APPLICATIONS AND IMPLICATIONS OF PHYLOGEOGRAPHY FOR CANID CONSERVATION

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ABSTRACT. Phylogeographic studies are currently used to infer historical demographic processes such as gene flow, determination of effective population sizes, colonisation dynamics, and population bottlenecks, as well as for the determination of species boundaries and the identification of possible conservation units. We present a review of the main contributions of this approach, and its applications and implications for canid conservation. Studies performed in canids have shown that the number of named subspecies is often larger than that of phylogeographic units. In recent times, the fragmentation of habitats has increased and one of the major concerns of conservation biologists is the occurrence of inbreeding. Large-sized canids have demonstrated to have enough physiological and behavioural plasticity to survive in open habitats, while smaller species that depend on closed habitats are more susceptible to fragmentation. Another process that strongly affects canids is hybridisation because contacts between wild and domestic populations have increased, leading to gene introgression into natural populations. Canids are a key element in food chains; thus, a precise knowledge of intraspecific subdivision is of the utmost relevance for their management before these carnivores disappear, with unknown social, economic, and ecological consequences. In this sense, phylogeographic studies constitute a fundamental tool.

RESUMEN. Aplicaciones e implicaciones de la filogeografía para la conservación de los cánidos. Los estudios filogeográficos son actualmente usados para inferir procesos demográficos históricos como el flujo génico, la determinación de tamaños efectivos de población, dinámicas de colonización y cuellos de botella poblacionales, así como la determinación de límites entre especies y la identificación de posibles unidades de conservación. Presentamos una revisión de las principales contribuciones de este enfoque y sus aplicaciones e implicaciones a la conservación de los cánidos. Estudios realizados en cánidos han mostrado que el número de subespecies nominales es con frecuencia mayor que el de las unidades filogeográficas. En épocas recientes, la fragmentación de los habitats se ha incrementado y una de las mayores preocupaciones de los biólogos conservacionistas es la ocurrencia de endogamia. Los cánidos de gran tamaño han demostrado poseer suficiente plasticidad fisiológica y comportamental para sobrevivir en hábitats abiertos, en tanto que las especies más pequeñas que dependen de hábitats cerrados son más susceptibles a la fragmentación. Otro proceso que afecta fuertemente a los cánidos es la hibridación, ya que han aumentado las zonas de contacto entre poblaciones silvestres y domésticas llevando a la introgresión de genes en el acervo génico de las poblaciones naturales. Los cánidos son un elemento clave de las cadenas alimentarias por lo que un conocimiento preciso de sus actuales subdivisiones evolutivas es de máxima importancia para su manejo antes de que estos carnívoros desaparezcan produciendo consecuencias.

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INTRODUCTION

Phylogeography is the analysis of the principles and processes that govern the geographic distribution of genealogical lineages (Avise et al., 1987; Avise, 2000). In this sense, this approach involves the study of the interaction between demographic aspects, the genetics of populations, and the dynamics of physical processes (geological or climatic) to address relevant questions in the areas of evolutionary biology, ecology, and conservation.

Phylogeography is currently being used as a tool for the inference of historical demographic processes such as gene flow, effective population size, colonisation sequences, and populational bottlenecks, and also for determining species boundaries and identifying possible conservation units (Avise et al., 1987; Avise, 2000, 2008; Vázquez-Domínguez, 2002; Freeland, 2005). In the latter aspect, studies of geographic distribution of genealogical lineages have been widely used to describe historical events such as habitat fragmentation or expansions in the distribution areas of species and populations, migration events, vicariance and extinction of genetic lineages, as well as other processes affecting population structure or causing speciation in a spatial or temporal context (Hardy et al., 2002). The comparative analysis of the phylogeographic patterns of different species allows the formulation of hypotheses about possible shared events such as vicariance or dispersion which then can be associated to geological, ecological and ethological processes (Arbogast and Kenagy, 2001; Zink, 2002; Lanteri and Confalonieri, 2003).

From its origins, phylogeography has been closely associated with analyses based on information of the mitochondrial genome (Avise, 1998). However in recent years, the utilisation of microsatellites (and other nuclear DNA loci) has drastically increased. These studies facilitate the interpretation of recent events due to the high rate of mutation of microsatellites. More generally, the use of different markers allows the understanding of phylogeographic patterns and processes at different evolutionary scales (Beheregaray, 2008). Thus, population genetics and phylogeographic studies may be considered as part of a continuum and not independent fields (Joseph and Omland, 2009).

Members of the Family Canidae are usually characterised by high mobility and vagility, ethological characteristics that result in low levels of geographical genetic differentiation (Dalén et al., 2005; Iyengar et al., 2005; Tchaicka et al., 2006). However, habitat fragmentation and loss have intensified genetic drift in small populations, accelerating differentiation and loss of genetic variability. The erosion of genetic diversity has occurred in many species, increasing extinction risk because of the maintenance of isolated subpopulations with reduced effective population numbers. High vagility may also be influential in promoting hybridisation and affecting genetic composition (Lehman et al., 1991; Jenks and Wayne, 1992; Wayne, 1992; Mercure et al., 1993; Wilson et al., 2000; Wayne and Brown, 2001), having an impact on interspecific gene flow and affecting the gene pools of species (Jenks and Wayne, 1992; Wilson et al., 2000; Wayne and Brown, 2001).

Phylogeographic concepts are increasingly being applied to the biological conservation of species (Beheregaray, 2008). We herewith present a review of the different contributions of this approach to the understanding of the formation of the mitochondrial genome (Avise, 1998). However in recent years, the utilisation of microsatellites (and other nuclear DNA loci) has drastically increased. These studies facilitate the interpretation of recent events due to the high rate of mutation of microsatellites. More generally, the use of different markers allows the understanding of phylogeographic patterns and processes at different evolutionary scales (Beheregaray, 2008). Thus, population genetics and phylogeographic studies may be considered as part of a continuum and not independent fields (Joseph and Omland, 2009).

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processes and patterns of the spatial distribution of genetic variability, and its applications and implications for canid conservation.

**EVOLUTIONARY SIGNIFICANT UNITS**

The patterns of variation of genetic and phenotypic characteristics and their interactions with environmental variation provide substrate that ultimately leads to geographical population differentiation (Avise, 2000; Coyne and Orr, 2004). In heterogeneous landscapes, restrictions to gene flow between populations may result not only from geographic barriers or isolation by distance (Ribera and Vogler, 2004; Knowles and Richards, 2005), but also because of non-vicariant events, among them, diversifying natural selection, when environmental conditions and selective regimes differ between different locations (Nosil et al., 2008). Most conservation biologists consider that sets of populations that show restricted gene flow with other equivalent units represent the highest level of the species geographical structure and require differentiated genetic management. (Fraser and Bernatchez, 2001). Such populations are called ‘evolutionary significant units’ (ESU) (Ryder, 1986). The concept of ESUs was developed to provide an objective approach for the protection of units below the species level, because subspecies delineations may be absent and often do not adequately reflect the geographical genetic structure existing within species (Avise, 1989).

Several large-scale phylogeographic studies have been developed in canids, allowing the identification of different patterns and levels of geographic genetic structuring. One of these studies analysed the genetic patterns in most of the distribution of the arctic fox (*Alopex lagopus*) using mtDNA D-loop sequences. Geographical differentiation was generally moderate in this species, reflecting ongoing frequent gene flow and/or recent isolation or divergence. However, high Fst values between Iceland and other populations demonstrated that the former were relatively more isolated from the rest (Dalén et al., 2005). Microsatellite population analyses of another Arctic emblematic canid, the grey wolf (*Canis lupus*), revealed high gene flow between continental and island populations (Carmichael et al., 2008). New methodological advances based on estimations from Bayesian analyses allow the estimation of rates and directionality of migration, growth, effective population sizes and extinction events (Sacks et al., 2005; Palstra and Ruzzante, 2008; Ilves et al., 2010, among others). For example, it is important to estimate such parameters to know if the Arctic species *A. lagopus* and *C. lupus* exhibit metapopulation dynamics where extinctions occur in islands that are then recolonised from the continent (Elmhagen and Angerbjorn, 2001), possibly facilitated by the ice that forms connecting bridges during winters (Johnston, 2002). The understanding of the population dynamics of a species is central to conservation programs because metapopulations do not occupy all adequate habitats as a consequence of the equilibrium between colonisation and extinction; thus, the conservation of seemingly unoccupied habitat may still be a priority (Hanski, 1998). Studies of mtDNA sequences comprising a large part of the geographic distribution of *C. lupus* demonstrated that North America, Europe and most of Asia populations represent a single mega-ESU, showing reciprocal monophyly with allopatric distributions. The exception to this lack of structuration are the Himalayan and Indian populations that form two haplogroups that can be considered independent ESUs each and isolated from the rest of the populations of North America, Europe and the rest of Asia (Pilot et al., 2010) (**Fig. 1**). In general, different studies of *C. lupus* have shown that the low structuring of their populations is not explained by evident geographic barriers and historical events (Carmichael et al., 2001; Geffen et al., 2004; Musiani et al., 2007). Nevertheless, in a study in northwestern Canada using microsatellites, Carmichael et al. (2001) found that genetic structuring may be influenced by prey specialization, suggesting that prey (caribou) migration could serve as a corridor for gene flow. In North America, Geffen et al. (2004), analysing populations using microsatellites and
Fig. 1. Haplotype network of Canis lupus. The rectangles limited by dashed lines indicate the three possible ESUs; mutational events are represented by circles on the lines that connect the haplotypes. It can be seen that wolves from Himalaya and India are separated by more than eight mutations. This figure corresponds to the work by Pilot et al. (2010) and was kindly provided by the senior author of that publication.

RFLP observed that genetic structuring could be correlated with the climatic characteristics of different regions. These data are consistent with the phenotypic variation shown by the species: colour and size vary from North to South (Gipson et al., 2002); these phenotypic characteristics could result in restrictions to gene flow, allowing genetic drift and/or natural selection to maintain genetic and phenotypic differentiated populations (Geffen et al., 2004). These findings led to the development of the ‘wolf ecotype’ concept, thus extending the ESU concept to...
consider not only the genetic composition of populations but also functional characteristics and local adaptations (Crandall et al., 2000). When *C. lupus* is analysed with mitochondrial markers at a reduced geographic scale, no significant structuring is observed, but when multilocus markers such as microsatellites are employed, small-scale geographical structuring related to prey availability and environmental characteristics is observed. Genetic structuring must be interpreted jointly with ecological and morphological data when defining ESUs. Furthermore, clinal variations of body size and coloration are common in homeothermic animals generating patterns known as Bergmann’s and Gloger’s rules, respectively, where larger and less pigmented individuals are found at higher latitudes (Bergmann, 1847; Gloger, 1883). Because this is a common pattern for mammals, it is possible that clinal variation of body mass or colour are not necessarily related with restrictions to gene flow between populations. Morphological analyses of the cranium, and the mandible, may allow the detection of functional characteristics and local adaptations subjected to natural selection, which in turn are important for the delimitation of ESUs (Cardini et al., 2003).

In the African continent, the analyses of the mitochondrial control region (D-loop), as well as eleven microsatellite loci of the African wild dog (*Lycaon pictus*), demonstrated genetic differentiation between all studied populations which, however, maintain genetic flow between them. Also, from mitochondrial data, two highly structured clades were found, one including genotypes exclusive of the southern populations and the second one formed by an exclusive eastern haplotype, the rest of haplotypes being shared by the southern and eastern populations. These genetic patterns where exclusive haplotypes are found in southern and eastern populations, could be the result of the Pleistocene/Holocene climatic transitions. Besides, the haplotypes shared by the southern and eastern populations could be the consequence of secondary migrations between these populations (Girman et al., 2001; Marsden et al., 2012). These information, added to that of previous work by Girman et al. (1993) who demonstrated that southern and eastern populations are genetically and morphologically distinct, support the classification of these two groups (eastern and southern) as different ESUs. Consequently, translocations of individuals from the south and the east are not advised, since possible adaptive differences may exist (Crandall et al., 2000). Since the eastern populations (Masai Mara and Serengeti) are in serious extinction risk, the locality of Selous would be a suitable alternative as a source for the introduction of individuals because it has genotypes typical of the eastern clade. Furthermore, captive populations showed typical southern genotypes, thus representing potential individuals for introduction in southern wild populations (Girman et al., 2001) (Fig. 2).

*Lycaon pictus* had a very large historical distribution inhabiting all African ecosystems south of Sahara, with the exception of tropical forests. However, its populations have dwindled dramatically after the arrival of Europeans and the subsequent growth of human populations (Creel and Creel, 1998); currently, the species is limited to isolated patches. In the study of Girman et al. (2001), which compared the genetic diversity of *L. pictus* with other phylogenetically close canids, no loss of genetic diversity could be detected using either mtDNA or microsatellite markers. However, this kind of approach is limited by the availability of populations for comparison and in fact, it is frequently difficult to verify the assumptions (Garza and Williamson, 2001). Recent analytical developments based on Markov chain Monte Carlo methods allow a robust characterization of historical variations of population size (Girod et al., 2011), which is central to *L. pictus* conservation because loss of genetic diversity may limit its evolutionary potential (Frankham et al., 1999) and reduce the ability of a population to produce a response to recently introduced pathogens and parasites (O’Brien and Evermann, 1989). The latter is probably the cause of the recent extinction of populations in the Kenya-Tanzania frontier (Sillero-Subiri et al., 2004).

The Crab-eating Fox (*Cerdocyon thous*) is one of the most common canid species in South America. This species has a disjunct
Fig. 2. Main localities and captive colonies of *Lycaon pictus* studied by Girman et al. (2001). The gray area represents the historical distribution of the species. Arrows indicate the direction of the reintroduction of individuals as recommended by the authors. Adapted from Girman et al. (2001).

distribution inhabiting northern Amazonia in Colombia and most of Venezuela (except for the southern region of the State of Amazonas), and to the south of Amazonia in northeastern, central and southern Brazil, Paraguay, central and northern Argentina, western Bolivia, and Uruguay (Sillero-Zubiri et al., 2004), showing a clear preference for open habitats (Bisbal, 1989; Trovati et al., 2007). Phylogeographic analyses of this species using nuclear and mitochondrial sequences in different Brazilian populations revealed two phylogenetic clades, one corresponding to the North, and the other to the Southern region of Brazil (Tchaicka et al., 2006). This structure may be related to habitat fragmentation during the Late Pleistocene; it is estimated that during the Last Glacial Maximum two isolated regions of high probability of occurrence existed for this species—one in northeastern Brazil in the Caatinga region, and the other in northern Argentina (Chacoan region) (Martinez et al., 2013). Despite the generalist ecology of *C. thous*, changes in the environment may have limited its dispersion possibly as a result of drastic vegetation changes. These observations suggest that the apparent cotinuity of present day biomes is deceiving, obscuring historical fragmentation (Martinez et al., 2013). The subdivision identified in *C. thous* does not correspond with the previously proposed subspecies by Cabrera (1931), who recognized three subspecies in Brazil, *C. t. entrerrianus* in the south, *C. t. azarae* in the center and north-east and *C. t. thous* in the north. Phylogeographic studies comprising the whole geographic distribution of *C. thous* are necessary to determine the taxonomic status of the subspecies and, with that information, define and delimit the ESUs, because at present genetic data from populations of northern Amazonia of *C. t. thous* and *C. t. aquilus* in Venezuela that show morphological
differentiation between them and the rest of subspecies, are practically non-existent (Bisbal, 1988; Machado and Hingst-Zahaer, 2009).

Eleven subspecies are traditionally recognized in the Asiatic wild dog (*Cuon alpinus*) (Cohen, 1978). Studies of mtDNA and microsatellite data revealed that only two phylogeographic groups exist; the accepted taxonomic status is inconsistent because a sufficient genetic structuring between the subspecies of the different regions is not observed. Besides, it was observed that the subspecies *C. a. sumatrensis* (restricted to Sumatra island) and *C. a. javanicus* (restricted to Java) could have originated from individuals introduced from India, although further studies are needed to confirm these findings (Iyengar et al., 2005).

The above mentioned studies corroborate that, in general, the number of geographical genetic units is smaller than the number of named subspecies of canids. The latter are primarily based on colour and size variations, Thus, phenotypic differentiation may respond to genetic or plastic variation associated with environmental gradients, whereas differentiation of presumably neutral genetic markers will tend to reflect historical fragmentation (or lack thereof) as well as historical and current levels of gene flow.

**INBREEDING**

Mating between relatives (inbreeding) may lead to reduction of reproductive potential and survival, as a consequence of the increase in frequency of homozygotes for deleterious or lethal alleles, which increases extinction risk. The harmful effects of inbreeding were for the first time documented in detail by Charles Darwin, who performed experiments in different plant species that exhibited auto-pollination and cross-pollination (Darwin, 1876). Different anomalies, such as lethal phenotypes in the first years of life, or genetic diseases have been reported in inbred individuals. When no major abnormalities are observed, endogamic depression is detectable as it results in low fertility and growth rates (Charlesworth and Willis, 2009).

In Scandinavia, *C. lupus* became extinct in 1960 but, around 1980, at least two wolves founded a new population in South-Central Scandinavia (Vilà, 2003). The first reproductive event occurred in 1983, and in 1991 a new wolf contributed genetically to the preexistent population. By 2005, this population consisted of about 135-153 individuals (Wabakken et al., 2005). Using microsatellite analyses, Lieberg et al. (2004) produced a pedigree of the population and observed a high level of inbreeding depression manifested in a reduction in the number of offspring and an increased level of vertebral fracture due to malformation (Räikkönen et al., 2006).

A clear case of inbreeding can be observed in the Ethiopian wolf (*Canis simensis*) that is distributed in the country’s highlands at about 3000 m in seven small, isolated populations. The subspecies *C. s. simensis* is found to the north of the Great Rift Valley, and *C. s. citerinii* occurs to the south (Gottelli et al., 2004). This species presents an imminent extinction risk. Studies using mtDNA and microsatellite sequences have shown that *C. simensis* has very low genetic variability. The isolation of the populations due to habitat seems to be the crucial factor influencing genetic diversity. These low genetic diversity values are consistent with an effective population number of a few hundred individuals. Anthropogenic perturbations have led to a reduction of population size, increasing the probability of allele fixation and of global loss of haplotypes and heterozygosity (Gottelli et al., 1994; Gottelli et al., 2004; Randall et al., 2010).

Canids in general show low levels of inbreeding because of their great dispersal potential, but in endemic species on islands the situation is very different due to the limitations to their dispersal. In North America, the island fox (*Urocyon littoralis*) is critically endangered and is only found in six islands of southern California (Wayne et al., 1991). Molecular studies indicated that the San Nicolas island population was invariant for all genetic markers, including multiple loci obtained by fingerprinting and 19 microsatellite loci (Gilbert et al., 1990; Goldstein et al., 1999). No other wild population approaches this lack of genetic variation. In the same way, the lesser island, San Miguel, revealed low levels of genetic variation
relative to the larger islands of Santa Catalina, Santa Rosa and Santa Cruz. Mitochondrial data suggested that the island of Santa Catalina may have been colonised several times from neighbouring islands (Roemer et al., 2002). These results indicate that each island should be considered as a different conservation unit.

In the coast of Chile, on the Pacific littoral of South America, lies Chiloé Island, where Darwin first observed a small endemic fox, _Lycalopex fulvipes_. Darwin’s Fox is the sole canid species on Chiloé island and has the smallest geographic distribution of all canids (Cabrera, 1958). Genetic analyses of this species using mtDNA confirmed that only three haplotypes exist and genetic variability is very low. It is estimated that less than 500 individuals exist, and none in captivity (Vilà, unpublished data).

Increased fragmentation of species habitat has occurred during the last 200 years, leading to the isolation of populations. Some canid species have shown enough physiological and ethological plasticity to survive in open habitats along with agriculture and cattle, thus maintaining gene flow between populations. Two different scenarios may be expected: 1) Canid species of large size are probably less susceptible to fragmentation but usually have low densities (Carbone and Gittleman, 2002). If fragmentation leads to an interruption of gene flow between populations, the resulting subpopulations will have reduced size and the effects of inbreeding will be evident in a few generations. 2) In contrast, species of smaller size that depend on closed habitats are more susceptible to fragmentation, thus inbreeding is favoured, but because of higher population densities the effects of inbreeding will be evident only after longer periods of time (Hartl and Clark, 2007).

**HYBRIDISATION**

Along the history of life on Earth, hybridisation has played a very important role in the evolution of biodiversity. On the one hand, hybridisation processes may produce novel gene combinations that could promote speciation and adaptive radiation generating new phenotypes on which natural selection can operate (Maváres and Linares, 2008). Conversely, hybridisation also can contribute to the extinction of species producing introgression of exotic alleles, causing possible exogamic depression and reducing species fitness (Templeton, 1986; Lynch, 1991; Edmands and Timmerman, 2003). Hybridisation between genetically distinct populations which are adapted to their local environments may modify the interaction between genes and environment producing less fit offspring, and lead to the disruption of coadapted gene complexes and exogamic depression. Since nowadays natural processes are strongly affected by anthropic action, hybridisation has become one of the main causes of extinction risk (Wolf et al., 2001).

Hybridisation, with and without genetic introgression has been frequently reported in Canidae (Lehman et al., 1991; Mercure, 1993; Roy et al., 1996; Sillero-Zubiri et al., 1996; Wayne et al., 1997; Wayne and Brown, 2001; Hailer and Leonardo, 2008; Kays et al., 2010; Wheeldon et al., 2010) leading in some cases to put the survival of species or populations at risk (Nowak, 1979; Wayne and Jenks, 1991; Gottelli et al., 1994; Roy et al., 1994).

Wolves and domestic dogs are very closely related, the latter having been domesticated from the former probably in several independent episodes of domestication (Vilà et al., 1997; Wayne and Ostrander, 1999). Both taxa differ in only 1.8% of their genome sequences and have identical karyotypes which greatly facilitate hybridisation (Mech and Beitani, 2003) and the production of fertile progeny in captivity and in nature, where they overlap. In the last few years, with the increase of human settlements, hybridisation between wolves and feral dogs has increased, resulting in introgression of dog genes into the wolf gene pool. Several phylogeographic studies have detected hybrids of _Canis lupus_ and domestic dog in nature. This situation has already been recorded in North America and several European countries (Dolf et al., 2000; García-Moreno et al., 1996; Randi et al., 2000; Randi and Lucchini, 2002).

In _C. simensis_, microsatellite analyses identified hybrid individuals in a population of the Sanetti plateau at the mountains of the Bale National Park. In this region, typical domes-
tic dog alleles were found in *C. simensis*, and typical *C. simensis* alleles, in some domestic dogs. Hybridisation between these two species seems to have no restrictions since offspring are produced through both species, indicating lack of hybrid inviability and/or sterility (Gottelli et al., 1994). In these conditions, domestic dogs not only hybridise with *C. simensis* but also compete with them for food resources, and may function as reservoirs of canine diseases (Sillero-Zubiri et al., 1996). Because population size of *C. simensis* is critical (300-500 individuals), hybridisation events may be sufficient to swamp the species genetic identity.

*Alopex lagopus* is classified as threatened and in risk of extinction in Sweden and Norway, respectively (Linnell et al., 1999; Gardenfors, 2000). One of the threats to this species is hybridisation with domesticated *A. lagopus* (Angerbjorn et al., 2004). Norén et al. (2005) analysed, by means of mtDNA and microsatellites, wild and captive *A. lagopus* individuals and observed strong genetic differentiation, possibly due to different geographic origins and selective breeding. Among the individuals from wild populations, some were related to the captive foxes, which suggests that occasional escapes from the farms do occur. *Alopex lagopus* is well adapted to its particular habitat (Angerbjorn et al., 2004) showing examples of local adaptations related to the time of reproduction, care of the young, and skin insulation and camouflage (Prestrud, 1991). Besides, the thickness of the fat layer of Scandinavian Arctic foxes seems to be adapted to fluctuations in food availability caused by the lemming cycles (Tannerfeldt and Angerbjorn, 1998). In view of this complex adaptive pattern, Norén et al. (2005) raised the hypothesis that hybridisation may lead to the loss of local adaptations, with the consequent risks for wild populations.

Wayne and Jenks (1991) analysing mitochondrial DNA, proposed that the red wolf (*C. rufus*) is a hybrid between the gray wolf (*C. lupus*) and the coyote (*C. latrans*), rather than a valid species or subspecies. This finding was used to criticise efforts made to reintroduce *C. rufus* in the wild (Gittleman and Pimm, 1991). However, evidence contradicting this hypothesis based on genetic, paleontologic and morphological data was almost immediately produced (Dowling et al., 1992a, b; Nowak, 1992). Nowadays, the taxonomic status of *C. rufus* is not clear, although recent evidences support *C. rufus* as a valid species (Sillero-Zubiri et al., 2004).

During the XXth century, putative hybrids between *C. rufus* and *C. latrans* have been reported. These cases are probably recent phenomena that resulted from anthropogenic modification of habitats or population decline caused by direct persecution (Nowak, 2002). In the United States, the state of Texas has historically been characterised by the presence of three morphologically distinct canid species that coexist sympatrically since the Holocene: the Mexican wolf (*Canis lupus baileyi*), the red wolf (*C. rufus*), and the coyote (*C. latrans*). Hailer and Leonard (2008) analysed these species using sequences of mtDNA control region and microsatellites located in the Y chromosome, in order to identify maternal and paternal lineages, respectively. In this study, the authors observed that one individual of *C. latrans* carried a mitochondrial haplotype of *C. l. baileyi*, and another one had Y chromosome alleles of the same species *C. l. baileyi*, evidencing that both males, and females of *C. latrans* may be involved in hybridisation events. Also, one individual of *C. l. baileyi* showed a mitochondrial *C. latrans* haplotype. A mitochondrial haplotype closely related with those of *C. latrans* was found in *C. rufus*. Another individual of this species had a Y chromosome haplotype possibly related to *C. latrans*. However, the lack of reciprocal specific monophyly between *C. latrans* and *C. rufus* as well as the lack of information of Y chromosome sequences of other canid species dificults the determination of the origin of the haplotypes. Hybridisation between *C. latrans* and *C. rufus* might have a strong impact on *C. rufus* populations (Adam et al., 2003; Fredrickson and Hedrick, 2006), while hybridisation between *C. l. baileyi* and *C. rufus* may have the same consequences on *C. rufus*, although presently both species are totally allopatric (Hailer and Leonard, 2008).

Molecular information has increased the ability to detect hybrids in recent years, but no easy answer exists in relation to whether
hybrids should or should not be protected. There are big economic interests that would benefit from any suggestion that a species is not taxonomically valid to argue that conservation efforts are not guaranteed and its habitat may be exploited (Nowak, 1995).

Naturally occurring hybridisation, whether leading to speciation or extinction, does not constitute a threat in itself for the involved species since it is part of their evolutionary history (Arnold, 1992), but it may turn into a problem if it is favoured by changes in habitat or in the composition of the species through anthropogenic activities. In the latter cases, immediate management action is required to avoid compromising the evolutionary histories and genetic integrity of the affected species (Allendorf et al., 2001).

**FINAL CONSIDERATIONS**

Phylogeographic studies have contributed to systematics in the recognition of species and subspecies boundaries, and more generally to identify genetic units in the wild. In recent years, the possibility of using a larger number of molecular genetic markers has increased. This will facilitate the understanding of the dynamics and the status of populations of different canid species. An increase of studies simultaneously using different mitochondrial and nuclear (autosomal and sex-linked) markers is decisive because the use of just mitochondrial markers may be telling only a part of the story, since mitochondrial inheritance is strictly maternal. More generally, multilocus analyses are far more informative than single-locus assays. Comprehensive sampling at the adequate level (ideally comprising the entire distribution of the focal species), involving different habitats and coupled to morphological studies for detecting adaptive characteristics represent the more adequate condition for the identification of actual evolutionary significant units. In turn, this information is critical to guide management and conservation policies. It is also necessary to study genetic variation of populations kept in natural preserves and ex-situ conservation centres. It will thus be possible to identify the potential of individuals for possible reintroductions, because knowledge of the genetic relationship with the wild populations is essential to avoid incorporating organisms with unwanted characteristics.

One of the main anthropic phenomena is the alteration and fragmentation of natural habitats which has an enormous impact on inbreeding and hybridisation processes. Thus, assessing the effects of habitat modification is of extreme relevance in conservation decision-making. In this sense, the genetic knowledge across the distributional range of species is essential, although many canids have been ignored in this respect. The majority of phylogeographic studies have been devoted to large-sized canids, while little has been done and is known about smaller-sized species (Schwartz et al., 2005). The latter may turn out to be more susceptible to habitat fragmentation. This is particularly evident for example, in species of the genus *Vulpes* in North America, Asia and Africa or species of *Lycalopex* in South America. In view of this, it is necessary to increase our knowledge of how different species are affected by restrictions to movements between different habitat fragments, and to what extent the different production matrices (e.g., cattle raising, agricultural lands, plantations, oil fields) interact and affect gene flow in many of these canid species.

Food chains are generally complex, and include both direct and indirect interactions between different trophic levels. Frequently this complexity confounds our best efforts to anticipate how wild populations will respond to human activities (Polis and Strong, 1996). Since canids either behave as top predators or have generalist habits, their loss represents a high impact in community structure. In view of this, the precise knowledge of their genetic diversity and subdivision, and demographic characteristics is of great relevance to guide their management before these carnivores disappear with negative social, economic and ecological implications.

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REFERENCES


GLOGER CL. 1833. Das Abändern der Vogel durch Einfluss des Klimas. August Schulz, Breslau, Germany.


PALSTRA FP and DE RUZZANTE. 2008. Genetic estimates of contemporary effective population size: What can they tell us about the importance of genetic stochasticity for wild population persistence? Molecular Ecology 17:3428-3447.


REICH DE, RK WAYNE, and DB GOLDSTEIN. 1999. Genetic evidence for a recent origin by hybridization of red wolves. Molecular Ecology 8:139-144.


REICH DE, RK WAYNE, and DB GOLDSTEIN. 1999. Genetic evidence for a recent origin by hybridization of red wolves. Molecular Ecology 8:139-144.


WILSON PJ. 2000. DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. Canadian Journal of Zoology 78:2156-2166.
