (1998–2006, figure 3), and his immediate progeny, including wolf 58, have as of 2009 produced an additional 45 offspring (figure 3). Of the 18 documented breeders in this pedigree, 12 were the immigrant or descendants of the immigrant. Since the immigrant began breeding in 1998, all three of the new alleles that he brought have become common. As of 2009, the frequencies of allele 224 at locus FH2226 and allele 146 at locus C05.377 are 0.33 and 0.27, respectively. Moreover, by the year 2003, immigrant allele 165 at locus MS34A replaced allele 163, becoming the only Y chromosome haplotype in the population. The molecular data and the constructed pedigree identified a new immigrant wolf whose origin was undetected from field observations. This unrelated male wolf immigrated into the ISRO population in 1997.

With the arrival of the immigrant, the inbreeding coefficient f dropped over the next 4 years from 0.81 to 0.09 ± 0.062 (s.e., figure 2a) and the average observed heterozygosity increased from 0.49 ± 0.023 (s.e.) in 1998 to 0.59 ± 0.032 (s.e.) in 2009. However, within 5 years of his arrival, he (wolf 93 in figure 3) began mating with his daughter (wolf 58) (event (a) in figure 3). Subsequently, two offspring of this parent–offspring mating began breeding with each other (wolves 135 and 147) when they established Paduka Pack (figure 1d) in 2007 (event (b) in figure 3). In 2003, the

breeders of East Pack were full sibs (wolves 62 and 102) who had been born to the immigrant and an unrelated, ISRO wolf (event *(c)* in figure 3). In other words, by 2002, two out of three breeding pairs were first-degree relatives and five of the population's six breeders were either the immigrant or an offspring of the immigrant. The dramatic success of the immigrant and his offspring subsequently led to high rates of inbreeding. *f* began rising sharply within 4 years (approx. one generation defined demographically) of the immigrant's arrival (figure 2*a*) and during the next 5 years, *f* had risen to 0.22 ± 0.023 (s.e.).

(d) *Structure analysis*

The population structure analysis clearly separated the ISRO wolf population from the mainland population. Specifically, the STRUCTURE results indicated the value of *K* with the highest log-likelihood was 2. For *K* = 2 all wolves from ON and MN grouped together with an average ancestry value of 97 per cent and all ISRO wolves grouped together with an average ancestry value of 94 per cent (see the electronic supplementary material, figure S1). Comparison of the immigrant's genotype to the mainland wolf population using a Bayesian clustering approach estimated 73 per cent of his ancestry traces to the mainland (see the electronic supplementary material, figure S1).

Importantly, 27 per cent of wolf 93's ancestry trace to the island. Given this result, we cannot exclude the possibility that this individual is a first-generation offspring from an immigrant rather than an immigrant himself. However, we genetically sampled all but one of the alpha wolves that lived in the population up to 11 years prior to the arrival of wolf 93 (1988–1999). We found no unique alleles among these wolves, indicating that wolf 93 is likely an immigrant and not the offspring of an immigrant.

(e) *Field observations*

Field observations made between 1997 and 2009 independently corroborated the genetic observations from the pedigree. Specifically, the immigrant first appeared in 1997 when an ice bridge connected ISRO and the mainland for several weeks. Field observations also indicated that the previous alpha male of the Middle Pack was replaced by a new alpha male between February 1997 and February 1998 [36], the same time when molecular genetic observations indicate the immigrant began reproducing in Middle Pack. Finally, in 1999, we collected a faecal sample from the alpha male of Middle Pack immediately after observing him defaecate. As expected, this wolf's genotype matched that of the wolf now known to be an immigrant.

The high fitness of this immigrant wolf was also associated with distinctive behaviour and physical appearance (figure 4). First, he was physically larger than most ISRO wolves. As alpha male of the Middle Pack, his high fitness was also reflected by his dominance over other ISRO packs. Specifically, he exhibited strong territorial behaviour that completely displaced West Pack (figure 1*b,c*), driving that pack to extinction by 1999. In 1999, Middle Pack had 10 wolves, the largest pack size observed in almost 20 years and that year he led the Middle Pack on major territorial incursions into



Figure 4. Several members of Middle Pack in 2006. The large, light-coloured wolf in the middle is the immigrant (wolf 93), and the wolf to his right, wearing a radio-collar is his daughter and mate (wolf 58).

the territory of the East Pack. All of these observations were reported prior to knowing that the alpha male of Middle Pack was an immigrant [37,38].

In addition and prior to knowing that wolf 93 was an immigrant, we observed that he turned very light in colour, almost white, as he aged (figure 4). While not uncommon among wolves in general, this had never been observed before on ISRO. Before knowing that wolf 93 was an immigrant, we reported two other whitish coloured alpha wolves [38], and in 2010, we observed a fourth light-coloured alpha. Each of these wolves is a descendent of wolf 93.

(f) *Demography*

Despite the immigrant's high fitness and success (figures 2 and 3), his arrival did not obviously benefit the population's demography. Specifically, key demographic rates did not increase significantly during the decade (1998–2009) following the immigrant's arrival (table 1). However, the power of a *t*-test at a significance level of 0.05 is very low for each of the vital rates that we observed. Specifically, for a hypothesized difference of 0.05 (an ecologically important difference for wolf populations [13]), the power is 0.16 for recruitment, 0.10 for survival and 0.09 for annual population growth rate. These low values of power indicate that concluding the immigrant had no effect on vital rates would be an unreliable inference.

Moreover, ecological conditions had deteriorated after the immigrant had arrived, inasmuch as the ratio of moose-to-wolves, an index of food availability, had been lower after the immigration of wolf 93 than before (table 1 and figure 5). The moose-to-wolf ratio declined when moose declined in response to severe winter, lack of food and an outbreak of moose ticks (*Derma-centor albipictus*) [39]. The demographic benefits of the arrival of the immigrant may well have been masked by these and other changing ecological conditions (see the electronic supplementary material, figure S2). In addition, any increase in wolf abundance or vital rates is limited owing to an unavailability of unoccupied habitat on ISRO.

4. DISCUSSION

(a) *Genomic sweep*

Our observations represent an example of what is aptly described as a genomic sweep. If the seven breeders at

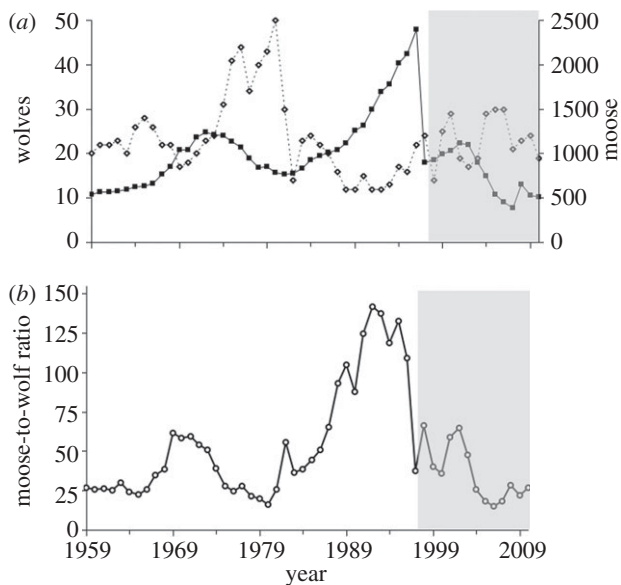


Figure 5. (a) Abundance of wolves and moose on ISRO, 1959–2009. The post-genetic rescue period is marked in a shaded area. (b) Ratio of moose-to-wolves on ISRO, before and after the genetic rescue. The post-genetic rescue period is indicated by the shaded area. Wolf vital rates did not respond after the genetic rescue, and the moose-to-wolf ratio was lower in the post-genetic rescue period (table 1). Diamonds, wolves; filled squares, moose.

the top of the pedigree (i.e. the immigrant and the six breeders with no ancestry to the immigrant) had equal fitness, their ancestries would each be $1/7$ (14.3%). The ancestry (or founder contribution) from a given individual is the expected proportion of genes carried by members of the population that have descended from that given individual [40]. A dramatic indication that the immigrant possessed higher fitness than the native ISRO wolves is by 2009 each individual traced, on average, 56 per cent of their ancestry to the immigrant (figure 2*b*), resulting in a genomic sweep with the maximum possible ancestry from the immigrant. In fact, the increase from 0 per cent ancestry in 1997 to nearly 50 per cent ancestry in 2003 took place in only slightly more than one generation. Of the six breeding wolves (at the top of the pedigree) with no ancestry from the immigrant, only two had ancestry in the population by 2009: wolf 99, the immigrant's first mate with 34 per cent ancestry, and wolf 67, a female that mated with a son of the immigrant with 10 per cent ancestry. The astonishing turnover in ancestry documented in figure 2*b* represents strong evidence that fitness was low among native ISRO wolves relative to the fitness of immigrant wolf 93.

If, hypothetically, the population had, by 2009, descended from just a single pair, then each of these wolves would have had an ancestry of 0.5 and the expected allele frequency for each of their four alleles would be 0.25. The ancestry from the immigrant (0.56) and frequencies of the unique autosomal alleles are even greater (0.33 and 0.27) than this extreme expectation, and the Y chromosome haplotype from the immigrant male is the only one now present. The explanation for this higher-than-expected maximum ancestry and allele frequencies for the autosomal alleles is the severe inbreeding among the descendants of the

immigrant that increased the contribution of his lineage relative to that of his mate (figure 3).

(b) Genetic rescue

Genetic rescue has been defined as occurring ‘when population fitness, inferred from some demographic vital rate or phenotypic trait, increases by more than can be attributed to the demographic contribution of immigrants’ [11]. By this definition and inferences based on hypothesis testing (table 1), we are unable to conclude that genetic rescue occurred in this case. Nevertheless, the statistical power of these tests is low. From one perspective, the appropriate conclusion is that reliable inferences about genetic rescue cannot be drawn because the sample size is too small. That is, a conclusion about whether or not genetic rescue has occurred in this population should be deferred until a much larger sample size has accumulated.

From the perspective of applied conservation, this provides a challenging circumstance. Specifically, statistical power depends on sample size and variability. If the assessment of genetic rescue is restricted to the assessment of vital rates, then sample size and power increase very slowly, i.e. one sample per year. Moreover, the variability of vital rates for ISRO wolves is relatively low [41]. These circumstances give reason to think that assessing genetic rescue would be difficult under certain conditions (i.e. ecological deterioration).

Our results reveal another under-appreciated challenge for assessing genetic rescue. This circumstance rises from defining genetic rescue in terms of fitness [11] and realizing that the concept of ‘fitness’ is more nuanced than is often appreciated [42]. In this case, the salient nuances are that fitness is a relational, not absolute, concept [43] and fitness depends on both a population's genetic constitution and its environment. For ISRO wolves, the immigrant's arrival was associated with both genomic sweep and deteriorating environmental conditions for wolves (figures 2 and 5). If one focuses on the relationship between the population's fitness before and after gene flow, then, indeed it is not obvious that genetic rescue occurred. However, if one compares the fitness observed after the immigrant's arrival in relation to what fitness would have been (in the face of deteriorating environmental conditions) had the immigrant not arrived, then it is plausible, perhaps likely, that genetic rescue benefitted fitness and demography.

By this understanding of fitness, genetic rescue could be difficult to document for any population that is exposed to environmental conditions that are deteriorating or limiting to a population's demography. For ISRO wolves, two environmental conditions limited demography. First, ISRO is an island, every portion of which had been occupied by a wolf pack prior to the immigration event. There was no opportunity for the population to expand into new habitat. The second limiting factor was the substantial decline in prey availability, as indicated by the decline in the ratio of moose-to-wolves (figure 5). While these circumstances are specific to ISRO, the general lesson is that there is reason to expect that the demographic benefits of genetic rescue may be masked if environmental conditions are limiting or deteriorating. This general circumstance is probably applicable to many populations of conservation concern. The significance of limiting environmental conditions for genetic rescue may have been

previously overlooked because the instances of genetic rescue receiving most attention involve environmental conditions that permitted population expansion (e.g. [8,10]).

These challenges for assessing genetic rescue—the relational nature of fitness and the time required for powerful tests of vital rates—might be overcome by reconsidering the definition of and standards for assessing genetic rescue. Consider, for example, that fitness ‘refers to the capacity of a variant type to invade and displace the resident population in competition for available resources’ [44] and that genetic rescue be quantified by this capacity. By these principles, the strength of genetic rescue’s influence is quantified by the dramatic increase in the ancestry of the immigrant(s) (figure 2). Moreover, the strength of genetic rescue defined in this way for this wolf population was at least as great as that observed in Florida panthers, a widely appreciated case of genetic rescue where the ancestry of five introduced Texas pumas was at most 0.410 [10,45].

The detection of inbreeding depression can be notoriously unreliable in the sense that false-negatives are likely, especially if the ultimate concern is inbreeding depression in overall fitness, not merely in a few components of fitness [46–48]. For this reason, there may be value in quantifying genetic rescue by changes in the inbreeding coefficient f occurring after an immigrant’s arrival. The value of doing so is that such changes can be reliably measured and there is as much reason to think f is associated with fitness as are a few selected components of fitness. By this standard, one can reliably infer that the impact of genetic rescue on the wolf population will be short-lived, in the sense that f will soon return to high levels (figure 2a).

Finally, skeletal remains have been recovered for only eight wolves that descended from the immigrant. Because the bone malformations identified in Rääkkönen *et al.* [14] include several different kinds, each of which is likely to have a different genetic basis, there is very little data at this point to know whether inbreeding depression has been reduced in these traits.

(c) Conclusion

The ISRO wolf population shows how gene flow from one individual into an inbred population can result in a genomic sweep. Within a few years of the immigration event, the population’s ancestry changed such that 90 per cent of the population’s genes were derived from the immigrant ‘superwolf’ and his first mate. Also, the new autosomal alleles that the immigrant contributed increased to high frequencies and the new Y chromosome haplotype he contributed quickly increased to 100 per cent. Importantly, these dramatic consequences of a genomic sweep were detectable only through the detailed inspection of molecular information and field observations.

The immigration event we observed also reduced inbreeding coefficients dramatically, but only for a short period of time. The benefits of gene flow were similarly short-lived in two other cases [3,49,50]. Collectively, these examples suggest that populations most in need of genetic rescue may derive only short-lived benefits because such populations tend to have much lower fitness than an immigrant and any F_1 progeny.

Our observations also highlight the need to consider more carefully the definition of and standards for assessing genetic rescue, especially in the presence of a deteriorating

environment or an environment that is limited both in resources and space. This is important because many populations of conservation concern face declining ecological conditions and reductions of available habitat, either of which are capable of masking the benefits of genetic rescue or potentially limiting the situations where genetic rescue occurs following immigration. Population viability seems to require long-term, comprehensive genetic restoration, not just the short-term benefits of genetic rescue [3,10], and an appreciation of how genetic and environmental factors interact to affect population viability.

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