

Progetto Lupo Piemonte

Ottobre 2005



## **Wolf Monitoring in the Alps**



**3<sup>rd</sup> Alpine Workshop**

**Entracque (Italia)**

**22 -23 July 2004**

**Wolf Monitoring in the Alps, 3<sup>rd</sup> Alpine Wolf Workshop, Entracque (Italia),  
22-23 July 2004**

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**Progetto Lupo Piemonte**

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Figure1: Valle Ellero, Wolf Tracks

**Francesca Marucco**

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## **Foreword**

### **Transboundary monitoring of a recolonizing wolf population in the Western Alps of Italy, France, and Switzerland**

Wolves were widespread in Italy, France, and Switzerland until the early 1900's when they were gradually extirpated in the Alps region. The last wolves were killed in the south-western Alps during the 1920-30s, but wolves survived along the Apennines range of central Italy. In the decades that followed, the importance of the wolf as part of a naturally functioning ecosystem came to be recognized. The wolf was listed as an endangered and strictly protected species in Europe after the Bern Convention in 1979 and the Habitat Directive in 1992. Today, ecological conditions and conservation efforts in Western Europe are improving and both wild ungulate and wolf populations are increasing. Wolves began naturally recolonizing the southwestern Alps at the beginning of 1990s. Genetic analysis conducted on wolf scat and tissue samples collected in the recently wolf recolonized areas in the Alps proved that wolves arrived through dispersal from populations in central Italy.

When these semi-isolated packs appeared progressively further from source wolf populations, questions arose regarding wolf origin, numbers, distribution, and the impact that these wolves could have on the domestic and wild animals in the Alps. Because of this in France from 1992-1993 the Minister of Environment, the Parc du Mercantour, and the ONCFS started a monitoring program, subsequently funded by two Life-Nature projects (1997-1999 and 2000-2002). At the same time in Italy the Piemonte Region and the European Community funded the "Interreg Progetto Lupo" from 1999-2001 and from 2002 to 2004 the Progetto Lupo Piemonte on the Italian Alps was exclusively funded by the Piemonte Region. In Switzerland the wolf monitoring was supported by OFEFP and conducted by KORA from 1999 to 2003.

This exceptional extended period of funding allowed the study of the wolf recolonization process in the Alps and the beginning and strengthening of a strict collaboration between the French, Italian, and Switzerland wolf monitoring groups, which constitute today the Wolf Alpine Group (WAG). The WAG is a technical group comprised of the research and management institutions of Italy (Regione Piemonte, Progetto Lupo Piemonte), France (ONCFS, PNM, Réseau loup), and Switzerland (KORA), in charge of the wolf monitoring in the Alpine area. The transboundary collaboration grew through the years from sporadic meetings to a formal series of yearly Alpine Wolf Workshops (AWW) where we met to update the situation of wolves in the Alps and organize a coordinated program of monitoring and exchange of data. This 3<sup>rd</sup> AWW held in Entracque (Italia) in July 2004 is the last but not the least appointment. The 4<sup>th</sup> AWW will be held in November 2005 in the Parc National du Mercantour. These appointments are fundamental to improve the transboundary monitoring, particularly important for the monitoring and management of the Alpine wolf population.

The large-scale wolf monitoring over the Alps is based on a combination of non-invasive methods, using both the more conventional non-invasive techniques such as snow-tracking and wolf-howling surveys, and the data from newly emerging DNA-based techniques. This combination of non-invasive techniques provided an optimal tool to monitor wolf pack dynamics and territories over large areas. The overall Italian, French, and Swiss wolf monitoring techniques are similar, but the sampling effort is different. In Italy a team of researchers developed an intensive wolf monitoring to follow the wolf

pack social history, population dynamics, intra and inter pack food habits, etc. over the years. In order to gather information on the predator-prey relationship and the success of livestock prevention methods we also recently started a GPS-radiotracking monitoring program. In France and Switzerland the main goals of a more extensive monitoring over the entire nations are to determine presence-absence of the species and the estimation of population size over a large-scale. In particular in France the monitoring is organized in a network of various observers, while in Switzerland is essentially carried out by game-keepers of the cantons concerned by wolf presence. Therefore, the Italian, French, and Swiss monitoring programs can be considered complementary.

The natural expansion of wolves in the Alps is a great challenge for conservation biologists and wildlife managers of each country because we try to achieve the goal of having a viable population, while minimizing the conflict that the species might generate. Such complex, large management issues require an understanding of the spatial and temporal dynamics of the wolf population. How large a population of wolves in the Alps must be to be viable and whether it is possible to allow any removal of wolves for damage control purposes on the French side of the Alps is controversial. Therefore these transboundary research and monitoring programs will provide quantitative understanding of the genetic, demographic, and territorial aspects of this Alpine wolf population, and will be fundamental to building an effective management strategy that will consider the Alps wolf population as one population.

*Francesca Marucco*



## **The Intensive Wolf Monitoring in Cuneo Province: Insights on Population Dynamics and on New Territories Formation**

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The natural return of the wolf (*Canis lupus*) in the Western Alps of Italy and France, far from known occupied wolf ranges in the Apennines Mountains, raised questions regarding the origin of these individuals, the number and distribution of the new alpine wolf population, and the impact which this wolf population could have on domestic and wild animals. Because of this the European Community and the Piemonte Region funded the “Interreg Progetto Lupo Piemonte” in the Alps from 1999 to 2001 in order to study the wolf recolonization process; to favour the development of measures aimed at preventing wolf attacks on livestock; and to establish a stable coexistence of wolves and human activities. Then the Progetto Lupo Piemonte has been funded by the Piemonte Region from 2002 to 2004.

The continuous research work from 1999 to 2004 allowed to document the origin, status, distribution, and the ecology of wolf packs in this recently recolonized area with the use of non-invasive techniques over the Piemonte Region.

The Progetto Lupo Piemonte is organized in 3 main actions:

1. wolf monitoring and research
2. monitoring of livestock depredations and improvement of prevention measures
3. human dimension study.

These actions are conducted in every Province of the Piemonte Region which is occupied by wolves; in particular the Cuneo, Torino, Alessandria, and Verbano-Cusio-Ossola (VCO) Provinces. In particular in the Cuneo Province, where there is the highest number of packs, an intensive research activity is conducted to investigate wolf population dynamics, habitat selection, prey selection, and develop a mark-recapture approach to estimate population size and survival rates of wolves using genetic tools. An intensive work is complementary to the extensive work conducted over the all region in order to answer more specific questions. A combination of 3 different non-invasive techniques (snow-tracking, wolf-howling, and genetic analysis on scat samples) and new technology from GPS tracking allowed us to monitor the transboundary packs in the Cuneo province.

From 1999 the collaboration between the Cuneo Province and the Mercantour National Park was fundamental to follow the evolution of the packs which have transboundary territories over the Maritime Alps range.

## Wolf distribution and pack territories

We evaluated wolves' exclusive presence in the area, pack size, and the presence of adjacent packs through winters using a combination of snow-tracking data (Table 1) and genetic results on scat samples.

	Winter			
	1999-2001	2001-2002	2002-2003	2003-2004
Km of followed wolf tracks	662,0	593,5	549,0	540,4

Table 1. Km of wolf tracks followed from winter 1999-2001 to winter 2003-2004 in the Cuneo Province.

During winter 2003-2004 in the Ligurian and Maritime Alps region we documented the presence of 7 wolf packs, 4 of them spent most of their time in the Cuneo Province and 3 of them used transboundary territories (Figure 1). We intensively followed the 4 packs which spent most of their time on the Italian side of the Alps: the Val Casotto Pack, Valle Pesio pack, Valle Stura Bassa pack, and Valle Stura Alta – Haute Tinée pack. The Valle Casotto pack and the Valle Stura Bassa pack are newly formed packs.

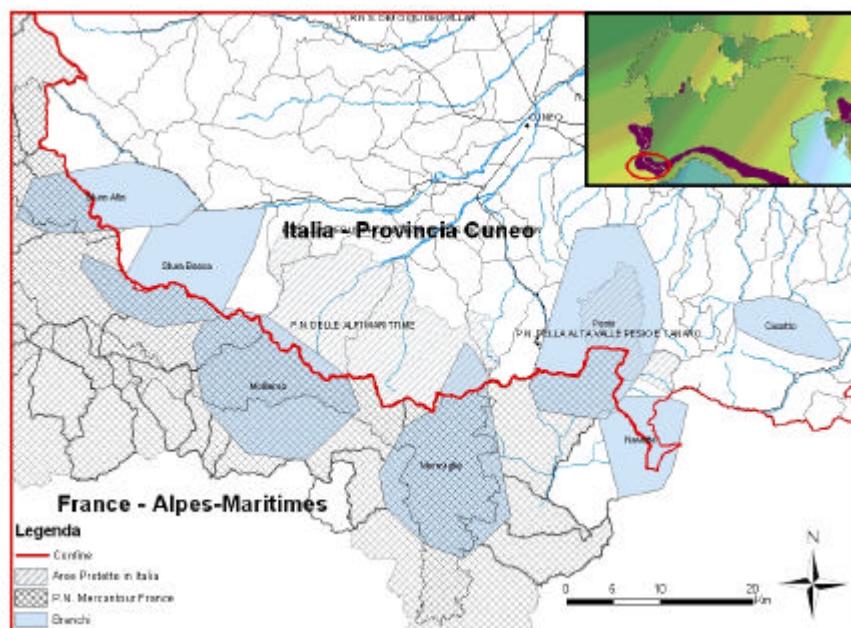


Figure 1. Wolf pack distribution in the Ligurian-Maritime Alps of Italy and France during winter 2003-2004..

## Estimation of the minimum number of wolves

We estimated the minimum number of wolves and the number of packs using a combination of snow-tracking data (Table 1) and genetic results on scat samples. The number of packs increased from 2002 to 2003 of 2 units (Figure 2).

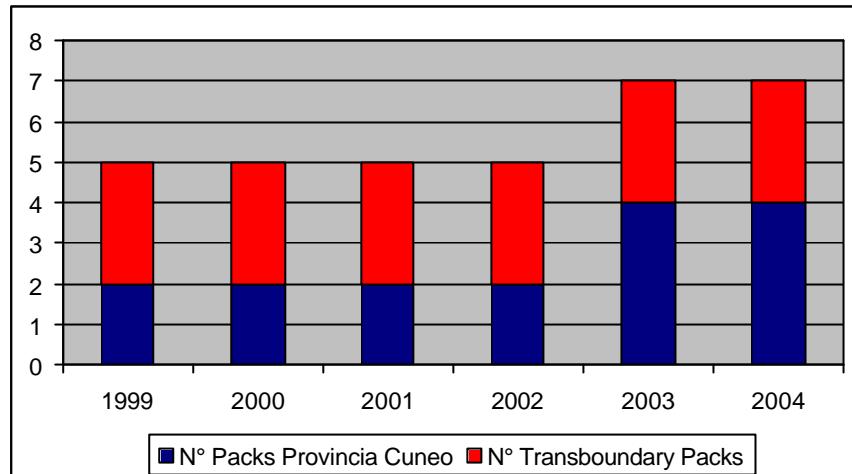


Figure 2. Number of packs detected in the Cuneo Province from 1999 to 2004.

The minimum number of wolves estimated during winter 2003-2004 ranged from 19 in the early part of the winter to 13 in the late part of the winter, considering only the packs which spent most of their time on the Italian side of the Alps (Figure 3). The number of wolves slightly increased from 1999 to 2004, especially after the formation of the new Val Casotto and Bassa Valle Stura packs. The number of wolves estimated in the early part of the winter is always greater than the one estimated in the last part of the winter, this suggests a high winter mortality or dispersal rate, and therefore indicates a difference in estimating the wolf population size in different times of the year. This should be an important issue for management purposes.

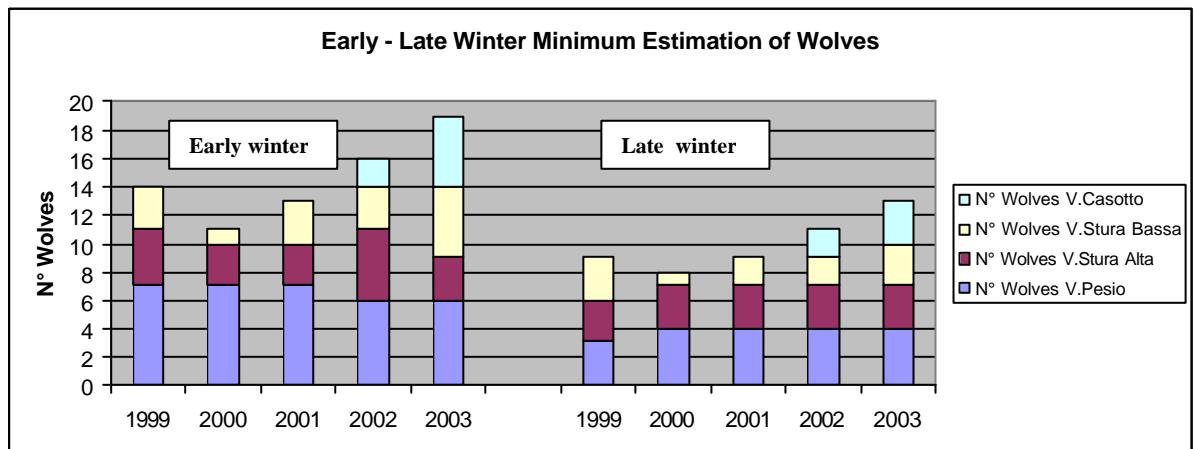


Figure 3. Early and late winter minimum number of wolves estimated from winter 1999-2000 to winter 2003-2004 in the Cuneo Province.

## Wolf population dynamics

We examined wolf pack dynamics using a combination of 3 non-invasive techniques. We followed the social history of individuals combining data from wolf howling surveys during the summer, snow-tracking surveys during the winter, and genetic analysis on scat samples collected over the entire year. Although there were on

average consistent packs of 3-6 individuals during each winter a high yearly turn-over of individuals within each pack occurred. From 1999 to 2002 we have genetically captured 50 different individuals using 6 polymorphic microsatellite loci, and documented 5 mortalities, 9 dispersals and 2 immigrations while intensively following 4 packs. We could not distinguish between dispersal and mortality on wolves that “disappeared” from packs, but reproduction was documented each summer for every pack since 1999 (Table 2).

Year	Valle Pesio Pack		Alta Valle Stura Pack		Val Casotto Pack		Bassa Valle Stura Pack	
	Reproduction	N° youngs	Reproduction	N° youngs	Reproduction	N° youngs	Reproduction	N° youngs
1999	x	3	x	2				
2000	x	3	x	1				
2001	x	2	x	1				
2002	x	1	x	3				
2003	x	2	x	2	x	3	x	2

Table 2. Reproductions and minimum number of pups documented per pack and per year in the Cuneo Province.

An important example is the Valle Pesio pack, which is the most intensively tracked pack. For this pack we could track and sample repeatedly almost each individual over the years, and we documented an high yearly turn over of individuals and a pack size which varied over the months (Figure 4). From 1999 to 2002, 2 wolf died, and 9 “disappeared”, of these 2 dispersed (Figure 4).

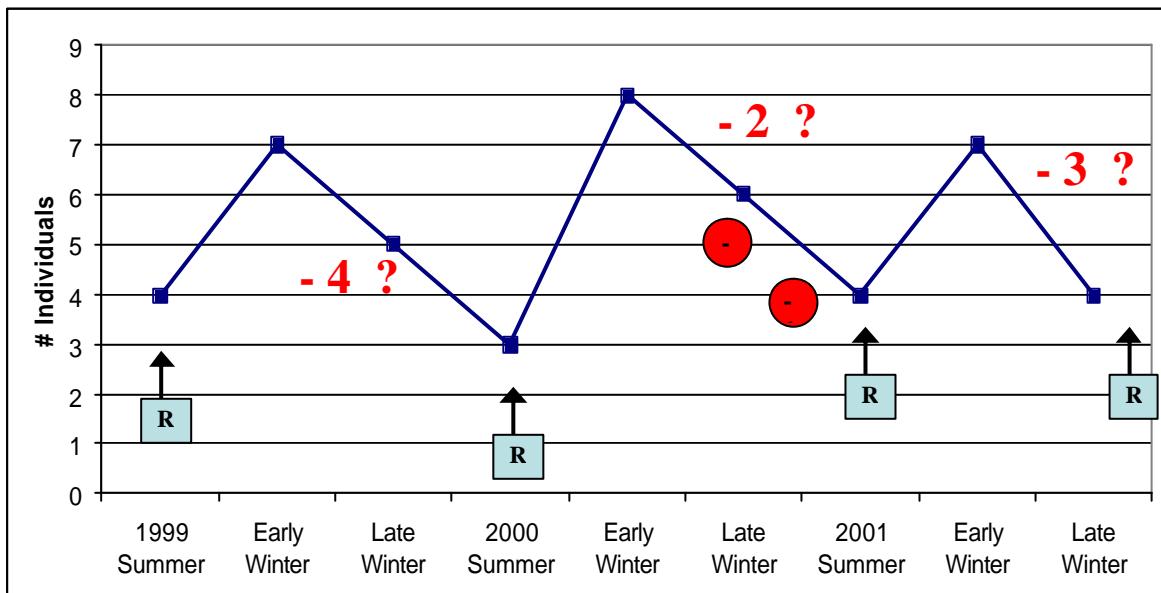


Figure 4. Number of wolves in the Valle Pesio pack over the months from 1999 to 2002. R= reproduction; red circle= one dead wolf.

In particular F31, a pup born during summer 2001 in the Valle Pesio pack, dispersed in VCO Province during winter 2002-2003 in an area distant a minimum straight distance of 225 km (see Wolf Monitoring in VCO). So far this wolf represents the furthest north wolf presence in the Italian Alps.

## **Wolf capture program**

In the wolf intensive study conducted in the Cuneo Province we started an experimental program of radiocollaring wolves. Modern GPS collars are used in order to follow individuals over the boundaries and along large distances. The main objectives are to improve the understanding of territory size, use, and selection, and to estimate wolf kill rates and improve the use of preventive methods for livestock depredations.

So far a female wolf, F7, from the Valle Pesio pack was captured in may 2004 and fit with a VHF-GPS collar. This wolf gave us interesting information, but she was killed after 3 weeks of GPS monitoring. The program is high time and effort consuming.

## **Conclusion**

The complex management issue of having a wolf viable population, while minimizing the conflict that the species might generate, requires an understanding of the spatial and temporal dynamics of the wolf population. The transboundary wolf monitoring is fundamental to follow the evolution of the wolf presence in the Alps. So far, the wolf population increased in the Cuneo Province, however the growth rate is lower than other wolf populations in the world. The minimum wolf population size estimated varies through the seasons; therefore to be conservative in management actions, we should consider the late winter wolf estimation due to the low winter apparent survival rate. Packs should be considered the fundamental units of the wolf recolonization process, and not single individuals. For wolf management and monitoring we should focus on numbers of wolf packs (or reproductive units) and not on wolf numbers. The local number of wolves in each pack can vary by months and years, but on average is constant; whereas the overall wolf population increases when new packs are formed. Therefore for a large scale wolf monitoring it is important to locate wolf packs and document reproduction to identify reproductive units. These quantitative understanding of the demographic, and territorial aspects of the Alpine wolf population, together with genetic information is fundamental to building an effective management strategy for long-term wolf conservation in the Alps.

## **Acknowledgement**

We are grateful to the Parco Naturale Alpi Marittime; Parco Naturale Valle Pesio e Tanaro; Mercantour National Park; il Corpo Forestale dello Stato; Comprensorio Alpino CN5 and CN4; all technicians, volunteers, agents, and rangers who collected data in field work with the Wolf Project Group.

## **Wolf presence in Turin Province**

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### **Introduction**

The Turin study area is a part of Western Alps covering about 2000 km<sup>2</sup>, and it consists of Monviso, Pellice, Chisone and Germanasca valley, Sangone valley, Susa valley and three Lanzo valleys (Viù, Grande and Ala) (fig. 1).

In the study area there are four Regional Natural Parks: Fascia Fluviale del Po tratto cuneese, Val Troncea, Gran Bosco di Salbertrand and Orsiera-Rocciavrè Natural Park.

The research in the Turin Province is part of regional monitoring wolf project, which involves different wolf research groups, Provincial Administrations, Regional Natural Parks, Forestry Administration. The wolf project, which has begun since 1999, has considered number of wolf packs, their reproduction success, and minimum number of wolves present in Piemonte region.

Previous research revealed the presence of three packs in Turin Province: two of them in Susa valley and one in Chisone and Germanasca valley (wolf-established zone). Some signs of wolf presence were also irregularly found in Pellice valley and no evidence of wolf presence was found in Lanzo and Monviso valleys (wolf non established zone).

At the moment three researchers with some students keep on doing daily the wolf monitoring in collaboration with rangers of Forestry, Natural Parks and Natural Service of the Turin Administration.

The wolf monitoring is carried out with different research methods: scat trails, snow tracking, genetic analysis and wolf howling.

Wolf signs were looked for in a total of 94 transects which were visited monthly.

Scat trails were distributed all over the study area especially in wolf-established area (Upper Susa Valley, Troncea and Germanasca Valley and Orsiera Zone).

Additionally, during winter, simultaneous snow tracking surveys were carried out in each zone of the study area, after 1-2 days of snowfalls, in order to detect wolf tracks, and search for wolf-signs. Genetic analysis on fresh scat permits to genotype individuals present in the area. Dott. Ettore Randi team of the Italian Wildlife Institute conducted genetic analysis on wolf scats, found all over Piemonte region (Cuneo province and Verbano Cusio Ossola province).

During summer wolf howling census enables to evaluate the reproductive success of packs, the litter size and to localize the home-site.

This report is the update of research from January 2003 to March 2004.

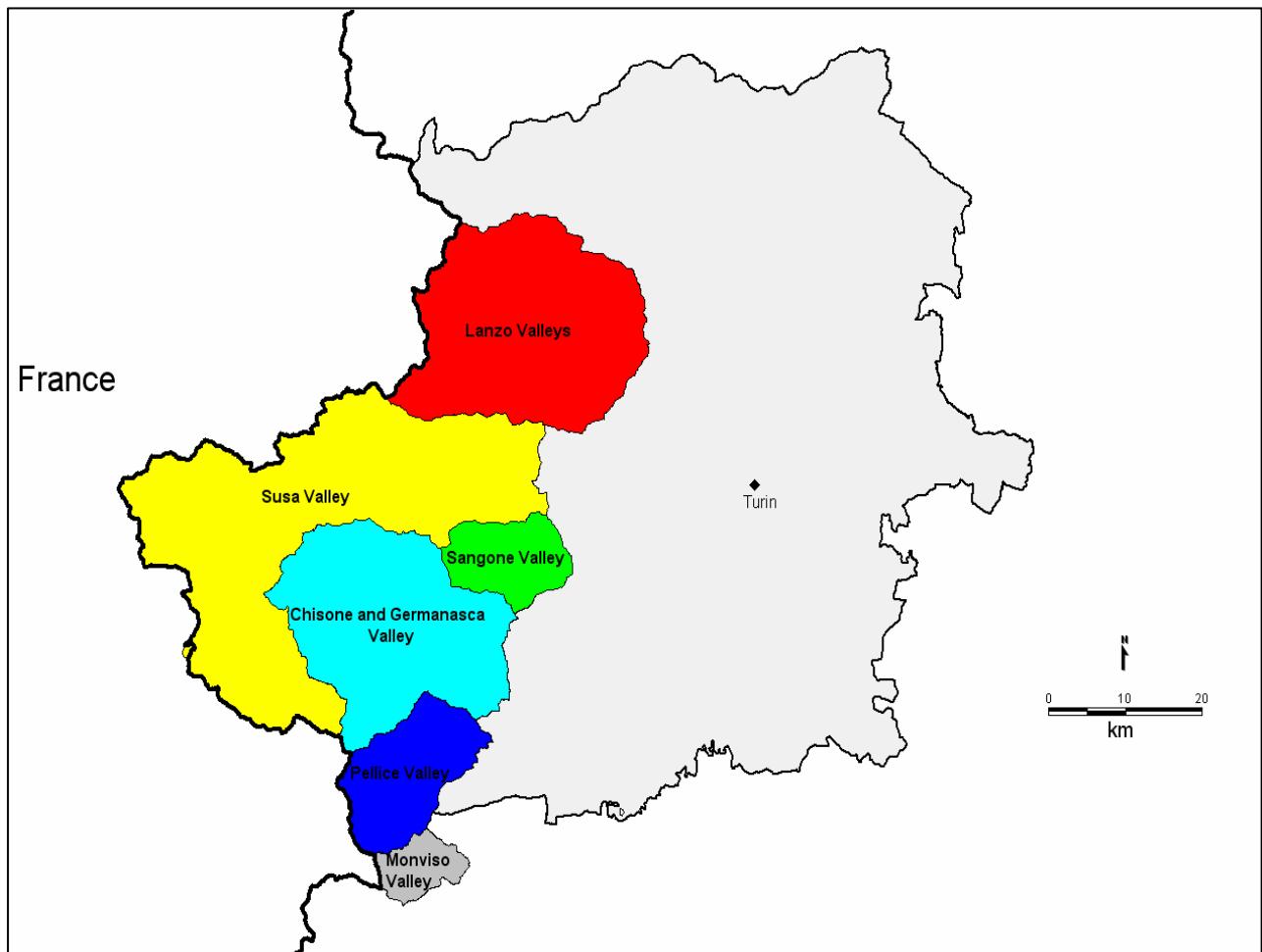


Figure 1- Turin province study area.

## Results

### Upper Susa Valley

In Upper Susa Valley there are two wolf packs: Bardonecchia and GB pack.

Wolf signs were localized near French border and inside the Gran Bosco di Salbertrand Natural Park.

We have detected two areas where wolf signs were concentrated in relation to two areas intensively used by wolves: Oulx-Bardonecchia-Cesana area and Oulx-Salbertrand-Susa area (fig. 5). We have not found different wolf signs distribution through the last three years. The table 1 resume findings in wolf surveys made in Upper Susa Valley from January 2003 to March 2004.

<b>Surveys</b>	<b>N. scat</b>	<b>Travelled distances (km)</b>	<b>N. scat/km</b>
<b>Regular transects</b>	259	2817	0.09
<b>Occasionally transects</b>	73	488	0.15
<b>Wolf tracks</b>	71	183	0.39
<b>Total</b>	403	3488	0.12

Tab. 1-Wolf surveys in Upper Susa Valley (jan 03-mar04).

The genetic results associated with wolf tracks, allowed to evaluate the minimum area used by Bardonecchia pack during winter 2002-03, that was 156 kmq wide.

Since signs of wolf presence were found near Italian border (Claviere, Mongenevre and Nevache) we think that Bardonecchia pack moved sometimes also to the French side, but genetic analysis with French side on fresh scats is required.

It was not possible to calculate this area also for GB packs, because there weren't sufficient genetic data. However thanks to previous data we think that GB pack used all territory of GB Natural Park, the western side of Orsiera Natural Park and the Chisone valley side of Upper Susa Valley.

The two packs (GB and Bardonecchia) reproduced in summer 2003 and the minimum litter size, estimated by wolf-howling technique, was three pups for each group.

However six pups in Bardonecchia group were documented by a film made in August 2003.

The distance between rendezvous-sites of different pack (GB, Bardonecchia and VT-VG packs) was about 17 kilometres and the howling pups responses of both pack were stable in the space for all over the summer.

Simultaneous wolf-howling census in French and Italian side was made in summer and the only reply of pups we obtained was localised in Susa valley.

In the last winter (2003-04) season in Alta Valle Susa six wolves died. Five of them were killed by traffic accident: 3 hit by car and 2 by train. Moreover one wolf skull was found and its cause of mortality is unknown. One wolf was hit by a car, but not killed.

More in general 8 wolves, that have been found since 2001 in the Turin province, were from Susa valley (tab.2).

We think that seven dead wolves belonged to Bardonecchia pack and only one to GB pack, but genetic analysis is required.

<b>Date</b>	<b>Mortality factor</b>	<b>Sex</b>	<b>Age</b>
17/12/01	train	M	<1 year
28/12/01	car	M	<1 year
19/11/03	train	M	<1 year
27/11/03	car	M	<1 year
14/01/04	car	F	<1 year
7/02/04	car	M	<1 year
9/02/04	train	M	<1 year
December 2003	unknown	unknown	<1 year

Tab.2- Dead wolves found in Upper Susa Valley since 2001.

All localisations of dead wolves were in the bottom of the valley along the main road and railway between Salbertrand and Bardonecchia.

Figure 2 and 3 shows the minimum number of wolves per Bardonecchia and Gran Bosco pack estimated through the last year, obtained by snow tracking, wolf howling, genetic analysis and documented observations.

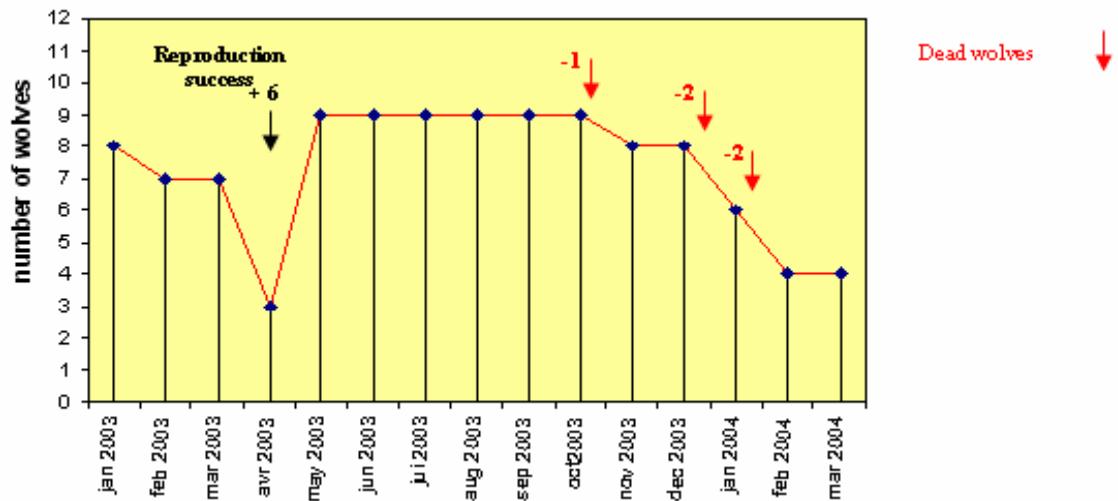


Figure 2- Minimum number of wolves in Bardonecchia pack from January 2003 to March 2004.

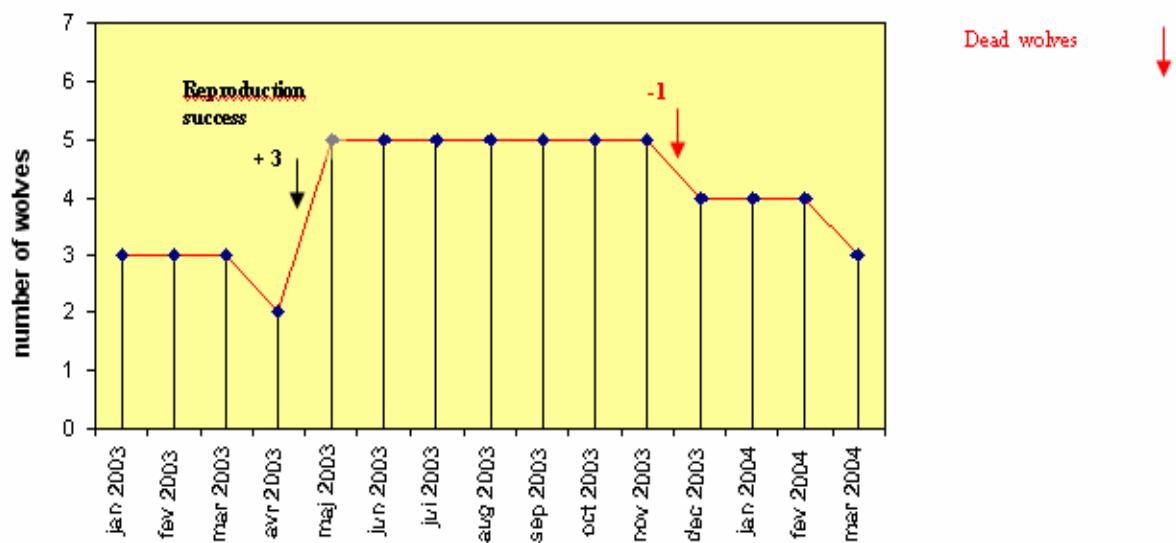


Figure 3 - Minimum number of wolves in Gran Bosco pack from January 2003 to March 2004.

### Troncea and Germanasca Valley

In the previous year we detected VT-VG pack in the central part of the zone, near and inside the Val Troncea Natural Park and Germanasca valley. On the contrary, in this last winter, the few wolf signs (scats and tracks) we found were concentrated in the northeastern side of the area, near Orsiera and Upper Susa Valley (fig.5).

The table 3 shows findings in wolf surveys made in VT-VG zone from January 2003 from March 2004.

The Abundance Kilometres Index of wolf transects decreased respect of the previous year, probably due to a different territory use by the pack.

We think that this switch to lower altitude and more accessibility valley is probably due to a different territory use by VT-VG pack, because of the abundance of snowfall in the last winter.

Only genetic analysis of fresh scats will confirm the presence of VT-VG pack in Chisone valley side of Orsiera.

Surveys	N. scat	Travelled distances (km)	N. scat/km
<b>Regular transects</b>	42	1429	0.03
<b>Occasionally transects</b>	16	305	0.05
<b>Wolf tracks</b>	18	58	0.31
<b>Total</b>	76	1792	0.04

Tab. 3-Wolf surveys in VT-VG zone (jan03-mar04).

The VT-VG pack reproduced in summer 2003 and the minimum litter size, estimated by wolf-howling technique, was three puppies.

Figure 4 shows the minimum number of wolves per VT-VG pack estimated through the last year, obtained by snow tracking, wolf-howling techniques, genetic analysis and documented observations.

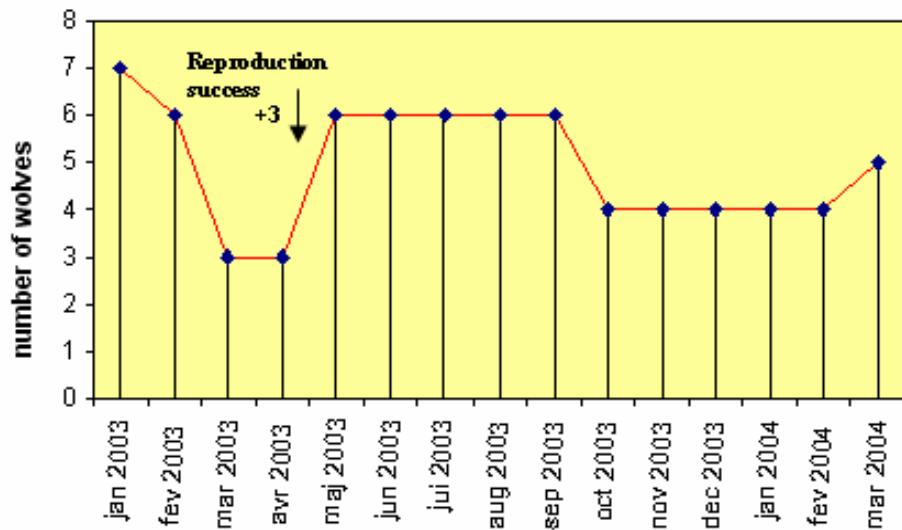


Figure 4 - Minimum number of wolves in VT-VG pack from January 2003 to March 2004.

## Orsiera Zone

The Orsiera Zone includes the Orsiera-Rocciavrè Natural Park, with Susa valley side and Chisone valley side.

The positive trend of wolf signs, found in the last two years, showed that the area is highly used by wolves (tab. 4 e fig.5), especially in the last winter, but we can't confirm the presence of a new fourth pack at the moment. Infact wolf tracks followed in the last winter confirm the presence of 2-3 wolves (with a mature female) in the Susa valley side; and 4-5 wolves in Chisone valley side of Orsiera.

Only genetic analysis could help us to understand if this area is used by new unit or by neighbouring packs (GB pack for Susa valley side and VT-VG pack for Chisone valley side).

Surveys	N. scat	Travelled distances (km)	N. scat/km
<b>Regular transects</b>	22	526	0.04
<b>Occasionally transects</b>	27	275	0.10
<b>Wolf tracks</b>	27	56	0.48
<b>Total</b>	76	857	0.09

Tab. 4-Wolf surveys in Orsiera Zone (jan03-mar04).

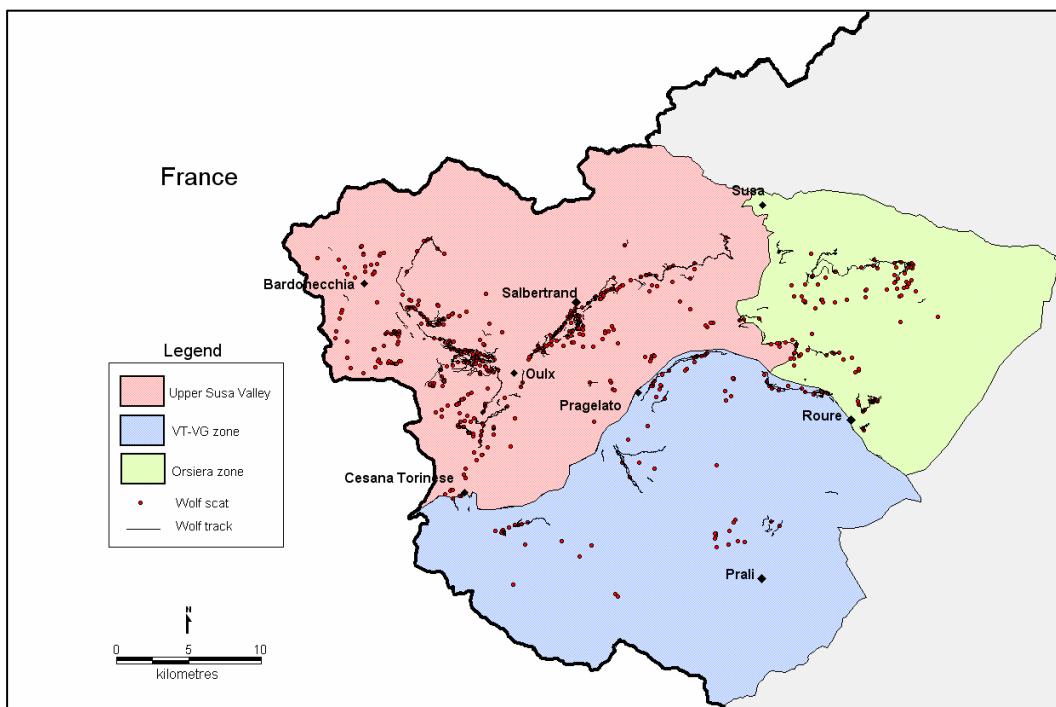


Figure 5- Wolf sign distribution in the wolf established zone (jan03-mar04).

### **Remaning study area (Monviso valley, Pellice valley, Lanzo valleys)**

In the rest of the study area we can summarize the wolf monitoring results in as follows.

At the moment in Lanzo valleys and Monviso valley there are no signs of wolf stable presence. However we can not discard temporary presences of the some individuals in these areas.

On the contrary in Pellice valley, especially in the last winter, the signs of presence of 2-3 wolves were found near the French border, in Carbonieri and Conca del Pra valley.

Genetic analysis on fresh scats, found in this last winter, could help us to understand if these wolves really belonged to the French Queyras pack.

### **Genetic analysis**

A sample of 68 scats found from January 2002 to July 2003 in Turin province, were analysed by the genetic laboratory of Italian Wildlife Institute. 42 scats were attributed to 22 wolf genotypes. Moreover no dog or dog-wolf hybrid genotypes were found.

The presence of GB pack was confirmed even in 2002, thanks to genetic results associated with wolf tracks. The lack of reproduction confirmation in GB pack in summer 2002, the presence of a neighbouring pack, like Bardonecchia, and the absence of genetic results made the unclear situation in the previous year, when we detected only one packs in Upper Susa Valley.

The table 5 shows the 22 genotypes detected in the Turin province from January 2002 to July 2003: 13 are females and 9 are males. 13 wolf genotypes were evidenced in winter 2001-02, and 15 in winter 2002-03.

<i>Genotypes</i>	<i>Sex</i>	<i>Winter 2001-02</i>	<i>Summer 2002</i>	<i>Winter 2002-03</i>	<i>Summer 2003</i>
F06 (n=4)	F				
F08 (n=4)	F				
F11 (n=1)	F				
F12 (n=2)	F				
F19 (n=1)	F				
F21 (n=1)	F				
F22 (n=1)	F				
M09 (n=3)	M				
M18 (n=3)	M				
F04 (n=1)	F				
F05 (n=2)	F				
M14 (n=2)	M				
M07 (n=1)	M				
M02 (n=4)	M				
M17 (n=2)	M				
M10 (n=1)	M				
M16 (n=1)	M				
F01 (n=1)	F				
F15 (n=1)	F				
F20 (n=1)	F				
M23 (n=1)	M				
F13 (n=2)	F				

present

Supposed present

Tab.5-Wolf genotypes in Turin province (jan01-ju103).

## **Conclusion**

Data collected in this last year confirmed the presence of three stable packs in Turin province: Bardonecchia, GB and VT-VG pack.

The hypothesis of a settlement of a new pack in Orsiera needs to be confirmed.

The occasional wolf signs we found in Pellice valley, probably belonged to a French pack, but more information are required.

The range of wolf packs size in Turin province is 3-9 wolves along the year and the mean wolf number is 4,9 in VT-VG pack, 4,1 in Gran Bosco pack and 7,3 in Bardonecchia pack. We found that the number of wolves per packs, more or less, is stable through years. Even if the death of five wolves in this last winter caused an important decrease in Bardonecchia pack size (from 9 wolves in summer to 4 wolves at the end of the winter).

We think that the main factor of this wolf mortality in winter is the high use of the bottom valley for hunting, especially in very snowy winters. Moreover wolves of Bardonecchia pack used to move between the two sides of the valley, in presence of the high-traffic road and railways along the Susa valley.

Piemonte Region and Provincial Administration of Turin (Servizio Tutela Flora e Fauna) are going to found a project, in the next year, to determine precisely corridors used by wolves, to cross the Susa bottom valley side, looking for possible action to reduce wolf mortality.

We think that more simultaneous surveys are necessary for the French and Italian side in the area of Susa and Pellice valleys in winter. For that reason we will increase the monitoring surveys in Pellice valley in the next year. Moreover genetic results of scats found in Italian and French side should be comparable to fully understand the wolf movements near the country border.

## **Acknowledgement**

We are grateful to Servizio Tutela della Fauna e della Flora of the Turin Province; Gran Bosco of Salbertrand, Val Troncea, Orsiera-Rocciavrè, Fascia Fluviale del Po tratto cuneese Regional Natural Parks; Forestry Administration, Consorzio Forestale Alta Valle Susa; Comprensorio Alpino TO1 and TO2; all agents and rangers who collected data in field work with the Wolf Project Group.

# **Wolf monitoring in the Verbano -Cusio-Ossola province**

Radames Bionda and Chiara Passalacqua

## **Introduction**

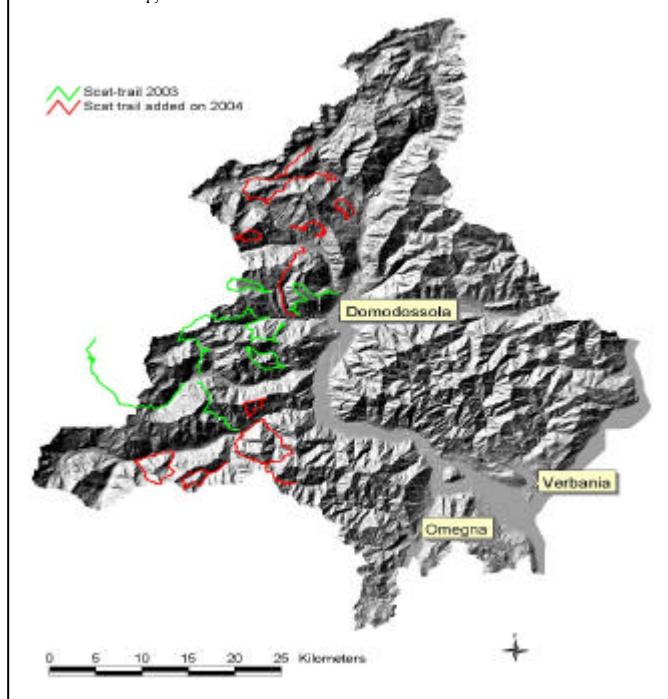
Wolf monitoring in the Verbano Cusio Ossola (VCO) province is carried out by Alpe Veglia Devero Natural Park, in collaboration with the “Office for Wildlife Protection ” of the Province of Verbano Cusio Ossola. We also keep continuously in touch with Urs Zimmerman, who is in care of the wolf monitoring in the Simplon area, CH.

We joined the Piedmont wolf project in autumn 2002 after an observation of a single individual in Bognanco valley and some predations on goats in Domodossola municipality. Before that, the presence of at least one wolf was confirmed by genetic analysis on the Swiss side of Bognanco valley in July 2002, few hundreds meters far from the Italian-Swiss border.

## **Methods**

The monitoring activity was based on a network of transects, which have been surveyed from the first year. During winter 2002-2003 we monitored a 100 km<sup>2</sup> area in Bognanco and Antrona valleys, by means of 9 transects of 69 km of total length. We then increased the total length of transects up to 119 km in summer 2003 and 231 km in summer 2004 (Fig. 1), so as to reach a total monitored area of 619 km<sup>2</sup>, which covers the valleys of Antigorio, Divedro, Bognanco, Antrona Anzasca and Strona. Monitoring effort has been more intensive in Bognanco and Antrona valleys, where the wolf presence had been reported, and less intensive in the other valleys. Snow-tracking and genetic analysis on scats collected were used to estimate wolf numbers and distribution. We also used wolf howling trying to confirm reproduction.

Fig. 1.Network of scat trail



## Results

During winter 2002-2003 transects have only been surveyed with good snow cover conditions at mid-low elevation. During 8 track sessions, 20,6 km of tracks were mapped and 5 scats were collected (Fig. 2). 3 scats where analysed by Italian Wildlife Institute and University of Losanna. All belonged to the same wolf, a female, which had also been sampled on the Swiss side of Bognanco valley the summer before (Urs Zimmermann, com. pers.; Fumagalli, 2003). During this winter we estimated the presence of at least 2 individuals in three track sessions, and one individual in 5. Track sessions carried out during the female's oestrus period (February 23rd) suggest that, during this time, the female was alone.

During summer 2003 14 scats were collected. 4 of them were analysed by Italian Wildlife Institute: DNA extraction failed in two cases, whereas it was successfully accomplished in the remaining two. One scat belonged to a wolf which had never been sampled before and one was from a dog.

Wolf howling was carried out in 7 stations but we had no reply.

Winter 2003-2004 was characterized by good snow conditions. 38,4 km of tracks were mapped during 34 track sessions (Fig. 2). Only once (November 2nd) the presence of two wolves was assessed. In all other cases we estimated the presence of only one individual. 12 scats were collected and no one has been analysed so far.

winters	km of snow track sessions	surveyed km	n sessions	tracked km	mean length	n of wolves	min. elev.	max. elev.
2002-2003	16	105	8	20,4	929 m (min=92; max =3306)	1 (5 cases), 2 (3 cases)	740	2250
2003-2004	48	370	34	38,4	1129 m (min=49; max= 4380)	1 (33 cases), 2 (1 case)	380	2005

Tab. 1. Results of winter monitoring

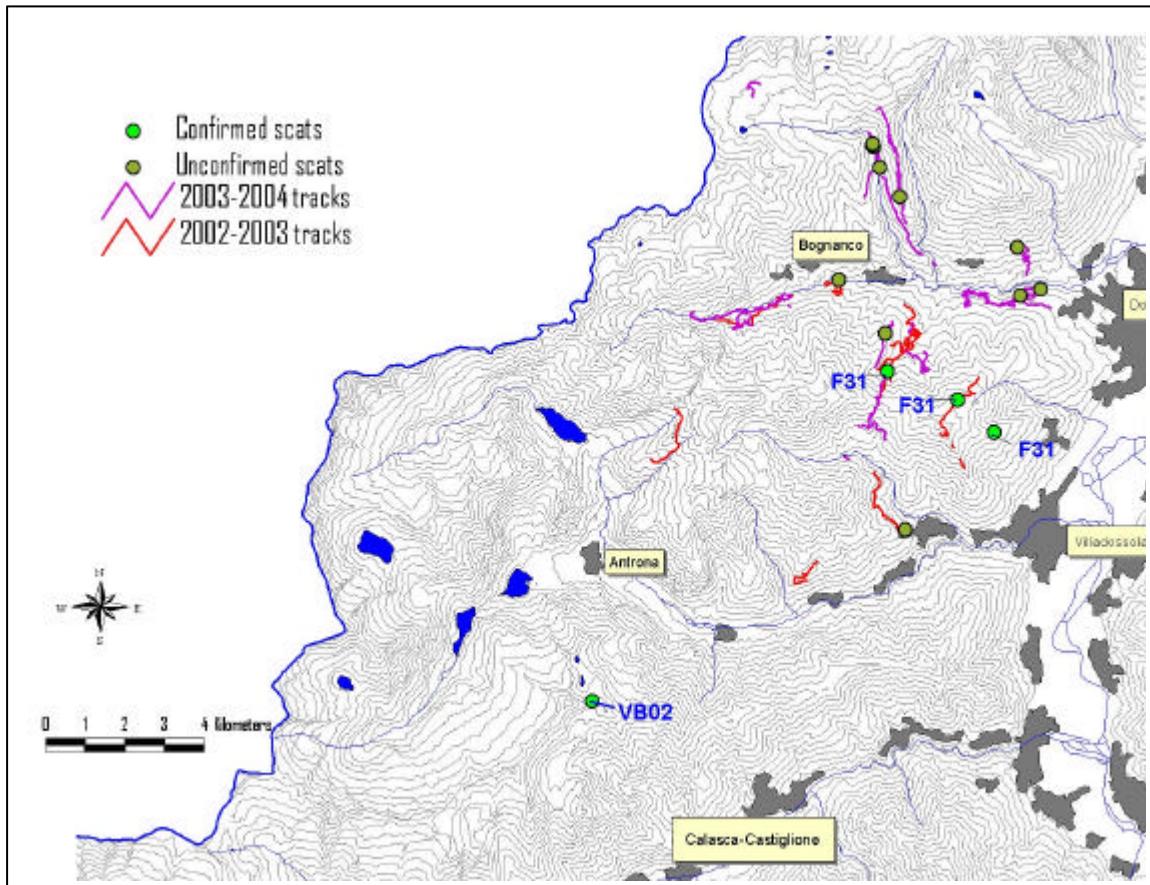


Fig. 2. Location of tracks and scats collected during winters 2002-2003 and 2003-2004

## Conclusions

From autumn 2002 to spring 2004 two different genotypes were sampled in the Verbano Cusio Ossola Province. Both belong to the Italian wolf population.

The snow tracking results confirmed the presence of at least two different wolves until autumn 2003. One was a female (3 samples), whereas the sex of the second wolf was unknown (one sample). The analyses made by Italian Wildlife Institute and University of Losanna on the same scats found in winter 2002-2003 confirmed that the female we tracked on the Italian side of Bognanco valley was the same sampled in Pontimia, on the Swiss side. This result was then confirmed by Fabbri, 2003, who discovered that this female had already been sampled in Valle Pesio (Cuneo province) in November 2001. Thus, she dispersed northwards over 212 km in no more than 7 months.

Pooling together data from snow tracking, confirmed scats and confirmed kills on livestock we obtained an area of wolf presence  $163,4 \text{ km}^2$  wide (Fig. 3).

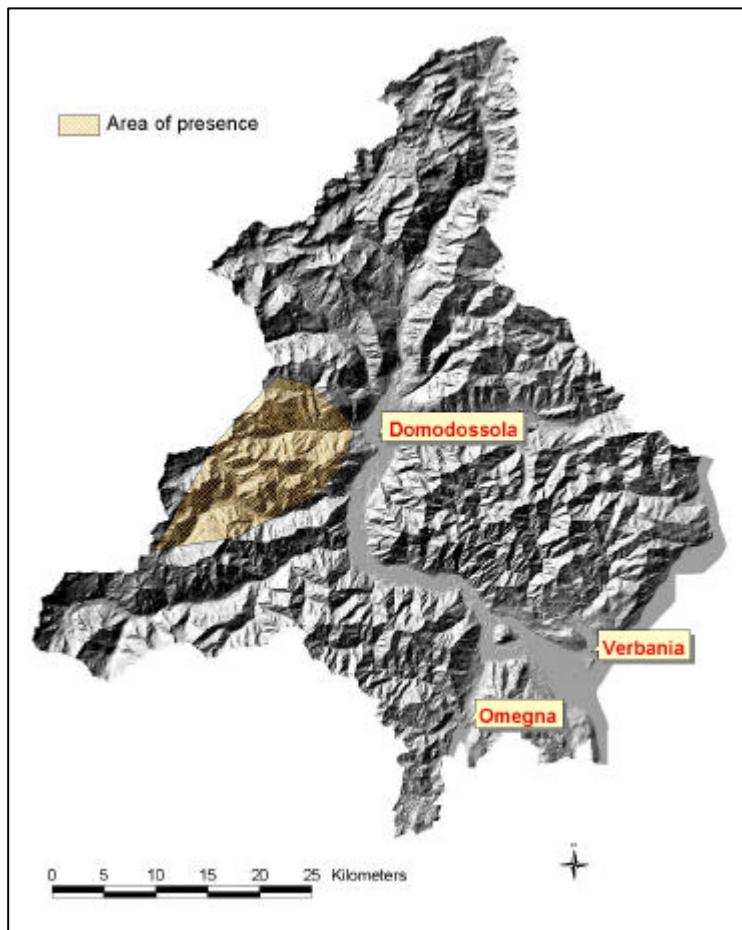


Fig. 3. Area of wolf presence in Verbano Cusio Ossola province

### Acknowledgements

We would like to thank every one who help us in monitoring activity, in particular Paolo Taffi and Flavio Bonzani; the head of Polizia provinciale corp dott. Marco Brondolo and all the agents, especially Osvaldo Lucini, Salvatore Attinà, Renzo Croppi and Renato Morelli; Urs Zimmermann for the constant exchange of information on wolf activity in the Swiss side; Francesca Marucco for her unvaluable comments on every stage of the activity.

### Literature cited

- Fabbri E., 2003. Analisi dei processi di colonizzazione del lupo italiano sulle Alpi occidentali: inferenze basate su analisi genetiche multilocus con campionamento non invasivo. Tesi di dottorato. Università Degli Studi di Ferrara Facoltà di Scienze Matematiche, Fisiche e Naturali.
- Fumagalli L., 2003. The wolf in the Swiss Alps: non-invasive genetic analyses over a 7 year-period. In Weber J.M.; Wolf monitoring in the Alps. 2<sup>nd</sup> Alpine Wolf Workshop, Boudevilliers (CH). 17-18 March 2003. Kora Bericht Nr. 18.

## **The French Wolf data in 2003-2004 : an update with population dynamics perspectives**

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Since the last workshop of the wolf alpine group was held in Switzerland (2003), we obtained additional data regarding the wolf monitoring together with some additional insights at population dynamic perspectives. The French wolf network still is the basic tool for collecting data in the field with 2 levels of sampling pressure: 1) an opportunistic sign survey involving 450 field technicians dispatched all around the French alpine range and 2) an intensive sign survey conducted along transects within each permanent wolf area (one or several wolves holding a territory during min. 2 consecutive years).

The sign survey consists to report each documented wolf sign using standardized field and analysing procedures, then to centralize them in a common database. As a special case, scats are collected for the purpose of genetic identification and diet analysis . Because the latter is not a priority investigation, updated results about the former topics will rather be described, as well as some suggestions about what could be investigated in common (i.e. international collaboration), especially considering the exploitation of the genetic results to study some aspects of population dynamics (e.g. dispersal rates, breeding parameters, survival rates).

The update about the last winter survey session will describe :

- 1) the use of the transversal dataset in order to set :
  - a. the wolf distribution in 2004 on the French side of the Alps,
  - b. the minimum estimated “packs-based” population size during 2003/2004 as calculated from the snow-tracking method (i.e. excluding dispersers),
  - c. the reproduction events that occurred in 2003 monitored with the wolf howling sessions.
- 2) the use of the longitudinal dataset available with the genetic tagging to distinguish the different wolf social units over space and time and to enlighten some dispersal patterns.
- 3) finally, a first overview about the opportunity to use the genetics tagging in Capture-Mark-Recapture models.

## **Wolf distribution, minimum wintering population size and reproduction events**

Since the last winter, two new permanent wolf area were confirmed (signs discovered during two consecutive winters) named “Taillefer-Oisans” in the northern alps and the second named “Haut Verdon-Ubaye” (Southern Alps) from which the wolf presence was only previously documented through summer damages on livestock (Figure 1). The first genetic identification reveals that these wolves were holding new genotypes never recorded somewhere else in the past.

One new area has been discovered for the first time in the very northern part of the alps (close to the Jura mountains) with damages to livestock and one confirmation with a genetic analysis made on scats. We also noticed an expansion toward the west with at least a temporary area of wolf presence in the west pre-alpine area. Wolf presence has been recorded in the Vanoise mountain chain (northern alps) where almost no signs were reported since 1999 despite the continuous year-to-year sampling effort (Figure 1).

The snowtracking sessions, implemented in each of the 13 permanent wolf areas, revealed an increase of the minimum wolf population size. The sum of maximum detected pack sizes was estimated between 37 to 41 resident wolves at the end of the winter 2003/2004 (all data from Nov 1<sup>st</sup> to March 31<sup>th</sup> included). The areas called “Canjuers” and “Monges” cannot be described as wolf packs because only one wolf has been recorded there.

In order to document the reproduction events, we investigated the wolf howling method as recommended during the 2<sup>nd</sup> alpine wolf workshop. An experimental study design was conducted during the 2 first years with stratification according to (1) knowledges (yes or not) of previous rendez-vous sites locations , (2) knowledge about the status of the pack (permanent documented area with potential reproduction or status not enough documented) and (3) known presence of pups from visual or acoustic contacts for the current year sampled (control sample). This first step aimed at testing our ability to detect a reproduction event as a function of the sampling effort and focused sampled area. These results will need to be assessed during at least 2 years of experimentation before concluding.

In 2003, 10 areas have been monitored. Six rendez-vous sites were documented (Figure 1). The absence of rendez -vous site in Haute-Tinée results from of the transboundary characteristic of this pack where the rendez-vous site was located on the Italian side (Marrucco *com pers*). This might well be the case for the pack “Clarée” for which reproduction has been discovered only in late autumn on the French side, whereas a reproductive event was detected all along the summer time in the Bardonechia Pack (It) (Avanzinelli, *com pers*). Unfortunately, no genetic data are available for these two areas to conclude if there is a single pack or two adjacent packs. Simultaneous repeated howling sessions would be useful for the next years to solve part of this question (i.e. simultaneous responses with pups identified on both sides of the border will mean two packs, whereas other combinations of responses or non-response will yield non-interpretable results).

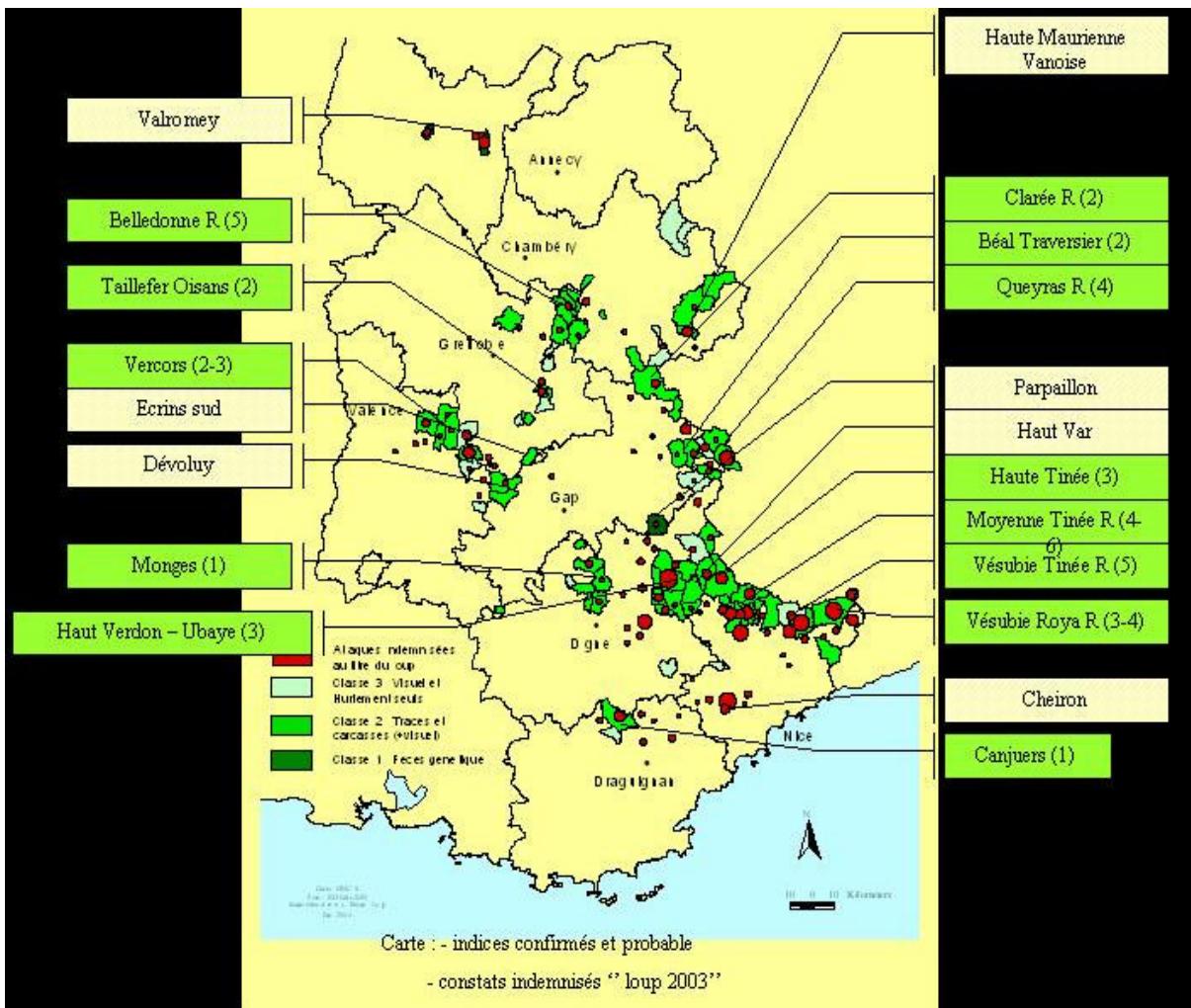


Figure 1 : Wolf distribution described according transversal signs survey in 2004 (dark to light green: 3 categories of declining signs quality rank from top –1- to low – 3- ) and damages to livestock (number of attacks for which the wolf responsibility could not be excluded). ® Reproduction documented in 2004. (n) Minimum pack size estimated with the snow-tracking method.

## First population dynamics results from non-invasive molecular tracking

P. Taberlet's team (LECA CNRS France) worked with E. Randi's (INFS-Italy) and L. Fumagally's (Lausanne Univ.- Switzerland) ones to provide a set of 6 common microsatellite loci. The results from the French side for all scats and hairs available (n=448 samples successfully genotyped, collected from 1994 to 2002 - table 1) allow for a reliable discrimination between social units (figure 2). As a whole since 1994, 107 different genotypes have been recorded (see Réseau loup, 2004) but some more cross-validations are currently performed to test for possible slight over-genotyping errors and to distinguish between the effects of sampling variability and/or population turn over on these results.

We spatially identified each pack but we consider that additional data are still necessary for a better understanding of boundaries and identification of the "Queyras", "Beal-Traversier" and "Clarée" packs.

Extraterritorial movements as well as dispersal movements have also been detected, which explain the temporal pattern of packs installation from south to north since 1994. The longest dispersal movement – 153 km as a straight line between 2 consecutive genetic recaptures - has been recorded from the "Vesubie-Tinée" pack to the "Belledonne" pack.

Next results in transboundary genetic investigated by Fabbri *et al* will be helpful to get a better balance between considering the alpine population as a single demographic unit instead of arbitrary administrative national units independent one from each other (which is definitely an "under characterized" biological status).

year	N analysis
1994	1
1995	14
1996	44
1997	88
1998	159
1999	107
2000	92
2001	80
2002	21 (in process)

Table 1 : Number of genetic analysis made on scats and hairs successfully genotyped with 6 microsatellite loci.

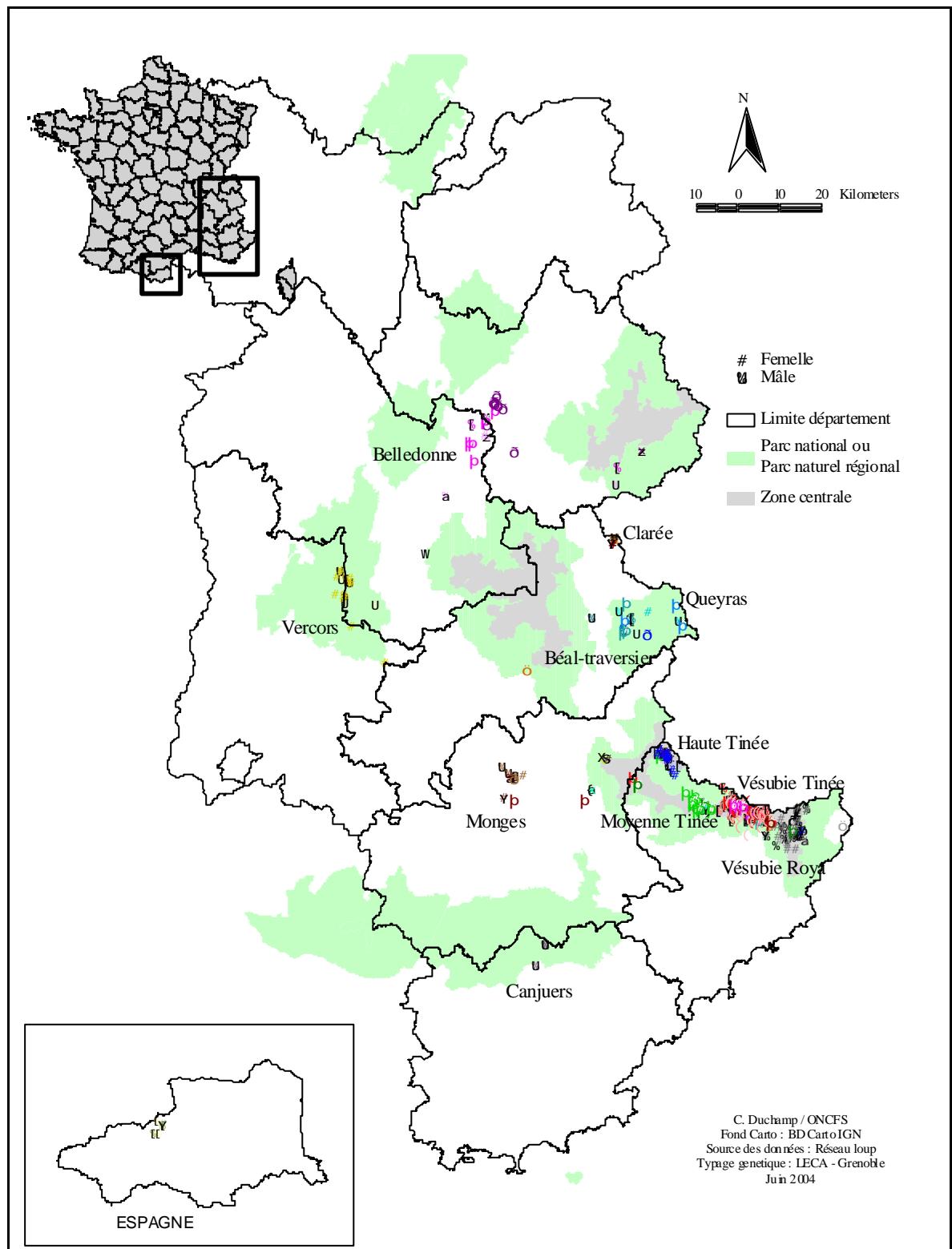


Figure 2 : Spatial distribution of wolf genotypes used to distinguish each social unit. Each symbol has been affected to one genotype with colour depending on where the animal was most often located : one wolf is considered to belong to one given pack if his genotype is found several times within the same area and never in the others. All the genotypes (Pyrenees included) are from the Italian lineage (from Duchamp *et al* 2004 in *LIFE project final report - France*).

## On the exploitation of wolf molecular tracking data to investigate population size estimators based on Capture-recapture models

The 448 genotyped samples have been used to evaluate different problems arising from applying this type of data to Capture-Mark-Recapture (CMR) modelling. The hypotheses underlying classical CMR models (Cormack-Jolly-Seber for open populations) are :

- equivalent probability of being recaptured for each animals according to their sex, social status ...and between marked and unmarked individuals.
- the number of marked individual is known every time (no errors in genotype reading)
- the sample of marked individuals is evenly distributed regarding the whole target population defined by gene flow and social relationships between individuals.

The exploration of whole French genetic datasets reveals a strong heterogeneity of the recapture probability. Indeed, 65% of the genotypes are captured only once, as opposed to few individuals re-captured from 10 up to 40 times during the 8 years tracking period (figure 3).

This strong heterogeneity in recapture rate translates in mark-recapture histories on one side a lot of individuals described by long runs of 0 following first capture (10000000) and on the other side as few individuals with a run of 1 (10111111). This heterogeneity enhanced violation of hypotheses 1 and 2 that may be due to differences in scat deposit rates between dominant and subordinate individuals, or different probability of capturing a resident or a transient individual, or errors in the genotype reading, or a combination of all these kinds of bias.

These data also showed that it is highly possible to miss some individuals for two or more consecutive years and recapture them only occasionally.

The transient hypothesis still needs to be tested as we know that during the expansion phase of wolf colonisation, the species is able to move over long distances and then, the target population is more likely to be the whole alpine population. Then the combination of all genetic data available, e.g. from France, Italy and Switzerland, is strongly needed. Part of these problems can be integrated into new approaches in CMR modelling that we will try to explore with the collaboration of J.D. Lebreton's team (CNRS France) which is one of the world-known research groups working with such modelling approach.

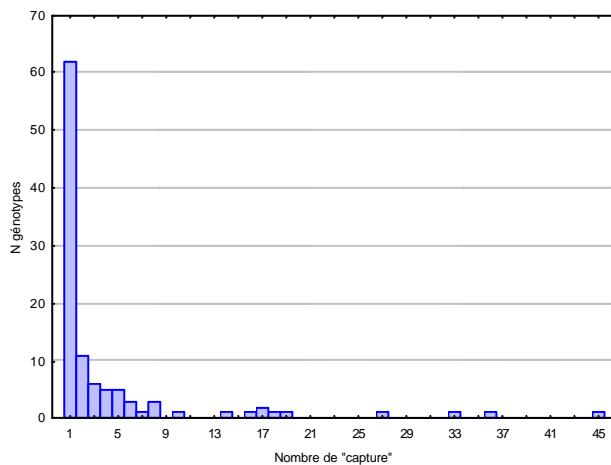


Figure 3 : distribution of the 107 genotypes according to the number of recapture that occurred during the 8 years molecular tracking period.

## **Conclusion**

The wolf expansion is still going on northward and westward in France. A better analysis of what is the target population regarding wolf conservation and management strategies is clearly needed. The first step defined during the first alpine wolf workshop, consisting in molecular methodologies standardization between countries is done, thanks to collaborations between the genetic labs involved. A common analysis and interpretation of all the data available is “on the road”. We now have to reach the same goal for the transversal signs survey in order to compile field data interpreted in the same way, in order to be able to produce a sound common map to describe each existing packs. That means we need first to establish a common standardization of data interpretations.

## **Acknowledgements**

All the data are provided by the “Réseau loup/lynx” involving more than 400 field technicians in charge to collect the data. The authors are grateful to all of them for implementing this team work. During the period 1997 – 2003, LIFE projects allow a financial support from the European community and the French Ministry of Environment.

## **Wolf monitoring in Switzerland, 1999-2003**

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Wolf monitoring program was launched in January 1999 by the Swiss federal agency for environment, forests and landscape, following the discovery, in November 1998, of a wolf carcass in Reckingen (Valais), and the finding, a month later, of several wolf signs in the neighbouring Simplon region. Wolf monitoring is essentially carried out by the wildlife management service of the cantons concerned by wolf presence. KORA is in charge of gathering the data at the national level and providing technical support when necessary.

Wolf observations may be of different types:

- 1) *Biological samples for genetic analyses*: scats, hair, regurgitations and saliva.
- 2) *Losses in the wolf population*: wolves legally and illegally shot, or found dead.
- 3) *Domestic animals killed by wolves*: wolf depredations confirmed by a cantonal wildlife management service or a competent person.
- 4) *Fortuitous observations*: wolf signs (i.e. tracks, wild prey), direct observations and howling.

Observations are categorized according to their reliability:

*Q1 observations*: dead wolves, genetic analyses, documented direct observations (i.e. picture).

*Q2 observations*: wild and domestic prey, and tracks confirmed by a competent person.

*Q3 observations*: unverified prey and tracks, undocumented direct observations, and howling.

A total of 545 wolf observations have been recorded in Switzerland from 1999 to 2003 (Table 1). Most of them belong to Q2 category (63.5 %) whereas Q1 observations represent approximately 5 % of the whole.

	<b>Q1</b>	<b>Q2</b>	<b>Q3</b>
<b>1999</b>	6	139	15
<b>2000</b>	6	139	12
<b>2001</b>	4	76	7
<b>2002</b>	2	45	16
<b>2003</b>	9	47	22
<b>Totale</b>	<b>27</b>	<b>446</b>	<b>72</b>

Table 1: Wolf observations (Q1 – Q3) recorded in Switzerland, 1999 – 2003.

Wolf presence is restricted to the Alps; no observation was reported from the Jura Mountains despite some rumours of a possible presence of the predator in this area. Sixteen Q1 observations originated from canton Valais (Figure 1) and mainly resulted from genetic analyses

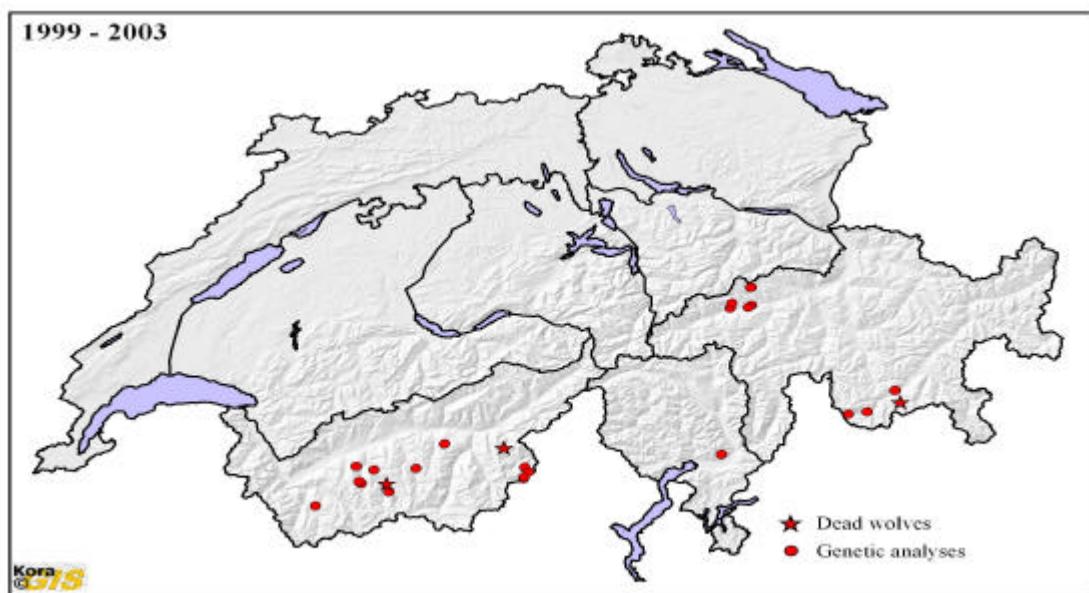


Figure 1: Distribution of Q1 observations, 1999 – 2003.

In addition, two wolves have been legally shot in this region during the monitoring period. Another individual has been shot in the canton Graubünden following recurrent depredations on livestock. Only one Q1 observation comes from canton Tessin. Excepted one individual – a scat found in 2000 in canton Graubünden revealed the wolf was from central Europe -, all identified wolves originated from the population of the Italian – French Alps.

Q2 observations have roughly the same distribution as Q1 (Figure 2).

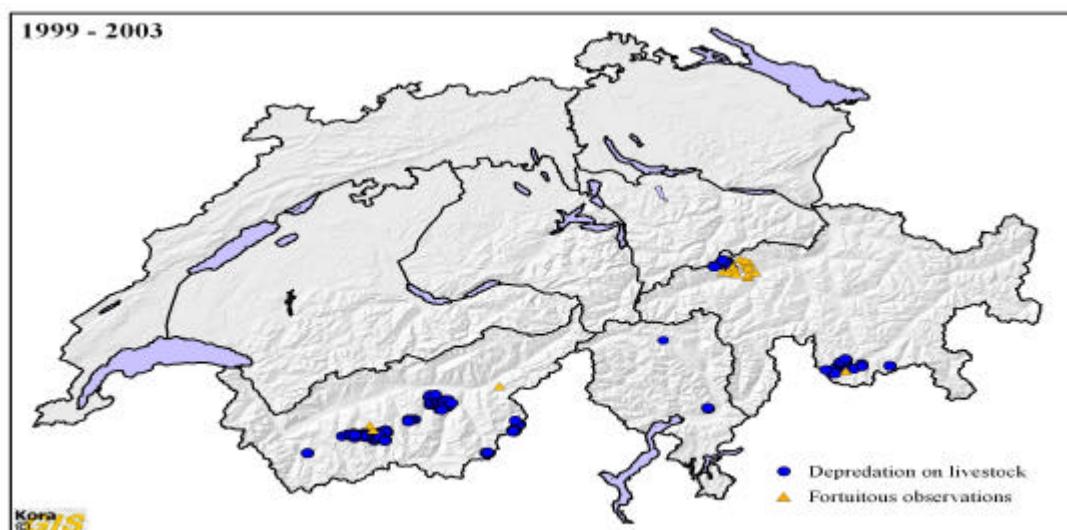


Figure 2: Distribution of Q2 observations, 1999 – 2003.

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With 335 observations, canton Valais is well ahead of Graubünden (102) and Tessin (9). However, most probable fortuitous observations were recorded in the second canton, possibly because of a more intensive monitoring effort from the local game-keepers. Unconfirmed fortuitous observations have not only been recorded in Valais, Graubünden and Tessin, but also in different regions north of the Alps (Figure 3).

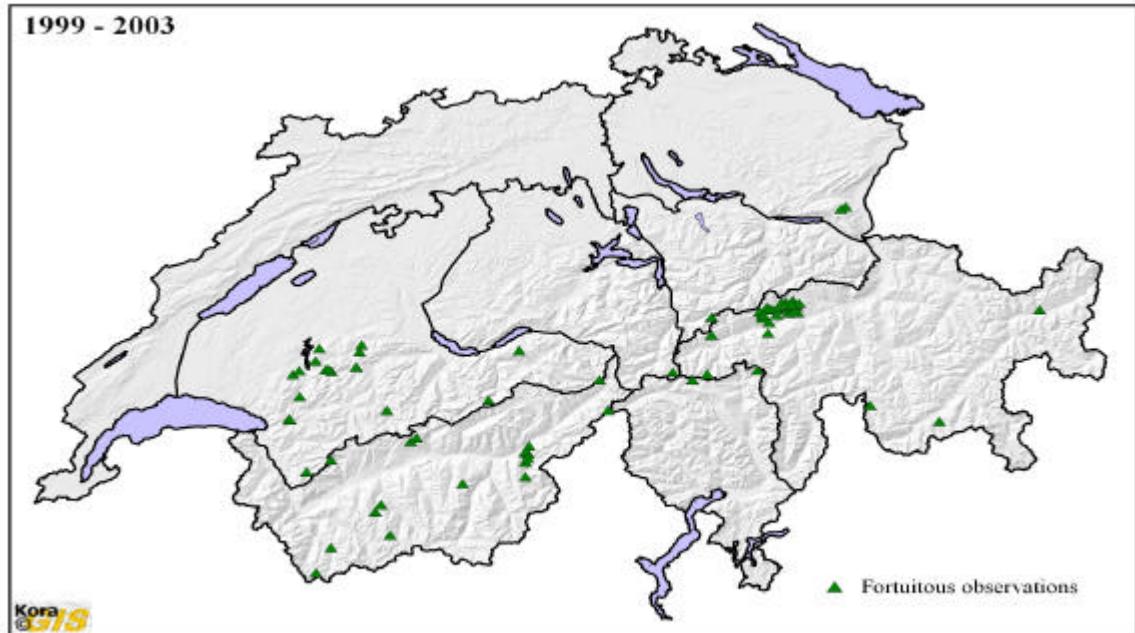


Figure 3: Distribution of Q3 observations, 1999 – 2003.

Although wolf presence could not be confirmed in canton Fribourg (north-western Alps), the various observations regularly collected there in 2000 suggest the temporary presence of at least an individual.

End 2003, wolf presence in Switzerland was limited to cantons Valais, Graubünden and Tessin (Figure 4).

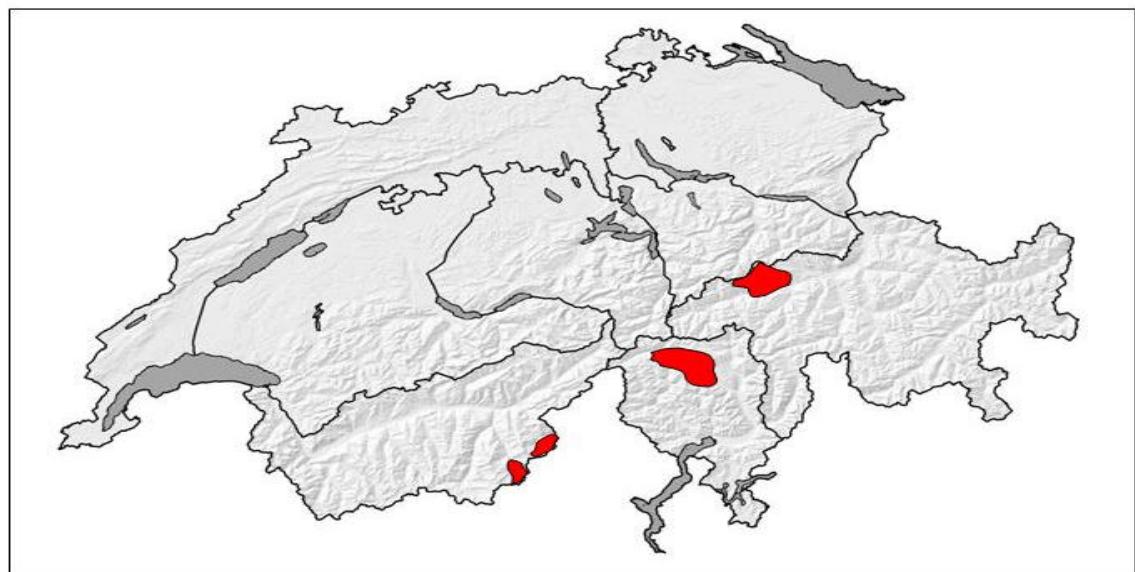


Figure 4: Areas with permanent wolf presence, December 2003.

A lone female commutes repeatedly from Italy to Switzerland in the Simplon region since 2002 and two other individuals could be settled in the Swiss Italian border area, south of the Simplon. A lone male is present in the Surselva region (GR) since 2002, and one male, at least, roams the Leventina valley (TI). Genetic analyses carried out in January 2004 have confirmed the Q2 observations collected in that area in December 2003. No reproduction has been recorded in the country so far.

# **Non Invasive Genetic Monitoring: Applications and Perspectives**

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## **Introduction**

The expansion of the Italian wolf population in the Alps has been monitored since 1999 by the Istituto Nazionale Fauna Selvatica, using non-invasive genetic methods.

The main aims of the research project are:

1. to implement reliable laboratory protocols for genetic analyses of non invasive samples;
2. to study wolf re-colonization process in the Alps;
3. to detect the presence of hybrids between wolf and free-ranging dogs,
4. to infer pack kin relationships and dispersal rates.

This communication is aimed to describe the most relevant aspects of genetic data collection protocols used in our non invasive project.

## **Non invasive sampling**

Proper management of small, vulnerable populations rely on estimates of parameters like population size, dispersal distance and immigration rate. Recently, non-invasive molecular techniques (the use of shed biological samples, such as faeces, urine, hairs) have emerged as a promising tools to estimate population data (Morin *et al.* 1996; Taberlet *et al.* 1999). A non-invasive approach allows collecting samples without disturbing or even handling the target animals. However, several authors have described problems associated with the genetic analysis of dilute DNA samples. A main problem is the risk of genotyping errors due to allelic drop-out and false alleles. Replication of genotyping experiments (the multiple tube approach, Taberlet *et al.* 1996) might allow detecting these errors. Moreover, it is very important to validate the data set (individual genotypes) by appropriate analytical and statistical methods

## **Laboratory analysis**

Each sample was extracted twice using guanidinium thiocyanate and diatomaceous earth protocols (Gerloff *et al.* 1995). Negative controls (no faecal material added to the extractions) were used to check for contaminations.

The strategy for the genetic analysis involves a preliminary screening to test the quality of DNA samples. The screening is done by amplification of the diagnostic part of the mtDNA CR, using primers L-Pro and H350 (Randi *et al.* 2000). PCR products are visualized in electrophoretic agarose gel, stained with ethidium bromide. PCR failures identify samples of low DNA quality, which are discarded. Usually about 25 % of the samples are discarded at this stage.

Individual genotypes are identified using a panel of 6 microsatellites loci (Fh2004, Fh2079, Fh2088, Fh2096, Fh2132, Fh2137 Francisco *et al.* 1996) that are variable enough to obtain a probability of identity (PID) lower than 1%, also if sibling individuals are sampled (Tab.1).

Amplification of long fragments (more of 500 nucleotide) often is not possible, due to DNA degradation in non invasive samples. To limit the rate of unsuccessful PCRs we have designed new primers to amplify shorter fragments.

All PCRs are performed in a room exclusively dedicated to excremental samples.

Our genotyping protocol involves several steps:

1. Four direct PCR amplifications for each sample that have passed the mtDNA test. The samples with less than 50% positive results for the six loci are then discarded.
2. The reliability of genotypes is tested by the software RelioType (Miller *et al.* 2002). RelioType is a program for assessing how reliable an observed multilocus genotype is and for directing further replication if it is not reliable enough.
3. All samples needing additional amplifications are replicated four time more.
4. Results of the new replicates are tested with RelioType again. Only the samples that reach a likelihood of 95% are considered reliable and identified with an individual code.
5. We determine the sex of all the individual genotypes amplifying a fragment of the ZFXY gene (Garcia-Muro *et al.* 1997), using wolf specific primers. PCR products are then digested by restriction enzyme HaeIII. Digestions produce two DNA fragments in males and one in females.

Locus	PID	PID sib
FH2079	0,369	0,593
FH2132	0,332	0,581
FH2096	0,325	0,572
FH2088	0,194	0,474
FH2004	0,177	0,478
FH2137	0,0675	0,37
Total	$9,2 \cdot 10^{-5}$	$1,6 \cdot 10^{-2}$

Tab. 1 - Probability of identity values for each locus used in the individual genotypic identification.

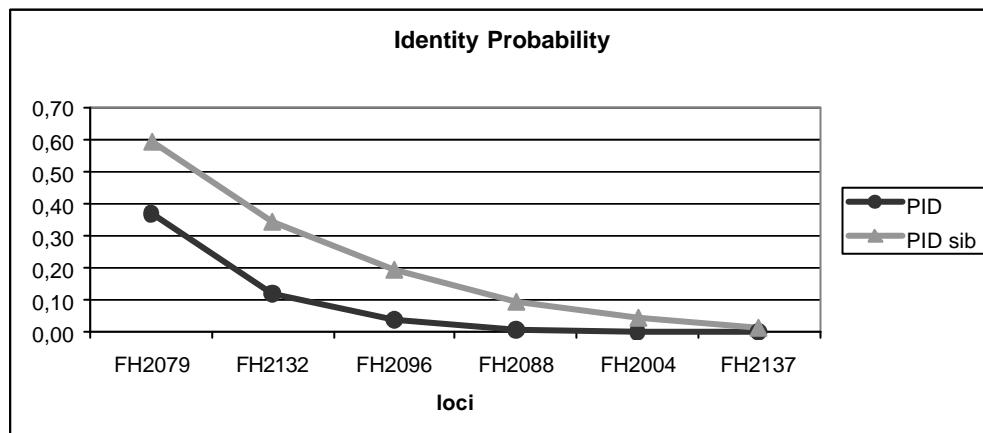


Fig.1- Trend of Identity Probability between random and related individuals. When increasing the number of loci, a reduction of identity probability is observed.

### Results: rate of success

Since the beginning of genetic non invasive monitoring we have analysed 412 samples which were collected from three study areas (Pesio Valley, Stura Valley and Turin province), as it is summarized in Table 2.

Study areas	N° extraction samples	N° samples analysed by 6 loci STR	N° genotyped samples
<i>Pesio Valley</i>	246	188 (76%)	101 (53%)
<i>Stura Valley</i>	263	195 (74%)	106 (54%)
<i>Torino</i>	50	29 (58%)	10 (34%)
<i>Total</i>	559	412 (73%)	217 (52%)

Tab. 2- Samples analysed since 1999 until 2003. Rates of success for study areas.

The average rate of success for all samples analysed is 52%, but if we consider the sampling seasons we can see there is a difference between the summer and winter samples. In fact, winter samples show a higher success rate. This difference is significant and demonstrates that winter samples perform better.

The protocol described above was performed in 2002 and allowed an improvement on genotyping rate from 45-37% to 62-72% (Table 3).

Study area	Lab work session	% Genotyped samples	Sampling season	% Genotyped samples
<i>Pesio Valley</i>	2000	45%	Summer	26%
			Winter	56%
	2002	62%	Summer	43%
			Winter	76%
<i>Stura Valley</i>	2000	37%	Summer	20%
			Winter	49%
	2002	72%	Summer	63%
			Winter	77%

Tab. 3- Rates of success divided for the study areas, lab work session and sampling season.

### International collaborations

In the previous Alpine wolf Workshop (Boudevilliers CH 17-18 March 2003) the necessity to standardize the methodology of wolf monitoring emerged, in particular for genetic labs involved in wolf monitoring in three countries. Thus we have pooled Italian, French and Switzerland genotypic data and have compared all wolf genotypes identified over the Alps.

This allowed us to check the data sets and create a common genetic database available for future research and collaborations. Now we have 104 wolf alpine genotypes for 16 microsatellites loci. By means of this genetic information it will be possible to better understand the alpine re-colonization, to perform studies on population genetics to monitor the dispersal cases, to identify the boundary wolf packs and to monitor future evolution of wolf alpine population structure.

## References

- Francisco et al 1996. A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. *Mamm. Genome* **7**, 359-362
- Garcia-Muro et al 1997. Sex-specific PCR/RFLPs in canine ZFX/ZFY loci. *Animal Genetics* **28**: 156.
- Miller et al. 2002. Assessing allelic dropout and genotype reliability using maximum likelihood. *Genetics* **160**:464-473.
- Morin PA, Woodruff DS 1996. Non invasive genotyping for vertebrate conservation. In: *Molecular Genetic Approach in Conservation* (eds Smith TB, Wayne RK), pp. 298-313. Oxford University Press, New York.
- Taberlet et al. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research* **16**:3189-3194.
- Taberlet P, Waits LP, Luikart G 1999. Non invasive genetic sampling: look before you leap. *Trends in Ecology and Evolution* **14**: 323-327.

## **General Discussion**

A list of topics to discuss was defined by the participants prior to the summit on the wolf monitoring over the Alps (AWW):

Topics :

- 1- Wolf monitoring and management of a transboundary alpine wolf population
- 2- Wolf monitoring in the Alps: should we focus on wolf numbers or wolf packs (reproductive units)?
- 3- Wolf estimation methods: descriptions, applications, limitations, and development of a unique protocol over the countries
- 4- Population size estimation with non-invasive microsatellite genotypes
- 5- Communication with administration and politics
- 6- Standardization of the observations of wolves into three classes of different quality (i.e. Q1 are "hard facts" observations), following the linx protocol by KORA, to allow a common interpretation and a comparison of the data.

The second day session of the 3<sup>rd</sup> Alpine Wolf Workshop was devoted for a general discussion which followed some of these points. Emphasis has been given on the following:

- ? The alpine wolf population should be considered one unique population and monitored and managed as such.
- ? We decided to consider reproductive units as the main units of the wolf population. A pack is defined as a group of wolves which either reproduced the previous summer or consisted of at least one male and one female present in the same area for 2 or more consecutive winters (e.g. potentially reproductive units) as recorded by non-invasive tracking.
- ? For large scale monitoring the standardization of the observations of wolves into three classes of different quality (i.e. Q1 are "hard facts" observations), following the linx protocol by KORA, should be carried out.
- ? Monitoring and research should be carried out at two levels: at a large scale and a local scale. Large scale monitoring is important for determining wolf distribution and numbers, local scale research is important for more specific questions, such as predator-prey relationships, studies on population parameters, etc. These 2 approaches should be complementary. Italy is conducting more detailed analysis, while Swiss and France more broad scale ones.
- ? Capture-mark-recapture (CMR) analysis are a great tool for wolf population size estimation using genetic results on scat samples. Problems in the analysis are related to a strong heterogeneity of the recapture probability. Improvement should be obtained if a common international dataset will be analysed and if genotyping errors minimized and quantified.
- ? The first goal defined during the first and second AWW, consisting in producing a common genetic database, is now obtained, thanks to collaborations between the genetic labs involved. Using this database and the results from all the monitoring work, the new goal after this meeting is to produce a common map showing the distribution of the wolf presence and the wolf packs over the Western Alps. The map showed below is the result of this common work of the WAG after the 3<sup>rd</sup> AWW and it is a great tool to show that the wolf alpine

population is one over the boundaries. This result is significant for wolf conservation and management.

