Animal Conservation

Animal Conservation. Print ISSN 1367-9430

Noninvasive monitoring of wolves at the edge of their distribution and the cost of their conservation

J. Echegaray[1,2,3] & C. Vilà[3]

1 Basque Wolf Group, Vitoria-Gasteiz, Spain
2 Center for Conservation and Evolutionary Genetics, Smithsonian Institution, Washington, DC, USA
3 Estación Biológica de Doñana-CSIC, Seville, Spain

Keywords
mitochondrial DNA; microsatellites; noninvasive monitoring; Spain; *Canis lupus*; dog; predator control.

Correspondence
Jorge Echegaray, Estación Biológica de Doñana-CSIC, Avd. Américo Vespucio s/n, 41092 Seville, Spain.
Email: echegaray@ebd.csic.es

Received 5 January 2009; accepted 10 September 2009

doi:10.1111/j.1469-1795.2009.00315.x

Abstract

Large predators are recolonizing areas in industrialized countries, where they have been absent for decades or centuries. As they reach these areas, the predators often encounter unwary livestock and unprepared keepers, which translates into large economic costs. The cost per individual may have important repercussions on the conservation and management of large predators. During the years 2003–2004, we collected 136 feces preliminarily identified as belonging to gray wolves *Canis lupus* along the north-eastern limit of the wolf range in the Iberia peninsula (Basque Country, Spain). Genetic analyses allowed us to identify the species of origin in 86 cases: 31 corresponded to wolves, two to red foxes *Vulpes vulpes* and 53 to dogs *Canis familiaris*. Among the wolves, we identified 16 different individuals. We estimated the cost of conserving wolves to be >€3000 per wolf per year, based on the cost of damage compensation and prevention during the 2003–2004 period. However, most of the wolf feces contained wild prey whereas dog feces contained mostly remains of domestic animals. This finding suggests that uncontrolled dogs could be responsible for some of the attacks on livestock, contributing to negative public attitudes toward wolf conservation and increasing its cost.

Introduction

Some large carnivores, including gray wolves *Canis lupus*, are coming back to many areas in industrialized nations (Boitani, 2003). As wolves return to areas that they have not occupied for generations, they encounter poorly guarded livestock, which often leads to predation on domestic animals and large economic losses (Sand *et al.*, 2006; Bostedt & Grahn, 2008). As a result, governments are both spending large amounts of money in damage prevention and compensation, as well as designating areas where predator populations are strictly regulated or eliminated (e.g. for Sweden, see Bostedt & Grahn, 2008; for Finland, see Ministry of Agriculture & Forestry, 2005). These policies slow down the potential growth of the predator population. Furthermore, feral and uncontrolled dogs *Canis familiaris* are common and are also capable of attacking livestock, especially sheep (Pimentel *et al.*, 2000). Their possible contribution to the depredation of livestock – and to the wolf's bad reputation – is usually not evaluated by managers due to technical difficulties and remains unrecognized by the public.

Over the last two decades, the densely populated Basque Country (7234 km^2, 295 people km^{-2}) in northern Spain has represented the eastern limit of the Iberian wolf population (Blanco & Cortés, 2002) (Fig. 1). The Iberian wolf population is composed of a minimum of 254 packs (Álvares *et al.*, 2005) and is distributed mainly in the north-western quadrant of the Iberian peninsula. The European mammal assessment considers the Iberian wolf population 'near threatened' because of human-induced threats and the lack of coordinated management (Boitani, 2000). In some parts of the Iberian Peninsula, wolves are protected, whereas in other areas they are considered a game species. Despite the geographic expansion of this wolf population in recent decades, wolves have not permanently settled in the Basque Country because they have been regularly eliminated.

Of the 1300 km^2 regularly occupied by wolves in the Basque Country, about 85% is in the province of Álava (3047 km^2), where the average human population density is relatively low (91 people km^{-2}). Herds of endemic latxa sheep, used to produce the highly appreciated Idiazábal cheese, are the most abundant livestock species (83 500 sheep occur in the entire province, 41% of them within the range of the wolf). Sheep are often free ranging and are not under continuous supervision by shepherds. These sheep are often reported to suffer attacks from wolves, which has led to conflict between farmers, managers and conservation agencies and groups.

Official accounts from the regional government of Álava showed that during 2003–2004, a total of 432 domestic animals were attacked in 154 separate incidents; 94% of these attacks were attributed to wolves (Aguirrezábal &

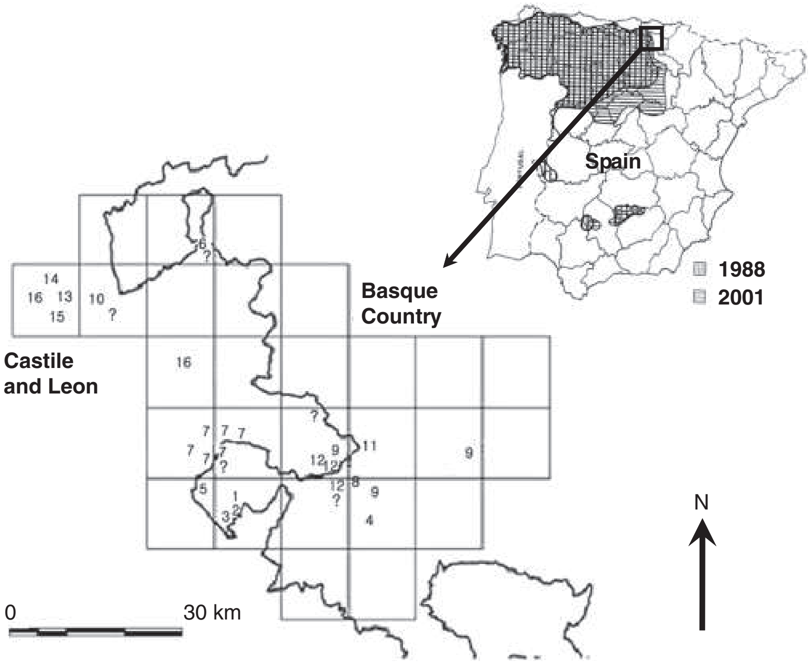

Figure 1 Study area in relation to the distribution of wolves *Canis lupus* in Spain in 1988 (vertical hatching) and 2001 (horizontal hatching) (Blanco & Cortés, 2002), and location of feces identified as corresponding to wolves. Each individual is marked with a different number. Question marks indicate unidentified individuals (incomplete genotypes). The line marks the limit of the Basque Country (the southern 2/3 marks the limit of the Basque province Álava, where the study was centered). Squares correspond to UTM grid cells of 10 × 10 km.

Sánchez, 2007). In response to these attacks, 27 collective drives were organized to hunt wolves during those 2 years and permits were awarded to kill wolves during wild boar hunts, resulting in the known death of two wolves.

Livestock farmers were compensated in all of the attacks attributed to wolves. Sheep accounted for 92% of the animals attacked, corresponding to 0.3% of all sheep in the region and about 80% of the costs. Direct compensation and prevention of wolf attacks in that 2-year period summed to €108 696. About 60% of these funds were invested in prevention activities, including the use and maintenance of large guard dogs. During this time period, only 10 attacks (affecting 30 animals) were attributed to dogs. Some groups feel that these costs are unsustainable (Askacíbar & Ocio, 2006).

We used noninvasive sampling of feces and the genetic identification of individuals to estimate the number of wolves living in and near the Basque Country during 2003–2004. We also used genetic methods to assign each feces to either wolf or dog and compared the occurrence of domestic and wild prey in their diets

Methods

During 2003–2004, we collected 136 feces along 690 km of transects in Álava and surrounding areas. The region surveyed included the area where all reported wolf attacks and sightings occurred during 1999–2002, and neighboring areas where the presence of wolves was probable. The sampling was opportunistic and some areas were more thoroughly explored and some transects were explored more than once. Feces were identified in the field as most likely corresponding to wolves based on size (diameter >2.5 cm) and the presence of large ungulate prey remains. Geographic coordinates were collected for each sample. Feces were kept dry and frozen at −20 °C until they were analyzed.

Genomic DNA was extracted using the QIAamp® DNA Stool Mini kit (Qiagen, Solna, Sweden). Partial mitochondrial DNA (mtDNA) control region sequences were obtained as described in Vilà *et al.* (1999). In order to identify the species of origin for each feces, sequences were compared with those reported in previous studies of wolves and dogs (Vilà *et al.*, 1997, 1999; Savolainen *et al.*, 2002) and with sequences deposited in GenBank (http://www.ncbi.nlm.nih.gov/).

Feces identified as corresponding to wolves were subsequently typed for 20 autosomal microsatellites as in Vilà *et al.* (2003): c2001, c2006, c2010, c2017, c2054, c2079, c2088 and c2096 (Francisco *et al.*, 1996), vWF (Shibuya *et al.*, 1994), u109, u173, u225, u250 and u253 (Ostrander, Sprague & Rine, 1993) and PEZ01, PEZ03, PEZ05, PEZ06, PEZ08 and PEZ12 (Perkin Elmer, Zoogen, Davis, CA, USA; see NHGRI Dog Genome Project, http://research.nhgri.nih.gov/dog_genome/). Sex determination was conducted following the protocol and markers of Seddon (2005). Because the amplification of DNA from feces can be heavily affected by allelic dropout (Taberlet *et al.*, 1997), each amplification was repeated six times and consensus genotypes were built for each sample. For a heterozygous genotype to be confirmed, it had to be observed in at least three replicates, four for a homozygote. Although these conditions were more stringent than those used in other noninvasive studies of carnivores (e.g. see Flagstad *et al.*, 2003; Hedmark *et al.*, 2004), it is still possible that allelic dropout affected some of the consensus genotypes. The number of different consensus genotypes was used as an estimate of the minimum number of wolves in the area.

With the help of a microscope, the contents of each feces genetically assigned to wolf or dog were identified following the key of Teerink (1991) and by comparison with reference collections compiled by the authors. Fecal analysis was conducted by the same person who conducted the genetic analysis (J. E.), but the two procedures were separated by a period of several months and knowledge regarding species identification was not considered.

Results and discussion

Previous studies identified a small number of maternally inherited mtDNA haplotypes in Iberian wolves (Vilà *et al.*, 1999 and unpubl. data) that were clearly differentiated from dog haplotypes (Vilà *et al.*, 1997; Savolainen *et al.*, 2002). We successfully extracted and sequenced mtDNA from 86 of the 136 feces sampled (63% success). A single Iberian wolf-specific haplotype was identified in 31 feces (corresponding to lu4, in Vilà *et al.*, 1999). Dog haplotypes were identified in 53 feces and 2 had red fox *Vulpes vulpes* sequences.

We then typed 20 canine autosomal microsatellite markers and molecularly sexed the wolf feces. The consensus genotypes for each of the samples successfully typed at a minimum of 12 loci revealed the presence of 16 individual genotypes, representing the minimum number of individual wolves in our study area (Fig. 1). The genetic profiles did not suggest the presence of any wolf–dog hybrid in the sample and all of them fit within the diversity observed in a larger survey of Iberian wolves (data not shown). In combination with the sex typing, these profiles indicated the presence of five males, seven females and four individuals of unknown sex. We decided not to estimate the number of wolves in the area with rarefaction curves (Kohn *et al.*, 1999; Eggert, Eggert & Woodruff, 2003) because field sampling was not random; we sampled the interior of the region more intensively than the periphery; we typically avoided resampling areas; most genotypes were observed only once; the population within the Basque Country was part of a much larger wolf population. These factors would collectively contribute to large confidence intervals in estimates of population size based on rarefaction curves.

If we assume that all wolves contributed equally to attacks on livestock, we can estimate the average cost of conserving a wolf by dividing €108.696, the total cost of damage prevention and compensation in 2003 and 2004, by 32 (16 wolves × years, assuming that all individuals are alive and in the area during the two years). This implies that each wolf costs the public *c.* €3397 per year. This amount would be lower if the number of wolves had been underestimated.

Given the small number of wolves and the large number of attacks, the diet of the Basque wolves appears be heavily dependent on domestic livestock, especially sheep. We investigated whether this was the case by comparing the remains of prey identified in both wolf and dog feces. Each feces contained only a single prey item. Among the prey items identified in 30 wolf feces (the remains in one wolf fecal sample were unknown), 22 contained wild prey (17 roe deer *Capreolus capreolus*, three wild boar *Sus scrofa*, one Eurasian badger *Meles meles* and one European hare *Lepus europaeus*) and eight contained domestic animals (four horses, three cattle and one sheep) (Fig. 2). Wild species represented 73% of all prey identified in wolf feces and sheep only 3%. Considering how rare attacks are on horses and cattle, it is possible that these food items were scavenged.

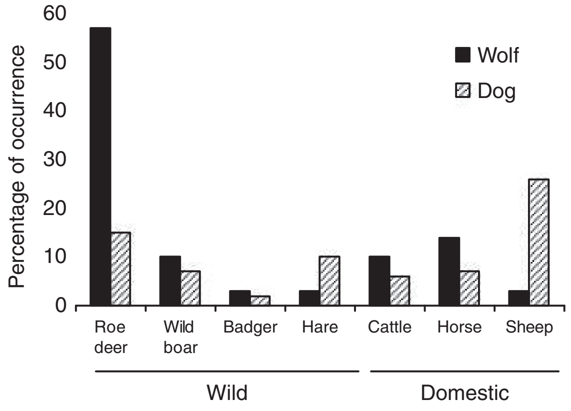

Figure 2 The per cent occurrence of wild and domestic prey in the feces of wolves *Canis lupus* ($n=31$) and dogs *Canis familiaris* ($n=39$) collected in the Basque Country, Spain, in 2003 and 2004.

Of the 39 prey items identified in dog feces (prey remains in 14 feces could not be identified), 14 (36%) contained remains of sheep and seven (18%) contained remains of either horses or cattle (Fig. 2). Domestic animals represented 54% of all prey identified in dog feces. Of the 39 prey items identified in dog feces (remains not identified for 14 feces), 14 (36%) corresponded to sheep (Fig. 2). Because we biased our sampling toward finding wolf feces, these data should not be considered to be indicative of the diet of all dogs in the area. Nevertheless, domestic animals, particularly sheep, are part of the diet of some dogs. Although our analyses cannot discern whether the consumption of sheep by dogs is a result of their scavenging carcasses or direct predation, they do suggest the possibility that some of the attacks on sheep could have been perpetrated by dogs.

Shepherd, hunting or feral dogs have been reported to prey on both wild and domestic species (Lowry & McArthur, 1978; Vos, 2000; Butler & Du Toit, 2002; Butler, Du Toit & Bingham, 2004). In the United Kingdom, where wolves are absent, 30 000 sheep and 5000–10 000 lambs are killed each year by dogs (Taylor *et al.*, 2005). These losses add up to €2.5 million per year. In a neighboring region of the Basque Country, 14% of the attacks on domestic animals initially attributed to wolves were refused compensation after a technical team determined that wolves were not the cause of the attacks (Resumen Plan Actuaciones Principado Asturias, 2005–2006). Wolves have been present in Basque Country since the 1980s (Blanco, Cuesta & Reig, 1992), but dogs were not considered as potential predators of domestic livestock there until 2003.

One reason why dogs often may not be considered predators of domestic livestock is probably related to how difficult it is to determine the predator responsible for an attack. Evidence left at a kill site or on the prey animal is often ambiguous (Bousbouras, 1997), especially if the carcass has been scavenged (Selva *et al*., 2005). Even experienced personnel using standardized protocols were unable to determine whether wolves or dogs caused 30% of the attacks on domestic livestock in nearby regions of Castille and Leon (Talegón, 2003). This area contains a large wolf population. In contrast, our estimate of the number of wolves in the Basque Country is much less than the number of dogs present in the area. Although the number of uncontrolled and feral dogs is unknown, there are at least 153 guard dogs within our study area, nearly 10 times the number of wolves we estimated.

Analysis of the diet of predators rarely allows separating predation from scavenging of carcasses (Fedriani & Kohn, 2001; Chavez & Gese, 2005). Similarly, an analysis of the evidence left at kill sites will rarely, if ever, be conclusive. The application of genetic methods to identify the species and individuals that may be preying on domestic species can be a valuable contribution to the development of comprehensive damage prevention and compensation programs (Bulte & Rondeau, 2005). Furthermore, genetic approaches may also aid in elucidating the role of feral or uncontrolled dogs in domestic animal depredation cases (Sundqvist, Ellegren & Vilà, 2008) and show authorities the importance of controlling feral dogs. Well-designed, respected and operational damage prevention and compensation programs are vital to minimizing depredations on livestock and reducing the conflict between natural predators and society (Sagor, Swenson & Roskaft, 1997; Boitani, 2000; Bisi *et al*., 2007). Here, we show that genetic methods are an important tool for developing such programs.

Because farmers in many areas only receive economic compensation for wolf attacks, biases can develop favoring the report of attacks by wolves or blaming them in cases of difficult assignment (Askacíbar & Ocio, 2006). Excessive blame placed on wolves encourages negative attitudes toward wolf recolonization, exacerbates conflict leading to further wolf population control and reduces the application of non-lethal measures that could protect both domestic animals and wolves (Bulte & Rondeau, 2005; Chavez, Gese & Krannich, 2005; Bisi *et al*., 2007).

Acknowledgments

This study was supported by the Biodiversity Area of the Environment Department of the Basque Government, the US National Science Foundation (OPP 0352634) and the 'Direcció General de Recerca' (2005SGR00090) of the 'Generalitat de Catalunya,' Spain. The 'Programa de Captación del Conocimiento para Andalucía' and Gas Natural SDG S.A. supported C.V. The Genetics Program, Smithsonian Institution, provided logistical support. We thank people of the Evolutionary Biology Department in Sweden for their help and assistance during the laboratory work, and also Andrés Illana, Alberto Hernando, Félix Martínez de Lecea, Juane Bayona, Juan Ángel de la Torre, Iratxe Covela and Diana Paniagua for their participation in the field and office work, and Jennifer A. Leonard for critical review of the paper.

References

Aguirrezábal, J.R. & Sánchez, N. (2007). Informe estadístico de la incidencia del lobo en el Territorio Histórico de Álava. Vitoria-Gasteiz (Spain). Diputación Foral de Álava (unpublished report).

Álvares, F., Barroso, I., Blanco, J.C., Correia, J., Cortés, Y., Costa, G., Llaneza, L., Moreira, L., Nascimento, J., Palacios, V., Petrucci-Fonseca, F., Pimenta, V., Roque, S. & Santos, E. (2005). Wolf status and conservation in the Iberian Peninsula. *International Congress Frontiers of Wolf Recovery*. *October 1–4, 2005*, *Colorado Springs*, *Colorado*.

Askacíbar, M. & Ocio, J.A. (2006). La ganadería extensiva y el lobo (*Canis lupus*) en Euskadi. *Sustrai* **78**, 56–60.

Bisi, J., Kurki, S., Svensberg, M. & Liukkonen, T. 2007. Human dimensions of wolf (*Canis lupus*) conflicts in Finland. *Eur. J. Wildl. Res*. **53**, 304–314.

Blanco, J.C. & Cortés, Y. (2002). *Ecología, censos, percepción y evolución del lobo en España: análisis de un conflicto*. Málaga: Sociedad Española para la Conservación y Estudio de los Mamíferos (SECEM).

Blanco, J.C., Cuesta, L. & Reig, S. (1992). Distribution, status and conservation problems of the wolf *Canis lupus* in Spain. *Biol. Conserv*. **60**, 73–80.

Boitani, L. (2000). *Action plan for the conservation of wolves (Canis lupus) in Europe*. Strasburg: Conseil d'Europe.

Boitani, L. (2003). Wolf conservation and recovery. In *Wolves. Behavior, ecology, and conservation*: 317–340. Mech, L.D. & Boitani, L. (Eds). Chicago: University of Chicago Press.

Bostedt, G. & Grahn, P. (2008). Estimating cost functions for the four large carnivores in Sweden. *Ecol. Econ*. **68**, 517–524.

Bousbouras, D. (1997). *Guide to identification of wounds inflicted on livestock by predators*. Arcturus & Balkan-Net, Thessaloniki (Greece).

Bulte, E.H. & Rondeau, D. (2005). Why compensating wildlife damages may be bad for conservation. *J. Wildl. Mgmt*. **69**, 14–19.

Butler, J.R.A. & Du Toit, J.T. (2002). Diet of free-ranging domestic dogs (*Canis familiaris*) in rural Zimbabwe: implications for wild scavengers on the periphery of wildlife reserves. *Anim. Conserv*. **5**, 29–37.

Butler, J.R.A., Du Toit, J.T. & Bingham, J. (2004). Free-ranging dogs (*Canis familiaris*) as predators and prey in rural Zimbabwe: threats of competition and disease to large wild carnivores. *Biol. Conserv*. **115**, 369–378.

Chavez, A.S. & Gese, E.M. (2005). Food habits of wolves in relation to livestock depredations in northwestern Minnesota. *Am. Midl. Nat*. **154**, 253–263.

Chavez, A.S., Gese, E.M. & Krannich, R.S. (2005). Attitudes of rural landowners toward wolves in northwestern Minnesota. *Wildl. Soc. Bull.* **33**, 517–527.

Eggert, L.S., Eggert, J.A. & Woodruff, D.S. (2003). Estimating population sizes for elusive animals: the forest elephants of Kakum National Park, Ghana. *Mol. Ecol.* **12**, 1389–1402.

Fedriani, J. & Kohn, M.H. (2001). Genotyping feces links individuals to their diet. *Ecol. Lett.* **4**, 477–483.

Flagstad, Ø., Walker, C.W., Vilà, C., Sundqvist, A.K., Fernholm, B., Hufthammer, A.K., Wiig, O., Kojola, I. & Ellegreen, H. (2003). Two centuries of the Scandinavian wolf: patterns of genetic variability and migration during an era of dramatic decline. *Mol. Ecol.* **12**, 869–880.

Francisco, L.V., Langston, A.A., Mellersh, C.S., Neal, C.L. & Ostrander, E.A. (1996). A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. *Mamm. Genome* **7**, 359–362.

Hedmark, E., Flagstad, Ø., Segerström, P., Persson, J., Landa, A. & Ellegren, H. (2004). DNA-based individual and sex identification from wolverine (*Gulo gulo*) faeces and urine. *Conserv. Genet.* **5**, 405–410.

Kohn, M., York, E.C., Kamradt, D.A., Haught, G., Sauvajot, R.M. & Wayne, R .K. (1999). Estimating population size by genotyping feces. *Proc. Roy. Soc. Lond. Ser. B* **266**, 657–663.

Lowry, D.A. & McArthur, K.L. (1978). Domestic dog as predators on deer. *Wildl. Soc. Bull.* **6**, 38–39.

Ministry of Agriculture and Forestry. (2005). *Management plan for the wolf population in Finland*. Helsinki: Publications of the Ministry of Agriculture and Forestry 11b/2005. Available at http://wwwb.mmm.fi/julkaisut/julkaisusarja/2005/MMMjulkaisu2005_11b.pdf (Accessed 1 January 2009).

Ostrander, E.A., Sprague, G.F. & Rine, J. (1993). Identification and characterization of dinucleotide repeat $(CA)_n$ markers for genetic-mapping in dog. *Genomics* **16**, 207–213.

Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *BioScience* **50**, 53–65.

Resumen Plan Actuaciones Principado Asturias. (2005-2006). *Propuesta de programa anual de actuaciones sobre la población de lobos en Asturias*. Oviedo: Principado de Asturias (unpublished report).

Sagor, J.T., Swenson, J.E. & Roskaft, E. (1997). The relationship between ships and bears. *Conserv. Biol.* **81**, 91–99.

Sand, H., Wikenros, C., Wabakken, P. & Liberg, O. (2006). Cross-continental differences in patterns of predation: will naive moose in Scandinavia ever learn? *Proc. Roy. Soc. Lond. Ser. B* **273**, 1421–1427.

Savolainen, P., Zhang, Y.P., Luo, J., Lundeberg, J. & Leitner, T. (2002). Genetic evidence for an East Asian origin of domestic dogs. *Science* **298**, 1610–1613.

Seddon, J.M. (2005). Canid-specific primers for molecular sexing using tissue or non-invasive samples. *Conserv. Genet.* **6**, 147–149.

Selva, N., Jêdrzejewska, B., Jêdrzejewski, W. & Wajrak, A. (2005). Factors affecting carcass use by a guild of scavengers in European temperate woodland. *Can. J. Zool.* **83**, 1590–1601.

Shibuya, H., Collins, B.K., Huang, T.H. & Johnson, G.S. (1994). A polymorphic $(AGGAAT)_n$ tandem repeat in an intron of the canine von Willebrand factor gene. *Anim. Genet.* **25**, 122.

Sundqvist, A.-K., Ellegren, H. & Vilà, C. (2008). Wolf or dog? Genetic identification of the predator from saliva collected at prey bite wounds. *Conserv. Genet.* **9**, 1275–1279.

Taberlet, P., Camarra, J.J., Griffin, S., Uhrès, E., Hannote, O., Waits, L.P., Dubois-Paganon, C., Burke, T. & Bouvet, J. (1997). Non-invasive genetic tracking of the endangered Pyrenean brown bear population. *Mol. Ecol.* **6**, 869–871.

Talegón, J. (2003). *Daños de lobos y perros en dos áreas de la provincia de Zamora con diferente manejo ovino*. Ciudad Real: Abstracts VII Jornadas de la SECEM.

Taylor, K., Anderson, P., Taylor, R., Longden, K. & Fisher, P. (2005). *Dogs, access and nature conservation*. Report no. 649, English Nature Research Projects.

Teerink, B.J. (1991). *Atlas and identification key hair of West-European mammals*. Cambridge: Cambridge University Press.

Vilà, C., Savolainen, P., Maldonado, J.E., Amorim, I.R., Rice, J.E., Honeycutt, R.L., Crandall, K.A., Lundeberg, J. & Wayne, R.K. (1997). Multiple and ancient origins of the domestic dog. *Science* **276**, 1687–1689.

Vilà, C., Amorim, I.R., Leonard, J.A., Posada, D., Castroviejo, J., Petrucci-Fonseca, F., Crandall, K.A., Ellegren, H. & Wayne, R.K. (1999). Mitochondrial DNA phylogeography and population history of the grey wolf. *Canis lupus*. *Mol. Ecol.* **8**, 2089–2103.

Vilà, C., Walker, C., Sundqvist, A.-K., Flagstad, Ø., Andersone, Z., Casulli, A., Kojola, I., Valdmann, H., Halverson, J. & Ellegren, H. (2003). Combined use of maternal, paternal and bi-parental genetic markers for the identification of wolf-dog hybrids. *Heredity*. **90**, 17–24.

Vos, J. (2000). Food habitats and livestock predation of two Iberian wolf packs (*Canis lupus signatus*) in the north of Portugal. *J. Zool.* **251**, 457–462.