

Ecology drives evolution in grey wolves

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ABSTRACT

Background: Grey wolves (*Canis lupus*) are a widespread, Holarctic species distributed across a wide variety of habitats, including deserts, dry plains, boreal forests, and the high arctic. They are generalist carnivores, feeding on a wide variety of species throughout their range. Wolves also disperse readily, with multiple records of over 1000 km. Despite this, surprising examples of differentiation between contiguous wolf populations have been described.

Questions: How can population differentiation arise and be maintained in a continuously distributed, generalist species? How might this structure impact evolution and conservation?

Data: Previously published genetic, morphological, and ecological data from ancient, historic, and modern populations of grey wolves from across their distribution.

Results: Genetic differentiation of wolf populations is tightly associated with habitat characteristics. This requires that dispersing wolves primarily disperse within their natal habitat. As wolves increase their range to occupy new areas, these tend to be colonized by small numbers of wolves often occupying similar habitats. The new arrivals quickly adapt to the new conditions and may be able to exclude other potential immigrating wolves. This limitation in the admixture between populations implies that populations in vanishing habitats (i.e. due to climate change or land use change) are likely to disappear with the habitats, resulting in the loss of locally adapted ecotypes. This cycle of repeated isolation and extinction has led to the observed low level of genetic diversity both within populations and within the species, lower than for other widespread canids.

Conclusions: Structure in wolf populations is generated through ‘isolation by environment’. This implies that the genetic diversity is more partitioned than expected within the distribution range of wolves, leading to a decrease in the effective population size and evolutionary potential of wolves within a given habitat, a threat when facing environmental changes. This has resulted in a pattern of local extinctions and reduced genetic variation.

Keywords: adaptation, *Canis lupus*, extinction, isolation by environment, phylogeography, population structure.

INTRODUCTION

Phylogeographic studies have found discontinuities of population structure in the distribution of the genetic diversity of the vast majority of widespread species (Sexton *et al.*, 2014). This

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structure is often associated with current or historical barriers to dispersal. Current barriers include areas of unsuitable habitat, roads, mountains, and so on (Riley *et al.*, 2006). Historical barriers refer to the shadow of past barriers, such as Pleistocene ice sheets or the past distribution of unsuitable habitat, which later disappeared allowing populations to expand and come into contact, but retaining the signal of differentiation from when they were isolated (Hewitt, 2000). Another common phylogeographic pattern, isolation by distance, arises in the absence of any barrier just through the limits that individual animals have to dispersal. In this case, animals that are geographically close are also more genetically related because individuals don't disperse far enough (relative to the distribution of the species) to keep the gene pool fully mixed. The genetic differentiation between these populations is mainly caused by the neutral process of random genetic drift in genetically isolated populations or along a gradient of decreasing connectivity, without necessarily implying adaptation to different environmental conditions. More recently, the importance of habitat in shaping phylogeographic patterns has been recognized, and is referred to as 'isolation by environment' (Wang and Bradburd, 2014). This opens the possibility for adaptation to local habitats to accumulate through natural selection without being 'swamped' by gene flow from animals in other habitats. In species with limited dispersal capability, it may be difficult to determine if different populations are in different habitats because they have some type of preference or selection for habitat type, or if they simply are not capable of regularly dispersing as far as the neighbouring habitats.

Grey wolves (*Canis lupus* L.) are a good model species to study the environmental factors that may influence genetic structure. Grey wolves are considered a generalist species, and have a very wide natural distribution throughout most of Eurasia and North America. This area includes many very different habitats, from the high arctic to dense forests, open steppe, and deserts. Wolves living in these different habitats have very different available prey, and hence very different diets; they also differ in water or refuge availability, requirements for thermal regulation, human pressure, competitors, and so on. Grey wolves have a particularly high dispersal capability, with movements of hundreds to over a thousand kilometres being recorded (Linnell *et al.*, 2005; Wabakken *et al.*, 2007). Wolves are able to cross geological features which act as barriers to other species, such as mountains, rivers, forests, open areas, and even narrow stretches of open ocean (Stronen *et al.*, 2014), as well as areas heavily populated or deeply transformed by humans, yielding very few physical environmental features that could be barriers. Furthermore, wolves of both sexes disperse (Kojola *et al.*, 2006). Concordant with these observations, little phylogeographic structure was found on a large scale (across their distribution) using mitochondrial markers (Vila *et al.*, 1999).

Some wolf populations have been identified as distinct. One of the first populations to be recognized as genetically distinct was the Mexican wolf. These wolves inhabit the southern edge of the species' range in North America, and are highly endangered following intense eradication efforts in the 1900s. First with mitochondrial DNA (Wayne *et al.*, 1992) and later with other markers (vonHoldt *et al.*, 2011), these wolves were shown to be different from other North American wolves. The differentiation and deep divergence in the mitochondria between Mexican and other North American wolves was used to suggest that they had a separate, earlier origin from Eurasia than the other North American wolves (Wayne *et al.*, 1992). Although this differentiation has been long recognized, the Mexican wolf is highly isolated from other wolf populations, as most of the wolves in the contiguous United States and southern Canada were eradicated. This presents the possibility that, as opposed to a discrete

structure, the observed pattern could be a remnant of isolation by distance, and only the extreme ends are left. Similarly, other small wolf populations that appear differentiated possibly as a result of their isolation are the Italian, Iberian, and the Scandinavian ones (Vilà *et al.*, 1999, 2003; Lucchini *et al.*, 2004). Interestingly, the differentiation of the current Scandinavian wolf population has arisen in a very short time (just over a decade) due to their extremely small population size (Vilà *et al.*, 2003), which shows that the degree of differentiation is not necessarily correlated with the time of isolation.

However, on a finer scale, strong population differentiation has been identified in continuously distributed wolves (Sharma *et al.*, 2004; Musiani *et al.*, 2007; Koblmüller *et al.*, 2009; Muñoz-Fuentes *et al.*, 2009; Pilot *et al.*, 2010; Jedrzejewski *et al.*, 2012). Differentiation between geographically adjacent populations has been observed across the range, in Europe, Asia, and North America. These patterns appear to be a widespread, general phenomenon, and not just a special case in one unusual circumstance.

RAPID EVOLUTION OF POPULATION STRUCTURE

There are now several detailed descriptions of population structure in wolves in areas where they are continuously distributed. The pattern was first suggested for North America (Carmichael *et al.*, 2001; Geffen *et al.*, 2004) and then supported by more detailed research on a smaller geographic scale both in Europe and North America. Two well-developed examples come from Canada. The first is from coastal British Columbia. There is a long, narrow strip of temperate rainforest along the Pacific coast of Canada. To the east of this forest is the Coastal Mountain Range, which has a continental habitat. Grey wolves are distributed continuously across both of these habitats. There is no anthropogenic or natural barrier between these two habitats. An average wolf could easily disperse a distance larger than the width of the temperate rainforest. Despite this, there is a strong genetic discontinuity in the wolves matching the habitat discontinuity. Significant divergence was observed over scales of <100 km where the habitat changed, but no significant divergence was found over five times that distance within the coastal forest habitat (Muñoz-Fuentes *et al.*, 2009).

Another example corresponds to the wolves across the Northwest Territories. In the North there are wolves that summer in the tundra where they den, raise their cubs, and feed on migratory caribou, following them south to the tree line in winter, where resident wolves live. All of these wolves spend the winter in/around the northern limits of the forest. Pronounced and significant genetic and morphological (coat colour) differentiation was reported for these two groups of wolves (Musiani *et al.*, 2007). The differentiation is found in genetic markers that are maternally, paternally, and bi-parentally inherited, so it reflects true population structure and not just a denning preference inherited from the mother (which could result in differentiation of maternally inherited genetic markers, but not paternally inherited markers). This is remarkable because the wolves mate in the winter, when all of the wolves are in the same area, ensuring that distance is not the driving force in differentiation between the two ecomorphs.

This pattern of genetic divergence in wolf populations associated with habitat is not only observed in North America. Genetic differentiation associated with different habitats or diets has also been reported in Eastern Europe, where the western edge of the Carpathian Mountains and the lowland boreal forest meet (Pilot *et al.*, 2010; Jedrzejewski *et al.*, 2012; Stronen *et al.*, 2013). The distance between these habitats is well within the dispersal capability of

the animals, and wolves inhabit both areas. However, the ungulate communities in the mountain and lowland forests are different, and a correlation between prey choice, genetic structure, and habitat was reported (Pilot *et al.*, 2012; Czarnomska *et al.*, 2013).

These examples demonstrate that population structure can arise quickly even in presumably large, continuous populations. These patterns must have arisen in the Holocene (in the last 10,000 years) because these regions (Canada and Poland) were largely glaciated in the last glacial cycle, and thus unavailable to wolves. They show that wolves can colonize new habitats as they arise, and that population patterns can arise (evolutionarily) very rapidly, on the scale of just a few thousand years. The differentiation between these populations is not only at neutral genetic markers. Some morphological differences, such as in pelage density or colour, or body size, have been recorded (Musiani *et al.*, 2007; Muñoz-Fuentes *et al.*, 2009; Anderson *et al.*, 2009). The biggest differences, however, may be in behaviour. Coastal wolves regularly eat salmon, although they avoid eating parts with higher accumulation of parasites (Muñoz-Fuentes *et al.*, 2009). Tundra wolves migrate (Musiani *et al.*, 2007). Wolves have a relatively long pre-dispersal life stage in which they are integrated in their natal social group and likely learn a lot about their habitat, how and what to hunt, how and what to eat. It seems likely that many of these behaviours in wolves could be learned. These behaviours could have a significant impact on the survival and fitness of individuals, so learned behaviours could be an important part of the differentiation between genetically isolated wolf populations.

NATURAL RECOLONIZATIONS

Wolf populations across their range in Europe, Asia, and America have been strongly negatively affected by land use changes and direct human persecution in historic times. The exact dates vary by location, primarily from about 500 years ago. In the last few decades, attitudes towards wildlife in general and wolves in particular have begun to change in many areas. This is reflected in a variety of legal changes in their status, in some cases reducing or eliminating eradication programmes, and in some cases leading to their protection. Paradoxically, legal protection has been easier to achieve in places where wolves were locally extinct. Changes in land use such as the abandonment of rural areas in favour of larger cities, both in North America and Eurasia, have reduced direct persecution and opened up more suitable habitat both for the wolves and their prey. The combination of these factors has allowed several populations of wolves to expand their distribution, or even to naturally recolonize areas where they were historically eradicated (Vilà *et al.*, 2003; Fabbri *et al.*, 2007; Wagner *et al.*, 2012; Hendricks *et al.*, 2015).

Grey wolves have now recolonized Germany (Wagner *et al.*, 2012), likely from adjacent Poland. As mentioned above, the wolves in this part of Europe form distinct genetic units associated with habitat. The habitat that the wolves have recolonized in eastern Germany is more similar to the lowland habitat of north and east Poland than the mountainous southeast. Genetically, the wolves from Germany fall in the more widespread lowland group in Poland, which may be connected to the larger populations in similar habitat across the North European Plain further east (Czarnomska *et al.*, 2013).

Two isolated populations of wolves survived in Western Europe, one in Iberia (Spain and Portugal) and the other in Italy. Following legal protection, the number of animals on both peninsulas increased (Ramirez *et al.*, 2006; Echegaray and Vila, 2009; Caniglia *et al.*, 2014). These two populations are of particular interest because the Iberian and Italian peninsulas were

two of the most important Pleistocene refugia for boreal taxa in Western Europe (Hewitt, 2000), and so may contain ancient lineages.

The population in Italy was restricted to the south-central Apennines during the mid-twentieth century, with a minimum in the 1970s, after which it was protected and expanded north (Lucchini *et al.*, 2004; Fabbri *et al.*, 2007). By the early to mid-1990s, the population recolonized the Alps in both France and Switzerland (Valière *et al.*, 2003). This new Alps population has also received immigrants from Croatia in the east (Fabbri *et al.*, 2014), and is still expanding. This new population seems to be the source for the even more recently established population in the Spanish Pyrenees (Sastre *et al.*, 2010). In the course of these expansions, the wolves necessarily must have dispersed across lowland and humanized areas, but they have only attempted to establish in alpine areas. This suggests some type of habitat selection by the dispersing wolves.

Natural recolonizations are also occurring in North America. Currently, wolves are recolonizing the Pacific Northwest region of the contiguous United States. This recolonization could be occurring from the ecologically more similar population of wolves in the coastal forests in Canada, which have also naturally recolonized Vancouver Island (Muñoz-Fuentes *et al.*, 2010), or from the geographically closer – and also expanding – Rocky Mountains population to the east (vonHoldt *et al.*, 2010). Genetic analyses of the potential source populations, historic Pacific northwest wolves, and the colonizers showed that both the animals now colonizing the region and the animals that historically inhabited the Pacific northwest forest are more closely related to the ecologically similar coastal wolves in Canada (Hendricks *et al.*, 2015). It is interesting that the current population, which is not a direct descendant of the historic populations because these were eradicated, is the most closely related genetically, as well as ecologically. This recolonization was natural, but in the case of assisted recolonizations, these are some of the criteria used in selecting appropriate founders. These studies emphasize the importance of selecting individuals for restocking from similar environments.

Some naturally recolonized populations ‘appear’ hundreds of kilometres away from the nearest wolf populations, and significant efforts have been made to determine the origin of these animals. For example, after decades of absence, a breeding pack of wolves appeared in southern Scandinavia in the 1980s, nearly 1000 km away from the nearest population of wolves (Vilà *et al.*, 2003). Controversy ensued, including accusations that zoo animals had been illegally released, and theories that they had actually survived and had not gone locally extinct. Genetic analyses of historic, captive, and wild animals from Finland were able to show that this was an example of a natural recolonization by animals that dispersed from Finland to a similar patch of forest in southern Sweden (Vilà *et al.*, 2003).

In all of these cases the dispersal events, many of which must have occurred over a very long distance, were not random. The dispersers appear to be selecting for natal-like habitat. This selection may be similar in different animals from the same source population, potentially facilitating the encounter of another disperser far from the natal range. The patterns of phylogenetic structure suggest that in prehistoric colonizations of new habitats, new wolf populations may have been able to increase in size to the point that they could effectively exclude immigrants from other habitats/populations, limiting the possibility of admixture between wolves from different sources. Given the human element in almost all habitats where wolves could be, it is not clear if these new populations will be allowed to reach such densities, or if the maximum density of wolves will simply be lower. Ongoing monitoring of these populations could uncover interesting and possibly different patterns

than those that developed in past populations. Could human pressure facilitate the exchange between populations by reducing the wolf population to densities below those required for the exclusion of new immigrants? Either way, this could become apparent on a conservation or management time scale (less than a century).

The large number of natural population expansions and recolonizations in the last few decades highlights the ability of wolves to disperse and survive in new areas, if they are allowed to. These expansions and recolonizations are not the result of intense, planned conservation or management actions. They are largely the result of a reduction in direct persecution of wolves (reduction of shooting, snaring, and poisoning).

ANCIENT ORIGIN OF EXTANT PATTERNS

At the other end of the time scale, some very deep patterns of differentiation, indicative of much more ancient isolation, have also been described. In India and the Himalayas, some very divergent mitochondrial lineages can be found in proximity to other wolf lineages (Sharma *et al.*, 2004; Aggarwal *et al.*, 2007). It seems that where there are these divergent lineages, no other lineages are present. Unfortunately, the precise distribution of these lineages is not clear.

Another example of a wolf population that appears to have been genetically isolated for a longer period of time is the Great Lakes wolves in eastern North America, associated with temperate broadleaf and mixed forest (Leonard and Wayne, 2008; Koblmüller *et al.*, 2009). In this case, the wolves appear to have incorporated mitochondrial DNA from their co-distributed sister species, the coyote (*Canis latrans*) (Lehman *et al.*, 1991). The coyote has a much larger genetic variation than the wolf (Koblmüller *et al.*, 2012), but the diversity in this wolf population is distinct, suggesting that sufficient time has passed since the introgression for new sequences to evolve. Dating this divergence suggests that the introgression occurred before the last glacial cycle (Koblmüller *et al.*, 2009).

Both of these examples illustrate apparently surprising longevity of populations, as the divergent haplotypes found in these populations appear not to have dispersed to neighbouring populations over long time periods. The precise limits of the distributions of these populations is not as well defined as for the more recent patterns described in the previous section. Despite this, they suggest that these patterns, which can appear quickly, may be able to endure for long periods if the conditions are right.

POPULATION EXTINCTIONS

The phylogeographic patterns described above suggest that wolf populations are tightly associated with specific habitats. However, habitats change through time. The last few hundred thousand years have seen particularly dramatic changes, and habitats that were widespread only 20,000 years ago, no longer exist anywhere. One example of this is the Pleistocene steppe, which was distributed over much of central and northern Eurasia and across Beringia into North America. This habitat was home to many now-extinct creatures such as mammoths (*Mammuthus columbi*), woolly rhinos (*Coelodonta antiquitatis*), lions (*Panthera atrox*), and camels (*Camelops* sp.). This habitat was also home to some species that still inhabit the same regions, such as brown bears (*Ursus arctos*), caribou (*Rangifer tarandus*), yak (*Bos grunniens*), and grey wolves. The Pleistocene wolves of Beringia, and perhaps across the steppe, were adapted to that habitat in that their diet was heavily

dependent on now-extinct species, and their tooth and skull morphology indicate specialization on those prey, and their tooth wear suggests that their behaviour was different from modern and recent North American wolf populations (Leonard *et al.*, 2007; Fox-Dobbs *et al.*, 2008; Baryshnikov *et al.*, 2009). Genetic analyses of the Alaskan population through time indicate that the current-day population does not derive from the late Pleistocene population. The data suggest that the late Pleistocene wolf population went extinct after the last glaciation, along with the populations of horses and other species upon which it preyed. Soon thereafter another population of wolves, with its origin in southern North America, established in Alaska. This could suggest that specialized populations of wolves are at risk of going extinct when their environment changes too much, even though the habitat may, in a broad sense, remain 'available' or 'appropriate' wolf habitat.

GENETIC DIVERSITY

Grey wolves have a fossil record that stretches back at least to the mid-Pleistocene in Europe, and soon thereafter in America (Tedford *et al.*, 2009). Wolves are widely distributed across most of Europe, Asia, and North America (Wozencraft, 2005). The generalist ecology and widespread distribution, both in historic times and in the fossil record, suggest that the global population size of wolves could have been high over much of their history. A large population size over long periods of time will yield a long-term high effective population size. The effective population size correlates with genetic diversity and potential for evolution. According to their widespread distribution range and large historic population size, one could expect grey wolves to have accumulated a large amount of genetic diversity. However, this seems not to be the case. Their sister species, the coyote, has a similarly long fossil record, but has a much more restricted distribution only in North America. Despite this, the genetic diversity in coyotes is much greater (Gray *et al.*, 2009; Koblmüller *et al.*, 2012). Also, the extant genetic diversity of wolves across their distribution has a much more recent common origin than the fossil record would suggest (Vilà *et al.*, 1999; S. Koblmüller *et al.*, submitted). So, where is all the 'missing' genetic diversity?

The local specialization and differentiation of wolf populations would result in drift acting independently in each differentiated population or ecomorph. This process can actually safeguard genetic diversity because different variants are likely to survive in different populations. The survival of the genetic variants is thus tied to the fate of the populations that carry them, and the habitat they live in. If there are large-scale environmental changes, many local populations could go extinct in a short period of time. If there is a high rate of local extinction, then this structure could result in a large amount of unique genetic variants being lost. After local extinction events, regions without wolves would be re-occupied by animals from surviving populations. A long-term pattern of population extinctions followed by recolonizations would reduce the genetic diversity present in the species through a series of population bottlenecks. Environments change, and have changed profoundly many times in the history of grey wolves. Along these lines, recent analysis of complete genome sequences for three wolves inferred a sharp decline in the effective population size of wolves about 20,000 years ago, coinciding with the last glacial maximum (Freedman *et al.*, 2014). This could suggest that many wolf populations went extinct at about the same time as the population in Alaska went extinct (Leonard *et al.*, 2007), resulting in a substantial reduction in the worldwide effective population size. Very soon thereafter the remaining wolf populations recolonized those areas. This corresponds well with the

observation that many of the well-characterized associations of population differentiation with habitat arose after the last glacial maximum.

Genetic diversity is important for the long-term health and evolutionary potential of a population or species. Some wolf populations have been documented to have very few founders, such as the Isle Royale and Scandinavian populations (Wayne *et al.*, 1991; Vilà *et al.*, 2003). A small number of individuals in a population can have multiple negative effects, such as problems in finding a mate (Allee effect) and inbreeding depression. Wolves exhibit strong social outbreeding in that they identify immediate family members and avoid mating with them (Smith *et al.*, 1997; vonHoldt *et al.*, 2008). A lack of appropriate mates could limit population growth (Vilà *et al.*, 2003), or force hybridization with other related species (Adams *et al.*, 2003; Muñoz-Fuentes *et al.*, 2010; Leonard *et al.*, 2014). A loss of genetic variation through drift in small populations can also lead to inbreeding depression, in which genetic based problems such as genetic diseases, reduced fecundity, and malformations, rise in frequency and threaten the survival of the population (Liberg *et al.*, 2005; O'Grady *et al.*, 2006; Asa *et al.*, 2007; Fredrickson *et al.*, 2007; Rääkkönen *et al.*, 2009). Thus, the repeated cycles of population extinction and recolonization in wolves could reduce their evolutionary potential if the cycles are too severe and/or too frequent.

FUTURE POPULATIONS

Some populations of wolves have been recently isolated through anthropogenic action, such as their eradication in most of the contiguous United States and southern Canada, which isolated the Mexican wolves from the other populations of wolves in North America. The small southern population is at risk of extinction from inbreeding depression. Populations of wolves in newly recolonized areas may be founded by very few animals (Wayne *et al.*, 1991; Vilà *et al.*, 2003). The probability of long-term survival of these populations is also slim if no new migrants arrive and add their genetic diversity to the population. So, despite the importance and history of isolation between wolf populations, given the very small effective population size in some cases, one of the current threats to their survival may be enforced isolation (Palstra and Ruzzante, 2008). Several wolf populations with small effective population sizes have been shown to suffer from inbreeding depression (Liberg *et al.*, 2005; Asa *et al.*, 2007; Fredrickson *et al.*, 2007; Rääkkönen *et al.*, 2009). Inbreeding depression effects can be reversed with the arrival of even a very small number of migrants through genetic rescue (Vilà *et al.*, 2003). Given the small size and isolation of some of the current wolf populations, proactive management measures or facilitating the survival of immigrants may be needed in some cases.

Right now there are still many populations of wolves that apparently retain sufficient genetic diversity to be viable on an evolutionary time scale despite the large population declines and local extinctions in historic times. However, many of these populations are subject to intensive hunting (Jędrzejewski *et al.*, 2005; Person and Russell, 2008; Robichaud and Boyce, 2010; Hindrikson *et al.*, 2013). High levels of hunting have multiple impacts on a population, including changes in social structure (Rutledge *et al.*, 2010), and may reduce effective population size, which increases drift and the loss of genetic diversity (Allendorf *et al.*, 2008). The more genetic diversity that is lost, the fewer options for true recovery and evolutionary survival exist, and the effects of hunting may be especially pernicious for populations where the effective number of breeders remains small.

If wolves are allowed to increase their population sizes, and if long-distance migrations are allowed, there is a very good chance that wolves could maintain population and genetic

diversity levels on their own without intense intervention or management. To choose this path we need to stop making wolves a scapegoat for other conservation and livestock problems. Wolves can and do consume domestic ungulates, but they are not always a major problem. If a real problem is an unsustainable level of attacks on sheep, then it is necessary to determine who is attacking the sheep, because it may not be primarily wolves (Sundqvist *et al.*, 2007; Echegaray and Vila, 2009). If a real problem is a declining number of wild ungulates in an area with increasing wolf population, the primary cause of the decline could be environmental change or anthropogenic activity (Vucetich *et al.*, 2005).

Degradation of the environment, which affects fundamental ecosystem services such as the cycling of water and nutrients, is the great challenge of our time. Rising to the challenge of conserving these ecosystem services will require action on many fronts. Detailed ecological studies of the response of the community and of individual species to the reintroduction of wolves are showing how important predators are to the balance of the entire system down to the plants and the rivers (Ripple and Beschta, 2012). Allowing the survival of predators such as wolves is one of those fronts. These important ecosystem 'services' would be difficult – or impossible – to replace through active management. Costs and benefits of the presence of a wild predator in an ecosystem can rarely be evaluated by a direct evaluation of the direct costs and revenues.

CONCLUSION

Grey wolves are a particularly good model to assess the impact of habitat characteristics on population formation and isolation in a species largely unconstrained by dispersal or habitat. Although the biology of wolves would predict no phylogeographic structure, strong patterns of differentiation have arisen repeatedly throughout their range. This suggests preferential gene flow within similar habitats and reduced gene flow across habitats, so the fate of populations becomes linked to the fate of habitats. If this pattern is strong enough, it will also cause the low levels of genetic diversity observed in the species today through repeated bottlenecks. As a result, the genetic diversity of the species is lower than would be predicted based on its fossil record. Populations of wolves are expanding and new populations are being formed through natural as well as assisted recolonizations. We are at a crossroads where we have to decide if we will allow wolves to reincorporate into ecosystems where they have been excluded, and where they fill important ecological niches as evidenced by the changes they precipitate. Together, these data strongly support the importance of ecology in the evolution of grey wolves, and the importance of grey wolves in Holarctic ecology.

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