ECOLOGY

The far-reaching effects of genetic process in a keystone predator species, grey wolves

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Although detrimental genetic processes are known to adversely affect the viability of populations, little is known about how detrimental genetic processes in a keystone species can affect the functioning of ecosystems. Here, we assessed how changes in the genetic characteristics of a keystone predator, grey wolves, affected the ecosystem of Isle Royale National Park over two decades. Changes in the genetic characteristic of the wolf population associated with a genetic rescue event, followed by high levels of inbreeding, led to a rise and then fall in predation rates on moose, the primary prey of wolves and dominant mammalian herbivore in this system. Those changes in predation rate led to large fluctuations in moose abundance, which in turn affected browse rates on balsam fir, the dominant forage for moose during winter and an important boreal forest species. Thus, forest dynamics can be traced back to changes in the genetic characteristics of a predator population.

INTRODUCTION

Detrimental genetic processes, such as loss of adaptive genetic variation (evolutionary potential), expression of deleterious recessive alleles, and insufficient levels of heterosis, can adversely affect both the vital rates and extinction risk of small populations (*1*, *2*). Beyond population-level effects, nascent evidence is beginning to show how genetic processes may also affect ecological communities and ecosystem processes (*3*). For instance, heritable phenotypes in fish (*Poecilia reticulata*) are thought to influence ecosystem processes, such as nutrient cycling, because fish phenotypes exhibit different patterns of diet selectivity and excretion rates (*4*). In addition, the genetic diversity of host plants (*Quercus* sp.) is thought to be positively associated with the diversity of endophagous insect communities because insect species are often specific to certain host species or hybrids (*5*).

If a keystone species is affected by detrimental genetic processes which limit the species' ability to perform its ecological function (e.g., predation, pollination), then it could have ecosystem-wide consequences. For example, if the keystone species is a top predator, then detrimental genetic processes could lead to changes in heritable traits, such as body size, behaviors, or vital rates, that subsequently influence the impact of predators on prey populations and thereby the vegetative community upon which prey populations depend (Fig. 1). However, little attention has been given to assessing the community- or ecosystem-wide consequences of genetic processes in top-level carnivores, in part, because the data necessary to do so are difficult to collect. Nevertheless, such assessments are valuable given that many animal populations, including top-level carnivore populations, are becoming increasingly fragmented and isolated to the point that loss of genetic diversity and inbreeding are major concerns (*6*, *7*). Moreover, there are numerous examples of detrimental genetic processes, often linked to inbreeding, causing population declines in small and isolated populations [reviewed in (*8*)].

In this study, we assessed how the genetic characteristics of a top predator population, grey wolves (*Canis lupus*) in Isle Royale National Park (IRNP), affected species in other trophic levels. Specifically, we examined the association between ancestry, an indicator of changes in genetic characteristics of the wolf population, and predation rate, the most direct approach of quantifying the ecological role of a top predator (details below). We then show how the association between ancestry and predation rate subsequently led to large changes in the abundance of moose (*Alces alces*), the dominant prey of wolves in IRNP and the only large herbivore in this system. Those changes in moose abundance subsequently led to changes in browse rates on balsam fir (*Abies balsamea*), the dominant winter forage of moose and a common and important species in boreal forests. Those changes in browse rate have important consequences for forest ecology by affecting the recruitment of saplings for a tree species that provides food and shelter for a wide array of wildlife species (*9*).

The wolf population in IRNP represents a model system to assess community-wide impacts of genetic processes in a top predator because the wolf population has been studied intensively and genetic relationships among wolves have been documented for the last three decades (*10*). Below we summarize the salient findings of earlier research which documents how the genetic characteristics of the Isle Royale wolf population changed over time and how the population showed signs of severe inbreeding and inbreeding depression until a genetic rescue event occurred in the late 1990s (*11*, *12*).

Wolves colonized IRNP in the 1950s. However, by the mid-90s, the population had an estimated inbreeding coefficient of 0.81 ± SE 0.09, a low effective population size ($N_e < 4$), and lost approximately 32% of its genetic diversity (heterozygosity) compared to mainland wolf populations (*11*, *12*). Moreover, inbreeding resulted in high levels of homozygosity for strongly deleterious recessive mutations (*13*). Those declines in the genetic health of the population were accompanied by a sharp increase in the number of wolves (up to 58%) exhibiting congenital bone abnormalities which are known to be associated with inbreeding in domestic dogs and to cause pain, paresis, paralysis, and affect mobility (*14*).

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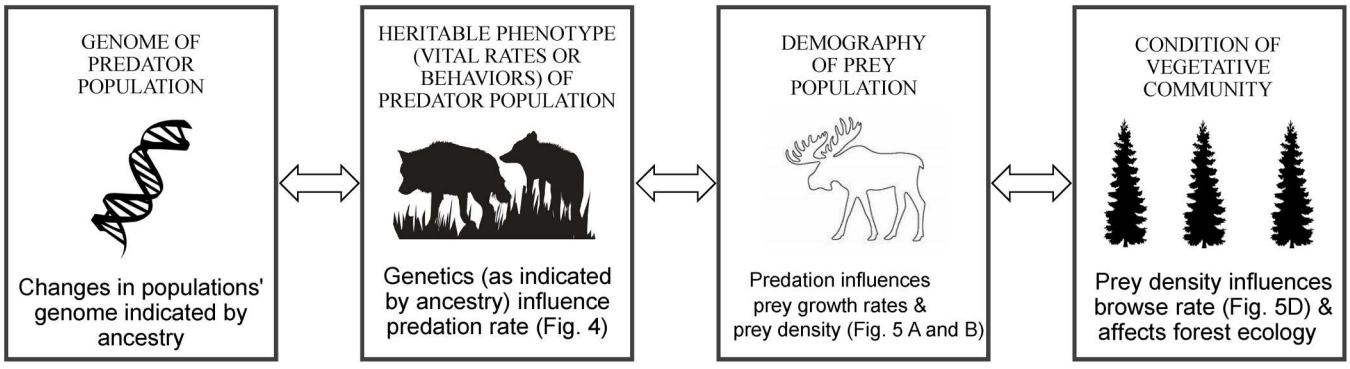

Fig. 1. Conceptual diagram of how predator genetics may influence communities. Abstracted chain of causation (upper text) from the genetic characteristics of a predator population (or keystone species) to widespread ecological effects. Evidence in support for that chain of events (lower text) for IRNP, where the ancestry of an immigrant wolf bolstered the wolf population's ability to kill prey (increased predation rates on moose), which led to changes in moose population growth rates and density and thereby moose browse rates on forest vegetation which ultimately changed the vegetative community.

In the late 90s, a male wolf, known as M93 (Fig. 2), migrated to IRNP across an ice bridge that temporarily connected Isle Royale to mainland Canada [for details, see the "Study system" section and (*11*, *12*, *15*)]. Shortly after M93's arrival, he became the breeding male of one of the three packs, which initially reduced levels of inbreeding in the population, and changed the genetic characteristics of the population [see figure 2 in (*12*)]. Specifically, M93 introduced several unique alleles into the population (not previously present in the native population) and genetic diversity (heterozygosity) increased back to levels that were comparable to mainland wolf populations (*12*). Thus, M93's arrival represented a powerful genetic rescue from the inbreeding depression that characterized the wolf population at that time (see also section S1) (*11*, *15*). However, the benefits of that genetic rescue started to wain approximately three generations later (after 2008) when inbreeding resumed. Specifically, M93 began mating with his daughter, and M93's offspring began to breed with each other (*12*). This inbreeding caused high mortality and low reproduction in M93's lineage, and the population declined to just two highly inbred individuals that were unable to successfully reproduce (for details see the "Study system" section and section S2). This combination of genetic, demographic, and behavioral data collected on wolves over several decades represents an unprecedented opportunity to investigate how genetic processes in a top predator influence predation rate and the subsequent impacts on prey dynamics and the prey's main food source.

Fig. 2. Immigrant male wolf M93. M93 immigrated to Isle Royale, presumably by crossing an ice bridge in 1997. M93 was identifiable throughout his life by his relatively large body size and distinctive light coat color. M93 is shown here, the light-colored wolf in front and center of the image. Photo credit: John A. Vucetich, Michigan Technological University.

Monitoring changes in the genetic characteristics of wild animal populations and how they influence population persistence or fitness related traits (e.g., reproductive success, and survival) is extremely challenging. Several different genetic statistics are commonly used, such as ancestry, the inbreeding coefficient, heterozygosity, effective population size, or the frequency of putative deleterious alleles (*15*–*17*). Although these genetic statistics are conceptually related, they may contain different genetic information about a population. Empirical evidence suggests that these genetic statistics are not always well correlated with each other or equally good predictors of fitness or population performance (*18*, *19*). The ability of genetic statistics to describe differences in population performance may depend partly on how they are estimated and also on aspects of the population's history and the precise mechanisms involved, such as how rapidly and recently populations have become inbred or whether strongly deleterious alleles have been purged in the past (*17*).

Given the history of the wolf population in IRNP and data available, changes in the genetic characteristics of the wolf population are best quantified using the ancestry statistic. Ancestry is a basic statistic in population genetics that can be calculated from a pedigree (for details see the "Genetic data" section) and captures information about how individuals in the population are related (*12*, *20*). More precisely, ancestry from a given individual, or group of individuals, refers to the portion of a population's gene pool which was inherited from that particular individual or group. For example, M93's ancestry was first detected in 1997, and over the next 10 years, his ancestry increased to a maximum value of 0.594 in 2008, which means that 59.4% of the population's gene pool was inherited from M93 (*11*). The rapid rise in M93's ancestry after 1997 reflects the fitness advantage of the immigrant's lineage over the native lineage of wolves (*11*). However, M93's ancestry declined after 2008 as the immigrant's lineage became increasingly and detrimentally inbred, such that his descendants suffered high mortality rates and low reproduction (see section S2). These two phases of

ancestry (a period of increase, followed by a period of decrease) highlight the value of assessing how ancestry may have affected ecological processes in different ways during those two periods of time (*21*). Other justification for focusing on ancestry is that it is the statistic most likely to reflect the influence of heritable traits (e.g., body size or aggressive behaviors) passed onto the population by M93, which may have influenced the wolf population's ability to kill prey. Furthermore, evidence from other small and isolated animal populations suggests that ancestry is an important predictor of fitness related traits, such as annual survival and reproductive success (*16*, *22*).

The ecological role of a top predator can be summarized using predation rate , which indicates the proportion of a prey (moose) population killed by predators and therefore represents a cause-specific mortality rate for the moose population. More precisely, predation rate is a synthesis of predation's key elements: kill rate which is the per capita rate at which predators acquire food, predator abundance , and prey abundance (*23*). Changes in the genetic characteristics of a predator population could influence predation rate via two main mechanisms:

1) Changes in the per capita kill rate which can be influenced by heritable traits, such as body size or specific behaviors, that influence a predator's success in hunting prey.

2) Changes in the survival and reproductive success of predators, which lead to changes in predator abundance. In principle, these demographic changes in the predator population could be due to the influence of heritable traits on kill rate or via some other genetic effects on predator vital rates. For example, cryptorchidism (undescended testicles) is a hereditary condition which reduces reproductive performance and has been observed in inbred carnivore populations (see also section S2) (*24*).

It is possible that the effect of genetic processes on either kill rate or predator abundance alone is too small to detect with statistical confidence but that the combined effect of genetics on both kill rate and predator abundance would become manifest with predation rate. Additional justification for focusing on predation rate is that prey population dynamics are more closely related to predation rate than to kill rate or predator abundance (*23*).

The study period for our analysis is 1997–2018, the period with the most detailed understanding of wolf genetics. Specifically, the study period begins the year that M93's ancestry was first detected and ends immediately before the National Park Service translocated wolves to IRNP to restore the wolf population (*25*). First, we used a path analysis to simultaneously evaluate the relationships between ancestry, predation rate (in winter), moose density, and browse rates in a single model, before using regression models to investigate each relationship in greater detail (see Materials and Methods).

RESULTS

The results of the path analysis supported our hypothesis that changes in the genetic characteristics of the wolf population were linked to changes in other trophic levels. More precisely, the path analysis revealed a strong positive relationship between ancestry and predation rate ($P < 0.001$); a strong negative relationship between predation rate and moose density ($P < 0.001$); and a strong positive relationship between moose density and browse rates on fir (Fig. 3).

Closer inspection of the relationship between ancestry and predation rate suggested it was phase dependent given that it was best described by different functions for the increasing and decreasing phases of ancestry (Fig. 4). Specifically, a phase-dependent model (i.e., linear model with separate slopes and intercepts for each phase) performed substantially better than a model with a single slope and intercept (ΔAICc = 25.3; see also fig. S1). The relationship between ancestry and predation rate also appeared to be nonlinear for the increasing phase, as indicated by the better performance of several nonlinear models (Table 1, see also fig. S2). The model taking account of both the phase-dependent and nonlinear features (depicted in Fig. 4) explained 87% of the variance in predation rate.

The ecological importance of the relationship between ancestry and predation rate is indicated by the influence of predation rate on moose population growth rates and ultimately moose abundance. Specifically, predation rate explained 49% of the interannual variation in the per capita growth rates of the moose population (Fig. 5A) and 76% of the interannual variation in moose density (Fig. 5B, see also table S1). Predation rate was still found to be the primary factor influencing moose population growth rates and density even after considering the potential role of other factors, such as winter severity (Fig. 5C and section S3).

The influence of predation rate on moose density also cascaded to influence browse rate on balsam fir (Fig. 5D). The relationship between moose density and browse rate was best characterized by an exponential model including a time lag of 3 years ($P < 10^{-3}$; Table 2). Moose density explained 68% of the variation in browse rates.

Associations between ancestry, predation rate, moose density, and browse rates were also evident in time series of those variables. Specifically, ancestry and predation rate rise, peak, and fall at about the same time, while moose density declines, bottoms out, and then rises (Fig. 6). Last, browse rates tended to decline from 2004 to 2010 and then increased after 2010, and moose density exhibited a similar pattern, except that the lowest moose density occurred 3 years earlier than browse rate (Fig. 6).

Table 1. Performance of models characterizing the relationship between ancestry and winter predation rate (mid-January to mid-March) during the increasing phase of M93's ancestry. The adjusted *R*-squared (R^2_{adj}) represents the squared correlation between the observed and predicted outcome values that has been adjusted to account for the number of predictors in the model. The root mean square error (RMSE) is the SD of the residuals and represents the model's prediction error (i.e., the average difference between the observed outcome values and the predicted outcome values). *k* is the estimated number of parameters in the model. Akaike information criterion (AICc) provides an indication of how well a model fits the dataset, which includes a penalty that is an increasing function of the number of estimated parameters in the model.

Model	R^2_{adj}	RMSE	Log-likelihood (*k*)	AICc
Linear	0.52	1.35	−18.94 (*k* = 3)	47.31
Generalize additive (GAM)	0.72	0.98	−15.42 (*k* = 4.06)	45.86
Exponential	0.58	1.27	−18.25 (*k* = 3)	45.92
Second-order polynomial	0.73	0.95	−15.06 (*k* = 4)	44.79

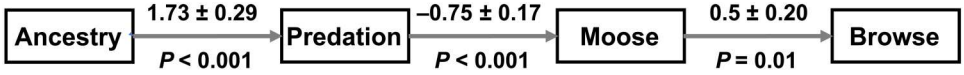

Fig. 3. Path analysis assessing the effect of predator genetics across multiple trophic levels. The genetic fitness of a predator (wolf) population, indicated by ancestry, was strongly correlated with predation rates on moose (estimated over a 2-month period in winter), which in turn led to changes in moose density and ultimately moose browse rates on forest vegetation. Numbers are the estimated coefficients with SEs and *P* values.

Table 2. Performance of models characterizing the relationship between moose density and browse rate under different time lags. The last model in this table is depicted in Fig. 5C. First, we built linear models to determine the most appropriate time lag. After identifying the most appropriate time lag, we investigated whether the functional shape of the relationship was best characterized by a linear or exponential function.

Time lag in years (functional form)	Slope	SE	*P* value	R^2_{adj}	ΔAICc
0 (linear)	0.12	0.05	0.03	0.30	9.06
1 (linear)	0.17	0.05	0.003	0.52	4.01
2 (linear)	0.20	0.05	0.003	0.53	3.95
3 (linear)	0.22	0.05	0.001	0.59	2.17
3 (exponential)	0.05	0.01	0.001	0.65	0

DISCUSSION

Our study adds to a small list of documented connections between ancestry and indicators of fitness and population performance in wild animal populations. For example, ancestry has been linked to annual survival in Florida panthers, *Puma concolor coryi* (*16*), and reproductive success in Pacific pocket mice, *Perognathus longimembris pacificus* (*22*). However, our study is unique for demonstrating that changes in a population's genetic characteristics, as indicated by ancestry, can also affect the dynamics of species in other trophic levels (Fig. 3). Previous work indicates that although a keystone species may be present, it may be unable to perform its ecological function (i.e., functionally extinct) due to various anthropogenic threats (*26*). Our study adds further insight by indicating that a keystone species' ecological function may also be limited by genetic processes. The generality of the concerns raised by these findings is indicated by observing that many keystone species exist at low population densities, making populations vulnerable to becoming isolated and suffering from deleterious genetic processes. Evidence indicates that isolation and habitat fragmentation, rising from a wide variety of common circumstances, adversely affect the genetics and fitness of animal populations (*6*, *27*). Many top predator populations around the world are becoming increasingly fragmented and isolated to the point that restricted gene flow is a major concern (*7*, *28*, *29*).

In the case of Isle Royale wolves, the patterns observed between ancestry and predation rate (initial increase in ancestry and predation rate, followed by a decline in ancestry and predation rate after 2008) likely result from the combination of several mechanisms. One potential mechanism is related to changes in the risk of wolves expressing deleterious recessive alleles. More precisely, earlier work suggests that before the arrival of the immigrant, the wolf population exhibited high levels of homozygosity for strongly deleterious recessive mutations, with the proportion of damaging homozygous genotypes being 38.4% higher in Isle Royale compared to nearby mainland wolf populations (*13*). However, the arrival of the immigrant (whose effect is measured by ancestry) may have initially reduced the risk of individuals expressing deleterious recessive alleles by introducing different genetic material into the population and increasing heterozygosity. Specifically, M93 is known to have introduced several alleles into the native Isle Royale population (i.e., alleles which were not present in the native population previously) and heterozygosity increased back to levels that were comparable to mainland wolf populations shortly after M93's arrival (*12*). It is possible that the decline in ancestry which occurred after 2008 may have been accompanied by a decline in the frequency of the unique alleles that M93 introduced into the population and could have led to an increase in homozygosity and the risk of expressing deleterious alleles.

A second potential mechanism may be related to genetically mediated changes in phenotypic traits, such as body size and aggressive behaviors, which are known to be highly heritable in wolves and influence aspects of individual fitness (*30*, *31*). Specifically, larger body size is advantageous for wolves preying on large prey (*32*) and for protecting their territorial boundaries (*33*). Aggression also influences territorial defense, social dominance, and ultimately reproductive success (*34*). Before M93's arrival, it is possible that inbreeding among the native Isle Royale lineage resulted in wolves with smaller body size given that inbreeding is known to reduce body size in many animal species, including wolves (*35*). M93 was notably larger than native Isle Royale wolves (Fig. 2), and he exhibited strong (aggressive) territorial behavior (*12*). Those heritable traits (large body size and aggression) likely contributed to M93 having a fitness advantage over the native inbred wolves (e.g., social dominance and higher hunting success) which led to the rapid spread of M93' genes and concomitant increase in heritable phenotypes among the wolf population—as indicated by the increase in M93 ancestry. However, the resumption of high levels of inbreeding among M93 lineage after 2008 may have resulted in a decline in traits such as body size. For example, wolf M183, the highly inbred descendent of M93 and the last surviving native male on IRNP, was notably smaller than wolves from nearby Canadian populations (see fig. S3).

It is plausible that Allee effects (low per capita growth rate at low population density) are a demographic mechanism underlying the observed decline in wolf abundance and predation rate after 2008. However, no strong evidence of Allee effects exists for this population as wolf abundance and the per capita growth rate of the wolf population are not positively correlated in IRNP, even at relatively low wolf densities (fig. S4). Allee effects can sometimes occur because individuals struggle to find mates when populations are at low densities (*36*). However, there is a very high probability of wolves encountering each other in IRNP, even at low densities, given that wolves readily travel across large parts of IRNP within a single day. Last, because wolves hunt in packs, it is plausible that an Allee effect, due to reduced hunting success at low densities

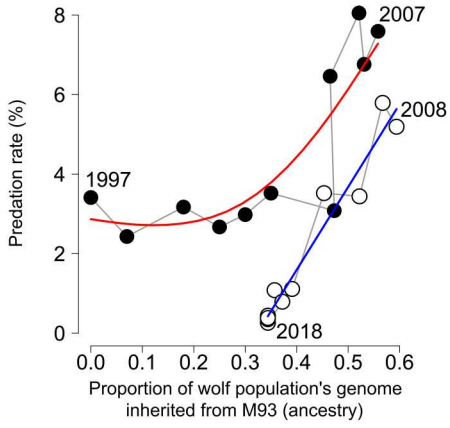

Fig. 4. Winter predation rate shown in relation to ancestry from M93. M93 was an immigrant wolf that genetically rescued the wolf population in IRNP. Winter predation rate represents the proportion of the moose population killed by wolves over a 2-month period (mid-January to mid-March). The period where ancestry from M93 is increasing (1997–2007) is indicated by filled circles and a curved red line (R^2_{adj} = 0.72). The period where ancestry from M93 is decreasing (2008–2018) is indicated by open circles and a blue line (R^2_{adj} = 0.94).

(as opposed to genetic deterioration), could explain the decline in predator abundance and predation rate after 2008. However, that alternative explanation is weakened by previous research, suggesting that hunting success does not measurably improve beyond three wolves (*37*, *38*). An Allee effect can also be caused by genetic deterioration (*39*). Thus, even if there were strong evidence of Allee effects in Isle Royale wolves, it would not necessarily undermine any of the inferences we make about our results.

Changes in ancestry and predation rate were closely linked to changes in moose population growth rates and ultimately moose density (Fig. 5A). Evidence supporting that claim comes from observing that predation rate explained three quarters of the interannual variation in moose density (Fig. 5B) and that moose density started to decline shortly after M93's arrival and the concomitant increase in predation rate (Fig. 6). The increase in ancestry and predation rate also coincided with another change in moose demography. Specifically, the average age of moose in the population showed a sustained increase between 1997 and 2007 [see figure 1C in (*40*)] because the increase in predation rate was predominately on moose calves, limiting recruitment rates (*41*). However, recruitment rates and moose abundance started to increase rapidly after 2012 as ancestry and predation rates declined.

The period ca. 2002–2012 was the decade with the lowest average moose density observed since observations began in 1959 (*42*). During that time, browse rates fell to less than 0.10 (Fig. 6F). Toward the end of that decade-long period, we observed many fir saplings growing taller than 175 cm (height beyond which apical leaders are on the cusp of being out of the reach of moose) on the western third of IRNP, which was especially affected by browsing (*43*). Dendrochronology indicates that such growth had not occurred during the previous century (*44*). However, as genetic deterioration in the wolf population led to a decline in predation rate and an increase in moose density, browse rates on fir more than tripled (Fig. 6). As this marked increase in browse rate occurred, very few fir saplings grew tall enough to escape apical browsing by moose. Continued observation is required to know how the island's forest ecology has been affected by the recent efforts to restore wolf predation in 2018–2019 (*25*). The long-term effects will depend on how quickly and how severely wolf predation is able to limit moose abundance in the near future. The degree to which balsam fir recovers has long-term implications for forest structure, because intensive moose browsing on regenerating trees (both on fir and many other species) has caused a gradual shift toward more open forests and savannas over the last few decades (*45*).

The salient point of this narrative, beginning with genetic rescue and ending with forest ecology, is to highlight how the genetics of a single individual predator can have impacts that flow through an entire predator population, through the prey population, and onto the forest and its ecological processes. While evidence from other systems demonstrates how the genetics of one or a few individuals can affect an entire population, our work goes further by demonstrating how those impacts can reverberate across trophic levels. In this regard, we have provided a compelling example that speaks to an important question, posed in (*46*), "Can heritable traits in a single species affect an entire ecosystem?" The effects described here are of broader relevance to the extent that keystone species, especially larger carnivores, increasingly live in fragmented populations and their health depends on occasional gene flow (*47*).

MATERIALS AND METHODS

Study system

IRNP is a remote archipelago, composed of a large island (544 km^2) and dozens of smaller islets, located in Lake Superior about 24 km away from mainland Canada. The wolf population was founded around 1950 by wolves from a mainland population northwest of IRNP. Wolves are the only predator of moose (*41*), and moose comprise more than 90% of wolf diets in terms of biomass (*48*). Although wolves also prey on beaver during certain times of the year, such as late spring and summer, moose remain the most important prey species for wolves in terms of biomass (*49*). The importance of wolf predation for understanding the dynamics of this moose population is widely appreciated (*23*). The indirect effects of wolves on forest vegetation have also been documented (*42*, *50*).

Previous research revealed that the IRNP wolf population has a history of severe inbreeding, inbreeding depression, low genetic diversity, and occasional gene flow facilitated by ice bridges that connected the island to the mainland during some winters (*11*). Retrospective genetic analysis and field observations revealed that a male wolf, known as M93, migrated to IRNP from mainland Canada across an ice bridge in either 1996 or 1997 (*11*, *12*, *15*). Specifically, field observations indicated that the alpha male of "Middle Pack" had been replaced by a different male with a distinctive appearance (larger body size and light coat color; see Fig. 2) during the winter of 1997. We collected a fecal sample from that distinctive alpha, immediately after observing him defecate, which confirmed he was an immigrant (*12*). Thus, M93 genotype is directly connected to a wolf with a particular social identity (alpha male of Middle Pack) that started reproducing in 1997. It is unlikely that M93 arrived earlier than 1996, because the only way to get to IRNP is via an ice bridge and there were ice bridges in 1996 and 1997 but no ice bridges in the years just before having first observed M93. Moreover, we had genetically sampled all but one of the alpha

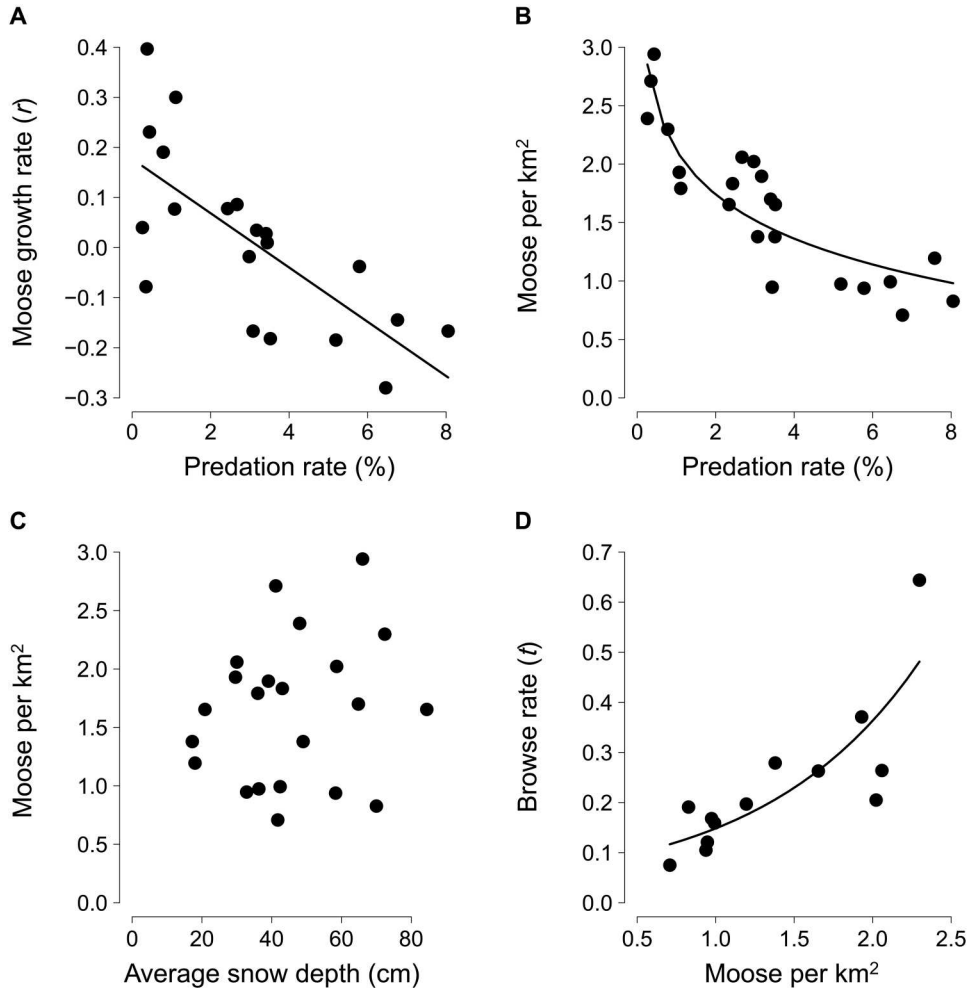

Fig. 5. Predation rate, moose abundance, snow depth, and browse rates on balsam fir. (**A**) The relationship between the per capita growth rate of the moose population in IRNP and predation rate by wolves on moose during a 2-month period in winter (R^2_{adj} = 0.50). (**B**) The relationship between the moose density and winter predation rate the previous year (R^2_{adj} = 0.75). (**C**) The relationship between moose density and snow depth the previous year (R^2_{adj} < 0.01). (**D**) The relationship between browse rate on balsam fir saplings and moose density 3 years earlier (R^2_{adj} = 0.65). Browse rate is the proportion of fir saplings between 0.5 and 3.0 m tall whose apically dominant leader was browsed.

wolves in the population between 1988 and 1999, and the absence of unique alleles among those wolves indicates that M93 was an immigrant and not the offspring of an immigrant (*12*).

Genetic data

A pedigree of the wolf population between 1997 and 2018 was constructed using 18 microsatellite loci derived from blood and fecal samples (*10*). The pedigree was used to estimate the proportion of ancestry from M93 each year. 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 pedigree of the wolf population in 2012 is given in (*11*). M93 initially mated with female F99 who had been born on Isle Royale. This mating produced 12 offspring, all of which by definition had 50% ancestry from M93. After F99 died, M93 mated with his daughter F58 and produced 21 offspring which all had 75% ancestry from M93, and by 2008, 59.4% of all genetic material in the population was inherited from M93 (*11*). This inbreeding caused high mortality and low reproduction in M93's lineage (for details, see section S2). By 2015, the only two surviving individuals in the population were M183 and F193 who were both descendants of M93. M183 and F193 were half-siblings and a father-daughter pair, and the two wolves never produced any offspring that survived past being a pup.

From the pedigree data, it was also possible to calculate the inbreeding coefficient (f) using the additive approach for the period 2002–2018 (*11*). The inbreeding coefficient is the probability that two homologous alleles in an individual are identical-by-descent, i.e., they are copies of an allele from a common ancestor. Thus, ancestry and the inbreeding coefficient may reflect different information about the genetic characteristics of a population. While it would be interesting to assess the combined influence of ancestry and f on predation rate, the data for f are limited. Specifically, pedigree data on f exist only for 2002–2018, and by 2003, inbreeding resumed. It would be statistically ill-advised to build a model of predation rate (with a sample size of only n = 16 years) that is a function of two covariates [ancestry and f; (*51*)]. Moreover, ancestry and f are

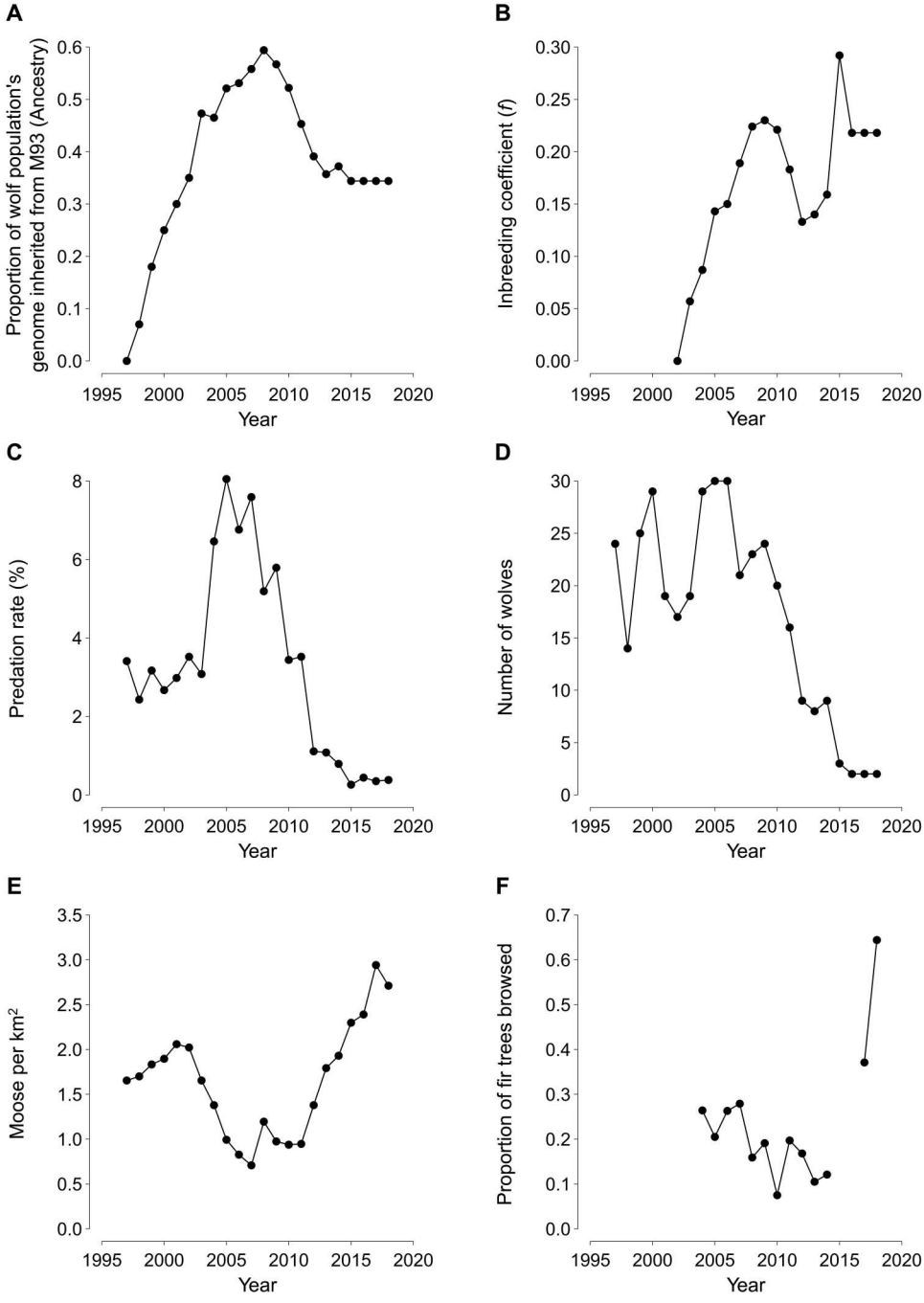

Fig. 6. Temporal trends in ancestry, the inbreeding coefficient, predation rate, wolf abundance, moose density and browse rates. Temporal trends between 1997 and 2018 in IRNP: (**A**) the ancestry of wolf, M93, an immigrant that genetically rescued the Isle Royale wolf population. Ancestry is the portion of the wolf population's gene pool which was inherited from M93; (**B**) the expected inbreeding coefficient (*f*) which represents the probability that two homologous alleles in an individual are identical-by-descent; (**C**) predation rate during a 2-month period in winter, which represents the proportion of the moose population killed by wolves each winter; (**D**) the number of wolves; (**E**) the density of moose; (**F**) moose browse rates on balsam fir saplings.

strongly correlated for the majority of the study period (2002–2014), until the wolf population collapsed to <4 highly inbred individuals (see section S4 and fig. S5). Thus, if both ancestry and *f* were included in the same model, then it may cause issues associated with multicollinearity. Consequently, we focus on assessing the relationship between predation rate and ancestry because of the longer time series in ancestry and because ancestry is more likely to reflect heritable traits (e.g., body size and behaviors) which were passed onto the wolf population by M93 that may have influenced the wolf population's ability to kill prey. Furthermore, evidence from other animal populations suggests that ancestry is a useful predictor of

fitness related traits (*16*, *22*). In section S4, we also show the relationship between *f* and predation rate (fig. S6).

Ecological data

Wolf abundance was estimated annually by counting the entire wolf population from a fixed-wing aircraft each winter (*52*). Confidence in census accuracy was provided by the frequent visibility of entire wolf packs at a single location and time and by making several complete counts during each winter survey. Moose abundance (N) was estimated annually, by cohort analysis and aerial surveys with a stratified sampling design that involves counting moose on 91, 1-km^2 plots from a fixed-wing aircraft (*40*). We then converted this estimate of moose abundance to an estimate of moose density across the island (area = 544 km^2). We also used these annual estimates of moose abundance to calculate the per capita growth rate (r) of the moose population, where $r = (N_{t+1} - N_t)/N$. We assessed temporal variation in r, in addition to changes in moose density, because previous work suggests that r is intimately connected to other elements of demography—including the population age structure—for long-lived species (*40*).

Each January and February, we estimated the number of moose killed by wolves over a period of ~44 days (median 44 days, interquartile range = [38, 47 days]) during aerial surveys (*53*). More precisely, we detected the carcasses of wolf-killed moose by direct observation and by following wolf tracks left in the snow. Several conditions reduce the risk of failing to detect the carcass of a moose killed by wolves. First, we searched for carcasses along the entire path of tracks that wolves left in the snow which allowed us to detect carcasses even when wolves had left the site. Second, packs typically spend 3 to 4 days consuming moose carcasses, and packs will return to those sites later in the winter, providing us with multiple opportunities to detect kills. Third, we regularly searched areas which packs had visited previously and conducted intensive, island-wide ground surveys to locate carcasses during the spring and summer months. The detection of wolf-killed moose was also facilitated by one or more wolves in each pack wearing radio-collars during most years throughout the study period.

We estimated a daily per capita kill rate as the number of wolf-killed moose divided by wolf abundance, divided by the number of days during which observations were made. From this daily per capita kill rate, we calculated the total number of moose killed by wolves over a 2-month period in mid-winter (mid-January to mid-March). To estimate predation rate, a cause-specific mortality rate for moose during winter, we divided the total number of moose killed by wolves during a 2-month winter period by the number of moose estimated to be in the population during winter. Because predation rate was calculated consistently throughout the study period, it is likely to represent a useful index of interannual variation in winter predation pressure. Furthermore, winter predation rates have been extrapolated into estimates of annual predation rate on recruited moose in other studies by making certain assumptions. These assumptions [described in detail in (*23*)] pertain to the relative proportion of total predation on recruited adults that occurs outside the time of year during which we sample. Note that our estimates of kill rate and predation rate are specific to wolves preying on recruited moose (>9 months old) and do not include instances where wolves prey on other species or on young calves.

We estimated browse rate on balsam fir saplings (0.5- to 3.0-m tall) for each year between 2004–2014 and 2017–2018. More precisely, we estimated browse rate as the proportion of fir saplings observed in a year whose apically dominant leader was browsed recently enough to not have yet begun growing another apically dominant leader. Each year's estimate of browse rate was based on at least 450 saplings, located on plots (mean = 37, SD = 17, range 20 to 92). Plots were located randomly throughout the eastern and western regions of Isle Royale (see fig. S7), although the random location was adjusted and constrained by the occurrence of fir and our ability to travel to sites by snowshoe or on skis from base camps in each region. Within each plot, we established transects with a total length of 300 m (covering an area of approximately 500 m^2), and every 3 to 6 m along the transect, we recorded the browse status of the nearest fir sapling.

Statistical analysis

All analyses were conducted using Program-R (*54*). First, we conducted the path analysis using the "laavan" package (*55*) to simultaneously evaluate the relationships between ancestry, predation rate, moose density, and browse rates in a single model. The results of that analysis are presented in Fig. 3.

Second, we use regression analysis to assess the relationship between ancestry and predation rate in greater detail. Because previous studies revealed that M93 ancestry exhibited both an increasing phase (i.e., positive growth between 1997 and 2007) and decreasing phase (i.e., negative growth between 2008 and 2018), we evaluated models that allowed for the relationship between ancestry and predation rate to be phase dependent (i.e., described by different functions for the increasing and decreasing phases of ancestry). We also evaluated models that allowed for the relationship between ancestry and predation rate to be either linear or best characterized by exponential, polynomial, or general additive models (GAMs; which do not make a priori assumptions about the specific functional form). We fitted GAMs using the "mgcv" package in Program-R (*56*). The results of that analysis are presented in Fig. 4 and Table 1.

Third, we assessed how changes in predation rate influenced r and moose density (moose per square kilometer). Specifically, we used linear regression models to assess the extent that interannual variation in r and moose density was associated with estimates of predation rate between 1997 and 2018. For the analysis focused on moose density, we also evaluated models that allowed for a time lag of 1 year and for a logarithmic relationship. The results of that analysis are presented in Fig. 5 (A and B) (see also table S1).

Fourth, we assessed how changes in moose density were associated with the intensity of moose browsing on forest vegetation. Specifically, we used linear models to assess the extent that interannual variation in browse rate was correlated with moose density between 2004–2014 and 2017–2018 (period when browse rate estimates are available). We built models predicting browse rate that allowed for time lags of up to 3 years because saplings were counted as browsed if they had not yet developed another apically dominant leader; however, it can take multiple years for replacement leaders to develop after being browsed. Moreover, time lags are commonly observed in data pertaining to trophic relationships (*57*, *58*). The results of that analysis are presented in Fig. 5D (see also Table 2).

Last, because winter severity may play an important role in determining moose population dynamics and predation dynamics, we carried out some auxiliary analyses. Specifically, we assessed the extent that interannual variation in r and moose density was

associated with mean annual snow depth—a useful indicator of winter severity in this region (*59*). In addition, we assessed how interannual variation in wolf abundance, annual predation rate, and browse rate were associated with snow depth. For details, see section S3 and table S1.

Supplementary Materials

This PDF file includes:

Figs. S1 to S7
Tables S1 and S2
References

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