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Long-distance wolf recolonization of France and Switzerland inferred from non-invasive genetic sampling over a period of 10 years

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Abstract

In the early 1900s, the wolf (*Canis lupus*) was extirpated from France and Switzerland. There is growing evidence that the species is presently recolonizing these countries in the western Alps. By sequencing the mitochondrial DNA (mtDNA) control region of various samples mainly collected in the field (scats, hairs, regurgitates, blood or tissue; $n = 292$), we could (1) develop a non-invasive method enabling the unambiguous attribution of these samples to wolf, fox (*Vulpes vulpes*) or dog (*Canis familiaris*), among others; (2) demonstrate that Italian, French and Swiss wolves share the same mtDNA haplotype, a haplotype that has never been found in any other wolf population world-wide. Combined together, field and genetic data collected over 10 years corroborate the scenario of a natural expansion of wolves from the Italian source population. Furthermore, such a genetic approach is of conservation significance, since it has important consequences for management decisions. This first long-term report using non-invasive sampling demonstrates that long-distance dispersers are common, supporting the hypothesis that individuals may often attempt to colonize far from their native pack, even in the absence of suitable corridors across habitats characterized by intense human activities.

INTRODUCTION

Several hundred years ago, wolf (*Canis lupus*) distribution covered the entire Northern Hemisphere, including all biomes (Mech, 1970). During the past 250 years, wolf populations in Europe underwent a continuous decline because of human persecution and degradation of ecological conditions (Breitenmoser, 1998), leading to the fragmentation of their geographic distribution. Eradication was complete in western Europe by the end of the nineteenth century, except for a few isolated populations in Italy (Ciucci & Boitani, 1991; Boitani, 1992) and in the Iberian Peninsula (Blanco, Reig & de la Cuesta, 1992). The last populations in the Alps disappeared before the end of the nineteenth century (Breitenmoser, 1998), and in France the last wolf was killed in 1939 (de Beaufort, 1987). In contrast, large populations have survived up to now in central and eastern Europe.

In the early 1970s, the lowest population size of the Italian isolate in the Apennines was estimated at approximately 100 wolves (Cagnolaro *et al.*, 1974; Zimen & Boitani, 1975). Since the end of the 1970s, the wolf has been legally protected in Italy (poison prohibition in 1976) and in Europe (Bern Convention in 1979). As a consequence, Italian populations increased and recolonized northwards through the entire Apennine range (Boitani, 1992; Randi *et al.*, 2000). Wolves reached the northwestern Apennines in 1985 (Meriggi, 1995) and southern French territories in Mercantour National Park some time before 1992 (two individuals observed; Houard & Lequette, 1993). From the early 1990s substantial field data, collected through a network of field observers, attested to wolf presence in France and Switzerland (Breitenmoser, 1998; Poulle, Lequette & Dahier, 1999). The comeback of a large predator in Alpine areas where it had disappeared several decades ago leads to huge sociological and economical controversies. In addition to legal-protection status, wolf expansion has been promoted by changes in ecological conditions (e.g. the increase of wild ungulate popula-

tions). Also, recent agricultural practices (e.g. extensive grazing, less guarding and large sheep flocks) in France and in Switzerland facilitate wolf attacks on livestock (Meriggi & Lovari, 1996), and generate conflicts of interest between stockbreeders and people in charge of wolf conservation (Breitenmoser, 1998). One of the key difficulties in managing conflicts between agricultural practices and wolf protection lies in certifying wolf presence and/or predation in a region. The wolf is a very elusive carnivore and direct observations are scarce. Furthermore, conventional analysis of field signs (tracks, scats, hairs, dead prey) do not allow the distinction between wolf and dog (*Canis familiaris*) with a high confidence level (e.g. T. Dahier, pers. comm.). Yet, reliable species identification can be achieved via non-invasive sampling and mitochondrial DNA (mtDNA) amplification and sequencing. Indeed, it has been shown that hair and faeces collected in the field can be used for this purpose (e.g. Höss *et al.*, 1992; Taberlet & Bouvet, 1992; see reviews in Morin & Woodruff, 1996; Kohn & Wayne, 1997; Taberlet, Waits & Luikart, 1999).

Recent studies have demonstrated that wolves from Italy share a single mtDNA control region haplotype. Such a haplotype has never been found in other genetic surveys of wolf populations or in domestic dogs worldwide (Vilà *et al.*, 1997; Randi *et al.*, 2000). As a consequence, mtDNA control region sequencing can be used to distinguish between wolf and dog, and to identify unambiguously the Italian among all the other wolf lineages. This approach represents a valuable tool to certify the presence of wolves in a region where field observations suggest a recent colonization process.

The purpose of this paper is (1) to demonstrate the usefulness of non-invasive genetic sampling as a routine management tool for lineage or species identification, and as a general approach to long-term studies of range expansions; (2) to identify the wolf lineage, allowing the distinction between wolves stemming from the Italian-expanding population and other populations; (3) to present a review of all wolf genetic identifications in France and in Switzerland for a better assessment of the recent colonization process.

MATERIALS AND METHODS

Sampling

A total of 256 unidentified samples (Table 1), including 190 faeces (74.2%), 40 hairs (15.6%), 22 tissues from dead animals (8.6%) and four regurgitates (1.6%), was collected in France and Switzerland between 1992 and 2001 by field correspondents of the French Wolf-Network (coordinated by the Office National de la Chasse) and the Swiss Wolf Project (coordinated by KORA, coordinated research projects for the conservation and management of carnivores in Switzerland). The geographic origins and sample types are given in Appendix 1. Before DNA extraction, tissue samples were stored in 80% ethanol, faeces and regurgitate samples were stored for several weeks or months at -20°C , whereas hairs were preserved dry in paper envelopes. In addition, we analyzed for reference purposes 21 wolf samples from different European populations and 15 dog samples from different breeds (Appendix 2).

DNA extraction

The 292 samples were processed between 1996 and 2001 in two different laboratories (Grenoble, France; Lausanne, Switzerland). DNA from tissue samples was extracted using the QIAamp tissue kit (QIAGEN), following supplier's instructions. DNA from faeces, regurgitate and hair was extracted in a physically separated facility dedicated only to low-DNA-content samples. DNA extractions from 150–200 mg of faeces were carried out using either the QIAamp tissue or the DNA stool kits (QIAGEN), and the regurgitates were processed using the QIAamp tissue kit (QIAGEN). For hair samples, standard Chelex method (Walsh, Metzger & Higuchi, 1991) was used. For each extraction experiment, control assays (blank extractions) were performed for monitoring cross-contamination among samples, possible human contaminations and PCR carry-over.

Table 1. Results of the genetic analyses for all unidentified putative wolf samples collected in the field (I, Italy; F, France; CH, Switzerland)

	Identified		Dog	Fox	Other	Total	Non-identified	Total
	Wolf							
	I/F/CH	Other						
Faeces	66		41	70	2	179	11	190
	65	1						
Hairs	11		7	3	1	22	18	40
	11	0						
Tissue	20		2	0	0	22	0	22
	16	4						
Regurgitate	2		0	1	0	3	1	4
	2	0						
Total	99		50	74	3	226	30	256

DNA amplification

We amplified each DNA extract using primers L15995 (called 'forward' in Taberlet & Bouvet, 1994) and H16498 (Fumagalli *et al.*, 1996), targeting the left domain of the mtDNA control region. Amplifications were carried out in a 25 µl reaction volume using 1U/tube AmpliTaq® Gold DNA Polymerase (Applied Biosystems), 1x PCR Buffer II (Perkin–Elmer), 2.5 µM MgCl₂ solution (Applied Biosystems), 100 µM each dNTP (PCR Nucleotide Mix, Roche Molecular Biochemicals), 200 µg/ml Bovine Serum Albumin (BSA, Roche Molecular Biochemicals) and 1 µM each primer. Template volumes were 5–10 µl for hair, faeces and regurgitate DNA extracts, and 2 µl for tissue samples. PCR amplifications were carried out on either a GeneAmp PCR System 9600 or 9700 (Applied Biosystems) for 35–55 cycles according to the number of target molecules in DNA extracts and using the following cycling parameters: initial step at 95°C for 10 min, and then 95°C for 30 sec, 50–54°C for 30 sec, 72°C for 60 sec. PCR products were visualized on a 1.6% agarose gel and purified using the QIAquick PCR Purification Kit (QIAGEN) following supplier's instructions.

DNA sequencing

Double-strand cycle sequencing was conducted using the ABI PRISM™ BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Unincorporated fluorochromes were removed using spin columns purification or ethanol precipitation. Electrophoresis of purified sequencing products was performed in a 5% polyacrylamide gel (Long Ranger™ gel, FMC), loaded on a ABI PRISM™ 377 DNA or 373XL Sequencer (Applied Biosystems). Both strands were sequenced.

Species and lineage identification

For species and/or lineage identifications, we compared the sequences obtained from unidentified samples with our wolf and dog reference sequences, and with the available homologous sequences in DNA databases using BLAST 2.0 at <http://www.ncbi.nlm.nih.gov/BLAST/>.

RESULTS AND DISCUSSION

Results are summarized in Table 1. In total, 226 samples (88.3%) could be successfully sequenced, leading to the identification of 99 wolf, 50 dog and 74 fox (*Vulpes vulpes*) samples, and three non-canid species (one *Cervus elaphus* hair, and two mustelid faeces). Thirty samples did not yield enough DNA during the extraction procedure and were not further analyzed. The success rate was much higher for faeces (94.2%) than for hairs (55.0%).

All but five wolf mtDNA sequences (94.9%) corre-

spond to a single haplotype identical to the Italian haplotype (W4 in Vilà *et al.*, 1997, or W14 in Randi *et al.*, 2000). Four out of the five remaining wolf sequences are identical or nearly identical with other known haplotypes: LS3 and D0500-01 samples correspond to W22 in Vilà *et al.* (1997); T3499-01 and T3499-02 samples (France) correspond to W16 in Randi *et al.* (2000), differing only by a single nucleotide substitution (transition). The last sequence (sample WCH-079) is close to several sequences of European and North American wolves from our reference data set or from GenBank (minimum divergence of three nucleotide substitutions). Accession numbers of all the mtDNA haplotypes produced in this study are given in Appendices 1 and 2.

Efficiency of non-invasive genetic sampling for species identification

Our study clearly demonstrates the usefulness of non-invasive genetic sampling for the identification of free-ranging canids, in particular those causing damage to livestock. Faecal and hair samples are often the only way to prove the presence of an elusive animal like the wolf. However, it appears that some samples cannot be analyzed (11.7%). We did not assess the causes of failure, but DNA degradation and the presence of PCR inhibitors are likely to affect the possibility of amplifying a relatively long mtDNA fragment (e.g. 342 base pairs for the wolf). The success rate we obtained (88.3%) should be considered as a minimum. Further experiments, such as additional DNA extractions from the same material and/or additional PCRs, would probably have increased the success rate, at least for faeces. However, we did not repeat unsuccessful DNA extractions in this study.

The PCR parameters we used for DNA extracts from hair and faeces are extremely sensitive (55 cycles) and could allow the amplification of a single target molecule. In such conditions, however, there is a high risk of contamination either by sample cross-contamination, or by PCR carry-over. These two potential error risks were monitored by appropriate laboratory procedures, including several negative controls (extraction negative controls, PCR negative controls) during the analysis, and by an appropriate laboratory design including a dedicated room only used for DNA extraction and PCR setup of ancient and/or low-DNA-content samples and a physical separation of pre- and post-PCR areas. Furthermore, when the identification of a wolf faeces sample was found far from known packs (e.g. samples FLV98 and F6699 found in Vercors and the eastern Pyrenees, respectively; see Appendix 1), experiments were systematically done independently at least twice from the extraction to the sequencing in order to rule out the possibility of contamination.

Nevertheless, as mtDNA is maternally inherited, and given that our approach is only based on mtDNA sequencing, we can only identify the maternal lineage. Because all members of the genus *Canis* are known to hybridize, a hybrid between a female dog and a male wolf will be identified as dog and not wolf (and vice

versa). However, several studies using both mtDNA (Wayne *et al.*, 1992; Randi, Francisci & Lucchini, 1995; Vilà & Wayne, 1999; Randi *et al.*, 2000) and allozyme markers (Randi, Lucchini & Francisci, 1993; Lorenzini & Fico, 1995) do not demonstrate serious introgression between dogs and wolves in Europe.

Reliability of lineage identification

As indicated in the Introduction, two recent studies involving more than 100 samples demonstrated that wolves from Italy exhibit a unique mtDNA control-region haplotype which has never been found either in domestic dogs or in other wolf populations world-wide

(Vilà *et al.*, 1997; Randi *et al.*, 2000). In such conditions, the identification of the Italian lineage is presumed unambiguous. Wolf samples collected in France or Switzerland and bearing the Italian mtDNA lineage indicate that the maternal ancestors of these individuals originate from Italy. Among the samples identified as wolves, we found five individuals bearing a haplotype different from the Italian lineage. Two that displayed an unusual confidence in humans before their capture near Montpellier (France) in 1999 exhibited a haplotype typical of southeast Europe, and thus probably originated from captivity. A third sample (a skull) was in fact a hoax set up by journalists, and possessed a North American haplotype, probably also coming from a

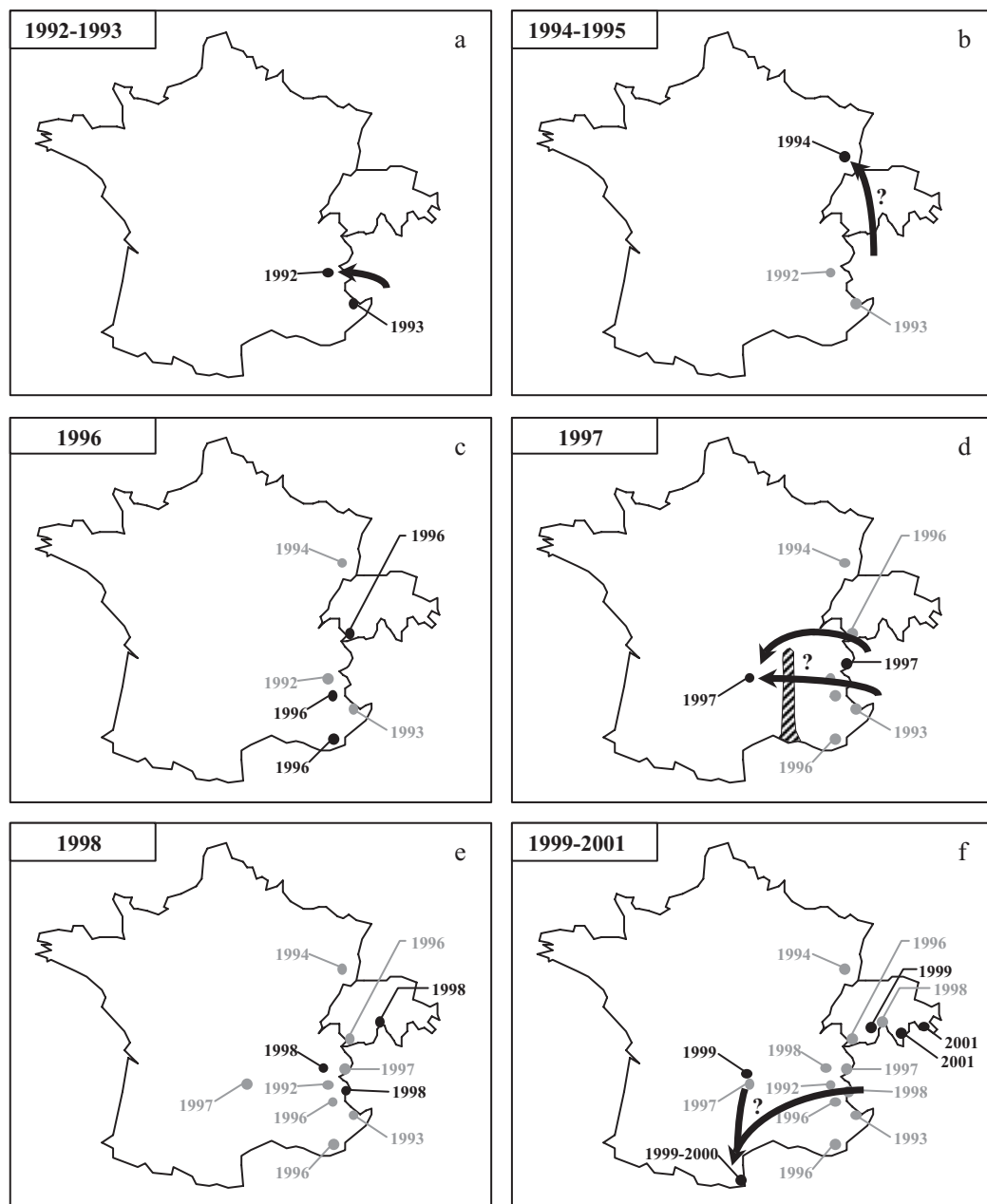


Fig. 1. Time of first genetic detection (black circles) of wolves of the Italian lineage in different regions of France and Switzerland. Arrows correspond to putative colonization routes. Cross-hatched areas indicate regions of high human activity, that might act as a barrier to dispersal.

captive population. A fourth sample (a skull found in the field) also bears the same North American haplotype, suggesting again a captive origin. For the last sample (faeces) collected in southeastern Switzerland, it is difficult to interpret reliably a single piece of data that might suggest either a current colonization from eastern Europe, or a captive source. It is clear that a precise knowledge of the genetic lineage could have a dramatic impact on management decisions, such as the possible removal of individuals of captive origin.

Wolf expansion in France and Switzerland

This study describes the use of long-term non-invasive genetic sampling to examine natural recolonization by a large carnivore. Our results show that the wolf has been present in the French and Swiss Alps since at least 1992 and 1996, respectively. Isolated data concerning wolf arrival in a particular region are often difficult to interpret, and provide relatively little information concerning the colonization process *per se* (see above). However, the unique combination of 10 years of field and genetic data enables the reconstruction of the pattern of expansion of the species in these countries (Fig. 1). The presence of wolves from the Italian lineage in locations far away from where packs have been identified seems to be the rule rather than the exception. In our data set, it is the case for sample LMFG found in 1992 in Aspres-les-Corps (Isère, France; Fig. 1(a)), sample LV3 found in 1994 in Vosges (France, Fig. 1(b)), sample LS1 found in 1996 in Valais (Switzerland, Fig. 1(c)), sample Lf.Cant. found in Massif Central (France) in 1997 (Fig. 1(d)), and finally sample F6699-01 found in 1999 in the Pyrenees (France, Fig. 1(f)). Wolves are characterized by a high dispersal potential, and seem to have a mechanism of expansion divided into two successive colonization steps (Mech, 1970). The first step is the dispersal of single, nomadic individuals, which explore new areas for mates. The second phase corresponds to the installation of packs, providing environmental conditions are appropriate. The establishment of the first pack in Mercantour National Park (France) occurred in 1992–93 (Houard & Lequette, 1993).

As the time between the arrival of the first long-distance dispersers and the presence of a stable pack can take several years (4 to 6 years according to Poulle *et al.*, 1999), it is likely that wolves were already present in the French Alps before 1992, the date of the first genetic identification of an 'Italian' wolf in the area. Given the current presence of wolves of presumed natural origin on both sides of the French Rhône valley, which is characterized by intense road traffic and a high level of urbanization, areas of high human activity do not seem to constitute a major barrier to wolf dispersal. This confirms the strong dispersal capability of this canid species (Mech, 1987), and suggests that natural corridors may not be as important as previously thought for dispersal. Our results are also consistent with the scenario of a natural return of wolves in France and

Switzerland from the expanding Italian population. Although our genetic analyses cannot rule out the possibility of illegal releases of captive 'Italian' wolves, we think that this is unlikely and is certainly the least parsimonious explanation, for a species recolonizing its traditional historical range, where it had been eradicated by man for a period of less than a century. Its present conservation status and the massive reconstitution of its wild ungulate prey populations, as well as its high dispersal potential, may explain its rapid and large-scale expansion since the 1970s. More generally, the results show that non-invasive genetic sampling can be used for long-term analyses of range expansions in wild species.

Perspectives

Since 1993, several hundred faeces samples have been collected in Mercantour National Park for studies on diet and health conditions, as well as for genetic analysis. In parallel with mtDNA sequencing for species and lineage identification, and thanks to improvements in non-invasive genetic techniques (Kohn *et al.*, 1999; Taberlet *et al.*, 1999), this large sample can now be subjected to nuclear DNA analyses (microsatellites). These markers will enable us to identify single wolves and assess their temporal and spatial distribution, to estimate the population size, and to track the evolution of pack composition.

The return of the wolf in portions of its former historical range represents a real challenge to predator and prey management. Molecular tools, together with field data, potentially provide a unique opportunity to understand thoroughly the ongoing colonization processes in a large carnivore species, and to establish a basic framework for a rational management in a controversial and sensitive sociological context.

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Appendix 1. List of samples analyzed for species identification. Sample type: F, faeces; H, hair; R, regurgitate; T, tissue. Country: CH, Switzerland; F, France. Genetic ID: D, dog; F, fox; O, other species; Wit: wolf of the Italian lineage; W: wolf from another lineage. Sequence lengths are 316 bp for dogs, 332 bp for foxes and 342 bp for wolves. Shorter lengths are indicated by * and the specified sequence length.

Code	Sampling date	Sample type	Country	Geographic origin	Genetic ID	Haplotype	Accession no.
F0400-16	23/03/00	F	F	Alpes de Haute Provence	D	D18 *290 bp	AF487730
F0401-01	02/02/01	F	F	Alpes de Haute Provence	D	D11	AF338782
F0599-12	13/07/99	F	F	Hautes Alpes (05)	D	D1	AF338772
F0600-01	18/11/00	F	F	Alpes Maritimes	D	D20 *292 bp	AF487733
F0600-02	18/11/00	F	F	Alpes Maritimes	D	D21 *301 bp	AF487734
F2600-10	25/08/00	F	F	Drôme	D	D11	AF338782
F2600-13	05/07/00	F	F	Drôme	D	D19	AF487731
F3800-03	??/??/00	F	F	Isère (38)	D	D10* 227 bp	AF338781
F6600-04	??/??/00	F	F	Pyrénées Orientales (66)	D	D3	AF338774
F6601-01	14/04/01	F	F	Pyrénées Orientales	D	D3	AF338774
F7499-01	23/07/99	F	F	Haute Savoie (74)	D	D8	AF338779
FCE98-2	25/11/98	F	F	Hautes Alpes (05)	D	D9	AF338780
FCI99-1	23/01/99	F	F	Isère (38)	D	D2	AF338773
FCI99-2	23/01/99	F	F	Isère (38)	D	D2	AF338773

Code	Sampling date	Sample type	Country	Geographic origin	Genetic ID	Haplotype	Accession no.
FCI99-3	02/01/99	F	F	Isère (38)	D	D1	AF338772
FCP98-5	06/01/98	F	F	Alpes de Haute Provence (04)	D	D4	AF338775
P3899-15	19/04/99	H	F	Isère (38)	D	D1	AF338772
P7399-14	25/03/99	H	F	Savoie (73)	D	D7	AF338778
P8301-01	06/05/01	H	F	Var	D	D22	AF487735
P8498-02	??/??/98	H	F	Vaucluse (84)	D	D3	AF338774
P8498-03	??/??/98	H	F	Vaucluse (84)	D	D1	AF338772
T0699-06	27/06/99	T	F	Alpes Maritimes (06)	D	D2	AF338773
T0699-07	27/06/99	T	F	Alpes Maritimes (06)	D	D2	AF338773
WCH-002	1998	F	CH	Graubünden	D	D1	AF338772
WCH-003	1998	F	CH	Graubünden	D	D1	AF338772
WCH-017	01/04/99	F	CH	Valais	D	D14	AF338785
WCH-028	28/10/99	H	CH	Valais	D	D2	AF338773
WCH-030	28/09/99	F	CH	Valais	D	D3	AF338774
WCH-037	05/03/00	F	CH	Vaud	D	D7	AF338778
WCH-042	09/07/00	F	CH	Valais	D	D23	AF487747
WCH-044	28/04/00	F	CH	Valais	D	D23	AF487747
WCH-050	17/07/00	F	CH	Valais	D	D8	AF338779
WCH-056	01/09/00	F	CH	Valais	D	D24	AF487748
WCH-057	01/09/00	F	CH	Valais	D	D2	AF338773
WCH-064	27/02/01	F	CH	Valais	D	D8	AF338779
WCH-065	27/02/01	F	CH	Valais	D	D8	AF338779
WCH-066	27/02/01	F	CH	Valais	D	D8	AF338779
WCH-067	27/02/01	F	CH	Valais	D	D23	AF487747
WCH-068	27/02/01	F	CH	Valais	D	D23	AF487747
WCH-069	27/02/01	F	CH	Valais	D	D24	AF487748
WCH-070	27/02/01	F	CH	Valais	D	D8	AF338779
WCH-071	27/02/01	F	CH	Valais	D	D25	AF487749
WCH-072	27/02/01	F	CH	Valais	D	D24	Nuc19
WCH-074	27/02/01	F	CH	Valais	D	D24	AF487748
WCH-075	27/02/01	F	CH	Valais	D	D25	AF487749
WCH-076	2001	H	CH	-	D	D26	AF487750
WCH-078	03/03/01	F	CH	Valais	D	D25	AF487749
WCH-086	26/08/00	F	CH	Valais	D	D8	AF338779
WCH-107	2001	F	CH	Valais	D	D27	AF487751
WCH-108	2001	F	CH	Valais	D	D27	AF487751
F0400-13	01/10/00	F	F	Alpes de Haute Provence	F	F7	AF338795
F0400-15	??/09/00	F	F	Alpes de Haute Provence	F	F15 *319 bp	AF487736
F0401-02	16/03/01	F	F	Alpes de Haute Provence	F	F18 *305 bp	AF487739
F0401-06	21/06/01	F	F	Alpes de Haute Provence	F	F19	AF487740
F0501-27	29/05/01	F	F	Hautes Alpes	F	F3	AF338791
F0501-32	19/05/01	F	F	Hautes Alpes	F	F20 *319 bp	AF487741
F0599-08	25/03/99	F	F	Hautes Alpes (05)	F	F3	AF338791
F2600-11	25/08/00	F	F	Drôme	F	F16	AF487737
F2601-20	04/07/01	F	F	Drôme	F	F22 *315 bp	AF487743
F3800-02	??/??/00	F	F	Isère (38)	F	F8	AF338796
F3801-03	29/01/01	F	F	Isère	F	F23 *320 bp	AF487744
F3801-16	26/03/01	F	F	Isère	F	F24 *320 bp	AF487745
F3801-17	29/01/01	F	F	Isère	F	F2	AF338790
F3899-01	09/05/99	F	F	Isère (38)	F	F2	AF338790
F3899-11	06/06/99	F	F	Isère (38)	F	F7	AF338795
F6600-05	31/08/00	F	F	Pyrénées Orientales	F	F17	AF487738
F6699-07	15/01/99	F	F	Pyrénées Orientales (66)	F	F6	AF338794
F7300-02	15/02/01	F	F	Savoie	F	F2	AF338790
F7301-03	18/05/01	F	F	Savoie	F	F3	AF338791
F7399-02	11/03/99	F	F	Savoie (73)	F	F3	AF338791
F7399-06	16/03/99	F	F	Savoie (73)	F	F3	AF338791
F7399-16	11/03/99	F	F	Savoie (73)	F	F2	AF338790
F7399-17	11/03/99	F	F	Savoie (73)	F	F3	AF338791
F7399-27	17/08/99	F	F	Savoie (73)	F	F4	AF338792
F7399-28	18/08/99	F	F	Savoie (73)	F	F3	AF338791
F7399-31	10/09/99	F	F	Savoie (73)	F	F4	AF338792
F7399-32	10/09/99	F	F	Savoie (73)	F	F3	AF338791
FCA98-1	04/12/98	F	F	Savoie (73)	F	F2	AF338790
FCA98-2	01/10/98	F	F	Savoie (73)	F	F2	AF338790
FCA98-3	04/10/98	F	F	Savoie (73)	F	F3	AF338791
FCE98-1	24/11/98	F	F	Hautes Alpes (05)	F	F2	AF338790
FCH98-2	13/12/98	F	F	Hautes Alpes (05)	F	F10	AF338798
FCH99-1	06/01/99	F	F	Hautes Alpes (05)	F	F2	AF338790
FCI99-6	03/03/99	F	F	Isère (38)	F	F2	AF338790
FCP98-1	01/12/98	F	F	Alpes de Haute Provence (04)	F	F9* 300 bp	AF338797
FCP98-2	04/12/98	F	F	Alpes de Haute Provence (04)	F	F5* 308 bp	AF338793
FCV98-1	02/12/98	F	F	Drôme (26)	F	F1	AF338789

Code	Sampling date	Sample type	Country	Geographic origin	Genetic ID	Haplotype	Accession no.
P0501-02	11/02/01	H	F	Hautes Alpes	F	F21 *320 bp	AF487742
P0600-02	23/01/00	H	F	Alpes Maritimes (06)	F	F13	AF338801
P7301-01	10/03/01	H	F	Savoie	F	F25 *308 bp	AF487746
WCH-013	17/01/99	F	CH	Valais	F	F2	AF338790
WCH-021	07/06/99	F	CH	Valais	F	F2	AF338790
WCH-023	14/07/99	F	CH	Valais	F	F2	AF338790
WCH-027	20/09/99	F	CH	Graubünden	F	F14* 181 bp	AF338802
WCH-031	06/11/99	F	CH	Valais	F	F2	AF338790
WCH-032	08/08/99	F	CH	Valais	F	F2	AF338790
WCH-033	18/10/99	F	CH	Valais	F	F11	AF338799
WCH-035	18/10/99	F	CH	Valais	F	F12	AF338800
WCH-038	17/05/00	F	CH	Valais	F	F2	AF338790
WCH-046	17/07/00	F	CH	Valais	F	F2	AF338790
WCH-047	14/07/00	R	CH	Valais	F	F2	AF338790
WCH-048	14/07/00	F	CH	Valais	F	F4	AF338792
WCH-049	24/04/00	F	CH	Valais	F	F2	AF338790
WCH-052	01/06/00	F	CH	Valais	F	F26	AF487752
WCH-053	01/06/00	F	CH	Valais	F	F27	AF487753
WCH-054	28/08/00	F	CH	Fribourg	F	F2	AF338790
WCH-063	27/02/01	F	CH	Valais	F	F2	AF338790
WCH-073	27/02/01	F	CH	Valais	F	F2	AF338790
WCH-077	08/03/01	F	CH	Ticino	F	F2	AF338790
WCH-082	01/04/01	F	CH	Valais	F	F2	AF338790
WCH-083	19/04/01	F	CH	Valais	F	F2	AF338790
WCH-085	15/08/00	F	CH	Valais	F	F4	AF338792
WCH-087	04/05/01	F	CH	Ticino	F	F2	AF338790
WCH-088	04/05/01	F	CH	Ticino	F	F2	AF338790
WCH-089	04/05/01	F	CH	Ticino	F	F2	AF338790
WCH-090	04/05/01	F	CH	Ticino	F	F2	AF338790
WCH-091	04/05/01	F	CH	Ticino	F	F2	AF338790
WCH-092	04/05/01	F	CH	Ticino	F	F2	AF338790
WCH-095	05/06/01	F	CH	Valais	F	F2	AF338790
WCH-101	20/09/01	F	CH	Ticino	F	F2	AF338790
WCH-103	21/09/01	F	CH	Valais	F	F2	AF338790
WCH-104	19/10/01	F	CH	Valais	F	F2	AF338790
WCH-105	19/10/01	F	CH	Valais	F	F2	AF338790
WCH-106	19/10/01	F	CH	Valais	F	F2	AF338790
Allevard	27/07/98	F	F	Isère (38)	Wit	W1	AF338803
Allevard2	07/09/98	F	F	Isère (38)	Wit	W1	AF338803
Besse	09/08/98	H	F	Isère (38)	Wit	W1	AF338803
Bramans	08/08/98	H	F	Savoie (73)	Wit	W1	AF338803
Clavans1	14/08/98	H	F	Isère (38)	Wit	W1	AF338803
Clavans2	14/08/98	H	F	Isère (38)	Wit	W1	AF338803
D0501-01	24/08/01	T	F	Hautes Alpes (05)	Wit	W1	AF338803
F0401-04	17/05/01	F	F	Alpes de Haute Provence (04)	Wit	W1	AF338803
F0401-05	18/05/01	F	F	Alpes de Haute Provence (04)	Wit	W1	AF338803
F0500-03	10/08/00	F	F	Hautes Alpes (05)	Wit	W1	AF338803
F0500-04	04/10/00	F	F	Hautes Alpes (05)	Wit	W1	AF338803
F0501-31	19/06/01	F	F	Hautes Alpes (05)	Wit	W1	AF338803
F2600-12	25/08/00	F	F	Drôme (26)	Wit	W1	AF338803
F2699-04	02/04/99	F	F	Drôme (26)	Wit	W1	AF338803
F2699-08	05/08/99	F	F	Drôme (26)	Wit	W1	AF338803
F2699-12	10/09/99	F	F	Drôme (26)	Wit	W1	AF338803
F2699-13	14/09/99	F	F	Drôme (26)	Wit	W1	AF338803
F3800-05	08/02/00	F	F	Isère (38)	Wit	W1	AF338803
F3800-15	16/07/00	F	F	Isère (38)	Wit	W1	AF338803
F3899-08	11/05/99	F	F	Isère (38)	Wit	W1	AF338803
F3899-18	03/09/99	F	F	Isère (38)	Wit	W1	AF338803
F3899-19	12/09/99	F	F	Isère (38)	Wit	W1	AF338803
F3899-21	19/09/99	F	F	Isère (38)	Wit	W1	AF338803
F6600-02	??/??/00	F	F	Pyrénées Orientales (66)	Wit	W1	AF338803
F6699-01	15/01/99	F	F	Pyrénées Orientales (66)	Wit	W1	AF338803
F6699-02	15/01/99	F	F	Pyrénées Orientales (66)	Wit	W1	AF338803
F6699-03	27/02/99	F	F	Pyrénées Orientales (66)	Wit	W1	AF338803
F6699-05	05/03/99	F	F	Pyrénées Orientales (66)	Wit	W1	AF338803
F7301-05	22/05/01	F	F	Savoie (73)	Wit	W1	AF338803
F7399-04	12/03/99	F	F	Savoie (73)	Wit	W1	AF338803
F7399-05	14/03/99	F	F	Savoie (73)	Wit	W1	AF338803
F7399-07	16/03/99	F	F	Savoie (73)	Wit	W1	AF338803
F7399-10	18/03/99	F	F	Savoie (73)	Wit	W1	AF338803
F7399-11	20/03/99	F	F	Savoie (73)	Wit	W1	AF338803
F7399-15	11/04/99	F	F	Savoie (73)	Wit	W1	AF338803
F7399-19	23/04/99	F	F	Savoie (73)	Wit	W1	AF338803

Code	Sampling date	Sample type	Country	Geographic origin	Genetic ID	Haplotype	Accession no.
F7399-21	05/04/99	F	F	Savoie (73)	Wit	W1	AF338803
F7399-23	12/06/99	F	F	Savoie (73)	Wit	W1	AF338803
F7399-29	30/08/99	F	F	Savoie (73)	Wit	W1	AF338803
FCI99-4	14/02/99	F	F	Isère (38)	Wit	W1	AF338803
FCP98-3	04/12/98	F	F	Alpes de Haute Provence (04)	Wit	W1	AF338803
FCP98-4	21/12/98	F	F	Alpes de Haute Provence (04)	Wit	W1	AF338803
FCP98-6	18/12/98	F	F	Alpes de Haute Provence (04)	Wit	W1	AF338803
FCV99-1	25/01/99	F	F	Drôme (26)	Wit	W1	AF338803
FCV99-2	24/01/99	F	F	Drôme (26)	Wit	W1	AF338803
FLI98-1	27/07/98	F	F	Isère (38)	Wit	W1	AF338803
FLI98-2	07/09/98	F	F	Isère (38)	Wit	W1	AF338803
FLV98-1	22/11/98	F	F	Drôme (26)	Wit	W1	AF338803
FLV98-2	22/11/98	F	F	Drôme (26)	Wit	W1	AF338803
LC.Canj	22/09/96	F	F	Var	Wit	W1	AF338803
LDAV1	01/11/97	F	F	Savoie (73)	Wit	W1	AF338803
Lf.Cant.	10/10/97	T	F	Cantal	Wit	W1	AF338803
LM1	08/06/95	T	F	Alpes Maritimes (06)	Wit	W1	AF338803
LM3	01/07/93	T	F	Alpes Maritimes (06)	Wit	W1	AF338803
LM4	02/09/95	T	F	Alpes Maritimes (06)	Wit	W1	AF338803
LM6	17/09/96	T	F	Hautes Alpes (05)	Wit	W1	AF338803
LM7	17/09/96	T	F	Alpes Maritimes (06)	Wit	W1	AF338803
LMFG	14/06/92	T	F	Isère (38)	Wit	W1	AF338803
LV3	16/06/94	T	F	Vosges	Wit	W1	AF338803
P0499-01	01/03/99	H	F	Alpes de Haute Provence (04)	Wit	W1	AF338803
P0601-01	19/05/01	H	F	Alpes Maritimes (06)	Wit	W1	AF338803
P0699-05	05/12/99	H	F	Alpes Maritimes (06)	Wit	W1	AF338803
P6399-01	17/07/99	H	F	Puy de Dôme (63)	Wit	W1	AF338803
P6399-02	17/07/99	H	F	Puy de Dôme (63)	Wit	W1	AF338803
P6399-03	17/07/99	H	F	Puy de Dôme (63)	Wit	W1	AF338803
P7399-18	20/04/99	H	F	Savoie (73)	Wit	W1	AF338803
Queyras1	15/06/98	F	F	Hautes Alpes (05)	Wit	W1	AF338803
Queyras2	18/06/98	F	F	Hautes Alpes (05)	Wit	W1	AF338803
T0501-01	29/01/01	T	F	Hautes Alpes (05)	Wit	W1	AF338803
T0699-02	16/04/99	T	F	Alpes de Haute Provence (04)	Wit	W1	AF338803
T3800-11	20/11/00	T	F	Isère (38)	Wit	W1	AF338803
Tiolache	22/11/98	F	F	Drôme (26)	Wit	W1	AF338803
Vomi1	01/01/97	R	F	Alpes Maritimes (06)	Wit	W1	AF338803
Vomi2	01/04/97	R	F	Alpes Maritimes (06)	Wit	W1	AF338803
LS1	18/06/96	F	CH	Valais	Wit	W1	AF338803
LS2	18/06/96	F	CH	Valais	Wit	W1	AF338803
TLS98-1	27/11/98	T	CH	Valais	Wit	W1	AF338803
WCH-001	27/11/98	T	CH	Valais	Wit	W1	AF338803
WCH-011	18/12/98	F	CH	Valais	Wit	W1	AF338803
WCH-012	19/12/98	F	CH	Valais	Wit	W1	AF338803
WCH-014	15/01/99	T	CH	Valais	Wit	W1	AF338803
WCH-018	03/06/99	F	CH	Valais	Wit	W1	AF338803
WCH-019	03/06/99	F	CH	Valais	Wit	W1	AF338803
WCH-024	14/07/99	F	CH	Valais	Wit	W1	AF338803
WCH-029	07/03/00	F	CH	Valais	Wit	W1	AF338803
WCH-036	16/10/99	F	CH	Valais	Wit	W1	AF338803
WCH-041	30/06/00	F	CH	Valais	Wit	W1	AF338803
WCH-043	30/06/00	F	CH	Valais	Wit	W1	AF338803
WCH-045	03/07/00	F	CH	Valais	Wit	W1	AF338803
WCH-055	25/08/00	T	CH	Valais	Wit	W1	AF338803
WCH-062	10/01/01	F	CH	Ticino	Wit	W1	AF338803
WCH-081	11/04/01	F	CH	Graubünden	Wit	W1	AF338803
WCH-084	16/04/00	F	CH	Valais	Wit	W1	AF338803
WCH-094	20/04/01	F	CH	Graubünden	Wit	W1	AF338803
D0500-01	11/05/00	T	F	Hautes Alpes (05)	W	W3	AF338805
LS3	??/??/??	T	?	?	W	W3	AF338805
T3499-01	05/06/99	T	F	Hérault (34)	W	W2	AF338804
T3499-02	05/06/99	T	F	Hérault (34)	W	W2	AF338804
WCH-079	20/04/00	F	CH	Graubünden	W	W11	AF487754
FCA98-4	19/09/98	F	F	Savoie (73)	O	O1	
FCA98-5	19/09/98	F	F	Savoie (73)	O	O2	
WCH-022	01/01/99	H	CH	Valais	O	O3	
F0599-15	10/08/99	F	F	Hautes Alpes (05)	-		
F2699-05	02/04/99	F	F	Drôme (26)	-		
F6600-01	??/??/00	F	F	Pyrénées Orientales (66)	-		
F6698-01	01/11/98	F	F	Pyrénées Orientales (66)	-		
F6699-04	01/03/99	F	F	Pyrénées Orientales (66)	-		
F7399-08	17/03/99	F	F	Savoie (73)	-		
F7399-09	17/03/99	F	F	Savoie (73)	-		

Code	Sampling date	Sample type	Country	Geographic origin	Genetic ID	Haplotype	Accession no.
F7399-12	23/03/99	F	F	Savoie (73)	-		
FCH98-1	14/06/98	F	F	Hautes Alpes (05)	-		
FCI99-5	14/02/99	F	F	Isère (38)	-		
P0499-02	??/??/99	H	F	Alpes de Haute Provence (04)	-		
P0600-01	23/01/00	H	F	Alpes Maritimes (06)	-		
P3800-01	??/??/00	H	F	Isère (38)	-		
P6699-06	05/03/99	H	F	Pyrénées Orientales (66)	-		
P7399-03	11/03/99	H	F	Savoie (73)	-		
P7399-13	23/03/99	H	F	Savoie (73)	-		
P8499-01	??/??/99	H	F	Vaucluse (84)	-		
PCA98-01	04/12/98	H	F	Savoie (73)	-		
PCA98-02	04/12/98	H	F	Savoie (73)	-		
PCH99-01	15/01/99	H	F	Hautes Alpes (05)	-		
PCI98-01	06/10/98	H	F	Isère (38)	-		
PLA98-01	17/11/98	H	F	Alpes de Haute Provence (04)	-		
PLI99-01	09/02/99	H	F	Isère (38)	-		
WCH-004	30/11/98	H	CH	Valais	-		
WCH-020	05/06/99	R	CH	Valais	-		
WCH-039	09/07/00	H	CH	Valais	-		
WCH-040	09/07/00	H	CH	Valais	-		
WCH-051	18/07/00	H	CH	Valais	-		
WCH-080	10/04/01	F	CH	Graubünden	-		
WCH-102	??/??/??	H	CH	?	-		

Appendix 2. List of samples used as reference. Species: D, dog; W, wolf. Sample type: F, feces; H, hair; T, tissue. Sequence lengths are 316 bp for dogs, 332 bp for foxes and 342 bp for wolves.

Code	Sampling date	Species	Sample type	Country	Haplotype	Accession no.
CB1 (beagle)	1996	D	H	France	D4	AF338775
CB2 (beagle)	1996	D	H	France	D4	AF338775
CB3 (beagle)	1996	D	H	France	D11	AF338782
CBA1 (German shepherd)	1996	D	H	France	D5	AF338776
CBA2 (German shepherd)	1996	D	H	France	D16	AF338787
CBAxG (German shepherd × groendhal)	1996	D	H	France	D17	AF338788
CC1 (cocker)	1996	D	H	France	D5	AF338776
CC2 (cocker)	1996	D	H	France	D5	AF338776
CH (Alaskan husky)	1996	D	H	France	D3	AF338774
CL1 (golden retriever)	1996	D	H	France	D13	AF338784
CL2 (golden retriever)	1996	D	H	France	D1	AF338772
CSHxL (samoyede-husky × g.retriever)	1996	D	H	France	D15	AF338786
LPort2	1997	D	F	Portugal	D6	AF338777
LPort3	1997	D	F	Portugal	D6	AF338777
Nick (samoyede)	1996	D	H	France	D12	AF338783
FESP9901	1999	W	F	Spain	W10	AF338812
FESP9902	1999	W	F	Spain	W7	AF338809
FESP9903	1999	W	F	Spain	W7	AF338809
LI1	1995	W	H/T	Italy	W1	AF338803
LI10	1996	W	H/T	Italy	W1	AF338803
LI2	1995	W	H/T	Italy	W1	AF338803
LI3	1995	W	H/T	Italy	W1	AF338803
LI4	1995	W	H/T	Italy	W1	AF338803
LI5	1995	W	H/T	Italy	W1	AF338803
LI6	1995	W	H/T	Italy	W1	AF338803
LI7	1996	W	H/T	Italy	W1	AF338803
LI8	1996	W	H/T	Italy	W1	AF338803
LI9	1996	W	H/T	Italy	W1	AF338803
LPol	1994	W	H	Poland	W9	AF338811
LPort	1997	W	F	Portugal	W6	AF338808
LRoum	1996	W	T	Romania	W8	AF338810
LWCRO10	1997	W	T	Croatia	W4	AF338806
LWCRO11	1997	W	T	Croatia	W5	AF338807
LWCRO12	1997	W	T	Croatia	W4	AF338806
LWCRO4	1997	W	T	Croatia	W4	AF338806
LWCRO6	1997	W	T	Croatia	W4	AF338806