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### Title

Genetics and extinction and the example of Isle Royale wolves

### Permalink

<https://escholarship.org/uc/item/2fd2600s>

### Journal

Animal Conservation, 22(3)

### ISSN

1367-9430

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### Publication Date

2019-06-01

### DOI

10.1111/acv.12479

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Peer reviewed

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5 Article type : Original Manuscript

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8 **Genetics and extinction and the example of Isle Royale**  
9 **wolves**

10  
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13  
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20  
21 **Keywords:** immigration, inbreeding, inbreeding depression, pedigree, runs of homozygosity,  
22 SNPs, extinction, *Canis lupus*

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**This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](https://onlinelibrary.wiley.com/terms-and-conditions). Please cite this article as [doi: 10.1111/acv.12479](https://doi.org/10.1111/acv.12479)**

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## 28 **Abstract**

29

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

44

45

## 46 **Introduction**

47

48 For nearly four decades, conservation biologists have been concerned with genetic impacts on  
49 the extinction of populations and species (Soule & Wilcox, 1980). These concerns focused on  
50 two main genetic issues, a short-term concern of avoiding inbreeding depression and a long-term  
51 concern of maintaining genetic variation so that future adaptation would be possible (Franklin,  
52 1980).

53

54 There is substantial and increasing evidence of inbreeding depression, that is, inbred  
55 individuals have lowered fitness compared to non-inbred individuals (Hedrick & Garcia-Dorado,  
56 2016). The increase in documented cases of large inbreeding depression appears partly due to the  
57 examination of other fitness components besides viability, such as fecundity and mating success,  
58 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

59 inbreeding, facilitating studies of inbreeding depression in the wild (Kardos *et al.*, 2016). For  
60 example in red deer (*Cervus elaphus*), given an inbreeding level equivalent to that in progeny  
61 from a mating between half sibs, the predicted lifetime breeding success was only about 10%  
62 that when there was no inbreeding (Huisman *et al.*, 2016).

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

74 Recently, examples of genetic rescue have been documented in organisms as diverse as  
75 butterflies (Roitman *et al.*, 2017), dogs (Stronen *et al.*, 2017), marsupials (Weeks *et al.*, 2017),  
76 pines (Hamilton *et al.*, 2017), fish (Robinson *et al.*, 2017), and rodents (La Haye *et al.*, 2017). In  
77 addition, meta-analysis has suggested that genetic rescue provided benefits in a very high  
78 proportion of the cases examined (Frankham, 2015) and that these benefits persist for several  
79 generations (Frankham, 2016).

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

88

## 89 **Methods**

90

## 91 **Background on the Isle Royale Wolf Population**

92

93 Isle Royale National Park is on an island in Lake Superior about 20 km from the nearest  
94 mainland point in Ontario, Canada. The wolf population there was founded about 1950, probably  
95 by two (or three) wolves from the mainland population in Ontario, Canada and Minnesota, USA  
96 (Adams *et al.*, 2011). For 60 years, the Isle Royale wolf population and the population of moose,  
97 their main prey, have been monitored and studied (Peterson *et al.*, 1998; Peterson & Vucetich,  
98 2016). It has been generally assumed that the Isle Royale wolf population during this period was  
99 nearly or completely isolated from the mainland population. However, there is evidence that  
100 additional immigration to Isle Royale has periodically taken place from the mainland population  
101 (Hedrick *et al.*, 2014).

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

110 However, in the past few years the numbers of Isle Royale wolves have dramatically  
111 declined from 19 in 2010 to only two from 2016 to 2018. These last two wolves are closely  
112 related and do not appear reproductive. In the five years during which these two wolves have  
113 been together, they only produced a single pup that died in less than a year. The physical  
114 appearance of the offspring was aberrant (Vucetich & Peterson 2015) and its expected  
115 inbreeding coefficient was 0.438 (Hedrick *et al.*, 2017).

116 Figure 1 is a photo of the two wolves observed in 2017, the adult female F193 below and  
117 the adult male M183 above. This photo shows the female snarling at the male and other photos  
118 taken over an hour of observation also show intense aggressive displays by the female with no  
119 change in her receptivity. This behavior strongly suggests that the female would not mate with  
120 this male. Given the history and circumstances of this pair, there is no reason to expect them to

121 successfully reproduce before they die. The decline, and now imminent extinction, of the Isle  
122 Royale wolf population provides an example of how genetic changes can result in extinction of a  
123 population.

124  
125 [insert Figure 1]  
126

## 127 **Molecular techniques and pedigree construction**

128  
129 We collected samples of DNA from blood from wolves that were live-captured and radio-  
130 collared, skeletal remains, and fecal samples at sites where wolves had fed on moose carcasses  
131 (the same protocol and microsatellite loci used by Adams *et al.*, 2011 were used here). The  
132 microsatellite genotype from each fecal sample was assigned to one of the wolf packs, based  
133 upon the pack territory where the kill site was located and the genotypes of other wolves in the  
134 pack detected at the same kill site. We determined the genetic identity of alpha wolves from  
135 direct observations and genetic exclusion. For example, alpha wolves can be identified in the  
136 field by their behavioral interactions with subordinate wolves. The genetic identity of some alpha  
137 wolves was determined when a sample of their feces was collected immediately after observing  
138 them defecate (see Adams et al. 2011 for more details). All family relationships assigned from  
139 field observations were tested genetically using exclusion.

140 We used the microsatellite genotypes and field observations to construct a pedigree of the  
141 Isle Royale wolf population for the years 1998-2018. We also determined, by direct observation  
142 of movement and behavior, territorial boundaries of the packs during these aerial surveys. The  
143 accuracy of observed numbers of genotypes representing offspring in each pack for each year  
144 was checked by comparing those numbers with the number of offspring observed during winter  
145 field season. All pedigree relationships assigned from field observations were confirmed using  
146 genetic exclusion. The pedigree was trimmed here to show primarily only the wolves from which  
147 the population is believed to have descended since the late 1990s and the two remaining wolves.

148 Using the relationship,

$$149 \quad f = \frac{H_0 - H_t}{H_0} \quad (1)$$

151  
152 where  $H_0$  is the heterozygosity from the ancestral Minnesota-Canadian population and  $H_t$  is the  
153 heterozygosity in the Isle Royale population, this expression gives an estimate of inbreeding  $f$   
154 resulting from genetic drift (e.g. Hedrick, 2011).

155 Estimates of genome-wide heterozygosity and  $F_{ROH}$  were derived from whole genome  
156 sequences generated by Robinson et al. (2018). Briefly, DNA from Isle Royale wolves was  
157 extracted from blood samples archived at Michigan Technological University and sequenced on  
158 the Illumina HiSeq 4000, generating paired reads 100 base pairs in length. The pipeline used to  
159 convert raw sequence data into high quality genotypes is described in more detail in Robinson *et*  
160 *al.* (2018). Briefly, raw reads were aligned to the domestic dog genome using bwa MEM (Li,  
161 2013) before removal of PCR duplicates and low quality reads. Base quality score recalibration  
162 and genotyping were performed with the Genome Analysis Toolkit (GATK, McKenna *et al.*,  
163 2010) and genotypes were filtered for quality and depth, leaving only high quality biallelic SNPs.

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

169

## 170 **Results**

171

### 172 **Before immigration**

173

174 Genetic variation in Isle Royale wolves before the immigration of M93 was estimated using both  
175 microsatellite loci and genomic SNP markers. First, the mean observed microsatellite  
176 heterozygosity for 20 wolves on Isle Royale before 1998 (0.430) was much lower than the mean  
177 observed heterozygosity for 35 mainland wolves from near the Minnesota–Canadian border  
178 (0.634), which represents the source population of Isle Royale wolves (Hedrick *et al.*,  
179 2014)(Table 1). From equation (1), the estimate of the inbreeding coefficient is  $f = 0.322$  (Table  
180 1). In other words, the significant loss in heterozygosity from the source population resulted in

181 the sizable inbreeding level of 0.322, indicating that the population was quite inbred before M93  
182 immigrated.

183

184 [insert Table 1]

185

186 Similarly, the mean per-site heterozygosity from the Minnesota-Canadian population is  
187 0.00160 and is significantly higher than that observed in the Isle Royale population in the  
188 animals born before 1998 of 0.00116 (Table 1)(Robinson *et al.*, 2018). Using these values from  
189 genomic markers and expression (1), then  $f = 0.275$ , further evidence that genetic drift had a  
190 quite high impact genetic variation and the inbreeding estimate.

191 Also using SNPs, the proportion of the genome that consists of ROH can be estimated,  
192 giving another measure of the inbreeding,  $F_{ROH}$ . In this case,  $F_{ROH} = 0.158$  in the Minnesota-  
193 Canada population and  $F_{ROH} = 0.370$  in the Isle Royale population before 1998 (Robinson *et al.*,  
194 2018). The relatively high value in the Minnesota-Canada is influenced by a high value for one  
195 of the wolves sampled, perhaps suggesting that she had some history of recent inbreeding. The  
196 difference between  $F_{ROH}$  for the Isle Royale population and putative ancestral population of  
197 0.212 gives a general estimate of the inbreeding accumulated on Isle Royale before 1998. These  
198 three different high estimates of inbreeding before the immigration suggests that the population  
199 fitness was low and that the population was a good candidate for genetic rescue but also  
200 susceptible to a genomic sweep.

201 Another indication of the low fitness in the Isle Royale wolf population, presumably the  
202 result of inbreeding depression, is the high rate of individuals with bone malformations (58%)  
203 (Räikkönen *et al.*, 2009; see also Robinson *et al.*, 2018), a level that has increased over time. For  
204 comparison, the incidence of similar malformations in outbred wolf populations in historic  
205 Scandinavia was 0%, contemporary Finland was 1%, and only 10% in modern inbred  
206 Scandinavian wolves (Räikkönen *et al.*, 2009). The fitness impacts of these malformations are  
207 not clear, but in dogs they have been implicated in quite debilitating syndromes (Morgan *et al.*,  
208 1993).

209

## 210 **After immigration**

211



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

216  
217 [insert Figure 2]  
218

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

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

235 After the immigration of M93, the level of inbreeding plummeted (Fig. 2a of Adams *et al.*  
236 *et al.*, 2011) and then rose quickly from 2003 to 2012 (Fig. 3 of Hedrick *et al.*, 2014). This was  
237 mainly due to inbreeding from M93 and again from the large number of progeny from the mating  
238 of M93 with his daughter F58 that resulted in identity-by-descent from M93 but not from the  
239 founder F99. For example, in 2009, 76% of the inbreeding in the population was from M93 (see  
240 Fig. 3 in Hedrick *et al.*, 2014).

241 After this, the most striking change was that by 2012 none of the 21 descendants of the  
242 father-daughter mating were alive and none of them had any surviving descendants. As a result,

243 both the ancestry and inbreeding from M93 greatly declined. Presumably, this change was the  
244 result of lower fitness of these descendants because they were homozygous due to inbreeding for  
245 detrimental variation originally brought into the population by M93. The loss of these 21  
246 individuals also greatly reduced the population size.

247 Much of the decline in overall inbreeding level from 0.230 in 2009 to 0.140 in 2013 is  
248 explained by the death of nine wolves with an inbreeding coefficient of 0.375, the result of two  
249 consecutive generations of close (first-degree) inbreeding (Hedrick *et al.*, 2014). All of these  
250 wolves had short lifespans (mean of 2.33 years compared to about 6 years for other unexploited  
251 wolf populations as indicated by the data in Fuller *et al.* 2003) and all of them had died by 2011.  
252 Specifically, of the wolves recruited into the pedigree between 2009 and 2011, there is a two-  
253 fold difference in inbreeding between the seven alive in 2012 (0.152) and the six not alive in  
254 2012 (0.292). None of these highly inbred wolves reproduced.

255 In the last few years, the population numbers of Isle Royale wolves have declined  
256 dramatically and there are only two wolves remaining, a male (M183) and a female (F193), in  
257 early 2018. These two adults are very closely related and are both father and daughter and half  
258 siblings because they have the same mother F160. They are in fact the most closely related pair  
259 of the four males and four females that were present in the population in 2013 (Hedrick *et al.*,  
260 2014). The expected inbreeding coefficient of an offspring from them was the highest of any  
261 possible pair at 0.438 and the expected relatedness between them is 0.734 (Hedrick *et al.*, 2017).  
262 Reflecting the reduction in M93 ancestry from the peak discussed above, the expected M93  
263 ancestry for M183 is 0.375 and for F193 is 0.3125.

## 265 Discussion

### 267 Genetics of the Isle Royale wolf population

268  
269 The decline of the Isle Royale wolf population, and now for all intents and purposes its imminent  
270 extinction, provides a detailed case study of how genetic changes can result in the extinction of a  
271 population. Because of the detailed examination of the Isle Royale wolf population, important  
272 genetic factors resulting in its imminent extinction have been documented and discussed here.  
273 First, because of the relative isolation of the population from immigrants and its relative small

274 size, the amount of genetic variation was significantly reduced compared to its source population  
275 and estimates of inbreeding levels were large,  $f = 0.322$  from microsatellites, and  $f = 0.278$  and  
276  $F_{ROH} = 0.370$  from two approaches using estimates from genomic data. In addition, the  
277 documented very high rate of bone malformations indicated inbreeding depression.

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

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

295 The putative pup of M183 and F193 seen in 2015, had an expected inbreeding coefficient  
296 of 0.438, but its actual inbreeding coefficient could have been considerably higher (or lower)  
297 because of the large 95% confidence limits around this pedigree estimate (0.311 to 0.565)  
298 (Hedrick *et al.*, 2017). This pup had an abnormal phenotypic appearance with a quite unusual,  
299 short tightly curled tail, appeared to have an unusual posture, and was relatively small. Further,  
300 field observations suggest that this offspring was short lived and died as a pup (Peterson &  
301 Vucetich, 2016). These malformations and the pup's short life suggest the negative impact of  
302 inbreeding depression on its phenotype and survival.

303 Genomic estimates of inbreeding based on analysis of runs of homozygosity identified  
304 with many thousands of SNPs are expected to be higher than inbreeding predicted from our

305 pedigree because of common ancestry of founders F99 and F67 in resident ancestors that are not  
306 included in the pedigree. For example, for the seven animals in which there is both a pedigree  
307 estimate of inbreeding  $f$  and  $F_{ROH}$ , the mean  $f$  is 0.091 and the mean  $F_{ROH}$  is 0.332 (Robinson *et*  
308 *al.*, 2018). The difference, 0.241, gives an estimate of the increase expected in inbreeding from  
309 unknown identity-by-descent found by genomic analysis over that predicted from the known  
310 pedigree. In other words, pedigree-based measures of inbreeding can provide basic guideposts,  
311 but realized values are likely higher due to unknown common ancestry.

312

### 313 **General relevance**

314

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

326 There have been a number of important cases where genetic rescue has resulted in  
327 substantial population recovery. In most of the well-known examples of genetic rescue, the  
328 population numbers initially increased and in general the populations appear to have higher  
329 viability than before the natural or artificial genetic rescue but other factors now appear to be  
330 limiting population numbers in some cases. For example, the translocations of prairie chickens  
331 (Westemeier *et al.*, 1998) appears to have been effective in increasing fitness and genetic  
332 variation and the population initially increased. However, the population is now limited by  
333 suitable habitat (Bouzat *et al.*, 2009) and the outlook is not as good. In the isolated Swedish  
334 population of adders (Madsen *et al.*, 1999), translocation initially resulted in enhanced  
335 population growth (Madsen *et al.*, 2004). However, the construction of human obstacles, a house

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

341 The introduction of Texas pumas into the Florida panther population resulted in dramatic  
342 population recovery (Hostetler *et al.*, 2010; Johnson *et al.*, 2010) although now the population  
343 appears potentially limited by available habitat and human-caused mortality. The outcome of the  
344 Florida panther genetic rescue might also be threatened by inbreeding and low effective  
345 population size in current and future generations (Hedrick, 2010). The natural introduction of  
346 one male wolf initially had important beneficial effects in Scandinavian wolves (Vilà *et al.*,  
347 2003) although a subsequent analysis documented significant inbreeding depression (Liberg *et*  
348 *al.*, 2005). Since then, two male wolves naturally migrated into the population and three wolves  
349 were translocated into the population from northern Sweden, resulting in both a lower inbreeding  
350 coefficient and higher reproductive success (O. Liberg, personal communication). Detailed  
351 examination of genetic rescue in Scandinavian wolves demonstrated that offspring of immigrants  
352 had higher breeding success than resident inbred individuals (Akesson *et al.*, 2016). Crosses  
353 between lineages in Mexican wolves resulted in higher fitness (Fredrickson *et al.*, 2007),  
354 numbers in the reintroduced population increased, but have plateaued in recent years. As yet  
355 there has been no detailed analysis determining how much genetic rescue or other factors, such  
356 as supplemental feeding of denning females, influenced this initial population increase and  
357 recent plateau.

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

367 The discussion of the genetic impacts on extinction in Isle Royale wolves illustrates a  
368 potential problem relying on a single genetic rescue event as an approach to overcoming  
369 inbreeding depression. Moreover, persistence of the Isle Royale population throughout its seven-  
370 decade existence was likely supported by multiple genetic rescue events (Hedrick *et al.*, 2014).  
371 In other words, although genetic rescue can alleviate problems associated with inbreeding  
372 depression over the short term, it can also generate problems itself, ones that in the case of the  
373 Isle Royale wolf population has resulted in its imminent extinction. Thus, the successful  
374 conservation of critically small populations would likely depend on alleviating the cause of  
375 having become critically small, such as habitat restoration, or periodic re-application of genetic  
376 rescue. The case study reported here is particularly significant because few documented  
377 instances of genetic rescue and the ensuing changes have been observed long enough or in  
378 sufficient detail to understand both the beneficial and detrimental effects of genetic rescue.  
379 Clearly, many additional case studies will be required before an overall perspective is developed.

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

## 386 **Acknowledgements**

387  
388 We appreciate the suggestion to write this article by Erik Olson and comments by Chris Funk,  
389 Bob Wayne, and several anonymous reviewers. We acknowledge support from the National  
390 Science Foundation (DEB-1453041), National Institute of Health (R35GM119856), National  
391 Park Service (CESU Task Agreement No. P11AC90808), National Geographic Society and a  
392 McIntyre-Stennis Grant (USDA-Nifa #1004363). R.O.P thanks the Robbins Chair in Sustainable  
393 Management of the Environment for partial support.

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

561  
 562

	Minnesota	Before 1998	$f$
Heterozygosity* (microsatellites)	0.634	0.430	0.322
Heterozygosity** (SNPs)	0.00160	0.00116	0.278
$F_{ROH}$ **	0.158	0.370	0.212***

563

564 \*These values are from Hedrick et al. (2014)

565 \*\*These values are calculated from the raw data in Robinson *et al.* (2018).

566 \*\*\*This  $f$  value is the difference in  $F_{ROH}$  between the Isle Royale sample prior to 1998 and the  
 567 Minnesota sample.

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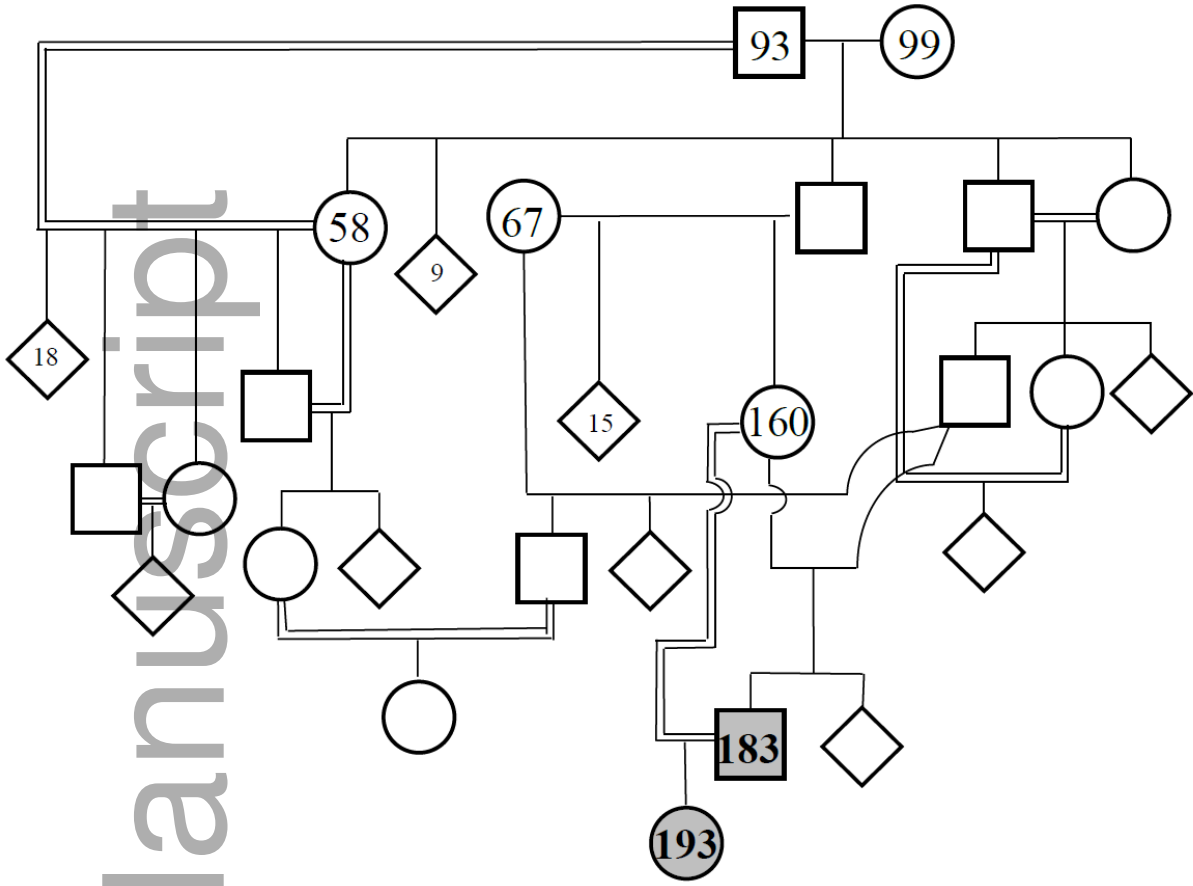
573 **Figure 1** Photo of the two remaining wolves on Isle Royale observed in 2017 with the female  
574 F193 below snarling at the male M183 above in response to his courtship advances.

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578  
579 **Figure 2** Pedigree showing the remaining two wolves, M183 and F193, as shaded and their  
580 known ancestors, M93, F99, and F67, in the Isle Royale population. Double lines indicate  
581 matings between relatives, squares indicate males, and circles indicate females. Notice that F160  
582 is the mother of M183 and F193 and that M183 is also the father of F193. Diamonds indicate  
583 multiple progeny, for example, the diamond on the left indicates 18 of the progeny from M93  
584 and his daughter F58.

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