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**Abstract:** The wolf *Canis lupus*, the most widespread of the four species of large carnivores in Europe, after centuries of population decline and eradication, is now recovering in many countries. Wolves contribute to regulating prey-predator dynamics and interact with human activities, mainly livestock farming and ungulate hunting. Although wolves are protected in most European countries, illegal or incidental killing is widespread. Wolf populations do not show any apparent phylogeographic structuring worldwide. Molecular and morphological studies of historical samples showed evidence of wolf ecomorph extinctions, coinciding with the great Pleistocene faunal turnover. Extant populations show recurrent long-range dispersal during cycles of expansion and recolonization. Demographically stable populations, in contrast, seem to be characterized by very limited gene flow. Despite the potential for dispersal and ecological flexibility, landscape genetic approaches have demonstrated the existence of genetically distinct wolf populations, which originated through habitat and prey specializations. Small isolated wolf populations may suffer from inbreeding depression, although selection for heterozygotes and the rescue effect can foster rapid population recovery. Population structure and dynamics is efficiently monitored by non-invasive genetic methods, which are also useful to identify wolf x dog *Canis lupus familiaris* hybridization. Despite technical advances and a better knowledge of wolf biology, wolf conservation is largely dependent on humans, and on the solution of conflicts with stakeholders.

## INVITED REVIEW

# Genetics and conservation of wolves *Canis lupus* in Europe

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## ABSTRACT

1. The wolf *Canis lupus*, the most widespread of the four species of large carnivores in Europe, after centuries of population decline and eradication, is now recovering in many countries. Wolves contribute to regulating prey–predator dynamics and interact with human activities, mainly livestock farming and ungulate hunting. Although wolves are protected in most European countries, illegal or incidental killing is widespread.

2. Wolf populations do not show any apparent phylogeographic structuring worldwide. Molecular and morphological studies of historical samples showed evidence of wolf ecomorph extinctions, coinciding with the great Pleistocene faunal turnover.

3. Extant populations show recurrent long-range dispersal during cycles of expansion and recolonization. Demographically stable populations, in contrast, seem to be characterized by very limited gene flow.

4. Despite the potential for dispersal and ecological flexibility, landscape genetic approaches have demonstrated the existence of genetically distinct wolf populations, which originated through habitat and prey specializations.

5. Small isolated wolf populations may suffer from inbreeding depression, although selection for heterozygotes and the rescue effect can foster rapid population recovery. Population structure and dynamics is efficiently monitored by non-invasive genetic methods, which are also useful to identify wolf  $\times$  dog *Canis lupus familiaris* hybridization.

6. Despite technical advances and a better knowledge of wolf biology, wolf conservation is largely dependent on humans, and on the solution of conflicts with stakeholders.

**Keywords:** Eurasian wolf, inbreeding, landscape genetics, phylogeography, population genetics

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## INTRODUCTION

The wolf *Canis lupus* is the most widespread large carnivore in Europe. Wolves were historically widespread in the entire continent, living in a variety of habitats from tundra to steppes and deserts. They were progressively eradicated throughout Western Europe in the 18th and 19th centuries, surviving only in

fragmented populations in Iberia and Italy (Breitenmoser 1998). Legal protection, socio-ecological changes in mountain and agricultural areas, and the recovery of wild ungulate populations, recreated the conditions for a natural expansion of wolves. The recovery has been spectacular in Italy, where in 40 years wolves have recolonized most of their historical range along the Apennine Mountains, reaching the south-western Italian and French Alps in 1992 (Fabbri et al. 2007). Wolves went extinct in Scandinavia by the 1960s. Two or three immigrants from Finland established a breeding pack in the 1980s, founding a new population of about 150 individuals (Vilà et al. 2003b). Currently, wolves in Europe might number c. 18000–20000 individuals (including in the European part of Russia; Linnell et al. 2007).

Wolves are top predators that play crucial roles in maintaining the structure and functions of natural ecosystems: they contribute to regulating prey–predator relationships and have positive cascade effects on the dynamics of ungulate–plant communities and on the density of smaller predators (Sergio et al. 2008). Wolves are highly mobile and have large individual territories; juveniles of both sexes are able to disperse over hundreds of kilometres (Ciucci et al. 2009). Wolf populations expand well across the boundaries of parks and protected areas in Europe. Their presence in heterogeneous landscapes raises conflicts with human activities due to real or feared livestock depredation and competition with hunters for wild ungulate prey. Wolves are protected in most European countries, and although some controlled hunting is allowed, almost everywhere, illegal or incidental killing is widespread, and wolf conservation remains problematic (Linnell et al. 2007).

The population dynamics and behavioural ecology of wolves have been investigated primarily through field methods such as direct observations, individual marking, radio-tracking and snow-tracking (Mech & Boitani 2003). These methods are difficult or exceedingly expensive when applied in the large territories which are used by large carnivores. Hence, the socio-ecological dynamics of large carnivores remain poorly known. The recent developments of population genetics and genomics, and non-invasive genetic sampling (NGS), now offer novel opportunities to study the dynamics of wolf populations, and implement long-term, wide-range and cost-effective monitoring programmes (Waits & Paetkau 2005). We can routinely utilize molecular genetic information to address questions about wolf behaviour, ecology, population genetics, evolution and domestication. Genetic information is used to design global conservation strategies at the European Union scale (Linnell et al. 2007), as well as to monitor local wolf populations (Aspi et al. 2009) or for forensic purposes (Caniglia et al. 2010b).

In this review, I will summarize the available information on wolf conservation genetics in Europe, with emphasis on: (i) the phylogeography of wolf populations; (ii) landscape genetics (ecotypes and local adaptations); (iii) the consequences of small population sizes, the founder effect and migration, and the detrimental consequences of inbreeding; (iv) monitoring wolf populations; (v) wolf domestication and wolf  $\times$  dog *Canis lupus familiaris* hybridization; and (vi) the use of molecular genetics in wildlife forensics. I conclude that results of genetic research are providing a deeper understanding of the evolutionary biology of wolves, and have the potential to reveal new perspectives on wolf conservation and management.

## PHYLOGEOGRAPHY OF EUROPEAN WOLF POPULATIONS

Phylogeography is the study of the geographical distribution of genetic lineages. Genetic divergence and coalescence of distinct lineages are often dated to the early Pleistocene or Pliocene and may have non-overlapping geographic distributions, resulting from isolation of the lineages in glacial refuges (Hewitt 2000). The coalescence times of all known wolf mtDNA lineages, c. 200000–290000 years ago (Vilà et al. 1999a), substantially pre-dates the Last Glacial Maximum (21000–17000 years ago), meaning that wolf mtDNA haplotypes originated before the last glaciation. Studies based on mtDNA sequences highlighted the absence of any large-scale genetic structure in wolf populations worldwide (Vilà et al. 1999a), but showed evidence of local substructure probably caused by recent restrictions to gene flow (Pilot et al. 2006).

European wolf haplotypes are split into two main clades (haplogroups I and II), which do not show any clear allopatric distribution pattern (Pilot et al. 2010). Both haplogroups are widespread in Europe, but have different frequencies in south-western (Iberian and Italian peninsulas) and eastern (Balkans, Eastern and North-Eastern Europe; see Fig. 1 in Pilot et al. 2010) wolf populations. Only one unique mtDNA haplotype occurred in the Italian wolves; two unique haplotypes were found in the Iberian Peninsula (Randi et al. 2000). All ancient western European wolf samples dating from between 44000 and 1200 years ago belong to haplogroup II, suggesting a long-term predominance of this haplogroup, which was replaced by haplogroup I only during the last few thousand years (Pilot et al. 2010). Parallel replacement of haplogroup II by haplogroup I has been reported in North American wolves (Leonard et al. 2007). Climate, ecological or prey composition changes might have caused the extinction and turnover of wolf genotypes and ecotypes in the past. The Apennine wolf haplotype belongs to the ancient haplogroup II. Bayesian coalescent analyses confirmed the ancient origin of Italian wolves, which could have been genetically isolated for thousands of generations south of the Alps (Lucchini et al. 2004). Generally, wolf populations cannot be assigned to distinct evolutionarily significant units (ESU) in Europe, possibly with the exceptions of the Italian subspecies *Canis lupus italicus* (Boitani et al. 2003) and the Iberian wolves.

## LANDSCAPE GENETICS: ECOTYPES AND LOCAL ADAPTATIONS

Some species of large and medium carnivores with widespread distributions have cryptic genetic structures that cannot be explained by obvious geographical barriers or historical factors (Carmichael et al. 2001, Rueness et al. 2003, Sacks et al. 2004, Dalén et al. 2005). Wolves can move across all kinds of terrain and cross natural or artificial barriers, allowing gene flow to occur and reducing genetic differentiation among populations. However, ecological and behavioural factors, such as diet and natal habitat, may limit dispersal, isolating neighbouring subpopulations. Carmichael et al. (2001) found that prey specialization constrained gene flow among contiguous wolf populations. Geffen et al. (2004) found that temperature and climate explained more genetic variation than geographical distances among wolf populations. The boundary between genetically distinct populations coincides with the ecological boundary between boreal coniferous forest and tundra or taiga habitats (Musiani et al. 2007).

Significant genetic differentiation resulting from prey specialization implies that restriction to gene flow might occur even in parapatric populations of highly mobile

vertebrates. Wolves in Eastern Europe showed non-random spatial genetic structure in the absence of physical barriers to movement (Pilot et al. 2006). Genetic differentiation was better explained by climate, habitat types and prey availability, as described by the frequency of red deer *Cervus elaphus* in the diet than by geographical distances. These findings led to the development of the concept of wolf 'ecotypes', that is, populations which are genetically and ecologically distinct because they have been evolutionarily adapted to living in different habitats (Muñoz-Fuentes et al. 2009). The 'ecotype' tangibly extends the concept of ESU (sensu Moritz 1994), including not only neutral genetic marker variation, but also distinct functional traits and unique local adaptations (Crandall et al. 2000).

#### **THE CONSEQUENCES OF SMALL POPULATION SIZE AND THE 'RESCUE EFFECT'**

Declining wolf populations in Western Europe have been fragmented and isolated for centuries. Consequently, they exhibit unique mtDNA haplotypes and reduced autosomal genetic diversity, caused by genetic drift and random fixation of alleles (Ellegren et al. 1996, Vilà et al. 1999b, Randi et al. 2000, Flagstad et al. 2003). The decline of genetic variability is correlated to the effective population size ( $N_e$ ), which is much smaller than census population size ( $N_c$ ) in wolf populations, reflecting the high variance in breeding success (Aspi et al. 2006, vonHoldt et al. 2008). Inbreeding is more likely to take place in small populations and may contribute to further decline and eventual extinction, through inbreeding depression (Liberg et al. 2005).

In Scandinavia, wolves form a highly bottlenecked population, which originates from three founders and has high inbreeding coefficients negatively correlated with reproductive success (Räikkönen et al. 2006). Following the arrival of a new immigrant in 1991, the heterozygosity increased and the population expanded to the current size of 135–152 wolves and 11 breeding packs, demonstrating 'genetic rescue' through immigration (Vilà et al. 2003b). Bensch et al. (2006) observed that the relationship between inbreeding and heterozygosity was weaker than expected. They found fewer homozygous wolves than expected, suggesting evidence for the selection for heterozygotes at recruitment. However, the results of Hagenblad et al. (2009), obtained using many more genetic markers, did not support these findings. The different number of markers and different statistical approaches could explain the discrepancies between the two studies.

#### **COLONIZATION GENETICS: FOUNDER EFFECT AND MIGRATION**

Natural colonization processes are poorly known. The genetics of colonization are usually dominated by initial bottlenecks if populations are founded by small numbers of colonists. Random drift might lead to further losses of genetic variability if colonies do not quickly expand, and do remain isolated from their source populations. Founder effect and isolation might reduce genetic diversity and the potential for adaptation, concomitantly increasing inbreeding and the probability of extinction.

Wolf colonization is sustained by long-distance dispersers, and colonizing wolves may not lose significant fractions of their original genetic variability (Valière et al. 2003, Aspi et al. 2009). Colonizing wolves in North America showed high genetic variation and dispersed over long distances, suggesting that the new packs were founded by multiple unrelated individuals, without any detectable bottleneck at

founding (Forbes & Boyd 1997). Sporadic but recurrent migration has increased heterozygosity and sustained population growth in Scandinavian wolves (Flagstad et al. 2003, Seddon et al. 2006).

Results from NGS programmes revealed that the Alps are being naturally recolonized by Italian wolves from the central Apennines (Lucchini et al. 2002, Valière et al. 2003). Fabbri et al. (2007) showed that wolves in the Alps have significantly lower genetic diversity than wolves in the Apennines, and are genetically distinct. The Alps were colonized by a few long-range migrating wolves. During the colonization process, there has been a moderate bottleneck; the gene flow between source and colonies was moderate, corresponding to 1.25–2.50 wolves per generation, despite high potential for dispersal. Bottleneck simulations showed that 8–16 effective founders are needed to explain the genetic diversity observed in the Alps. Migration from the Apennines to the Alps was unidirectional and male-biased.

### MONITORING WOLF POPULATIONS

Wolf populations are organized in familial packs, each composed by a single breeding pair, their offspring of the year, and occasional older offspring or unrelated individuals migrating from other packs, the adoptees (Mech & Boitani 2003). Kinship structure and inbreeding avoidance influences reproductive strategies, ultimately affecting the dynamics of local populations. vonHoldt et al. (2008) reconstructed the genealogy of 200 wolves in the reintroduced Yellowstone National Park, USA, population. They described complex pack dynamics, including: (i) formation of new packs by pack splitting, involving unrelated males and females; (ii) immigrant males mating with females breeding in their natal packs after the death of dominant males (see also Jedrzejewski et al. 2005); (iii) breeding of an immigrant male with multiple unrelated females in a pack; (iv) extra-pack copulations of subordinate females with immigrant males; and (v) substitutions of existing dominant breeders. Inter-pack dispersal was common and male biased: no females immigrated into existing packs.

NGS projects can provide relevant data in European landscapes, where wolves cannot be easily observed or captured. Spatial and temporal distributions of individual genotypes and kinship analyses led to the identification of two distinct packs in an early phase of wolf colonization of the Italian Alps (Lucchini et al. 2002). The packs included some unrelated individuals (the putative reproductive parents and immigrants), plus closely related wolves (their putative offspring), and ranged in separate but adjacent areas. In a long-term NGS monitoring project of the wolf population in the central Apennines, Caniglia et al. (2010a) identified and mapped the territorial ranges of 31 wolf packs in a study area of c. 15000km<sup>2</sup>. Twenty-four complete genealogies were reconstructed. In 16 packs, a single breeding pair was detected for up to five consecutive years, while in the other eight packs there were from one (in seven packs) to three (in one pack) breeding pair changes. In five cases, only one parent was replaced; in another five cases, there was a complete turnover. In four of these latter cases, the new breeding female was one of the previous pair's offspring. Five pairs were composed of related individuals: four father–daughter matings were detected, and one pair consisted of two cousins. One multiple breeding event, in which the same male mated with two females, was detected. There were 37 dispersers (26 males and 11 females); 14 of them established themselves in a new pack and became breeders, two joined existing packs and 11 founded their

own new packs. These results show that pack dynamics are complex and can be reconstructed using NGS methods.

NGS data are also used to estimate the abundance of wolf populations. Wolf populations are intrinsically open to immigration. Genotyping errors, heterogeneity in sampling efforts and variable individual marking behaviour can produce biased demographic estimates due to capture heterogeneity. Because the assumption of homogeneous detection probability is violated, accurate model selection is needed. Marucco et al. (2009), Cubaynes et al. (2010) and R. Caniglia, E. Fabbri, S. Cubaynes, O. Gimenez, J.-D. Lebreton and E. Randi (unpublished data) showed that demographic models which ignore individual detection heterogeneity may underestimate wolf population size by 27%–30%.

### **WOLF DOMESTICATION AND HYBRIDIZATION**

Dogs were probably the first domesticated animals. Molecular data suggest multiple domestication events in Asia (Vilà et al. 1999a, Savolainen et al. 2002, Pang et al. 2009, vonHoldt et al. 2010). Despite the deep genetic changes in behaviour, physiology and morphology due to domestication of dogs, dogs and wolves are still able to breed successfully in captivity and in the wild (Vilà & Wayne 1999). The fear of extensive hybridization between declining wolf populations and widespread free-ranging domestic dogs in Europe is a major concern for conservation biologists (Randi 2008). Hybridization is a two-step process: (i) wolf  $\times$  dog cross-breeding generates first generation hybrids ( $F_1$ ), which might reproduce among themselves, originating hybrid swarms or even new species (Hailer & Leonard 2008, Koblmüller et al. 2009); (ii) hybrids might backcross with wolves generating introgression into the wolf populations. Wild wolf  $\times$  dog hybrids have been identified in North America (Hailer & Leonard 2008) and in Europe (Anderson et al. 2002, Randi & Lucchini 2002, Vilà et al. 2003a, Verardi et al. 2006). However, their diffusion is poorly known, the biological consequences of introgression are controversial (Anderson et al. 2009) and official guidelines to manage hybrids do not exist (Allendorf et al. 2001).

Analyses of diagnostic mtDNA haplotypes failed to detect introgression of dog mtDNA in European wolf populations, suggesting that hybridization is rare or strictly unidirectional, or that  $F_1$  hybrids cannot backcross into wolf populations (Vilà & Wayne 1999, Randi et al. 2000; an exception is reported by Muñoz-Fuentes et al. 2010, in Vancouver Island wolves). Randi and Lucchini (2002) analysed allelic variation at 18 unlinked canine microsatellites (short tandem repeats or STRs) in a sample of Italian canids. Only one animal with an unusual black coat colour out of 107 wolves (0.9%) was genetically admixed, suggesting that recent hybridization was negligible. However, small panels of unlinked STRs have reduced detection power, and the occurrence of undetected hybridization or past introgression events was not definitively ruled out. In order to trace hybridization events further back in time and infer the population of origin of chromosomal blocks, linked STR loci were analysed in Italian canids (Verardi et al. 2006). Results indicated that 11 out of 220 wolves (5%) were admixed, a proportion that is significantly higher than previously estimated from unlinked markers. Bayesian clustering showed that, despite some admixture, wolf and dog gene pools remain sharply distinct, suggesting that hybridization was not frequent, or that introgression in nature is counteracted by behavioural or selective constraints.

Introgression can be highly selective, affecting only some parts of the genome, while other genomic regions remain virtually untouched. An example of putative selective introgression is provided by coat colour variation in wolves. White wolves are common in the Arctic regions, while black wolves occur in Western North America (Musiani et al. 2007). Black wolves were never reported in Europe, except in Italy, where black wild canids have been observed since 1976 (Boitani 1983). Mutations at coat colour genes, or hybridization with other species (coyotes *Canis latrans* in North America; Roy et al. 1994) or with free-ranging domestic dogs (Anderson et al. 2009), could have generated morphological variants in wolves. Candille et al. (2007) discovered that melanism in dogs is prevalently controlled by the *CBD103* gene (corresponding to the *K*-locus), which codes for a  $\beta$ -*Defensin* protein. The melanistic mutation is a three-nucleotide deletion, which has been detected in 50 different black dog breeds, and is widespread in Western North American wolves and in coyotes (Anderson et al. 2009). Molecular analyses suggest that this melanistic deletion in wolves derived from domestic dog introgression, and could have risen to high frequency under positive selection (Anderson et al. 2009, but see Hedrick 2009). The melanistic mutation is widespread in wolves and in feral dogs in Italy (E. Randi, unpublished data). The identification of coat colour mutations offers new informative markers for detecting hybridization and introgression.

#### FORENSIC APPLICATIONS

The return of wolves to regions that were not occupied for generations often leads to predation on livestock. Damage prevention, compensation policies and selective hunting to regulate wolf presence in some areas are not always efficient. Moreover, free-ranging dogs may predate on livestock. Wolf or dog predations are not easily identified, and kills are usually attributed to wolves. In consequence, illegal wolf killing is widespread in Europe, and remains perhaps the major threat to wolf survival. Sundqvist et al. (2008) were able to collect DNA from saliva traces close to bite wounds following a canid attack on sheep in Sweden. The saliva samples originated from a single dog, demonstrating that predators can be identified from bites and showing that wolves are not the only predator responsible for livestock kills.

In 2008, police confiscated a necklace made of 10 canine teeth from a man living in a small village in the northern Italian Apennines, and recovered in the same area a male wolf carcass without the entire muzzle. DNA was extracted from the wolf carcass and from dental pulp samples obtained by slow drilling of the roots of the teeth. The samples showed the same mtDNA diagnostic Italian wolf haplotype. STR and molecular sexing led to the identification of six different individuals represented by the teeth in the necklace, three males and three females; the males shared the same Y-linked wolf haplotype. The six genotypes were matched with a large database of European wolf and dog genotypes, including wolves from the entire Italian range distribution. The profile of one tooth matched the genotype of a female wolf, non-invasively sampled twice in 2007 in an area 10km from the suspected poacher's house. Another tooth profile matched the genotype of the wolf found dead (Caniglia et al. 2010b).

#### CONCLUSIONS

Wolves in Eurasia and North America show no apparent large-scale population structure, nor consistent signatures of past allopatric fragmentation. Past population



dynamics have been dominated by range expansion in Eastern Europe, but it is impossible to identify specific post-glacial dispersal routes. In consequence, wolf populations are genetically admixed throughout Eastern Europe. Late Pleistocene wolves from Alaska and Eastern Europe had unique mtDNA haplotypes and were morphologically distinct (Leonard et al. 2007). Beringian wolves, specialized hunters and scavengers of the extinct Pleistocene megafauna, went extinct during the great faunal turnover at the end of the Pleistocene. The extinction of Pleistocene wolves suggests that climate changes and prey specialization might play crucial roles in shaping the adaptation processes of modern wolves. Combining modern and ancient DNA data to reconstruct the dynamics of historical wolf populations may increase the understanding of the dynamics of wolf populations in relation to habitat and prey variations, in an era of global climate and ecological changes.

Wolves are flexible and opportunistic predators, but they adapt to local conditions, evolving into ecological specialists. Ecotypic divergence, despite potential gene flow, may be an important mode of differentiation in large wolf populations, while drift may be much more important in small populations with only a few founders. The concept of wolf ecotypes tangibly extends the concept of the ESU, including not only neutral genetic marker variation, but also distinct functional traits and unique local adaptations. The migratory wolves of the North American tundra or taiga represent a unique ecotype adapted to the Arctic, and specialized for predation on migratory barren-ground caribou *Rangifer tarandus*. The coastal salmon-eating wolves of British Columbia are another unique ecotype, and are genetically and ecologically different from the adjacent inland populations. Differential ungulate prey selection might have constrained wolf dispersal and gene flow, thus determining the observed genetic population structure in Eastern Europe. Habitat types and the composition of ungulate communities may lead to differences in hunting strategy and habitat-biased wolf dispersal. How long did these behavioural mechanisms need to evolve? The wolf-caribou migratory system, salmon-eating and selective red deer predation have probably taken thousands of years to evolve and, consequently, these ecotypes could not be easily replaced if they should go extinct. Global climate and ecological changes could reduce and fragment tundra habitats, thus threatening the wolf-caribou migratory system. Unique adaptations for tundra life may be lost. If habitat specialization is a major factor driving and/or maintaining divergence, then ecological factors may drive genetic differentiation in other species as well.

A primary concern of conservation genetics is to understand the connections between inbreeding and effective population size. In a short period of time, inbreeding depression causes fitness declines that may threaten the survival of small populations. Low genetic variation also predictably threatens the evolutionary dynamics of small populations. Wolves are prone to inbreeding depression (Liberg et al. 2005, Räikkönen et al. 2006). In captive or small populations, mate choice and inbreeding avoidance mechanisms may not be possible, so that the negative consequences of inbreeding are not avoidable. Large or fast-growing populations, in contrast, might avoid inbreeding depression thanks to selection for heterozygotes. Understanding if selection may act to maintain heterozygosity in small populations is a relevant, although still unsolved, issue in conservation genetics.

Just a few unrelated and outbred immigrants may suddenly increase heterozygosity and population growth rates. This finding adds to the knowledge on the genetics

of endangered wolf populations, and helps with the design of efficient management plans for small natural or reintroduced wolf populations. Founding populations, as large as possible and composed of genetically unrelated individuals, should be introduced in areas where several wolf packs can coexist in adjacent territories, facilitating connectivity.

The structure and dynamics of wolf packs is complex and may vary in different ecological conditions. Packs are not always simple familial groups including a breeding pair and their offspring. Dispersal patterns, mortality of the breeders and a variety of mechanisms, such as the splitting or budding of packs, integration of adoptees, multiple matings of the dominant male with two females within a pack, occasional consanguineous matings and the selection for heterozygotes, can modify the structure of local wolf populations. Inbreeding avoidance seems prevalent whenever possible, but sometimes wolves are forced to mate with relatives, especially in exploited populations with high mortality rates.

Wolf dispersal is poorly understood. Sex bias and variable dispersal ranges predictably affect heterozygosity and inbreeding levels, and have consequences on recolonization patterns and population viability. Socio-ecological predictions may be tested using NGS methods. Spatial patterns of pack distribution and dynamics in large areas, and accurate identification of immigrants are fundamental to assessing the potential for gene flow. NGS might help to identify structural details of wolf social behaviour, which could be used to understand the determinants of wolf population dynamics in different habitat or prey-availability conditions. Molecular analyses of wolf scats can produce diverse information on individual capture-recapture histories that are used to estimate population abundance; DNA from prey remains can reveal which individuals were predated and thus provide direct evidence of natural selection and wolf parasites.

Limited numbers of unlinked markers are enough to identify distinct wolf and dog populations and individuals, and their hybrids. The use of linked markers and genomic data sets promises dramatic improvements in the identification of admixed individuals. Analyses of linkage groups will lead to straight identifications of chromosomal segments deriving from each parental population, and to the estimation of the onset of admixture processes. The estimation of times to hybridization will allow the testing of alternative hypotheses of past vs. recent hybridization events that, consequently, will be placed in their own historical and ecological framework. Hybridization and introgression occur naturally among many plant and animal species, and are important factors of evolutionary change. However, hybridization and introgression can cause the extinction of species threatened by translocations, diffusion of ferals or biological invasions. The occurrence of a wolf  $\times$  dog admixture is of great conservation concern in Europe. The introgression of dog genes may reduce the viability of wolf populations with the destruction of adaptation, and provide an increase in aggressive behaviour and livestock depredations. Hybrid policies, however, are still poorly implemented. Risks of hybridization should be reduced by preventing the diffusion of free-ranging dogs, which is simple in principle, but complex in practice due to legislation constraints in some European countries. Wildlife forensic methods can help to detect admixed individuals and populations, and identify the predator species from prey. Extensive genetic databases can support wolf monitoring and management programs to obtain detailed information on wolf presence, distribution range, population size and structure. The

complete sequencing of the dog genome (Lindblad-Toh et al. 2005) has led to the identification of approximately 19000 genes, and more than 2.1 million single nucleotide polymorphisms, which represent invaluable tools also for wolf population studies. Information from the dog genome is being used to generate genotyping microarrays (Gray et al. 2009, vonHoldt et al. 2010) and tools for quantitative analyses of gene expression (Saetre et al. 2004).

Wolf conservation in Europe, independent of technical advances and deeper knowledge of its biology, is mainly dependent upon improving interactions between wolves and humans. The conditions needed for effective conservation strategies can only be met alongside human tolerance for wolves and the solution of conflicts with stakeholders and with human activities in human-dominated regions. The coexistence between wolves and humans is not easy to obtain.

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