


Genetics and extinction and the example of Isle Royale wolves

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Introduction

For nearly four decades, conservation biologists have been concerned with genetic impacts on the extinction of populations and species (Soule & Wilcox, 1980). These concerns focused on two main genetic issues: a short-term concern of avoiding inbreeding depression, and a long-term concern of maintaining genetic variation so that future adaptation would be possible (Franklin, 1980).

There is substantial and increasing evidence of inbreeding depression, that is, inbred individuals have lowered fitness compared to non-inbred individuals (Hedrick & Garcia-Dorado, 2016). The increase in documented cases of large inbreeding depression appears partly due to the examination of other fitness components besides viability, such as fecundity and mating success, and partly due to measuring inbreeding depression in more natural environments. In addition, new genomic approaches to estimate inbreeding have provided greater resolution for quantifying inbreeding, facilitating studies of inbreeding depression in the wild (Kardos *et al.*, 2016). For example, in red deer *Cervus elaphus*, given an inbreeding level equivalent to that in progeny from a

Abstract

Genetic factors have long been a concern in the extinction and viability of species with the short-term effects focusing on inbreeding depression. Genetic rescue has been suggested as a means to overcome the detrimental effects of inbreeding depression. However, it has been difficult to document the genetic dynamics over time of genetic rescue, inbreeding depression and other genetic relationships in endangered species. We show here using a detailed pedigree and genomic data that genetic rescue in the gray wolf *Canis lupus* population on Isle Royale had only a temporary positive effect reducing inbreeding depression and then the genetic changes from the immigration event resulted in a population decline and now imminent extinction of the population. Examining the genetic details of this situation shows how genetic dynamics after the initial positive effects of genetic rescue have passed can return a small population to a path toward extinction. Thus, the successful conservation of critically small populations would likely depend on alleviating the cause of having become critically small, such as habitat restoration, or periodic re-application of genetic rescue in a manner that does not result in negative genetic dynamics.

mating between half sibs, the predicted lifetime breeding success was only about 10% that when there was no inbreeding (Huisman *et al.*, 2016).

One approach to overcome the impact of inbreeding depression is genetic rescue (Tallmon, Luikart & Waples, 2004; Whiteley *et al.*, 2015), that is, the introduction of genetic variation from unrelated individuals to an inbred population. Hedrick & Fredrickson (2010) provided guidelines about when genetic rescue might be beneficial. They also suggested that the effects of genetic rescue could be short-lived and in some cases even result in genetic swamping of the target population by ancestry from the immigrants potentially resulting in subsequent low effective population size. These guidelines were primarily developed for endangered species like the Mexican wolves *Canis lupus baileyi* and Florida panthers *Puma concolor coryi* when potentially different management options, such as releases from captivity and translocation, are available. However, they are also useful to provide an understanding when genetic rescue occurs because of natural migration as in the Isle Royale wolf population.

Recently, examples of genetic rescue have been documented in organisms as diverse as butterflies (Roitman

et al., 2017), dogs (Stronen *et al.*, 2017), marsupials (Weeks *et al.*, 2017), pines (Hamilton *et al.*, 2017), fish (Robinson *et al.*, 2017) and rodents (La Haye *et al.*, 2017). In addition, meta-analysis has suggested that genetic rescue provided benefits in a very high proportion of the cases examined (Frankham, 2015) and that these benefits persist for several generations (Frankham, 2016).

In spite of these developments, the connection that genetics is a primary cause of extinction in wild populations is generally difficult to document and even downplayed by some conservation biologists. However, if there is detailed genetic information about the relationships of individuals in a wild population, then documentation of genetic problems is potentially possible. In addition, recent developments in genomic analysis now provide insight into genetic changes that were previously not known (Kardos *et al.*, 2018). Here, we present and synthesize current genetic data on the gray wolf population in Isle Royale National Park, Michigan, USA in an effort to understand its imminent extinction using both pedigree and genomic data.

Materials and methods

Background on the Isle Royale wolf population

Isle Royale National Park is on an island in Lake Superior about 20 km from the nearest mainland point in Ontario, Canada. The wolf population there was founded about 1950, probably by two (or three) wolves from the mainland population in Ontario, Canada and Minnesota, USA (Adams *et al.*, 2011). For 60 years, the Isle Royale wolf population and the population of moose, their main prey, have been monitored and studied (Peterson *et al.*, 1998; Peterson & Vucetich, 2016). It has been generally assumed that the Isle Royale wolf population during this period was nearly or completely isolated from the mainland population. However, there is evidence that additional immigration to Isle Royale has periodically taken place from the mainland population (Hedrick *et al.*, 2014).

Most importantly, it was discovered in 2008 from genetic examination of scats that a male wolf known as M93 (M indicates male), or Old Grey Guy, migrated to Isle Royale from nearby mainland Ontario, Canada, probably in 1997, across the ice bridge present that year (Adams *et al.*, 2011). He was behaviorally dominant over resident wolves, mated with a native female for several years, and their descendants quickly dominated the genetic ancestry of the population. This genetic rescue event plausibly prevented the population from going extinct in the late 1990s and early 2000s because it occurred when wolf demography was poor due to inbreeding and a collapse in the moose population food supply.

However, in the past few years, the numbers of Isle Royale wolves have dramatically declined from 19 in 2010 to only 2 from 2016 to 2018. These last two wolves are closely related and do not appear reproductive. In the 5 years during which these two wolves have been together, they only produced a single pup that died in less than a year. The physical appearance of the offspring was aberrant (Vucetich &



Figure 1 Photo of the two remaining wolves on Isle Royale observed in 2017 with the female F193 below snarling at the male M183 above in response to his courtship advances.

Peterson, 2015) and its expected inbreeding coefficient was 0.438 (Hedrick *et al.*, 2017).

Figure 1 is a photo of the two wolves observed in 2017, the adult female F193 (where F indicates female) below and the adult male M183 above. This photo shows the female snarling at the male and other photos taken over an hour of observation also show intense aggressive displays by the female with no change in her receptivity. This behavior strongly suggests that the female would not mate with this male. Given the history and circumstances of this pair, there is no reason to expect them to successfully reproduce before they die. The decline, and now imminent extinction, of the Isle Royale wolf population provides an example of how genetic changes can result in extinction of a population.

Molecular techniques and pedigree construction

We collected samples of DNA from blood from wolves that were live-captured and radio-collared, skeletal remains and fecal samples at sites where wolves had fed on moose carcasses (the same protocol and microsatellite loci used by Adams *et al.* (2011) were used here). The microsatellite genotype from each fecal sample was assigned to one of the wolf packs, based upon the pack territory where the kill site was located and the genotypes of other wolves in the pack detected at the same kill site. We determined the genetic identity of alpha wolves from direct observations and genetic exclusion. For example, alpha wolves can be identified in the field by their behavioral interactions with subordinate wolves. The genetic identity of some alpha wolves was determined when a sample of their feces was collected immediately after observing them defecate (see Adams *et al.*, 2011 for more details). All family relationships assigned from field observations were tested genetically using exclusion.

We used the microsatellite genotypes and field observations to construct a pedigree of the Isle Royale wolf population for the years 1998–2018. We also determined, by direct observation of movement and behavior, territorial boundaries of the packs during these aerial surveys. The accuracy of

observed numbers of genotypes representing offspring in each pack for each year was checked by comparing those numbers with the number of offspring observed during winter field season. The pedigree was trimmed here to show primarily only the wolves from which the population is believed to have descended since the late 1990s and the two remaining wolves.

Using the relationship,

$$f = \frac{H_0 - H_t}{H_0} \quad (1)$$

where H_0 is the heterozygosity from the ancestral Minnesota–Canadian population and H_t is the heterozygosity in the Isle Royale population; this expression gives an estimate of inbreeding f resulting from genetic drift (e.g. Hedrick, 2011).

Estimates of genome-wide heterozygosity and F_{ROH} were derived from whole genome sequences generated by Robinson *et al.* (2018). Briefly, DNA from Isle Royale wolves was extracted from blood samples archived at Michigan Technological University and sequenced on the Illumina HiSeq 4000, generating paired reads 100 base pairs in length. The pipeline used to convert raw sequence data into high-quality genotypes is described in more detail in Robinson *et al.* (2016, 2018). Briefly, raw reads were aligned to the domestic dog genome using bwa MEM (Li, 2013) before removal of PCR duplicates and low quality reads. Base quality score recalibration and genotyping were performed with the Genome Analysis Toolkit (GATK, McKenna *et al.*, 2010) and genotypes were filtered for quality and depth, leaving only high-quality biallelic single nucleotide polymorphisms (SNPs).

Genomic heterozygosity was defined as the total number of heterozygous genotypes divided by the total number of called genotypes. Runs of homozygosity (ROH) were identified using VCFtools (Danecek *et al.*, 2011) and ROH spanning regions with fewer than 50 variant sites were excluded. The proportion of the genome that consists of ROH can then be estimated, giving another measure of inbreeding, F_{ROH} .

Results

Before immigration

Genetic variation in Isle Royale wolves before the immigration of M93 was estimated using both microsatellite loci and genomic SNP markers. First, the mean observed microsatellite heterozygosity for 20 wolves on Isle Royale before 1998 (0.430) was much lower than the mean observed heterozygosity for 35 mainland wolves from near the Minnesota–Canadian border (0.634), which represents the source population of Isle Royale wolves (Hedrick *et al.*, 2014) (Table 1). From equation (1), the estimate of the inbreeding coefficient is $f = 0.322$ (Table 1). In other words, the significant loss in heterozygosity from the source population resulted in the sizeable inbreeding level of 0.322, indicating that the population was quite inbred before M93 immigrated.

Similarly, the mean per-site heterozygosity from the Minnesota–Canadian population is 0.00160 and is significantly

Table 1 The heterozygosity of microsatellites and SNPs in a sample of wolves from Minnesota and from Isle Royale before 1998 and the estimated inbreeding coefficient f before 1998 and on the bottom row is the estimated inbreeding coefficient from ROH (runs of homozygosity) for these two groups and difference between them in the rightmost numerical column

	Minnesota	Before 1998	f
Heterozygosity ^a (microsatellites)	0.634	0.430	0.322
Heterozygosity ^b (SNPs)	0.00160	0.00116	0.278
F_{ROH} ^b	0.158	0.370	0.212 ^c

SNP, single nucleotide polymorphism; ROH: runs of homozygosity.

^aThese values are from Hedrick *et al.* (2014).

^bThese values are calculated from the raw data in Robinson *et al.* (2018).

^cThis f value is the difference in F_{ROH} between the Isle Royale sample prior to 1998 and the Minnesota sample.

higher than that observed in the Isle Royale population in the animals born before 1998 of 0.00116 (Table 1) (Robinson *et al.*, 2018). Using these values from genomic markers and expression (1), then $f = 0.275$, further evidence that genetic drift had a quite high impact genetic variation and the inbreeding estimate.

Also, using SNPs, the proportion of the genome that consists of ROH can be estimated, giving another measure of the inbreeding, F_{ROH} . In this case, $F_{ROH} = 0.158$ in the Minnesota–Canada population and $F_{ROH} = 0.370$ in the Isle Royale population before 1998 (Robinson *et al.*, 2018). The relatively high value in Minnesota–Canada is influenced by a high value for one of the wolves sampled, perhaps, suggesting that she had some history of recent inbreeding. The difference between F_{ROH} for the Isle Royale population and putative ancestral population of 0.212 gives a general estimate of the inbreeding accumulated on Isle Royale before 1998. These three different high estimates of inbreeding before the immigration suggests that the population fitness was low and that the population was a good candidate not only for genetic rescue but also susceptible to a genomic sweep.

Another indication of the low fitness in the Isle Royale wolf population, presumably the result of inbreeding depression, is the high rate of individuals with bone malformations (58%) (Räikkönen *et al.*, 2009; see also Robinson *et al.*, 2018), a level that has increased over time. For comparison, the incidence of similar malformations in outbred wolf populations in historic Scandinavia was 0%, contemporary Finland was 1% and only 10% in modern inbred Scandinavian wolves (Räikkönen *et al.*, 2009). The fitness impacts of these malformations are not clear, but in dogs they have been implicated in quite debilitating syndromes (Morgan *et al.*, 1993).

After immigration

After M93 immigrated on to the island in 1997, his genetic dominance emerged very quickly. To understand the genetic dynamics of this change, the pedigree in Fig. 2 can be used which shows the two remaining wolves, M183 and F193, as shaded and their known ancestors, M93, F99 and F67.

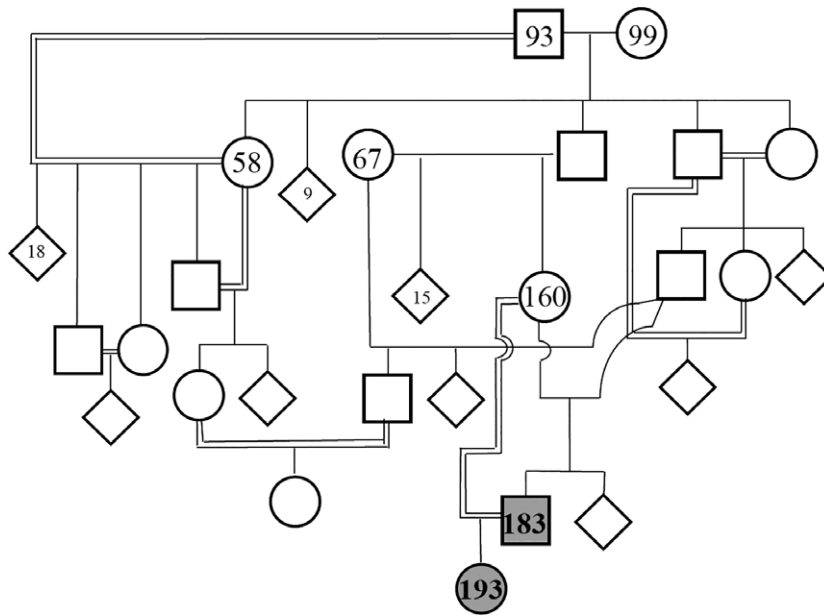


Figure 2 Pedigree showing the remaining two wolves, M183 and F193, as shaded and their known ancestors, M93, F99 and F67, in the Isle Royale population. Double lines indicate matings between relatives, squares indicate males, and circles indicate females. Notice that F160 is the mother of M183 and F193 and that M183 is also the father of F193. Diamonds indicate multiple progeny, for example, the diamond on the left indicates 18 of the progeny from M93 and his daughter F58.

This successful reproduction of M93 resulted in a rapid increase in the proportion of genetic ancestry from him and a ‘genomic sweep’ where the proportion of all genes in the population that can be traced back to him increased quickly to an expected value of 59.4% of the population in 2008 (Hedrick *et al.*, 2014). From 2005 on, all the ancestry in the Isle Royale population has been descended from only three individuals; the male immigrant M93, F99, and F67, another female population resident. In other words, genetic rescue had a strong beneficial influence for about a decade (about 2.5 wolf generations) after the arrival of M93, followed by a return of genetic problems attributable this time to the reduction of diversity in the gene pool due to the elimination of ancestry from other individuals except his initial mate and one other female.

A major factor causing the very high proportion of M93 ancestry was the result of him, after his first mate F99 died, mating with his daughter F58. This father–daughter mating produced 21 progeny total in five litters from 2003 to 2007. Ordinarily only 50% of the ancestry in progeny would be from one parent of a pair but in this case, any progeny from the father–daughter mating would be expected to have 75% of their ancestry from M93. In 2008, 9 of 24 individuals were progeny from the father–daughter mating, resulting in the particularly high M93 ancestry that year.

After the immigration of M93, the level of inbreeding plummeted (Fig. 2a of Adams *et al.*, 2011) and then rose quickly from 2003 to 2012 (Fig. 3 of Hedrick *et al.*, 2014). This was mainly due to inbreeding from M93 and again from the large number of progeny from the mating of M93 with his daughter F58 that resulted in identity-by-descent

from M93 but not from female F99. For example, in 2009, 76% of the inbreeding in the population was from M93 (see Fig. 3 in Hedrick *et al.*, 2014).

After this, the most striking change was that by 2012 none of the 21 descendants of the father–daughter mating were alive and none of them had any surviving descendants. As a result, both the ancestry and inbreeding from M93 greatly declined. Presumably, this change was the result of lower fitness of these descendants because they were homozygous due to inbreeding for detrimental variation originally brought into the population by M93. The loss of these 21 individuals also greatly reduced the population size.

Much of the decline in overall inbreeding level from 0.230 in 2009 to 0.140 in 2013 is explained by the death of nine wolves with an inbreeding coefficient of 0.375, the result of two consecutive generations of close (first-degree) inbreeding (Hedrick *et al.*, 2014). All of these wolves had short lifespans (mean of 2.33 years compared to about 6 years for other unexploited wolf populations as indicated by the data in Fuller, Mech & Cochrane, 2003) and all of them had died by 2011. Specifically, of the wolves recruited into the pedigree between 2009 and 2011, there is a twofold difference in inbreeding between the seven alive in 2012 (0.152) and the six not alive in 2012 (0.292). None of these highly inbred wolves reproduced.

In the last few years, the population numbers of Isle Royale wolves have declined dramatically and there are only two wolves remaining, a male (M183) and a female (F193), in early 2018. These two adults are very closely related and are both father and daughter and half siblings because they have the same mother F160. They are in fact the most

closely related pair of the four males and four females that were present in the population in 2013 (Hedrick *et al.*, 2014). The expected inbreeding coefficient of an offspring from them was the highest of any possible pair at 0.438 and the expected relatedness between them is 0.734 (Hedrick *et al.*, 2017). Reflecting the reduction in M93 ancestry from the peak discussed above, the expected M93 ancestry for M183 is 0.375 and for F193 is 0.3125.

Discussion

Genetics of the Isle Royale wolf population

The decline of the Isle Royale wolf population, and now for all intents and purposes its imminent extinction, provides a detailed case study of how genetic changes can result in the extinction of a population. Because of the detailed examination of the Isle Royale wolf population, important genetic factors resulting in its imminent extinction have been documented and discussed here. First, because of the relative isolation of the population from immigrants and its relative small size, the amount of genetic variation was significantly reduced compared to its source population and estimates of inbreeding levels were large, $f = 0.322$ from microsatellites, and $f = 0.278$ and $F_{ROH} = 0.212$ from two approaches using estimates from genomic data. In addition, the documented very high rate of bone malformations indicated inbreeding depression.

Second, this lowered fitness contributed to the great genetic success of male M93 who migrated in to the population in 1997. His immigration resulted in a short-lived genetic rescue and then in a genomic sweep in which in 2008 his ancestry was 59.4% of the population. Finally, this great genetic success resulted in a substantial cost because he produced 21 inbred progeny with a daughter, all of whom died without contributing any surviving descendants. This, and other close inbreeding, resulted in individuals with low fitness and a subsequent decline in the population size. In other words, a series of genetically related events likely ultimately resulted in a great reduction in the Isle Royale wolf population and now its imminent extinction.

Furthermore, the initial progeny from M93 and his mate F99, such as his daughter F58, might have had higher fitness than other wolves on Isle Royale because some recessive detrimental alleles accumulated in the Isle Royale population were covered up as heterozygotes in these initial offspring. In fact, M93 mated with daughter F58 even though unrelated females were present in the population, suggesting that she was more fit than the other resident females without M93 ancestry. The success of these offspring could have initially increased the frequency of detrimental variants brought in by M93 but then with inbreeding these detrimental alleles were subsequently expressed as homozygotes and resulted in lowered fitness. In sum, the genetic benefit of M93 was dramatic but short-lived.

The putative pup of M183 and F193 seen in 2015, had an expected inbreeding coefficient of 0.438, but its actual inbreeding coefficient could have been considerably higher

(or lower) because of the large 95% confidence limits around this pedigree estimate (0.311–0.565) (Hedrick *et al.*, 2017). This pup had an abnormal phenotypic appearance with a quite unusual, short tightly curled tail, appeared to have an unusual posture, and was relatively small. Furthermore, field observations suggest that this offspring was short-lived and died as a pup (Peterson & Vucetich, 2016). These malformations and the pup's short life suggest the negative impact of inbreeding depression on its phenotype and survival.

Genomic estimates of inbreeding based on analysis of runs of homozygosity identified with many thousands of SNPs are expected to be higher than inbreeding predicted from our pedigree because of common ancestry of females F99 and F67 in resident ancestors that are not included in the pedigree. For example, for the seven animals in which there is both a pedigree estimate of inbreeding f and F_{ROH} , the mean f is 0.091 and the mean F_{ROH} is 0.332 (Robinson *et al.*, 2018). The difference, 0.241, gives an estimate of the increase expected in inbreeding from unknown identity-by-descent found by genomic analysis over that predicted from the known pedigree. In other words, pedigree-based measures of inbreeding can provide basic guideposts, but realized values are likely higher due to unknown common ancestry.

General relevance

It has been generally difficult to document the impact of genetic factors on extinction in natural populations. However, endangered species often face significant genetically related threats, including lowered fitness due to loss of genetic variation, increase in detrimental variants and inbreeding. One way to overcome the impact of lowered fitness is genetic rescue in which the natural or artificial introduction of individuals from outside the population provides genetic variation that subsequently results in higher fitness (Tallmon *et al.*, 2004; Whiteley *et al.*, 2015; Hedrick & Garcia-Dorado, 2016). Because many populations have become small and isolated in recent decades due to human impacts, genetic rescue is likely to become a management action or natural event of great importance for rare or endangered species in the future. Also, because many of the known examples are from recent years, the longer term impact of genetic rescue has not been documented or examined.

There have been a number of important cases where genetic rescue has resulted in substantial population recovery. In most of the well-known examples of genetic rescue, the population numbers initially increased and in general the populations appear to have higher viability than before the natural or artificial genetic rescue but other factors now appear to be limiting population numbers in some cases. For example, the translocations of prairie chickens (Westemeier *et al.*, 1998) appear to have been effective in increasing fitness and genetic variation and the population initially increased. However, the population is now limited by suitable habitat (Bouzat *et al.*, 2009) and the outlook is not as good. In the isolated Swedish population of adders (Madsen *et al.*, 1999), translocation initially resulted in enhanced

population growth (Madsen, Ujvari & Olsson, 2004). However, the construction of human obstacles, a house and a brick wall, have dramatically reduced population numbers (Madsen & Ujvari, 2011). Genetic rescue in the population of bighorn sheep living in the National Bison Range resulted in significant population increase (Hogg *et al.*, 2006) and recent genomic analysis has examined what loci might have been involved (Miller *et al.*, 2012) although recently up to 70% of the population has been lost due to pneumonia (Heyler, 2018).

The introduction of Texas pumas into the Florida panther population resulted in dramatic population recovery (Hostetler *et al.*, 2010; Johnson *et al.*, 2010) although now the population appears potentially limited by available habitat and human-caused mortality. The outcome of the Florida panther genetic rescue might also be threatened by inbreeding and low effective population size in current and future generations (Hedrick, 2010). The natural introduction of one male wolf initially had important beneficial effects in Scandinavian wolves (Vilà *et al.*, 2003) although a subsequent analysis documented significant inbreeding depression (Liberg *et al.*, 2005). Since then, two male wolves naturally migrated into the population and three wolves were translocated into the population from northern Sweden, resulting in both a lower inbreeding coefficient and higher reproductive success (O. Liberg, personal communication). Detailed examination of genetic rescue in Scandinavian wolves demonstrated that offspring of immigrants had higher breeding success than resident inbred individuals (Akesson *et al.*, 2016). Crosses between lineages in Mexican wolves resulted in higher fitness (Fredrickson *et al.*, 2007), numbers in the reintroduced population increased, but have plateaued in recent years. As yet, there has been no detailed analysis determining how much genetic rescue or other factors, such as supplemental feeding of denning females, influenced this initial population increase and recent plateau.

The general positive evaluation of genetic rescue, along with the increasing likelihood of lowered fitness in small, isolated populations make detailed evaluation of genetic rescue particularly important. Only in populations where individuals are identified and their genetic relationship to others in the population is known can the reasons for the success or failure of genetic rescue be evaluated in detail. Two such examples besides the Isle Royale wolf population are that of the translocation of Texas pumas into the Florida panther population and the natural immigration of wolves from the north into the Scandinavian wolf population. In both of these populations, continued monitoring of the genetic success or failure of individuals with known relationships appears fundamental to understanding the outcome of genetic rescue.

The discussion of the genetic impacts on extinction in Isle Royale wolves illustrates a potential problem relying on a single genetic rescue event as an approach to overcoming inbreeding depression. Moreover, persistence of the Isle Royale population throughout its seven-decade existence was likely supported by multiple genetic rescue events (Hedrick *et al.*, 2014). In other words, although genetic rescue can alleviate problems associated with inbreeding depression over

the short term, it can also generate problems itself, ones that in the case of the Isle Royale wolf population has resulted in its imminent extinction. Thus, the successful conservation of critically small populations would likely depend on alleviating the cause of having become critically small, such as habitat restoration, or periodic re-application of genetic rescue. The case study reported here is particularly significant because few documented instances of genetic rescue and the ensuing changes have been observed long enough or in sufficient detail to understand both the beneficial and detrimental effects of genetic rescue. Clearly, many additional case studies will be required before an overall perspective is developed.

During the final review of this paper, the National Park Service has begun relocating wolves from the mainland to Isle Royale. Their plan allows for translocating up to 20–30 wolves from the mainland over the next 3–5 years to re-establish a breeding wolf population on Isle Royale. At this point, it is not clear how, or if, the translocated wolves will interact with the two remaining closely related wolves.

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