Wolf monitoring in the Alps



2nd Alpine Wolf Workshop Boudevilliers (CH) 17 – 18 March 2003



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Wolf monitoring in the Alps 2nd Alpine Wolf Workshop, Boudevilliers (CH), 17 – 18 March 2003



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Jean-Marc Weber (ed.)

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Foreword

During the last decade, wolf range has expanded in the Alps, and nowadays the species occurs in Italy, France and Switzerland. Some packs and lone individuals have established their home range along the state borders, and regularly move from one country to another. This situation clearly emphasizes the need for collaboration between the different teams involved in wolf study and conservation in the alpine region. In order to define the bases of this collaboration, a first meeting – the 1st Alpine Wolf Workshop (AWW) – was organized by the French Wolf LIFE Project, and held in Briançon (F) on 5-6 November 2001.

The discussions were fruitful, but expectedly it was not possible in a couple of days to determine precisely the way collaboration should be implemented (*i.e.* data collection, evaluation and analyses), hence the decision to meet on an annual basis. Since then, however, the contacts and the exchanges between the national wolf projects have been strengthened. For instance, the genetic labs involved in wolf monitoring in the three countries pooled their data to improve individual identification of wolves, and give a better understanding of the re-colonization process in the Alps.

No doubt that an important step has been made so far. However, there is still a long way to go particularly regarding wolf management and conservation in the Alps. Although it was decided during the 1st AWW not to address directly political and management issues within our technical group, improvement of communication and collaboration between the alpine countries could help the implementation of a common wolf management plan for these countries. This would certainly be an asset for wolf conservation in the Alps. In this context, the 2nd AWW held in Boudevilliers (CH) in March 2003 should provide further essential elements for the construction of an efficient international exchange platform.

Jean-Marc Weber



Participants of the 2nd Alpine Wolf Workshop in Boudevilliers (CH) in March 2003.

1. Wolf monitoring in Switzerland

Jean-Marc Weber

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1.1. Introduction

As a consequence of wolf (*Canis lupus*) recovery in the French and Italian Alps, wolf presence has been regularly recorded in Switzerland since mid-1990s. To follow the re-colonization process, an extensive monitoring has been carried out primarily in the Swiss Alps from 1998 onwards.

Unlike in France, wolf monitoring in Switzerland is not organized in a network of various observers, but is essentially carried out by the game-keepers of the cantons concerned by wolf presence. The survey of wolf spatial and temporal distribution is based on different elements. Hard facts, such as wolves shot or found dead, and documented observations (*e.g.* with cameratraps) are of prime importance to assess wolf presence in an area. Wild and domestic prey, tracks and scats are also taken into account as long as they are confirmed by a competent person, and compensated (domestic prey). Unconfirmed tracks, scats, prey and direct observations or howling may be useful to some extent, but they have not been considered to describe wolf status hereafter.

1.2. Wolf status

Wolf recovery in Switzerland is at an early stage: no breeding has been recorded so far, and most observations are occasional, scattered and involve lone males (Figure 1). The first evidences of wolf presence were reported from the canton Valais. In 1994, an individual settled in the Val Ferret-Val d'Entremont area (1), but its tracks were lost after a gamekeeper had shot it early 1996. Two and half years later, a young male was found dead in Reckingen (2). The necropsy confirmed that the animal had been illegally shot. In February 1999, another male was run over by a snow-plant on the Simplon pass road close to the Swiss-Italian border (3). Summer 1999, the presence of wolf was recorded in the Val d'Hérens following several attacks on livestock (4). This individual, a male, was eventually shot in summer 2000 like another depredating wolf in the Turtmanntal, a nearby valley (5). According to genetic analyses, two other males have also been roaming in the Val d'Hérens in 2000 (6, 7). In 2001, signs of wolf presence were reported from canton Ticino (8) and in the Graubünden. A male was shot for repeated depredation on livestock during the same year (9), and a scat of another individual was found (10). Finally, 2002 brought the first evidence of the presence a wolf female in the country (11). Interestingly, she apparently established her home range on both Swiss and Italian territories. A male was regularly observed in the Surselva region throughout the year $(12)^1$. All wolves had an Italian lineage at the exception of wolf #10. Genetic analyses of the scat suggest an East European origin. However, no new evidence was found to confirm this result.

1.3. Depredation on livestock

In most cases, wolf presence has been noticed thanks to depredation on livestock (Figure 2). The first damages were recorded in the Val Ferret-Val d'Entremont area, where 118 domestic animals, mostly sheep, were killed by a wolf between 1995 and 1996 (Landry 1997). From 1998 to 2002, wolf damages occurred on a regular basis in the Swiss Alps. More than 130 wolf attacks on livestock were recorded, and almost 420 domestic animals killed during that period (Table 1). Wolf depredation may occur at any time during the year, but most attacks took place in July and August, when sheep are left unattended on the alpine pastures. The simultaneous presence of at least two wolves in the Val d'Hérens-Turtmanntal region could explain the peak in depredation observed in 1999-2000.

In the absence of any damage compensation program, the livestock killed by the wolf in 1995/1996 was partly compensated by environmental NGOs. However, a governmental compensation program was implemented in the following year. Since then, 182'751 euros have been distributed to farmers in order to cover the loss of domestic animals (Table 1).

Table 1: Confirmed wolf depredation on livestock in Switzerland, 1998-2002.

Year	Number of wolf attacks	Livestock killed	Compensation (Euros)
1998	10	31 ^A	21'800
1999	33	138	72'177 ^B
2000	52	137	73'502 ^C
2001	23	74	12'415
2002	15	38	2'857
Total	133	418	182'751

^A Including 2 fallow deer and 2 mouflons kept in captivity.

^B In addition, 128 sheep announced missing were compensated.

^C In addition, 105 sheep killed by a large canid (wolf not confirmed but not excluded) were compensated.

¹ Genetic analyses ('microsat') performed on another scat found in the area suggested the presence of a second individual. However, the replication of the analyses - with a higher number of μ satellites - did not confirmed it.

1.4. Future of wolf monitoring

The presence of a lone (?) wolf commuting regularly between Italy and Switzerland shows the necessity to develop close collaboration and set up regular contacts among the alpine countries. Moreover, the fact that regular depredation on livestock occurs on the ItalianSwiss border stresses the need of such an international co-operation. Clearly, wolf conservation in the Alps requires a common management strategy supported by an effective monitoring and productive scientific research.

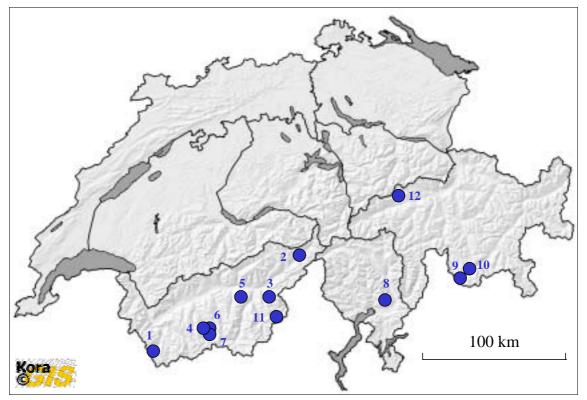


Figure 1: Wolf presence in Switzerland, 1994-2002.

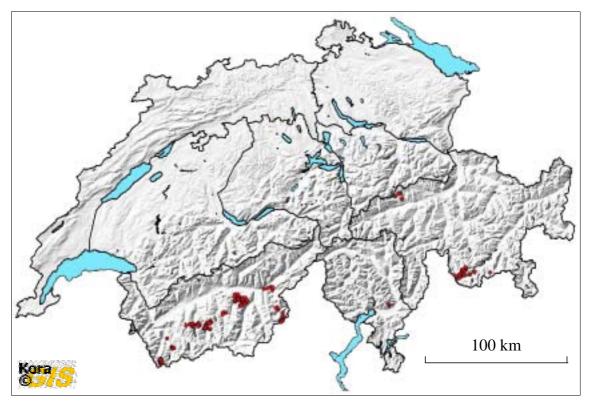


Figure 2: Distribution of confirmed wolf depredation in Switzerland, 1995-2002.

2. Wolf in Switzerland: political context and expectations

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Since 1998, wolves were regularly observed in Switzerland. Most often, their presence was noticed because they attacked livestock and killed or injured many sheep and sometimes goats. Between 1998 and 2002, there were approximately 630 sheep killed by wolves (see Wolf monitoring in Switzerland, this report) and the Confederation together with the Cantons compensated around 185'000 € to the owners. In spite of these compensations, which are based on the Federal Law on Hunting and on the Protection of Mammals and Birds Living in the Wild, the resistance against wolf return to Switzerland is strong and in some regions it is still growing, especially among sheep farmers. To understand the reason of this, it is important to know more about the system of sheep farming in Switzerland.

In 2001, there were about 12'000 sheep farmers owning approximately 420'000 sheep. However, most of these owners do not make a living from their animals but breed them among other animals or as a hobby. Out of these 420'000 sheep, some 240'000 were brought in 1'145 flocks to high pastures in the Alps above the forest limit during summer. On these pastures, sheep (and goats) are most vulnerable to wolf attacks because most flocks are not herded by shepherds or protected by any other measures (they are usually controlled only once a week or so by the owner), so they can move wherever they want on the pastures. Furthermore, they are often very small (Figure 3). The re-colonisation of wolves in the Alps with its effects on livestock requires significant changes in the system of sheep farming, e.g. implementation of damage prevention measures such as electric fences, shepherds and guarding dogs. Of course, such measures are costly and the agricultural payments for those small flocks are by far to low to cover these costs (only flocks larger than approximately 650 - 700 sheep gain enough money to pay a shepherd out of the agricultural payments). Therefore, the change of today's extensive and relatively cheap system of sheep farming during summer to a more intensive and much more expensive system is one cause for the resistance among sheep and goat farmers. Other reasons are related to the wolf's myth but are not addressed in this paper.

The above mentioned resistance is present on several levels:

• In the field: Many sheep farmers do not want to implement any prevention measures, even when their flocks have already been previously attacked by wolves and even when the extra-costs for damage prevention are covered by the Confederation. Applying those measures means often to them accepting the wolf, an animal that has been, according to

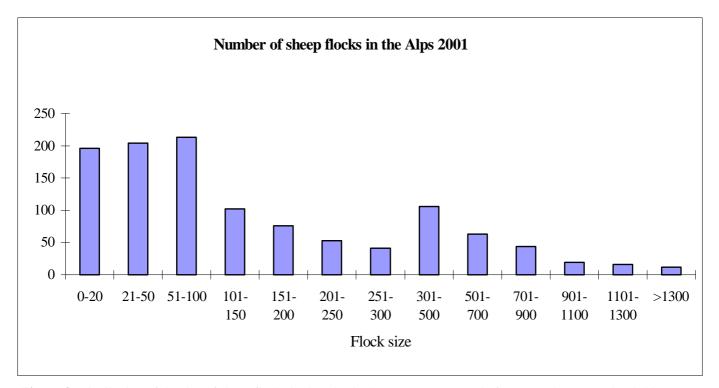


Figure 3: Distribution of the size of sheep flocks in the Alps in the year 2001. A total of 243'539 sheep were herded in 1'145 herds (Source: Swiss Federal Agency of Agriculture).

their opinion, illegally reintroduced by biologists, naturalists, environmentalists and other urban dreamers.

- At the regional and cantonal levels: In some regions, lobbying groups have been created (e.g. Komitee contra Wolf) and fight against the wolf by producing hand-outs, letters to the editor, books, internet-sites, articles for newspapers, press conference and demonstrations. Some communes and regional Members of Parliament try also to forbid guarding dogs by communal or cantonal laws. Should they be successful, then it would no longer be possible to protect sheep flocks effectively and thus, all wolves causing repeated damages to livestock could be killed according to the national and international laws (Convention of Bern, Art. 9).
 - At the national level: A member of the Council of States proposed a motion demanding the end of full protection for wolves in Switzerland, hence allowing hunting of this species. Should the motion be accepted by both Councils of Switzerland, then most probably Switzerland would have to quit the Convention of Bern. The Council of States accepted the motion in December 2001 whereas the National Council rejected it in June 2003. Consequently, the wolf remains fully protected in the country.

However, the National Council asked for an adaptation of the National Wolf Management Plan in the way that today's sheep farming system in the Alps could be conducted without unreasonable restrictions due to the wolf. This management plan will be widely discussed during next winter and should therefore be implemented in spring 2004.

To improve the acceptance of local people towards the wolf in the alpine region, and especially among livestock owners, the Swiss Federal Agency of Environment, Forest and Landscape (SFAEFL) promotes and supports financially livestock protection measures. Furthermore, a national co-ordination of livestock protection process led by an agricultural consultation service should be active very soon. Besides, several regional centres run by farmers and in charge of implementation of preventive measures are or will be installed. A group of shepherds for emergency cases, e.g. wolf attacks in new areas, is maintained. All those activities are financed by the SFAEFL. However, in the long term, damage prevention should become a task of the agriculture and not of the environmental protection as it is today.

A very important point is the exchange of knowledge about the status of wolf population in the neighbouring countries such as France and Italy. To know more about the dispersal of those animals would help to argue against the never-dying rumours that wolves have been illegally reintroduced. However, the SFAEFL is convinced that the present report on the 2nd Alpine Wolf Workshop will improve the collaboration among researchers and that the good personal relationships among the participants will contribute to the improvement of monitoring and hence to a much better understanding of the dynamic of wolf populations in the Alps. Therefore, these Alpine Wolf Workshops should be organised regularly in the future.

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3. The wolf in the Swiss Alps: non-invasive genetic analyses over a 7 year-period

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3.1. Introduction

Molecular genetics techniques allow the identification of biological samples and their assignment to a species or a population. Within the context of the ongoing recolonization of the Alps by the wolf (*Canis lupus*), such a genetic approach was shown to be useful as a routine management tool not only for identifications, but also for long-term survey of range expansion.

The wolf was extirpated from the Swiss Alps before the end of the 19th century, mainly due to human persecution and habitat degradation. The fragmentation of its geographic distribution lead to the survival of only few isolated populations in Western Europe, namely in the Italian and Iberian peninsulae. After legal protection and better conservation efforts, the declining Italian population increased in the 1970s, and started to recolonize northwards the Apennine range first, then the southwestern Alps, reaching the latter sometime at the end of the 1980s (e.g. Boitani 1992; Breitenmoser 1998). The comeback of this predator lead to important economical and sociological controversies (e.g. attacks on livestock), and needs the application of adapted management and conservation decisions. Since conventional, morphological analysis of field signs (tracks, scats, dead prey) does not allow the distinction of canids with a high confidence level, molecular techniques can offer a unique and reliable mean for unambiguous identifications.

In this report, based on the analysis of samples collected over a 7 year-period in the Swiss Alps, we demonstrate the efficiency of non-invasive genetic sampling for species and lineage identification, for the identification of the origin of Swiss wolves, for the description of the re-colonization dynamics, and for individual identification.

3.2. Mitochondrial DNA sequencing for species and genetic lineage identification

A total of 128 unidentified biological samples were collected in the field from June 1996 to January 2003. These samples included 107 faeces (84%), 13 hair samples (10%), 5 tissue from dead animals or blood (4%), and 3 regurgitates (2%). DNA from tissue and regurgitates, and from faeces samples was extracted using the QIAamp tissue and the DNA stool kits (QIAGEN) respectively. For hair, a standard Chelex® method was used (Walsh et al. 1991). We amplified each DNA extract using primers L15995 (Taberlet & Bouvet 1994) and H16498 (Fumagalli et al. 1996), targeting a portion of the mitochondrial DNA (mtDNA) non-coding control region. For each extraction and amplification experiment, control assays were performed for monitoring potential contaminations. After amplification and purification of DNA products, double-strand cycle sequencing was conducted using the ABI PRISMÔ BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). Sequencing products were loaded on a ABI PRISMÔ 377 DNA or 373XL Sequencer (Applied Biosystems). Both strands were sequenced. For species and lineage identification, the obtained sequences were compared with our wolf and dog reference sequences and with the available homologous sequences in DNA databases. Results are summarized in Table 2. In total, 119 samples (93%) could be successfully sequenced, leading to the identification of 32 wolf, 30 dog (C. familiaris) and 52 fox (Vulpes vulpes) samples, and five non-canid species.

				Identified			Non-identified	TOTAL
	Wol	Volf Dog		Dog Fox	Other	Total		
	Italian lineage	Other						
Faeces	27		27	50	1	105	2	107
	26	1	27	50	1	105	2	107
Hair	1		2	1	2	7		12
	1	0	3	1	2	7	6	13
Tissue	4		0	0	1	~	0	-
	4	0	0	0	1	5	0	5
Regurgitate	0		0	1	1	2	1	2
	0	0	0	1	1	2	1	3
TOTAL	32		30	52	5	119	9	128

Table 2: Results of the mtDNA sequencing analysis for all unidentified putative wolf samples collected in the field.

Nine samples did not yield enough DNA during the extraction procedure, and were not further analyzed. The success rate was much higher for faeces (98%) than for hair (46%).

All but one wolf mtDNA sequences correspond to a single haplotype. This haplotype is unique to the Italian populations and has never been found either in domestic dogs or in other wolf populations worldwide (Vilà *et al.* 1997; Randi *et al.* 2000). The remaining wolf sequence is close to several sequences of European and North American wolves from the reference datasets, therefore the interpretation of its origin (e.g. captive origin, natural colonization from eastern Europe) is difficult.

The unique combination of 7 years of field and genetic data enables the reconstruction of the pattern of expansion of the species in the Swiss Alps (Figure 4). The data confirm a high dispersal potential for the species, and suggest that natural, suitable corridors may not be as important as previously thought (*e.g.* the crossing of the Rhône valley, an area characterized by intense human activities).

The results clearly demonstrate that: (i) it is possible to identify free-ranging canids from non-invasive genetic sampling; (ii) the Italian haplotype can be undoubtedly identified; (iii) arrival dates, frequencies and localizations are consistent with the scenario of a natural re-colonization from the expanding Italian population. It must be noted that hybridization between wolves and dogs can not be detected by mtDNA analysis, in particular when mating occurs between male dogs and female wolves.

3.3. Microsatellite genotyping for individual and sex identification

All samples identified as wolves of Italian origin by mtDNA sequencing (see above) were subjected to nuclear DNA analysis (microsatellite genotyping), in order to identify single individuals and assess their spatial and temporal distribution. A major limitation when analyzing DNA from non-invasive biological material is the low concentration and poor quality of DNA. Whereas mtDNA is present in thousands of copies in a single cell, nuclear DNA occurs in only two copies, and therefore many samples will not be suitable for molecular analysis. Furthermore, in order to avoid the detection of incorrect genotypes when using a highly diluted extract, several independent amplifications of each sample are needed (multi-tube approach; Taberlet *et al.* 1999).

Overall, 31 samples were assayed (84% faeces, 3% hair, 13% tissue). Six canine microsatellites and one Y-

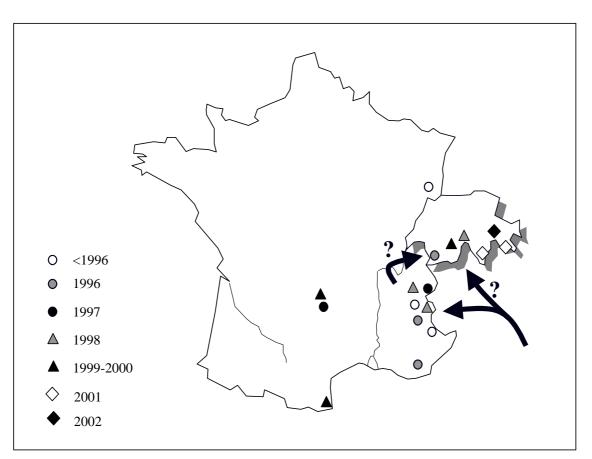


Figure 4: Time of first genetic detection of wolves of the Italian lineage in the Swiss and French Alps, with arrows corresponding to putative colonization routes (modified after Valière *et al.* 2003).

chromosome locus were analyzed; each locus has been independently typed 8 times.

Table 3 shows that between 1996 and January 2003 samples from at least 11 different individuals were collected in Switzerland. It should be noted that this corresponds to a minimal estimation, since only 65% of samples were successfully genotyped (13% partially genotyped, 22% not genotyped), and since faeces and hair were generally sampled in the field only after attacks on livestock. Only three individuals were found across several years, and the majority of individuals were males. The latter could be explained by the fact that within the Swiss Alps, which represent the northeastern edge of the colonization wave, essentially dispersing males are present and packs are still not established (J.-M.Weber, pers. comm.).

Overall, the results show that non-invasive genetic sampling can be used for long-term analyses of range expansions in wild species. Furthermore, this approach is of conservation significance, since it has important consequences on management decisions. As a perspective, in an ongoing collaboration with P. Taberlet (Univ. J-Fourier, Grenoble, France) and E. Randi (INFS, Bologna) we are analyzing with an increased number of microsatellite loci (16) dozens of samples identified as wolves and collected within the French, Italian and Swiss Alps. This will enable the estimation of parameters such as the population size, the parentage relationship among individuals and the evolution of pack composition.

3.4. Acknowledgements

We would like to thank the Swiss Federal Agency of Environment, Forest and Landscape for funding, the KORA (in particular J.-M. Weber) for the coordination of all field correspondents, all organizations and agencies which participated in the sampling, P. Taberlet (Grenoble University) who coordinated the laboratory analyses, and K. Parker, C. Ossola and N. Duvoisin for technical help.

Table 3: Number of different wolves (*a* to *k*) inferred from microsatellite genotyping on biological non-invasive samples collected between VI.1996 and I.2003 in the Swiss Alps (M: male, F: female; VS: Valais, TI: Ticino, GR: Graubünden; Ti: tissue, Fa: faeces, Ha: hair).

ID	Code	Sex	Date	Locality	Sample	Remarks
а	WCH-001	М	25.11.98	VS	Ti	poached
b	WCH-012	М	19.12.98	VS	Fa	
	WCH-014	М	04.01.99	VS	Ti	killed by a vehicle
с	WCH-018	М	03.06.99	VS	Fa	
	WCH-019	М	03.06.99	VS	Fa	
	WCH-024	М	14.07.99	VS	Fa	
	WCH-036	М	16.10.99	VS	Fa	
	WCH-029	М	07.03.00	VS	Fa	
	WCH-084	М	16.04.00	VS	Fa	
d	WCH-041	М	30.06.00	VS	Fa	
	WCH-043	М	30.06.00	VS	Fa	
е	WCH-045	М	03.07.00	VS	Fa	
f	WCH-055	М	25.08.00	VS	Ti	legally shot
g	WCH-058	М	26.11.00	Italy, border TI	Fa	partial genotype
	WCH-059	М	26.11.00	Italy, border TI	Fa	partial genotype
	WCH-060	М	26.11.00	Italy, border TI	Fa	partial genotype
	WCH-061	М	26.11.00	Italy, border TI	Fa	partial genotype
h	WCH-109	М	29.09.01	GR	Ti	legally shot
i	WCH-112	F	23.07.02	VS	Fa	
	WCH-134	F	06.01.03	Italy, border VS	Fa	
	WCH-135	F	27.01.03	Italy, border VS	Fa	
j	WCH-129	М	31.12.02	GR	Fa	
k	WCH-133	М	21.01.03	GR	На	

4. News about the wolf status in France: 2002-2003 update

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4.1. Wolf monitoring in France

The overall monitoring is made of two sub-processes, each one being applied with a given spatial scale in order to answer two kinds of questions:

- A large scale sign survey (i.e. a coarse grained spatial monitoring over very large areas), implemented through a national network of ca. 450 trained wildlife managers, dispatched all over the Alps (>2 Millions ha) in order to collect and check any information about wolf occurrence (prey remains, scats, tracks, urine deposits, sightings, howling). Such transversal survey aims at (i) distinguishing between permanent and temporary wolf areas of presence, (ii) documenting reproduction cases, as well as compiling all the monitoring data to investigate the yearly variations in wolf spatial distribution. Each damage on livestock in the Alps is also controlled for several technical criteria to enable compensation whenever the wolf responsibility is involved.
- A small scale sign survey (i.e. a fined grained temporal investigation over restricted areas) conducted locally by wildlife managers who monitor wolf areas previously identified, based on intensive snow tracking, wolf howling, scats collection, to estimate changes in minimum group sizes and to distinguish social units. Scats are used for molecular tracking and diet analysis.

All data are compiled in a national database implemented by the Office National de la Chasse et de la Faune Sauvage (ONCFS).

4.2. The status of wolves in 2002-2003

The survey of wolf recovery spatial pattern indicates a constant increase in the distribution from Mercantour northwards, yielding in 2003 a total of 11 permanent wolf areas (most likely including packs or lonely individuals, to be next checked using with the longitudinal genetic dataset – i.e. recapture over time of given genotypes). A permanent wolf area is defined when signs of presence are reported during two consecutive winters (pack stability period, Fuller 1989), provided signs other than visual contacts are also available. The current distribution of wolf group sizes is presented in Figure 5. One can also notice that visual contacts and damages to livestock are the first signs reported outside

already-known wolf distribution. Three different wolves belonging to the Italian lineage have also been detected in the Pyrenees (SO of France) about 450 km away from the closest known wolf area (Mercantour but also from the Massif Central, where two additional individuals with an Italian lineage have been found dead). In the Pyrenees, there is another network made of observers first trained for the purpose of brown bear monitoring, which may contribute to improve the detection of signs of presence from other carnivorous species. There is no such organized monitoring between the Alps and the Pyrenees, which can explain why so few data are found in between, together with the characteristic long-distance dispersal of the species. In the next future, defining the kinship relations between all the genotypes already identified (Italia, France, Switzerland) will be of a great help for a better understa

nding of the colonization process.

4.3. Wolf population dynamics

The minimum number of wolves alive in each permanent area is monitored using snow tracking, and molecular tracking, in combination with capture recapture applications (spatial dynamics of colonization, survival rates, population size), and diet analysis.

Among more than 1200 scats collected since 10 years, 289 collected outside the Mercantour National Park have been analysed with μ satellites techniques (Laboratoire d'Ecologie Alpine, CNRS 5551, Grenoble). Another set of scats from the Mercantour is currently analysed for individual identification. The first results indicate that:

- 50 individuals have been detected during the last 5 years outside the Mercantour Park.
- few of them are found several times (i.e. a low recapture rate over years -18%).
- only 1 animal has been recaptured in 2 different areas.

To explain this low recapture rate, the 3 following hypotheses may be involved, separately or in interaction:

- a high turn over of individuals (characteristic of expanding wolf populations with long distance dispersal, and high mortality rates).
- a sampling pressure too low to yield large recapture rates, particularly for a species characterized with low density, long distance dispersal, and large home ranges (up to 300 km²).
- an unknown but presumably larger than expected total population size (especially if lonely wolves such as dispersers and floaters are included) that may enhance the dilution effect on recapture rates.

4.4. Running exchanges and expectations for collaborations

To get a better understanding of the colonization process, an exchange of genotypes already identified over the Alps by the different research teams is needed in order to investigate pre & post colonization process. Following recommendations originally formulated during the previous Alpine Wolf Workshop, the 3 genetic labs (I, F, CH) have initiated collaborations. Applying CMR methods to non invasive genetics will yield pow erful results, provided there is some kind of methodological standardization between the countries involved (sampling method, cost ...). The first step should be the set up of common rules to interpret data about wolf presence, such as (i) what should be recorded for data compilation, and (ii) which classification key should be used to assess data reliability.

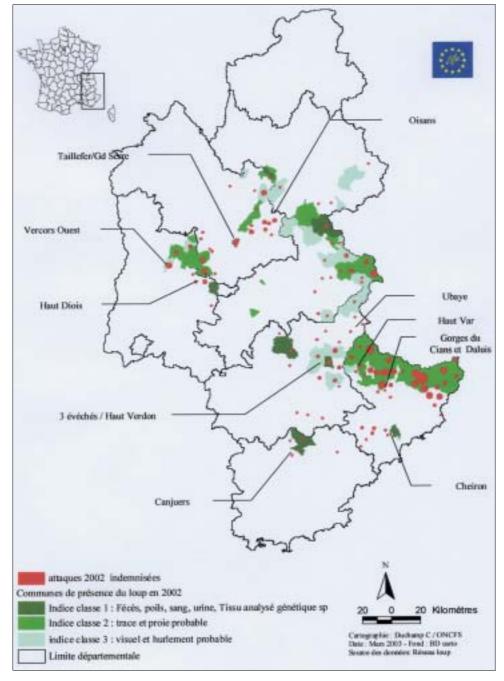


Figure 5 : Communal wolf distribution in 2003 and amount of damages to livestock (red dots are proportional to the number of attacks attributed to wolf / Source: Réseau loup ONCFS.

5. The wolf monitoring program in the province of Cuneo

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5.1. Introduction

The wolf monitoring program in Piemonte region started in May 1999. The study area includes the alpine territory of the provinces of Torino and Cuneo.

In the province of Cuneo there has been an extensive monitoring study from valle Gesso-Vermenagna (the territory of Alpi Marittime Natural Park) to valle Varaita, and an intensive one of a single wolf pack located in the Alpi Liguri (from valle Corsaglia to valle Pesio), coordinated by Francesca Marucco.

In this report we present the main results of the extensive wolf monitoring program in the province of Cuneo from 1999 to 2002. This research has been conducted with the collaboration of Lorenzo Manghi, Alessandra Tropini and the rangers of Alpi Marittime Natural Park. The main objectives of this study were to assess:

• Wolf numbers and their distribution;

- Genetic identity of the local wolves (study coordinated by Dr. Randi);
- The feeding ecology of the species;
- The impact of wolf depredation on livestock.

5.2. Methods

In the study area we have defined a system of about 680 km of transects to look for signs of wolf presence. The sampling effort has been more intensive (each transect covered every 7 – 30 days) where the species has already been reported, that is valle Gesso-Vermenagna and valle Stura, and less intensive (each transect covered every 30 - 40 days) but anyway constant in valle Maira and valle Varaita. For logistic reasons we could not maintain this systematic effort in valle Varaita and valle Maira during last winter (2001 – 2002).

The following research techniques were used to estimate wolf number and distribution: snow-tracking, wolf-howling and genetic analysis of the scats collected.

5.3. Results

In valle Maira and valle Varaita we did not find any wolf presence and, on the basis of our sampling effort, we can exclude a stable wolf presence in these valleys.

In the remaining part of the study area we delimited 3 distinct areas of wolf presence: one in the southeastern part of the territory of Alpi Marittime Natural Park, the second one in the northeastern part of the park and two adjacent lateral valleys of valle Stura, and the last in Valle Stura.

In the southeastern part of the park, on the basis of snow-tracking sessions, we followed 5 animals the first winter and at least 2 - 3 wolves the remaining ones.

Wolf movements were followed for a total of 108 km over an altitudinal range between 1200 - 2700 m (Table 4).

Considering that wolf movements were mainly located around the French border we hypothesized that the activity on the Italian side was related to the wolf pack mainly located on French side in valle Roya (Figure 6).

In the northwestern part of the park, we estimated the presence of 2 - 3 animals and wolf movements were followed for a total of 139.2 km (Table 5). Also in this case the animals showed transboundary movements between French and Italian side so we think that they could be the same wolves found in the area of Vésubie-Tinée (Figure 6).

In valle Stura we followed at least 3 animals the first winter and 4 the following ones. The reproduction of the pack was documented each year using wolf howling.

Wolf movements were followed along 602.7 km. Considering the outermost locations of wolf tracks we estimated a wolf activity area to about 270 km² (Table 6). The wolf activity area includes the territory between Colle della Lombarda and Colle del Puriac and the high valle Tinée on the French side, where it is impossible to exactly define the situation (Figure 7).

It is interesting to compare the results, concerning numbers of animals estimated on the basis of snowtracking, with results coming from the genetic analysis of the scats collected.

Over 141 scats genetically analyzed (considering mithocondrial and nuclear DNA analysis), all but one has been classified as a wolf scat. The different genotypes identified belong to the Italian wolf population.

A total of 14 genotypes have been identified from July 1999 to May 2001 over the study area (9 males and 4 females, 1 not determined).

Six genotypes have been continuously sampled during the whole study period, 8 only during one year but not the following one.

In the first year (July 1999 – October 2000), among 45 scats considered, we identified 7 genotypes in valle Stura, 1 in NW part of the territory of the park, and 1 in the SW. The second year (march 2001 – october 2001), over 15 scats considered, 5 animals were identified in valle Stura (4 previously sampled + 1 new) and 1 in NW part of the park (the same wolf just identified in 1999). Last year (November 2001 – May 2002), among 59 scats considered, 7 animals were sampled in valle Stura (5 previously identified + 2 new), 2 in the NW part of the park (1 previously identified +1 new) and 1 animal in the SW part of the park (not previously sampled).

For the NW and the SW part of the park, the difference found between the number of animals estimated by the means of snow-tracking and by genetic analysis

			Wolf m		
Winter	# sessions	# animals	km tracked	Alt. min	Alt. max
1999 – 2000	3	5	8.2	1350	2350
2000 - 2001	4	3	26.8	1230	2700
2001 - 2002	13	2-3	73	1200	2700

Table 4: Snow-tracking sessions in the southeastern part of the territory of Alpi Marittime Natural Park.

Table 5: Snow-tracking sessions in the northwestern part of the territory of Alpi Marittime Natural Park.

			Wolf m	ovements	
Winter	# sessions	# animals	km tracked	Alt. min	Alt. max
1999 – 2000	3	2	6.6	1650	2600
2000 - 2001	3	2-3	13.3	1650	2600
2001 - 2002	17	2 - 3	119.3	1200	2800

Table 6: Snow-tracking sessions in valle Stura.

			Wolf n	novements	
Winter	# sessions	# animals	km tracked	Alt. min	Alt. max
1999 – 2000	15	3	89.6	1080	2872
2000 - 2001	32	4	192	900	2750
2001 - 2002	42	4	321.1	800	2750

can be clearly related to the low number of scats genetically analyzed in these 2 areas.

In valle Stura the understimation of the number of animals based on snow-tracking, is related to the fact that, in this valley, there is an area of territory overlap between different packs.

As shown in Figure 8, between Colle della Lombarda and Bagni di Vinadio, 3 wolves (W8, W16, W36) could be the same individuals found in the Moyenne Tinée area, overlapping their territory with the reproducing pack of valle Stura (W11, W12, W13, W35).

We have to consider that it is not possible to recognize the different animals on the basis of the tracks, so the help of genetic analysis is of fundamental importance.

5.4. Conclusions

The present results underline that the same animals move between French and Italian side. A common management strategy should then be developed by the different alpine countries. From a methodological point of view this research shows how the integration between different research techniques is a fundamental way to better interpret the results obtained.

Of course, the results of genetical analysis relative to the French side will help to clarify the situation of wolf presence on the Alpi Marittime.

In conclusion this study highlights some different aspects (e.g. winter habitat selection by wolves, territory overlap between neighbouring packs, turnover of individuals) which should be further investigated.

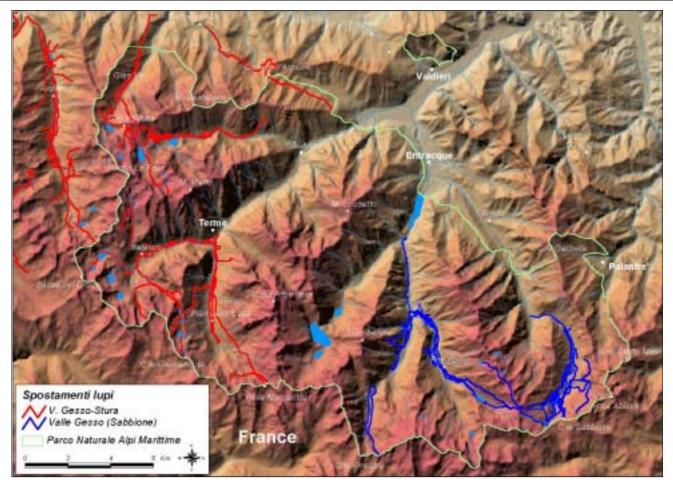


Figure 6: Wolf movements in the territory of Alpi Marittime Natural Park and surroundings area during the winter seasons (1999 - 2002). The blue lines are relative to the wolf movements in the south-western part of the park, the red lines are relative to the wolf movements in the north-eastern part of the park.

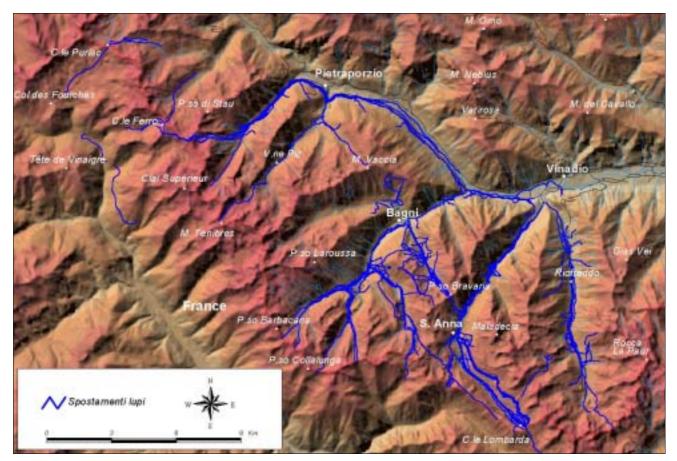


Figure 7: Wolf movements in valle Stura during the winter seasons 1999 – 2002.

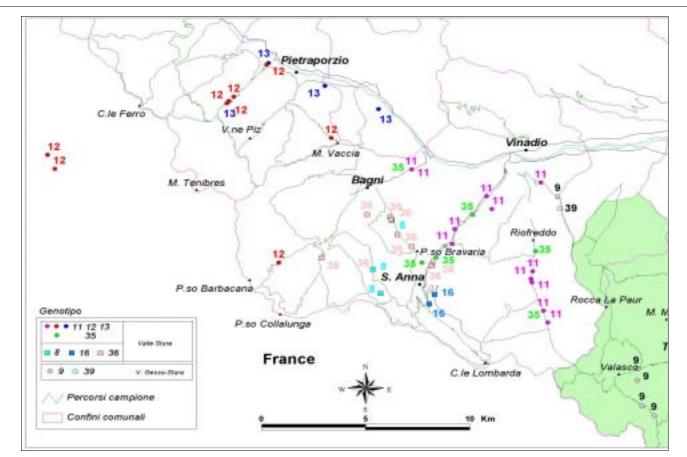


Figure 8: Localization of the genotypes sampled in valle Stura and the north-western part of the territory of Alpi Marittime Natural Park (nov 2001 – may 2002).

6. The wolf in province of Turin, Italy (December 2001-February 2003)

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The wolf monitoring in province of Turin was carried out in 2002 as part of a regional project (Piemont Region) by the Province of Turin (Fish and Wildlife Service) in collaboration with Regional Parks, State Foresters and the Forest Consortium Alta Valle Susa.

The field research was coordinated by Marco Apollonio and realized by Elisa Avanzinelli, Andrea Gazzola and Paola Bertotto, supported by provincial rangers and park officers in the study area (Val Troncea Regional Park, Orsiera- Rocciavrè R.P., Chianocco and Foresto preserves, Gran Bosco di Salbertrand R.P.).

This report refers to data collected between December 2001 and February 2003. On the basis of the results obtained in previous years, the study area was divided into an area of stable presence and an other of occasional presence.

Area of stable presence comprises: the Susa valley (where an intensive study is in progress), the Chisone valley and the Germanasca valley. In other valleys (Lanzo, Pellice and Po), signs attributable to wolves have not been so far regularly detected. The biggest monitoring effort is concentrated within the area of stable presence in order to determine the number of resident packs, to estimate their size and to evaluate their movements.

Different methods were employed to detect wolf presence in Turin province:

- search for signs of presence on sampling transects;
- summer count by wolf-howling;
- snow-tracking.

Moreover a strong collaboration with public administrations involved in territory control and management (Provincial Fish and Wildlife Service, Regional Parks wardens, State Foresters, etc.) allowed to set up a network for information exchange.

The area of stable presence of wolves was divided into three zones: 1) Alta Valle Susa; 2) Val Troncea-Val Germanasca; 3) Orsiera.

Altogether 600 km of fixed transects and 750 km of occasional transects were travelled, covering over $10,000 \text{ km}^2$. The distribution of collected scats and the collection rate can contribute to understand wolf use of the areas (Table 7).

In Valle Susa and Val Troncea-Val Germanasca signs of presence were abundant and regular throughout the study period. Tracks on the snow in winter were discovered and followed only in Alta Valle Susa and Val Troncea-Val Germanasca. The maximum number of individuals detected along a single trail was 7 wolves. The Alta Valle Susa pack was established close to the Italian-French border (Vallée Etroite and Vallée de la Clarée). Just once an incursion in French territory was recorded through the Col des Acles.

In summer, wolf-howling simultaneous surveys allowed to verify pack reproduction, to estimate litter size and to locate rendezvous sites (Table 8).

Two packs were detected, one in Valle Susa and the other in Val Germanasca. Rendezvous sites of the two packs were about 20 km apart. In summer, Valle Susa pack was located in different places on the Italian side of the Chamberton massif. In most occasions pups were detected. The maximum distance between consecutive pup locations was 4 km in August. The rendezvous site used by the Val Troncea-Val Germanasca pack was discovered in August and was left by the pack in the first week of October.

Fresh scats collected in the field are going to be analysed by Dr. Randi's laboratory, and the only genetic analysis so far realized relied on hair and blood spots found on the snow. In the lab of the University of Sassari (Dr. Massimo Scandura), this DNA source allowed to genotype 7 individuals over 10 microsatellite loci, five attributable to the Valle Susa pack and two to the Val Germanasca-Val Troncea pack. Two young wolves, killed by vehicles in December 2001 in Valle Susa, were also analyzed. Microsatellite analysis revealed that they could be siblings and strongly related to Alta Valle Susa genotypes.

In summary, the study confirmed a stable presence of two wolf packs in Province of Turin.

The Alta Valle pack used the high part of Valle Susa, in proximity of the Italian-French border (Bardonecchia, Cesana Torinese). The Val Troncea – Val Germanasca pack moved between Val Chisone and Val Germanasca.

Even if definitive data from genetic analyses are needed, we believe their territories may share at least a part of the mountain chain separating Valle Susa and Val Chisone (Figure 9 and Table 9).

Signs found in the Orsiera-Rocciavrè Regional Park are difficult to attribute in absence of genetic data.

Wolf diet was evaluated by scat content analysis. The occurrence of wild ungulates was high, demonstrating that they are preferred prey in Province of Turin (Figure 10).

In Valle Susa main prey are red deer, roe deer, chamois and livestock (in summer). Diet analysis over three years (1999-2002) showed that red deer is the main prey in winter, followed by roe and chamois. In summer domestic ungulates become the third food source for wolves, followed by chamois. Use percent-

age of the three main wild prey showed a considerable seasonal variation: the use of chamois, for instance, was double in summer than in winter.

Age class analysis of wild ungulates detected in wolf scats highlights a selection by wolves of young red deer in winter and of roe deer fawns, red deer calves and chamois kids in summer. The availability of chamois kids in summer and their vulnerability might explain the larger use of this species with respect to winter.

In order to point out the role of wolves in ungulate mortality, we compared the relative frequency of wild ungulates in their diet to that found in data of mortality by disease, starvation, predation and traffic accidents during last three winters. The relative frequency of wild ungulates in wolf diet and in predation data (wolf kills) did not differ significantly. Red deer was the ungulate species most affected by predation (diet and kills), whereas roe deer by disease, starvation and traffic accidents. On the contrary chamois, the most abundant species in the area, was not particularly affected by wolf predation. Huggard (1993) pointed out that factors influencing prey selection by wolf are: habitat overlap between predator and prey, profitability of prey, risk of being injured by prey, encounter rate and probability of successful kill. Also prey body size may play an important role (Meriggi et al., 1996). According to these factors, wolves in our study area are likely to select positively red deer for being the largest ungulate in the valley, gregarious (average winter group size = 6.38 heads), and for using winter areas alternating forests and pastures at low altitudes. Roe deer is a medium-sized prey species, frequenting dense woods with considerable undergrowth; it is elusive and lives mainly as solitary. Chamois frequents high pastures and rocky areas, less accessible to the predator; it is gregarious and well adapted to climate and topography of alpine regions.

Data presented in this report represent the preliminary outcome of an ongoing research, which intends to improve the knowledge of wolf behaviour in alpine habitats.

Table 7: Collection rate in different areas of the study area in Turin Province between December 2001 and February 2003.

Area	No. of scats	Overall travelled distance (km)	No. of scats/km
Alta Valle Susa	508	3,633	0.138
V. Troncea –V. Germanasca	214	1,772	0.120
Orsiera	40	635	0.060
Other areas	5	573	0.008

Table 8: Wolf-howling in Province of Turin, summer 2002.

Area	No. of nights	No. of sessions	No. of re- plies	Max no. pups	Max no. adults	Estimated no. of wolves
Alta Valle	31	127	7	3	2	2 ad + 3 pups
V. Troncea – V. Germanasca	23	79	3	3	2	2 ad + 3 pups
Orsiera	14	34	0	0	0	0

Table 9: Pack size in 2002 – 2003 in Province of Turin.

	Pack size winter $2001 - 02$	Litter presence summer 2002	Pack size winter 2002 – 03
Alta Valle pack	6	yes	7
VT-VG pack	5	yes	7

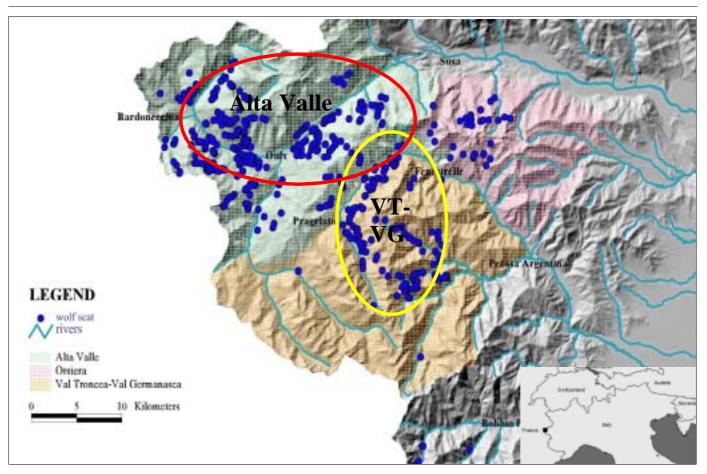


Figure 9: Wolf packs in Province of Turin (digital map, CSI Piemonte).

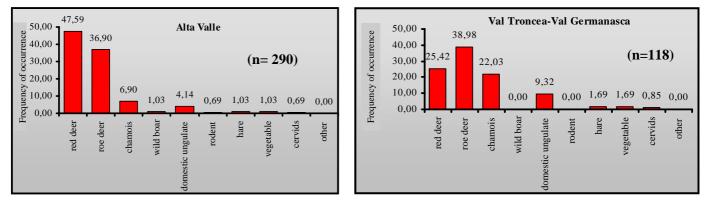


Figure 10: Diet composition in two areas of the Province of Turin, 2002.

7. The Italian wolf genetic monitoring project

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7.1. Introduction

The Istituto Nazionale per la Fauna Selvatica (INFS) is managing a non-invasive genetic monitoring project of the Italian wolf population since 1999. Main goals of this research project are: a study of the expansion of the Apennine wolf population and the recolonization of the Alps; a monitoring of the dynamics of genetic diversity during the ongoing recolonization process; an assessment of the genetic structure of the Italian wolves and their population dynamics.

We have set up two main study areas, one located in the western Italian Alps, and the other along the north Appenines (Figure 11). These projects are managed in collaboration respectively with the Piemonte and Emilia-Romagna regions. We have standardized sampling and laboratory procedures involved in the two different projects, with the aim to obtain homogeneous data sets. In this communication we describe the main aspect of the data collection protocols that are used in our projects.

7.2. Field collection

We use mainly DNA extracted from scat samples. Most of the samples are collected in winter during snow-tracking sessions. This strategy makes it possible to sample not only the dominant individuals that mark with high frequency and are easy to find during all seasons, but also juveniles that usually don't mark. Moreover, sampling on snow allows associating faeces sampled along each snow track to individuals that belong with high probability to the same pack. This information is very important since it is the only way to infer the association of different individuals without direct observations.

The age of the samples vary from one day to onetwo weeks. We ask our field collaborators not to col-

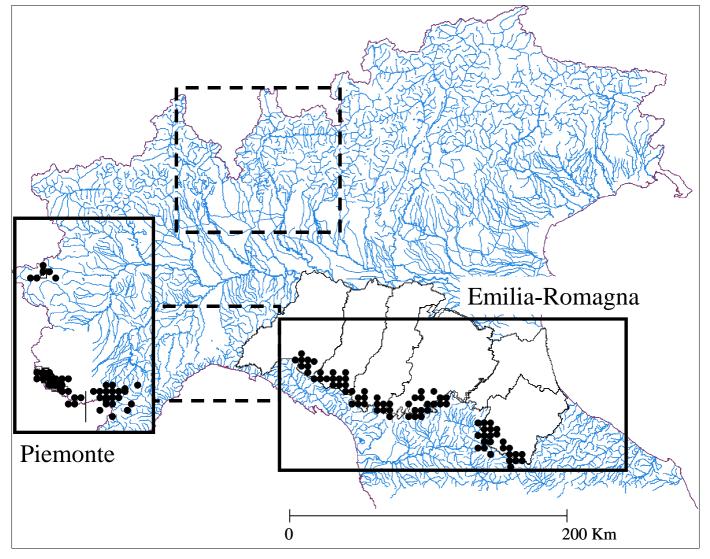


Figure 11: Non-invasive genetics of the Italian wolf population. Map of the two study areas.

lect older samples, since the laboratory work and the quality of the final data strongly depend on the quality of the DNA extracted from the samples. Samples collected in the field are individually stored into 50 ml plastic tubes containing 95% ethanol, and preserved at -20° C until shipped to the laboratory. The shipping is made at room temperature since DNA is stable for several days in ethanol.

7.3. Laboratory analysis

Each sample is extracted twice using a guanidinium tiocyanate and diatomaceus earth protocol (Gerloff *et al.* 1995). DNA is eluted in 200 μ l of TE buffer. The strategy for the genetic analysis involves a preliminary screening to test for the quality of the DNAs. The screening is done by PCR amplification of a fragment of 400 mitochondrial DNA nucleotides. This fragment includes the hypervariable region of the D-loop, which is diagnostic for the Italian wolf mtDNA haplotype (Randi *et al.* 2000). PCR products are visualized in electrophoretic agarose gel, which are stained with ethidium bromide. PCR failures identify samples of low DNA quality, which are discarded from the following analysis. If required, PCR products could be sequenced to identify the Italian wolf haplotype.

Individual genotypes are identified using a panel of 6 microsatellites (Table 10) that are variable enough to obtain a probability of identity (PID) lower than 1%, also if sibling individuals are sampled. Microsatellite amplifications are also screened for the frequency of allelic dropout (DOP) and occurrence of false alleles (Table 10). DOP is the failure of amplification of one allele at heterozygote loci due to scarcity of DNA. The result of DOP is that a true heterozygote is detected as false homozygote. False alleles are errors made during the first PCR cycles, leading to the generation of alleles that does not exist in the samples. DOP errors are more frequent, while false alleles are relatively rare. Our genotyping approach involves several steps:

- 1. Direct PCR amplifications (45 cycles) with four replicates for all the samples that have passed the mtDNA test. Samples with less than 50% of the six loci successfully typed are discarded from successive analyses.
- 2. Genotypes typed at six loci are screened with the software Reliotype (Miller *et al.* 2002), which computes an estimate of the reliability of each genotype based on the frequency of the errors that occured during the replicates (DOPs) and the allele frequency in the reference population. The program indicates how many times it is necessary to replicate each homozygote to reach a 95% reliability of each genotype.
- 3. All loci needing additional amplifications are replicated four times more, using eminested PCR. The eminested PCR consist in a double amplification, a

first one with 35 cycles, and a second one performed using 2 μ l of the first PCR, one of the internal primers and 45 cycles.

4. Results of the new replicates are added to the data set and Reliotype is run again.

We also determine the sex of individual genotypes amplifying a fragment of the ZFXY gene (Garcia-Muro *et al.* 1997) using wolf specific primers. PCR products are digested with HaeIII, and analyzed in an ABI 3100 automated sequencer. Digestions produce two DNA fragments in males and one in females.

When all the genetic analysis are completed and the genotypes have passed the reliability test at the 95% we code each different genotype with an univocal code and map its location on a electronic map (G.I.S) as shown in Figure 11. Examples of the data collected using non-invasive genetic analysis is shown in Figures 12 and 13. Using non-invasive genetic analysis we were able to identify several genotypes from the two study areas. Some of the genotypes were sampled only once while others were sampled several times, identifying probably those individuals that are stable on the territory. We detected also the sex of the different individuals. This is only an example of how non-invasive genetic analysis can be applied the study of the wolf biology. Other important data that could be obtained are the relatedness among the different genotypes, the association of particular genotypes along snow tracking (Lucchini et al. 2002).

7.4. International collaboration

The wolf is a very important key species for the Alpine environment since it is the most important large predator. Very poor information is available on its biology, especially on its population dynamics. No information is available on reproductive success, juvenile dispersal and pack turnover for the Italian wolf population. Wolves in Italy are able to survive in high human density territory, being able to expand and re-colonize the Alps from where they were eradicated one century ago. However, the population is not safe from extinction risks. International collaboration is the only way to preserve the wolf. Studies limited to a single country will be of limited utility for the wolf conservation in the Alps.

Table 10: In this table are indicated the allele size range, the expected heterozygosity (He) for each locus used for genotyping, the probability of identity without (PID) and with (PIDsib) the correction for sibling individuals, the percentage of DOP and false alleles averaged across all loci.

Locus	Range	He	PID	PID (sib)	DOP	False alleles
А	106 – 176	0.63				
В	232 - 294	0.80				
С	248 - 264	0.66				
D	92 - 100	0.66				
E	138 - 188	0.81				
			2 50 10-6	r c1 10 ⁻³	1.40/	50/

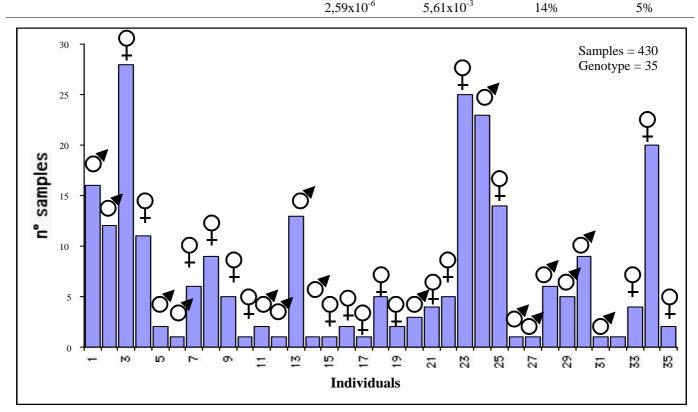


Figure 12: Frequency of wolf genotypes identified in the Alps (Piemonte region).

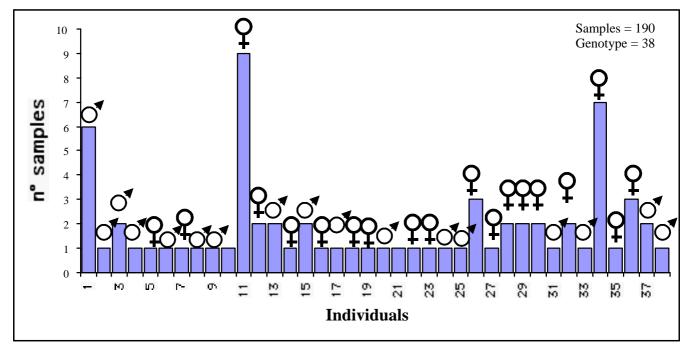


Figure 13: Frequency of wolf genotypes identified in the Apennines (Emilia Romagna region).

8. General discussion

Part of the second day's afternoon session was assigned to a general discussion. Its purpose was to define the objectives and basic needs of wolf monitoring in the Alps on one hand, and lay the foundations of an effective collaboration between our three countries on the other hand. Emphasis has been put on the following points:

- Non invasive genetic sampling is a powerful monitoring tool, particularly when it is coupled to "capture-marking-recapture" methods. However, as each country has a different approach (*e.g.* compilation and interpretation of data) and organization of wolf monitoring, some kind of methodological standardization would be necessary. It was also suggested that an evaluation of the different approaches could be useful. In this context, a first step has already been made this year: the genetic labs of the three countries have pooled and compared all wolf genotypes identified over the Alps.
 - Sampling should be made at two levels: at a large scale and at a local level. Aims of large scale sampling are to study wolf population dynamics on the long term and understand the colonization process of the Alps through kinship investigation. Local

sampling could be used to assess wolf presence in an area in relation to current management issues (*e. g.* depredation on livestock).

- Piemont region plans to capture and equip wolves with radiotransmitters as soon as possible. At the moment, wolf radiotracking is not a priority in France and in Switzerland.
- Monitoring at a large scale requires logistic and financial support. An "Interreg" project between Italy and France, and Italy and Switzerland could provide it. Piemont region strongly supports this idea. In this regard, an Interreg project between Italy and France is already in preparation. The project has four main objectives: monitoring and scientific research, depredation on livestock, human dimension, and information and education of the public. Switzerland welcomes the proposal, but thinks that neighbouring regions (*e.g.* Lombardia and Valle d'Aosta) should also be involved. Future meetings will be organized to work out a common proposal.

The 3rd Alpine Wolf Workshop will be held next year in the Parco Alpi Marittime.

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