RESEARCH ARTICLE

Genetic rescue in Isle Royale wolves: genetic analysis and the collapse of the population

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Abstract While genetic rescue is known to benefit population viability, the duration of that benefit is poorly understood. We document what appears to be the waning benefit of genetic rescue after approximately 2–3 generations for the wolf population in Isle Royale National Park. The fitness benefit of genetic rescue declined because of inbreeding and population abundance declined when the inbred individuals exhibited low reproduction and survival. Only detailed studies of other cases will reveal what aspects of these dynamics represent general features of genetic rescue. We also present evidence indicating that numerous past immigration events have likely gone undetected. This finding is of particular significance because the Isle Royale wolf population has maintained good population viability for decades even though it was small and thought to be isolated from the mainland population of wolves. Past gene flow also suggests that human-assisted gene flow is necessary to conserve the ecosystem services associated with predation, since climate warming has reduced the frequency of ice bridges and with it the only opportunity for unassisted gene flow.

Keywords Ancestry · Gene flow · Heterozygosity · Inbreeding · Pedigree · Relatedness

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Introduction

Endangered species often face significant genetically related threats, including lowered fitness due to loss of genetic variation, increase of detrimental variants, and inbreeding. One escape from 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). Genetic rescue has resulted in dramatic population recovery when Florida panthers (Johnson et al. 2010), adders (Madsen et al. 1999), bighorn sheep (Hogg et al. 2006) and prairie chickens (Westemeier et al. 1998) from other populations were introduced into low fitness populations. The natural introduction of one male wolf had important beneficial effects in Scandinavian wolves (Vilà et al. 2003) and wolves on Isle Royale in Lake Superior (Adams et al. 2011).

Because many populations have become small and isolated in recent decades due to human impacts, genetic rescue will probably become a natural phenomenon or management action of great significance. However, because many of the known examples are from very recent years, the longer term impact of genetic rescue has not been documented or examined. In fact, Hedrick and Fredrickson (2010) advocated thorough monitoring after the introduction of outside individuals to determine how long the initial positive effect remained and if any possible negative side effects, such as lower effective population size or inbreeding and subsequent lowered fitness, resulted.

The Isle Royale wolf population has already offered some very important lessons and insights about genetic rescue. In particular, genetic rescue from the immigration of single male resulted in a "genomic sweep" and the ancestry of this individual increased quickly to over 50 %

in the population (Adams et al. 2011). Also, because genetic rescue took place during a period of low prey (moose) numbers, the wolf population size did not increase over that before the genetic rescue (Hedrick et al. 2011).

Here we present further analysis of this case study that helps better understand what may be some general features of genetic rescue over time. In Adams et al. (2011), we concluded the immigration of a single male increased the fitness in the population and implied that this would be beneficial for the population. However, that benefit might have been temporary because in the last several years the population has greatly declined and the extraordinary initial success appears to have subsequently diminished in its favorable impact. Here, we conduct a more thorough analysis of the genetic impact of this genetic rescue event and extend that analysis forward in time to the present day to provide a more complete understanding of genetic rescue as an example for other studies.

Study system

The Isle Royale population was founded about 1950 by wolves from the mainland population (about 20 km away at the nearest point) living northwest of Isle Royale and it has been assumed that the Isle Royale wolf population was completely isolated from this population. For over 50 years on Isle Royale, both wolves and moose, their main prey, have been monitored and studied (Peterson et al. 1998; Vucetich and Peterson 2004). From genetic examination of scats, it was discovered in 2009 that a male wolf known as M93 (M indicates male) migrated from nearby mainland Ontario, Canada in 1997, probably across the ice bridge present that year (Adams et al. 2011). He was behaviorally dominant over resident wolves and his pack and first mate and their descendants quickly dominated the genetic ancestry of the population. By 2008, 59.4 % of the genetic ancestry in the population was from him (see below), resulting in a genomic sweep of the population. Although this success was a strong indication of increased fitness from genetic rescue, the environment during this period was poor because of deteriorating prey base of moose and population numbers did not increase substantially (Hedrick et al. 2011). From 2005 on, all the ancestry in the Isle Royale population was descended from three individuals, the male immigrant M93), and two females, F99 (F indicates female) his first mate, and F67, another female population resident.

In the last few years, the population numbers of Isle Royale wolves have declined and in 2012 and 2013, there were only 9 and 8 wolves, respectively, the lowest numbers ever recorded. The year 2012 was also remarkable for

being the first year since records were kept (1971) that no reproduction was detected.

Methods

We constructed a pedigree for the years, 1998–2013, based on 18 microsatellite loci that were derived from samples of feces and blood of wolves. For methodological details, see Adams et al. (2011). Our analysis here is based on temporal trends in a variety of genetic indicators derived either from this pedigree or from microsatellite heterozygosity. In particular, we calculated the proportion of ancestry (a_i) from each of the i wolves with known descendants from 1999 on. To do this for each individual offspring, half the ancestry was assigned to the known male parent and half to the known female parent. The inbreeding coefficient was calculated using the additive approach (Ballou 1983). In addition, the proportion of the inbreeding coefficient attributable to each of the known ancestors for the pedigreed population was determined using gene-drop simulation (MacCluer et al. 1986).

The observed individual heterozygosity (H_{Oi}) for 18 microsatellite loci was calculated for nearly all of the 99 individuals in the pedigree (four individuals had genotypes for less than 18 microsatellite loci and two individuals first seen in 2012 were not genotyped for these same loci). The expected individual heterozygosity for a given year was calculated as the product of the ancestry from founder i in that year, the observed heterozygosity of that founder, and the complement of the inbreeding coefficient ($1-f$) in that year as

$$H_E = (1 - f) \sum a_i H_{O.i} \qquad (1)$$

To better understand why there was no reproduction in 2012, we analyzed the relatedness between potential mates. A new measure of relatedness that takes into account past inbreeding (Hedrick and Lacy 2014), can be calculated using the different modes of identity-by-descent of four homologous genes possessed by two individuals (Jaquard 1971; Lynch and Walsh 1998). Given the probability of the nine different identity-by-descent modes Δ_i, the new measure of relatedness between individuals x and y is

$$r_{xy} = \Delta_1 + \Delta_7 + \frac{3}{4}(\Delta_3 + \Delta_5) + \frac{1}{2}\Delta_8 \qquad (2)$$

We compare this to another measure of relatedness (Wright 1922) that also takes into account past inbreeding, generally known as the coefficient of relationship (Crow and Kimura 1970)

$$r_{xy}^* = \frac{2f_{xy}}{\left[(1 + f_x)(1 + f_y)\right]^{1/2}} \qquad (3)$$

Fig. 1 Pedigree for Isle Royale wolves where *squares* indicate males, *circles* indicate females, and the number in them are the identification number of an individual. *Diamonds* indicate other progeny with the number in them indicating the number of progeny. *Shaded* individuals indicate the ones known alive in 2012 and *double lines* indicate matings between first-degree relatives
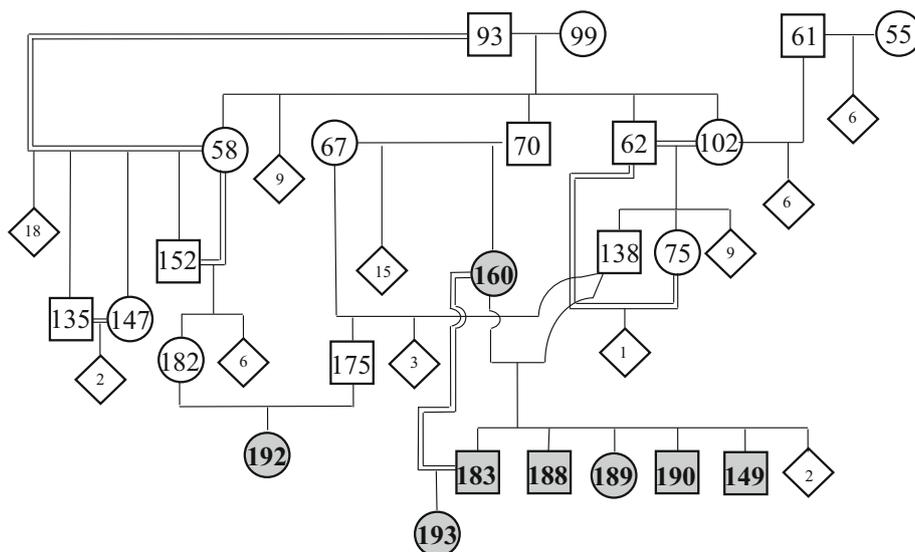
where f_x, and f_y, are the inbreeding coefficients in individuals x and y, and f_{xy} is the inbreeding coefficient of an offspring from individuals x and y.

In an isolated population, the expected heterozygosity in generation t (H_t) is predicted to decline as a function of the effective population size N_e and the number of generations from an initial value of H_0 as

$$H_t = H_0 \left(1 - \frac{1}{2N_e} \right)^t \qquad (4)$$

(Hedrick 2011). The influence of gene flow from outside the population can be incorporated using the island model of Wright (1940) as

$$H_t = 1 - \left[\frac{1}{2N_e} + \left(1 - \frac{1}{2N_e} \right)(1 - H_{t-1}) \right](1 - m)^2 \qquad (5)$$

given that the rate of gene flow per generation into the populations is m.

Results

Pedigree analysis and recent genetic change

Figure 1 gives the updated pedigree through 2013 and some salient, context-providing features of that pedigree are the following. In 2012, there were nine individuals alive and eight were identified genetically (indicated by shaded symbols in Fig. 1). The four males and female F189 present in 2012 are full sibs. Ancestry from female F67 continues in the population because F160 is the daughter of F67. Eight individuals were identified from aerial surveys in 2013 and seven of them were detected through their
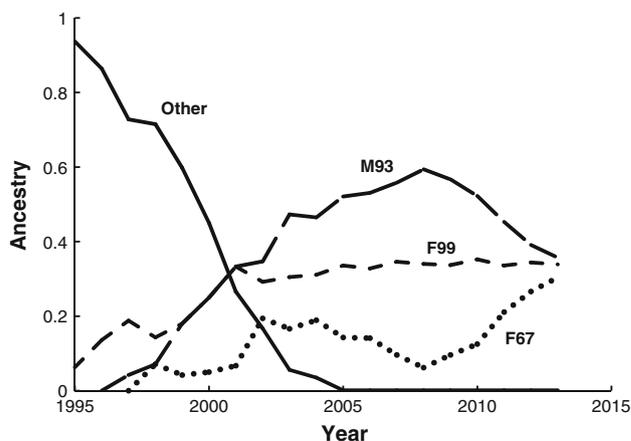

Fig. 2 Ancestry over years in the Isle Royale wolf population descended from M93, F99, F67, and Other wolves

fecal DNA. These wolves were the same as those detected in 2012, except M183 was not detected.

In the early years of the pedigreed population, ancestry from three wolves (male immigrant M93, his first mate F99, and female F67) generally rose, while the ancestry from other wolves (including M61 and F55) declined to zero by 2005 (Fig. 2). Within a decade (~ 2.5 generations) of the arrival of M93, his ancestry had risen to 59.4 % in 2008, greater than the ancestry of all other individuals combined. His ancestry rose to such a high level in part because he mated with his own daughter (F58) to produce 21 offspring. Between 2008 and 2013, the ancestry of M93 declined, while the ancestry of F67 increased and the ancestry of F99 remained about constant. By 2013, the ancestry of those three wolves had become approximately equal.
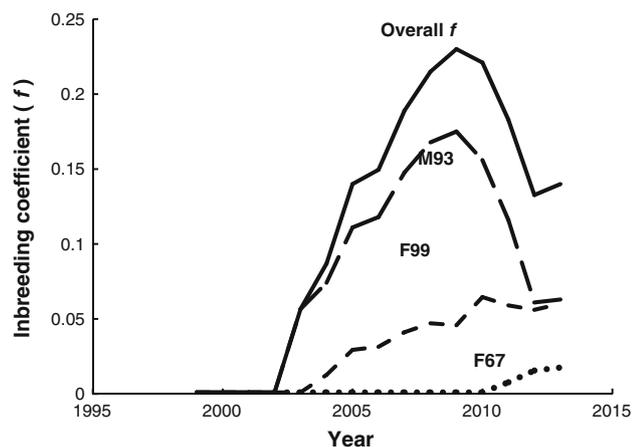
Fig. 3 The overall level of inbreeding (*f*) in the Isle Royale wolf population over time and the identity-by-descent attributable to wolves M93, F99, and F67

Table 1 The matings between first-degree relatives, the inbreeding coefficient of their progeny (some of these parents were already inbred), and the number of progeny produced (seen in the annual survey in January and February) from each mating

Father	Mother	Type of first-degree mating	Inbreeding coefficient	Number progeny
M93	F58	Father–daughter	0.25	21
M152	F58	Mother–son	0.375	6
M183	F160	Mother–son	0.3125	1
M135	F147	Brother–sister	0.375	2
M62	F75	Father–daughter	0.375	1
M62	F102	Brother–sister	0.25	11

Table 2 The nine wolves with an inbreeding coefficient of 0.375 due to two consecutive generations of matings between first-degree relatives, the year they were first and last seen in the annual count in January and February (birth and death years here, respectively), and the matings in these two generations

Wolf	Generation 1	Generation 2	Birth	Death	Life span
M177	Brother (M62)–sister (F102)	Father (M62)–daughter (F75)	2007	2008	2
M181	Father (M93)–daughter(F58)	Mother (F58)–son (M152)	2008	2008	1
F182	Father (M93)–daughter(F58)	Mother (F58)–son (M152)	2008	2010	3
M179	Father (M93)–daughter(F58)	Brother (M135)–sister(F147)	2008	2010	3
F180	Father (M93)–daughter(F58)	Brother (M135)–sister(F147)	2008	2009	2
M141	Father (M93)–daughter(F58)	Mother (F58)–son (M152)	2009	2010	2
F184	Father (M93)–daughter(F58)	Mother (F58)–son (M152)	2009	2011	3
F185	Father (M93)–daughter(F58)	Mother (F58)–son (M152)	2009	2011	3
M187	Father (M93)–daughter(F58)	Mother (F58)–son (M152)	2010	2011	2

The basis for using years of detected in samples of fecal DNA as a basis for estimating lifespan is given in Marucco et al. (2012)

The level of inbreeding in the pedigreed population started to increase in 2003 and reached a maximum in 2009 of 0.230 (Fig. 3). In the past several years, the level of inbreeding has declined, reaching levels of 0.133 and 0.140 in 2012 and 2013, respectively. This decline has resulted primarily from the short lives and low reproductive contribution of the highly inbred wolves in the population (see below).

The inbreeding (identity-by-descent) experienced by the population can be attributed to either M93, F99, or F67 (Fig. 3). A very large proportion of the inbreeding in the early years is attributable to the immigrant M93, primarily because of the highly successful mating of M93 with his daughter F58. This father-daughter mating resulted in identity-by-descent from M93 but not from F99. For example, in 2009, 76 % of the inbreeding was from M93 and only 24 % from F99. Recently in 2012 and 2013, the level of inbreeding from M93 and F99 was nearly identical. Only since 2011 was there any inbreeding attributable to F67.

Detailed inspection of the pedigree reveals additional insight. In particular, there have been six matings between first-degree relatives, two father-daughter matings, two mother-son matings, and two brother-sister matings, which have produced progeny (Table 1). Overall these matings between close relatives produced 42 offspring, 44.7 % of the known 94 progeny produced in the pedigreed population.

Much of the decline in overall inbreeding level from 2009 to 2013 (Fig. 3) is explained by the death of nine wolves with an inbreeding coefficient of 0.375, the result of two consecutive generations of close inbreeding (Table 2). All of these wolves had short lifespans (mean of 2.33 years) and all of them had died by 2011. Specifically, of the wolves recruited into the pedigree between 2009 and 2011, there is a two-fold difference in *f* between the seven alive in 2012 (0.152) and the six not alive in 2012 (0.292). None of these highly inbred wolves reproduced.

The increase in the ancestry of F67 and concomitant decrease in the ancestry of M93 is explained by inspecting the pattern of inbreeding that occurred between 2008 and 2013. Because the male immigrant M93 is unrelated to resident females F99 and F67, any offspring between them have an inbreeding coefficient of zero. With the recent increase of the ancestry of F67, some matings between individuals with only F67 ancestry and with only M93 and

Table 3 The inbreeding coefficient f_{xy} of an offspring from a mating between male x and female y, the inbreeding coefficient f_y of the female (the males all have $f_x = 0.125$), the relatedness, r_{xy}, of Hedrick and Lacy (2014), and the coefficient of relationship, $r_{xy}*$, of Wright (1922) for the four males and four females known to be present in 2012

Male (x)	Female (y)	f_y	f_{xy}	r_{xy}	$r_{xy}*$
M149, M183, M188, M190	F160	0.000	0.312	0.595	0.589
M149, M183, M188, M190	F189	0.125	0.344	0.617	0.611
M149, M183, M188, M190	F192	0.125	0.219	0.396	0.389
M149, M188, M190	F193	0.312	0.328	0.567	0.540
M183	F193	0.312	0.438	0.734	0.720

F99 ancestry have resulted in offspring with inbreeding coefficients of zero, consequently resulting in a decline in the average inbreeding in recent years.

Insight about mating behavior and reproduction in 2012 depends on understanding the relationships among individuals that were alive in 2012. In particular, the four males present in 2012 were full sibs (Table 3). The four females were F160 (mother of the full sibs), F189 (a full sib of the males), and two other females F192 and F193. All four full-sib males are equivalent when mating with females F160, F189, and F192. However, because M183 was the father of F193, a potential mating between them requires separate evaluation (see below).

If one of the four males were to have mated with F160 or F189, or if one of the three males excluding M183 were to have mated with F193, then the inbreeding coefficient of their offspring (f_{xy}) would be greater than 0.3 (Table 3). The high f_{xy} value from F160 would be because she is the mother of the four full-sib males, and the high f_{xy} value from F189 would be because she is a full sib of the four males. If F192 were to have mated with any of the four males, their progeny would have a lower inbreeding coefficient ($f_{xy} = 0.219$). The lower value of f_{xy} for an offspring of F192 is because she is not a descendent of F160 and is the granddaughter of F67. Finally, if M183 were to have mated with his daughter F193, their progeny would have a higher inbreeding coefficient ($f_{xy} = 0.438$).

Of the 16 possible matings that could have occurred in 2012, four represent matings between the least related wolves and matings that would have produced offspring with the lowest f (matings between F192 and any of the males, see Table 3). In fact, the F192 and M190 pair ($r_{xy} = 0.396$ and $r_{xy}* = 0.389$) were the only pair of wolves to display signs of courtship and mating behavior during the 2012 mating season (February). Nevertheless, we were unable to detect any sign of any pups having been
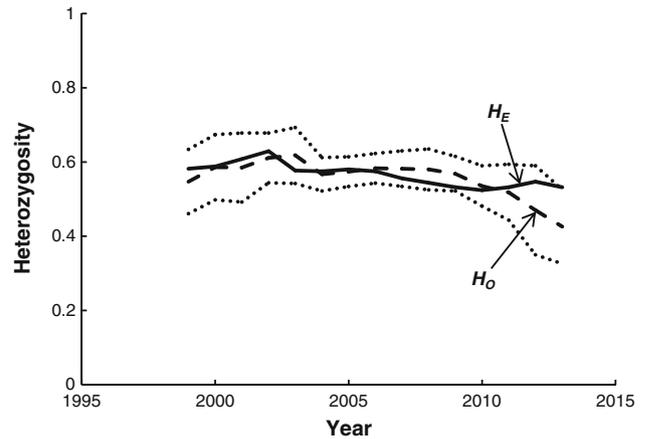

Fig. 4 The observed heterozygosity (H_O) (*broken line*) for 18 loci for Isle Royale wolves from 1999 to present and 95 % confidence limits (*dotted lines*) and the expected heterozygosity (H_E) (*solid line*) based on the observed heterozygosity of the five ancestors, their ancestry, and level of inbreeding for each year

born in 2012. However, aerial surveys conducted in January, 2014 suggested that this pair had given birth to two or three pups that had survived at least to 9 months of age (Vucetich and Peterson 2014).

Observed and expected microsatellite heterozygosity

The observed heterozygosity is relatively stable for the early years, 1999–2006, with a mean value of 0.589 (Fig. 4). More recently, however, H_O declined to 0.426. The variance and standard errors of the observed heterozygosity among individuals was influenced both by the variance in inbreeding among individuals and the variance in observed heterozygosity among the five ancestors of the pedigreed population (the observed individual heterozygosities of the ancestors were $H_{O93} = 0.833$, $H_{O99} = 0.500$, $H_{O67} = 0.500$, $H_{O61} = 0.444$, and $H_{O55} = 0.389$). Expected heterozygosity was a good predictor of H_O throughout most of the study period. However, by 2013, H_O had become significantly lower than H_E (Fig. 4).

High heterozygosity in the Isle Royale population

The Isle Royale population would have been expected to have lost most of its original heterozygosity if it had been isolated throughout the approximate 12 generations (assuming the generation length is 4 years, see Peterson et al. 1998 for justification) that passed from the time of its founding around 1950 until the arrival of M93 in 1997. More precisely, 81.7 % of the original heterozygosity is expected to have been lost (Fig. 5), according to the estimate for N_e of 3.8 (Peterson et al. 1998) based on Eq. (4).

By contrast, the population appears to have lost only 32.2 % of its heterozygosity. This estimated loss is based on
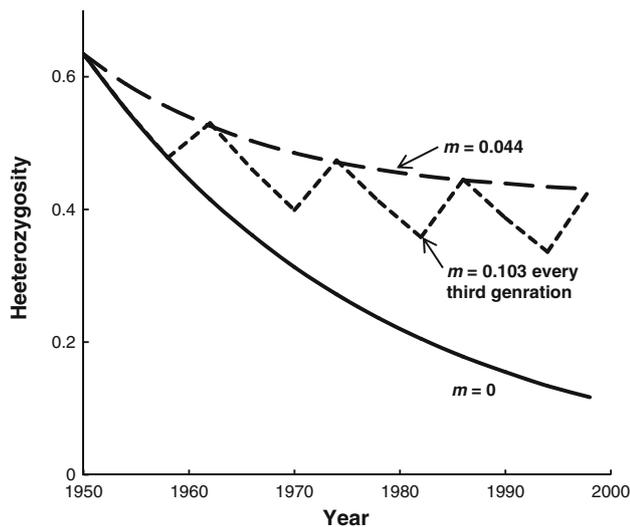
Fig. 5 The expected change in heterozygosity when there is no gene flow ($m = 0$), there is a constant rate of gene flow each generation ($m = 0.044$), and there is gene flow every third generation of $m = 0.103$

Table 4 Movements of wolves and other canids between Isle Royale and the mainland by year from the 1940s to 2013, characteristics of the migrants and whether they reproduced

Date	Description of animals	Reproduction	Citation
Late 1940s	Founding pair (or more)	Yes	Mech (1966)
1967	Four black wolves	Yes	Wolfe and Allen (1973)
1977	Wolf	Chased off island	Peterson (1979)
1980	Wolf (black? pup)	Yes (implied)	This study
1996–1998	Coyote-like canid	No (lone animal)	This study
1997	Wolf (M93)	Yes	Adams et al. (2011)

comparing the mean observed heterozygosity for 20 wolves on Isle Royale before 1998 (0.430 ± 0.022) to the mean observed heterozygosity for 35 mainland wolves from near the Minnesota–Canadian border (0.634 ± 0.020), which represents the source population of Isle Royale wolves.

A possible explanation for the discrepancy is undetected gene flow. For example, if the rate of gene flow had been 0.044 each generation, then heterozygosity is expected to have declined to the value observed in 1998 (Fig. 5). This rate of gene flow represents about 1 in 23 individuals being a migrant, which corresponds to about one migrant per generation, because the mean population size on Isle Royale for the period 1959–1998 is 23.4 individuals. The observed heterozygosity could also have resulted from higher levels of gene flow occurring less frequently, such as $m = 0.103$ every third generation which corresponds to about 2.3 migrants every third generation (Fig. 5).

The possibility of undetected gene flow prompted us to review field observations from the four decades prior to the arrival of M93 for clues suggesting the occurrence of such events (Table 4). First, several wolves, including four that were black, arrived over an ice bridge in 1967. One of the black wolves was observed living in 1968 in the single pack that comprised the population at that time. Behavioral observations indicated that he subsequently became the alpha male in 1971 and 1972 (Wolfe and Allen 1973). Other field observations indicated that two pups had been recruited into the population in 1971 and another four in 1972 (Peterson et al. 1998). Because no black pups were observed, the black wolf was presumed, at the time, not to be the father. Contemporary understanding for the inheritance of coat color in wolves (Anderson et al. 2009),

however, indicates that it would not be unexpected to observe only gray pups from the mating between a gray mother and a black father, if that father had been a heterozygote for the gene determining coat color. About two generations later, in the summer of 1980, a National Park Service employee photographed a wolf pup that also might have been black. After this photograph is retrieved from the Park's archives and inspected, we are likely to know more about this event.

These field observations of black wolves are noteworthy because the arrival of M93, a gray-colored wolf, was only detected from genetic observations made more than a decade after his arrival (Adams et al. 2011). Because of his gray appearance, his arrival would have been undetectable from field observations alone. Black wolves are uncommon in the Great Lakes region, representing approximately only approximately 3 % of the population (Mech and Frenzel 1971, Mech and Paul 2008). The rarity of black wolves and the detection of two, and possibly three, black wolves in the four decades of observation prior to 1998 suggests the plausibility of additional undetected gene flow involving gray-colored wolves.

Gene flow is possible only in the presence of ice bridges that form during some winters. These bridges are sometimes present for a few days and other times they persist for several weeks. Travelling at a typical speed (Mech 1994), a wolf could cross an ice bridge in three to 6 hours. In 2008, two radio-collared wolves disappeared shortly after an ice bridge had formed (unpubl. field notes). Similarly in early 2014, female F189 disappeared after an ice bridge had formed and was subsequently was found dead on the mainland. A pack of wolves was also observed chasing a pack mate onto an ice bridge in 1977 and airborne observers followed the egress event halfway to the mainland (Peterson 1979).

During 3 years (1996–1998) following a period when ice bridges had been present in 1994, 1996, and 1997, a lone animal that appeared to be a coyote was observed on Isle Royale (unpubl. field notes). Populations of fox and coyote are thought to have been established on Isle Royale early in the 20th century by migrants that would have crossed an ice bridge but the coyote population was driven to extinction shortly after the wolf population was established in the late 1940s. Mech (1966) recounts several instances that likely involved individual wolves crossing ice bridges during the first half of the 20th century. Collectively, these observations further suggest the plausibility of undetected movement and consequent gene flow for wolves (or other canid species).

Inbreeding measures the level of identity by descent and if migrant and resident wolves did not have recent common ancestors, then their progeny could have an inbreeding level of zero. Further, it would potentially take some time for inbreeding to accumulate because the closest mating in wolves to produce inbred offspring is either a parent—offspring or a mating of siblings, both of which would take at least two generations (8 years). If there was an earlier unrelated migrant (like M93), then the inbreeding level could potentially decline back to low levels.

As a result, undetected gene flow should also influence the level of inbreeding the population had exhibited just prior to the arrival of M93. For that time, f had been estimated as 0.801, using an indirect estimator that assumed the absence of gene flow (Peterson et al. 1998; Adams et al. 2011). Another indirect, but useful, basis for estimating f before the arrival of M93 that accounts for having observed higher than expected heterozygosity resulting from undetected gene flow is

$$f_t = 1 - \frac{H_t}{H_0} \qquad (6)$$

Replacing H_t in this equation with the heterozygosity for Isle Royale wolves prior to 1998 (0.430) and H_0 with the heterozygosity for 35 wolves living near the Minnesota–Canadian border (0.634), yields $f_t = 0.332$ for the time prior to the arrival of M93. The expected pattern change in f given these two initial inbreeding points, and the known remainder composition of the population is given in Fig. 6.

Discussion

General conclusions

The success of immigrant M93 and his influence on the Isle Royale wolf population was remarkable. Within a generation of his arrival, the inbreeding coefficient had declined from 0.332 to 0.036 (Fig. 6). He sired 34 offspring, 21 of
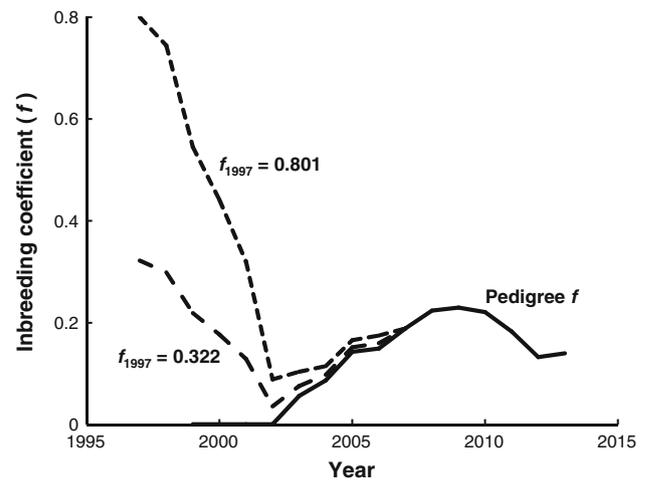
Fig. 6 The mean inbreeding coefficient (f) over time from the individuals in the known pedigree and that expected given that in 1997 $f = 0.801$ (as assumed in Peterson et al. 1998; Adams et al. 2011), and assuming that $f = 0.322$ as estimated from observed heterozygosity on Isle Royale and the mainland in 1997

which were with his daughter and within 2.5 generations, and his ancestry had risen to 59.4 % (Fig. 2). This high ancestry level is further supported by molecular genetic data showing that the only variant of the Y chromosome present in the population today was inherited from M93 (Adams et al. 2011). In addition, he was a heterozygote for two new alleles (allele 224 at locus FH2226 and allele 146 at locus C05.377) that increased to frequencies of 33 and 27 % in the population by 2009, bracketing the expected value of 28 % (= 56 %/2) expected from the pedigree for that year (Adams et al. 2011). This rise in ancestry is, to our knowledge, faster and greater than any previously documented for a wild vertebrate population and as a result, the impact of the immigrant represents a genomic sweep of the population.

Demographic benefits, such as increased rates of recruitment or survival that might have resulted from the arrival of M93 were masked by a severe collapse in food supply that coincided with his arrival (Adams et al. 2011). As a result, his impact on population fitness was indicated by the dramatic rise in his ancestry (Hedrick et al. 2011). In recent years, however, the ancestry of M93 has declined to 33.9 % and became approximately co-equal with two other individuals. That decline in ancestry coincided with a decline in mean inbreeding level (f) and observed heterozygosity (H_O) (Figs. 3, 4). Ordinarily, a decline in f is associated with an increase in H_O. However, if f declines as a result of increased ancestry from an unrelated individual with lower H_O, then the pattern we observed would be expected. Specifically, ancestry from F67 increased as ancestry from M93 decreased (Fig. 2), and F67 had a lower

H_O than did M93 ($H_{O67} = 0.50$ and $H_{O93} = 0.833$). This observation represents another possible mechanism by which f and H_O might not be strongly associated (Balloux et al. 2004).

Figure 5 and the estimated microsatellite heterozygosity of the population indicate that it had been highly inbred before the arrival of M93. The genomic sweep and rapid rise in ancestry of M93 in years following his arrival also indicated the population had low fitness. The more recent decline in his ancestry was associated with selection against his inbred descendants and resulted in an increase in ancestry from the individuals with lower fitness which had been present before his immigration (F99 and F67). That dynamic is likely associated with the decline in demographic performance for both wolf abundance and recruitment that occurred during the same period.

Ordinarily, one would not expect a decline in mean f (as occurred between 2008 and 2013) to be associated with a decline in demographic performance. Specifically, mean f rose prior to 2008 because a large portion of the individuals alive at that time were the result of two successive generations of close inbreeding ($f = 0.375$) but they also had high ancestry from M93. Those wolves had short lifespans, did not reproduce, and died between 2008 and 2011 (Table 2). Their deaths caused mean f to decline and contributed importantly to the decline in population abundance and recruitment rate.

The low fitness of those inbred wolves might have been attributable to the expression of recessive deleterious alleles inherited from M93. Any migrant from a large outbred population (like the Canadian wolf population) is expected to carry a number of such detrimental alleles. Given successive generations of close inbreeding, these alleles would have contributed to the decline of the population because those inbred wolves with high ancestry from M93 represented a large portion of the population. As examples in other wolf populations, both the Swedish wolf (Liberg et al. 2005) and the Mexican wolf (Fredrickson et al. 2007) populations were segregating for multiple detrimental variants that greatly reduced the fitness for inbred individuals. Bijlsma et al. (2010) observed similar dynamics of deleterious alleles in experimental populations of *Drosophila*.

That those inbred wolves represented a large portion of the population is a legacy of the success of M93. Being so successful and bringing about a genomic sweep, allowed for the possibility of recessive deleterious alleles to increase in frequency with little chance of being purged (Hedrick 1994). Ultimately, the apparently short-lived benefit of migrant M93 might have been associated with how very successful his lineage had become. His success was largely attributable to the degree the Isle Royale population had, at the time of the arrival of M93, been

suffering from low fitness. In any event, the beneficial effect of M93 appears to have begun waning about two or three generations after his arrival. The details of this short-lived benefit illustrate potential predictions about the nature of genetic rescue (Hedrick and Fredrickson 2010).

The apparent failure of the Isle Royale wolf population to reproduce in 2012 is remarkable for being the first time since such records have been kept (1971) that no signs of reproduction were detected. Recent genetic analyses offer new insight on that failure. In 2012, the population was organized into two social groups: the West-end Duo was a newly formed and young pair and failed to produce pups. Because those wolves are closely related ($r = 0.39$) and the inbreeding coefficient of their offspring would have been relatively high ($f = 0.22$), one would not be surprised if their reproductive output is lackluster. Nevertheless, that pair is still alive and their lifetime reproductive output remains to be seen.

The other wolves present formed the Chippewa Harbor Group, which was comprised of five individuals. In February, 2012, those wolves had not shown any signs of courtship or even signs of hierarchy that accompany courtship. We now know that this group was comprised of a mother (F160) and her four offspring. One might expect inbreeding avoidance given that every pairing of wolves in that group was a first-order relationship (full siblings or parent-offspring). Moreover, the alpha male of Chippewa Harbor Pack (M138) had died just 2–3 months prior to the 2012 mating season and the pairing of wolves M138 and F160 had successfully reproduced in previous years. M138 died prematurely along with two other wolves when they drowned in a flooded mine shaft. If M138 had not died (along with two other wolves), then the survival and recruitment rates in 2011–2013 would almost certainly have been higher, and the population would not have declined from 16 to 8 wolves. That mine shaft tragedy—an artifact of 19th century mining and consequently an anthropogenic influence on the population—appears to have been an important contributor to the current high risk of population extinction.

High heterozygosity

The higher than expected heterozygosity that we observed appears as though it can be explained by previously undetected gene flow (Fig. 5; Table 4). While inbreeding avoidance and selection for heterozygotes (against homozygotes) might, in principle, also explain higher than expected H, additional considerations suggest otherwise. First, prior analyses indicated that inbreeding avoidance is relatively weak in the Isle Royale population (Geffen et al. 2011). Moreover, matings between first-degree relatives represent 6 of the 11 reproductively successful pairings that

we observed in the pedigree, and 45 % of the 94 progeny that survived long enough to be recruited into the population were the result of first-degree matings. Much of the inbreeding resulted from immigrant M93 mating with his daughter (F58) and F58 mating with her son (M152). Overall, inbreeding avoidance appears not to have been strong enough to overcome the impact of small population size. One potential exception is the recent pairing of M190 and F192, involving the least related wolves present (Table 3).

Selection appears not to have consistently favored heterozygotes. For example, observed and expected heterozygosity were similar during the early years of the study period, and H_O declined relative to H_E during the most recent years (Fig. 4). Additional insight rises from considering temporal trends in mean inbreeding level, which increased as the ancestry of M93 increased; then both began to decline in 2008 (Figs. 2, 3). Because M93 had relatively high heterozygosity, those trends are consistent with selection for higher heterozygosity followed by selection against individuals with higher heterozygosity, but happened to be more inbred. Overall, selection appears to have been against individuals with high f. Moreover, f is not significantly correlated with microsatellite heterozygosity ($r = 0.105$, $P = 0.51$, for individuals with $f > 0$), in large part because M93 who was responsible for a large portion of inbreeding (Fig. 3) had relatively high heterozygosity. Because the impact of M93 appears unprecedented, these kinds of dynamics may be unusual. Selection for heterozygosity was reported in a study of 31 microsatellite loci for the inbred population of Swedish wolves (Bensch et al. 2006), but not in a subsequent analysis of 237 loci for the same population (Hagenblad et al. 2009).

Climate change

The genetic health of the Isle Royale population has very likely been maintained by periodic gene flow, which is only possible during winters in which an ice bridge has formed. Moreover, the frequency of ice bridges has steadily declined throughout the past five decades. The mean expected probability of observing an ice bridge during the 1960s was 0.67 while for the past decade, the mean expected frequency is only 0.16 (Vucetich and Peterson 2014). Lake Superior is expected to be largely ice free by 2040 (Austin and Colman 2007). The effect of anthropogenic climate change on the health of the Isle Royale wolf population has significant policy implications because Isle Royale is a National Park and one of the few places on earth inhabited by an un-persecuted top predator, an un-hunted large ungulate population, and a forest that is not commercially logged. Whether the National Park Service decides to actively conserve the wolves of Isle Royale through genetic rescue will set an important precedent for whether they will mitigate the threat

of climate change in instances where doing so is feasible (Vucetich et al. 2012, a, b).

Comparisons to other organisms

As we suggested in the introduction, because many populations have become small and isolated due to human impacts, genetic rescue will probably become of greater importance for rare and/or endangered species. In most other well-known examples of genetic rescue, the population numbers have increased and in general the populations appear to have higher viability than before the natural or artificial genetic rescue. For example, the translocations of prairie chickens (Westemeier et al. 1998) appears to have been effective in increasing fitness and genetic variation and the population initially increased, although now the population is struggling and appears to be limited by suitable habitat (Bouzat et al. 2009). In the isolated Swedish population of adders (Madsen et al. 1999), translocation initially resulted in enhanced population growth (Madsen et al. 2004). However, the recent construction of a house and a brick wall, unrelated to genetic rescue, have dramatically reduced population numbers (Madsen and Ujvari 2011). 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.

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 comm.). Crosses between lineages in Mexican wolves resulted in higher fitness (Fredrickson et al. 2007) and recently numbers in the reintroduced population have increased. However, as yet there has been no detailed analysis determining how much genetic rescue or other factors have influenced this population increase. 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).

These examples of genetic rescue suggest that its benefits can be mediated by other ecological factors. In particular, beneficial effects of genetic rescue were straightforward for the Swedish wolf population where habitat was not limiting and where genetic rescue occurred on multiple occasions. However, the effects of genetic rescue appear to have been limited in several other cases where habitat was limited or had become degraded. The Isle Royale case represents

another kind of complexity. Here, the beneficial effects of genetic rescue were not detectable because of a sudden diminishment in food supply that coincided with genetic rescue (Adams et al. 2011; Hedrick et al. 2011) and ultimately limited by the geographical constraints on the size of the population.

The case study reported here is significant because few documented instances of genetic rescue have been observed long enough or in sufficient detail to understand how long one can expect the beneficial effects of genetic rescue to persist. Clearly, additional case studies will be required before an adequate understanding is developed.

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