WOLF POPULATION STATUS IN THE ALPS: PACK DISTRIBUTION AND TREND UP TO 2016 - WITH FOCUS ON YEAR 2015-2016 – WAG REPORT

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1. FOREWORD

Wolves have been naturally recolonizing the south-western Alps since the late 80's (Lucchini et al. 2002; Valiere et al. 2003) through dispersal from the north Apennine wolf subpopulation. A moderate bottleneck occurred during the recolonization process, and gene flow between the Apennines and the Alps was moderate too (corresponding to 1.25-2.50 wolves per generation) (Fabbri et al. 2007). Bottleneck simulations showed that a total of 8-16 effective founders explained the genetic diversity observed in the Western Alps in 2007 (Fabbri et al. 2007). So far the genetic diversity of the alpine population showed a separation of the Italian haplotype from the other subpopulations of eastern Europe and Caucasus area (Pilot et al, 2014). Therefore, the levels of genetic diversity in the current expanding alpine wolf population may depend on future successful migrants from the Apennines, as well as those coming from the Dinaric, Carpathian and central Europe wolf populations (Fabbri et al. 2013).

Following the Guidelines for Population Level Management Plans for Large Carnivores, the wolf population in the Alps has been identified as a unique population segment (Linnell, et al. 2008). Although it is only genetically connected to the Italian wolf population in the Apennines (Fabbri et al. 2007), this population segment of wolves in the Alps is functionally autonomous enough as a demographic entity to produce a numerical increase (Maresco et al. 2011; Marucco et al. 2009). The level of habitat suitability in the Alps (Falcucci et al. 2013) as well as the single narrow corridor allowing wolves to disperse from the Apennines to the Alps (and reciprocally) is a component to define the two different population segment (Kaczensky et al. 2013) as well as the ecological and socio-economic contexts (Linnell et al. 2008) that strongly differ between the two regions.

Because they live at low density over large territories of about several hundred square km, packs may extend beyond administrative borders, and dispersers are able to move over hundred kilometres. The need for standardized monitoring techniques among countries to actually monitor wolves at the population level in the Alps is then obvious.

The last WAG population status update in 2012 recorded 35 wolf packs and 6 pairs over the Alps, the great majority of them located in the Western part between Italy and France. In fact only two packs where not located in this region, but in Switzerland and Slovenia, together with the first scent marking pair in the Central Alps (WAG 2014). The wolf alpine population is expanding to the Central-Eastern part, as well as the Dinaric population is also expanding

northward (Kaczensky et al., 2013). Long distance movements documented via GPS collars (Potocnik et al. 2015) and with investigations conducted on genetic structures of Alpine *vs* Dinaric populations (Fabbri et al. 2013) turned to evidence the first signs of reconnection between the three European populations (Italian, Dinaric, Carpathians) in the Central-Eastern Alps (WAG 2014). Indeed the DNA_{mt} investigations in Austria and East of Italy documented the presence of different haplotypes previously known as region-dependant within the same area, which may consequently improve the genetic diversity of the alpine population segment over time (WAG 2014). For France (Cubaynes et al. 2010) and Italy (Marucco et al. 2009) population size estimations using "capture recapture" models based on non-invasive genetic samples have been conducted. However, this approach is not yet applicable to the entire population due to incongruities in datasets and methodological constraints of genetic procedures over labs.

The information provided in these reports on the wolf population over the Alps is issued from the work of the Wolf Alpine Group (WAG). The WAG (see chapter 2) gathered experts of Italy, France, Switzerland, Austria, Slovenia and Germany in charge of wolf monitoring and management in the Alpine area. Associate research groups (especially genetic labs involved) also contribute to the WAG (see § 2). Despite large inputs of collaboration between labs to define common protocols and a common set of microsatellite markers, direct comparisons of genetic results obtained in different labs are still not always possible (see Annex 1). The used standard technology so far is based on relative comparisons of allele lengths, thus direct comparisons between labs would require dynamic calibrations. To solve this issue a research project was initiated by the WAG providing the genetic reference labs with a joint set of samples containing allele DNA sequences of the whole population (Fumagalli 2012). Although technically available, the process faced the strong limitation of maintaining the bridges any time a new allele is documented in the population. A new approach based on new generation sequencer and DNA massive

sequencing is then on the course to solve those discrepancies (Taberlet et al, in prep) in the way to enable a large scale molecular tracking straightforward (see Annex 1).

With this last WAG report we would like to present the last population status update evaluated for year 2015-2016. The dataset used in this report comes from Centro Gestione e Conservazione Grandi Carnivori - Regione Piemonte (who is coordinating the Italian program), Regione Valle Aosta, Parco Nazionale Gran Paradiso, Regione Lombardia, Provincia Autonoma di Trento e Bolzano, Regione Veneto e Friuli Venezia Giulia for Italy; from Wolf/Lynx network driven by the Office National de la Chasse et de la Faune Sauvage (ONCFS) for France; from Carnivore Ecology and Wildlife Management (KORA) for Switzerland; from Bavarian Environment Agency (LFU) for Germany; from the Veterinary Medicine Vienna for Austria and from University of Ljubljana for Slovenia.

The present report originated from the agreements had at the 8th « Wolf Alpine Group » workshop conducted in Bormio (IT). The aims of the workshop were (1) to exchange scientific knowledge on wolf distribution and demography over the Alps; (2) to evaluate and implement minimum standards to assess a robust output of the wolf population status according to the available data sources; (3) to continuously improve methodological approaches designed to monitor distribution and demography of the alpine wolf population and update the evaluation of the wolf population status in the Alps for year 2015-2016.

2. The WOLF ALPINE GROUP (WAG)

In 2001, a first wolf monitoring Workshop was organized in France gathering experts from France, Italy and Switzerland concerned with the recently recolonization of the wolf population over the alpine areas. The main objective of the workshop was to set up an effective collaboration among the three countries to exchange scientific data to effectively monitor the wolf population in the Alps as a whole. This was the start for the Wolf

Alpine Group from which significant progress have been recorded and a strong collaboration among experts has been settled up, particularly regarding information exchanges and common/practical methodologies (WAG, 2003). Monitoring standards (e.g. definitions of wolf packs, minimum requirements to document pack presence), common genetic approaches and practical exchanges of basic data have been defined; taking into account differences between countries in data collection, institutional organisation, levels of monitoring, and specific objectives for population monitoring. The WAG has to be considered a scientific independent group, which can address specific scientific requests from different platforms (see WAG, 2008 for an example) dealing with large carnivore management (e.g. WISO Platform of the Bern Convention, European Commission). Twelve years after the first discussions, the Wolf Alpine Group met for the 7th time in Jausiers (France) on the 19-20th of March, 2013, with the main goal of making an update of the wolf population status in the Alps within the different countries (WAG 2014). Moreover, some methodological approaches (especially exchanges of genotyping results) have been discussed to solve the discrepancies between technological changes and result updates. After defining the population segment of interest and according to previous results and future goals, Austria and Slovenia joined the group covering now the entire alpine range in 2013. A WAG logo has been defined in April 2014. In 2015 the 8th "Wolf Alpine Group" Workshop has been organized in the Stelvio National Park, Bormio, Italy on 27th-28th October, in the framework of the Life Wolfalps Project, which allowed the hosting of the event. In the first day we discussed and agreed on transboundary monitoring standards for the wolf alpine population (see chapter 3), fundamental to produce products like this population status report and a map of population occurrence. We then decided to update the distribution of wolf packs and pairs over the Alps given the agreements, which resulted in the present report. On the second day a discussion was conducted among the genetic labs involved in the genetic analysis on

biological samples from wolves in the Alps, with the ultimate goal of continuing to have a joint genetic approach to monitor the wolf population over the Alps in the future, also if techniques are further evolving. This is important to keep comparing genetic data within the WAG, trying to move forward with new genotyping approaches. A report on this part of the meeting has been produced (Schwartz et al. 2015), and it is attached as an Annex 1 to this report.

WAG members at the 8th « Wolf Alpine Group » Workshop, 2015 October 27th& 28th - Bormio – ITALY

3. METHODS

3.1. Monitoring standards and techniques for the wolf population in the Alps

Similar monitoring techniques are applied over the Alpine countries, which basically consist in sign surveys, snow-tracking sessions in winter and wolf howling sessions in summer, all associated with standardized non-invasive molecular tracking and photo-trapping. According to the levels of wolf occurrences, the combination of these monitoring tools vary between countries.

In France and in the Italian Alps, standard sampling design protocols are implemented using a network of experts to perform systematic and opportunistic signs collections, snow tracking in winter, and wolf howling in summer where packs/pairs are present, all associated with standardized non-invasive genetic molecular tracking of about 600 up to 1000 samples yearly and opportunistic phototrapping (for details in strategies and

protocols see Duchamp et al. 2012 for France and Marucco et al. 2012; Marucco et al. 2017 for Italian Alps). A few pilot studies using GPS marked wolves are also used for specific purpose on wolf space use (Italy, Slovenia) or predator-prey studies (France). The LIFE project WolfAlps (2013-2018) developed a standardized and unified wolf monitoring strategy with standard criteria and a network for the Italian Alps from west to east, together with Slovenia (Marucco et al. 2014), now applied over the all Italian Alps and coherent with the WAG criteria.

Because of the lower wolf occurrence in Switzerland, and no territorial wolves in the German and Austrian part of the Alps data collection relies on a passive monitoring (collecting second hand information) which includes dead wolves, damages to livestock, and chance observations (tracks, killed wild preys, pictures). In Switzerland the active monitoring is mainly based on the opportunistic collection of genetic samples and opportunistic camera trapping in areas where (potential) pairs or packs have been documented (detection of reproduction and minimum pack size). On very rare occasions snow tracking is conducted in areas of packs. In Switzerland, 349 genetic samples were collected for the monitoring period considered in this report. The majority of the samples consisted of saliva taken opportunistically from prey carcasses (54.7%), followed by faeces (37%) and urine (5.2%) (see Zimmermann et al. 2010, Manz et al. 2014, Zimmermann et al. 2015, Fumagalli 2018 and http://kora.ch/index.php?id=158&L=1 for details in strategies and protocols for Switzerland; Kaczenski et al. 2009, Reinhardt et al. 2015 for details in strategies and protocols in Germany and Austria). In Italy and France howling surveys are conducted to document reproduction in areas where a pack or a pair has been documented. In Slovenia, an intensive and systematic monitoring is applied since 2010 in the SE part of the alpine area (~700 km^2), including snow tracking, wolf howling, photo trapping and molecular tracking, In the rest of the Slovenian alpine area, only opportunistic sampling for DNA analyses and recording of signs of presence are performed (see Potočnik et al. 2014 for details in strategies and protocols

in Slovenia). A LIFE Project Slowolf (2010-2013) allowed additional captures and GPS monitoring for few wolves, of which three were roaming in or dispersed into the alpine area.

During the last WAG workshop in Bormio, the WAG agreed on additional monitoring standards definitions, and criteria to better harmonize the data collection and interpretation, fundamental to produce our common evaluation of the wolf population size and distribution over the Alps, which are further detailed but not yet finalized (chapter 3.2 and 3.3). However more discussion is needed to further harmonize data interpretation criteria, and this will be continued in the WAG workshops of the future years.

3.2. Wolf signs, and their categorization

The sign classification were first developed in the framework of SCALP (Status and Conservation of the Alpine Lynx Population), a conservation initiative that among other things developed standardised criteria for presentation and interpretation of lynx-monitoring data (Molinari-Jobin et al. 2012). These criteria have been adapted to wolves with adjustments and adopted by other countries in Europe(e.g. Kaczenski et al. 2009, Marucco et al. 2014, Reinhardt et al. 2015). In the following, we define the agreed SCALP criteria required for standardised monitoring of wolves in the Alps.

A few preconditions apply:

- For the evaluation of field data at least one experienced person must be available.
- "Experienced" in this regard means having extensive field experience with wolf.
- All observation must be checked for genuineness (i.e. the possibility of intentional deception must be ruled out).

The letter "C" stands for "category". The numbers 1, 2 and 3 below have nothing to do with the observer's qualifications but are used to denote the level of validation for an observation as follow:

C1: Hard evidence = Hard fact, i.e. evidence, that unambiguously confirms the presence of a target species.

C2: Confirmed observation = Indirect signs confirmed by an experienced person as being caused by the target species. The experienced person can either confirm the signs himself in the field, or based on documentation by a third party.

C3: Unconfirmed observation = All observations that are not confirmed by an experienced person or observations which by their nature cannot be confirmed (e.g. sightings without morphological details).

False observations are not considered and ruled out.

Tab 1. Wolf signs, and their C categorization (this categorization is the generalized one, and it might have country's adaptations described in Kaczenski et al. 2009 for Germany and Austria, Marucco et al. 2014 for Italy, Zimmermann et al. 2010 for Switzerland, Duchamp et al 2012 for France)

C1	C2	C3
Captured or rescued alive animals	**Tracks** with typical trend/pattern, assessed by an expert, followed for at least 100 m	Tracks followed for less then 100 m in snow or single footprint
Dead animals	**Scats,** checked by an expert , or if collected along a C2 snow track	not associated with snow tracks and/or without DNA evidence
Whatever DNA evidence confirms the biological sample (i.e. scats, hairs, urine, saliva)	**Predation signs** with bites and/or consumption description with expert check or if combined with other C2 data	Heavily eaten kills, livestock depredations not technically described or not combined with other C2 data
Telemetry	**Wolf howling**, Howl with wolf pups presence checked by expert	Howls Single and sightings not supported by photos or videos
High quality video and photos	Medium quality video and photos with expert assessment	Inappropriate documentation provided by third party. Bad quality videos and pictures preventing animal description

3.3 Agreed definitions and Criteria for data interpretation:

Tab 2. Definitions used in wolf monitoring over the Alps and Agreed Criteria for data interpretation

	DEFINITION	DATA NEEDED
Year	Biological year for wolves: from reproduction to next reproduction	From the 1st of May to the 30th of April
Single resident wolf	Single wolf holding a territory for more than one biological year	At least C1 (identification of the same individual) collected at least in two consecutive biological years apart
Pair	Only 1M +1F holding a territory and travelling together but not (yet) having reproduced.	C1 that confirms the pair (video/foto), or at least 2 independent C2 showing the pair travelling together (tracks) confirmed by at least one C1 (genetics)
Pack	Reproductive unit identified by either pup occurrences or by at least ≥ 3 individuals travelling together and holding a territory within (at least) two consecutive biological years (i.e. a potentially reproductive units) (i.e. potential reproductive units).	Either reproduction confirmed with C1 or C2; or at least 2 independent C2 showing the pack travelling together (tracks), or ≥ 3 individuals confirmed by at least one C1 (genetics / photo / video)
Wolf Occurrence (Cell)	10x10 km cell (EU grid) where the species has been detected on the yearly basis	At least 1 C1 or 2 C2
Representation of the Territory	Area hold by the resident wolf/wolves to point it's approximate localization over space	Circle of about 250 km2 (9 km radius) centered on the centroid of the MCP constructed on the collected C1-C2 wolf signs

3.4 Mapping wolf occurrence

European wolf occurrence is updated every 5 years by LCIE. All validated wolf signs of presence are projected on a EU 10*10 grid cell dispatching regular vs occasional wolf presence

within a 5 year temporal windows (see Kaczensky et al. 2013 for details). This validated dataset on wolf occurrence is reported in the previous WAG map (WAG 2014), and now it is used in this present WAG map, where the LCIE codes now used mean:

- **1 = Permanent with reproduction** (presence confirmed in >= 3 years within the last 5 years OR in >50% of the time AND reproduction confirmed within the last 3 years)
- **2 = Permanent without reproduction** (presence confirmed in >= 3 years in the last 5 years OR in >50% of the time AND no reproduction confirmed in the last 3 years)
- **3 = Sporadic** (highly fluctuating presence) (presence confirmed in <3 years over the last 5 years OR on <50% of the time)

Also in this representation, there have been country adaptations, in order to present data on permanent presence with the best available data (details in Kaczenski et al. in prep).

3.4 Wolf packs, distribution and population trends

We considered changes in the number of wolf packs as the biologically meaningful measure of population trend and distribution, such as in other wolf population monitoring systems worldwide (Mech and Boitani, 2003). The biological year is defined from May 1th to April 30th the year after (Tab 2), corresponding to the wolf reproduction period.

The wolf distribution is represented by three categories (Tab 2) as pack, pair, a solitary wolf, all based on temporal recurrences that depict evidences to exist longer than 1 year for solitary ones. A pack is defined as the main wolf reproductive unit documented either by pup occurrences or identified by at least ≥ 3 individuals travelling together holding a territory within (at least) two consecutive biological years (i.e. a potentially reproductive units). A pair is defined as, at least, one male and one female as recorded by non-invasive molecular tracking or C1 pictures / videos marking their territory. A solitary wolf is considered to have settled its territory if detected at least in two consecutive

biological years. Therefore, dispersers are not reported either in the territories of the map or in the population trend evaluation, but are included if detected in the wolf occurrence layer as sporadic occurrence. Packs are defined as "transboundary" (Tr) once hard evidences are documented with genetic matches, or as "likely transboundary" (LTr) without hard facts but based on the interpretation of the sign presence spatial distribution.

This 2015-2016 update then analysed the wolf population structure with the data recorded from 2015 May 1st up to 2016 April 30th.

4. LAST 2015-2016 RESULTS AND DISCUSSION

The previous population status update in 2012 recorded 35 wolf packs and 6 pairs over the Alps, the great majority of them located in the Western part between Italy and France. In fact, only two packs where not located in this region, but in Switzerland and Slovenia. In addition the first pair was confirmed in the Central Alps (WAG, 2014).

In 2015-2016, the wolf alpine population has expanded to the Central-Eastern part. In the same time the Dinaric population has also expanded northward, while the population has increased in density in the Western part of the Alps of Italy and France. In those areas wolves have reached hills and expanded beyond the alpine chain. However, the focus of this report is still the alpine wolf population, which considers the Alps defined after the Alpine Convention.

A positive trend of the number of wolf packs is documented over the years showing an additional increment in 2015-2016. The number of packs and pairs is still increasing over the countries, with the main population increase occurring in the Western Alps of Italy and France, where wolf pack density is further increasing (Fig. 1). This last update in 2015-2016 recorded **65 wolf packs and 12 pairs** over the Alps, with the great majority of them located in the Western part between Italy and France (Fig. 1 and 2). In particular, in Italy we documented 27 packs, 8 pairs and 5 single wolves; in France 31 packs, 3 pairs and 3 single wolves; in

Switzerland 1 pack, 1 pair and 3 single wolves; in Slovenia 2 packs and in Austria the first single resident wolf. Moreover, we could document one transboundary pack between Switzerland and Italy with C1 data (i.e. compared genotypes) and 3 likely transboundary packs between Italy and France documented with spatial distribution of presence signs but not genetic proof (Fig. 1 and 2). The map evidences the international dimension of the wolf alpine population.
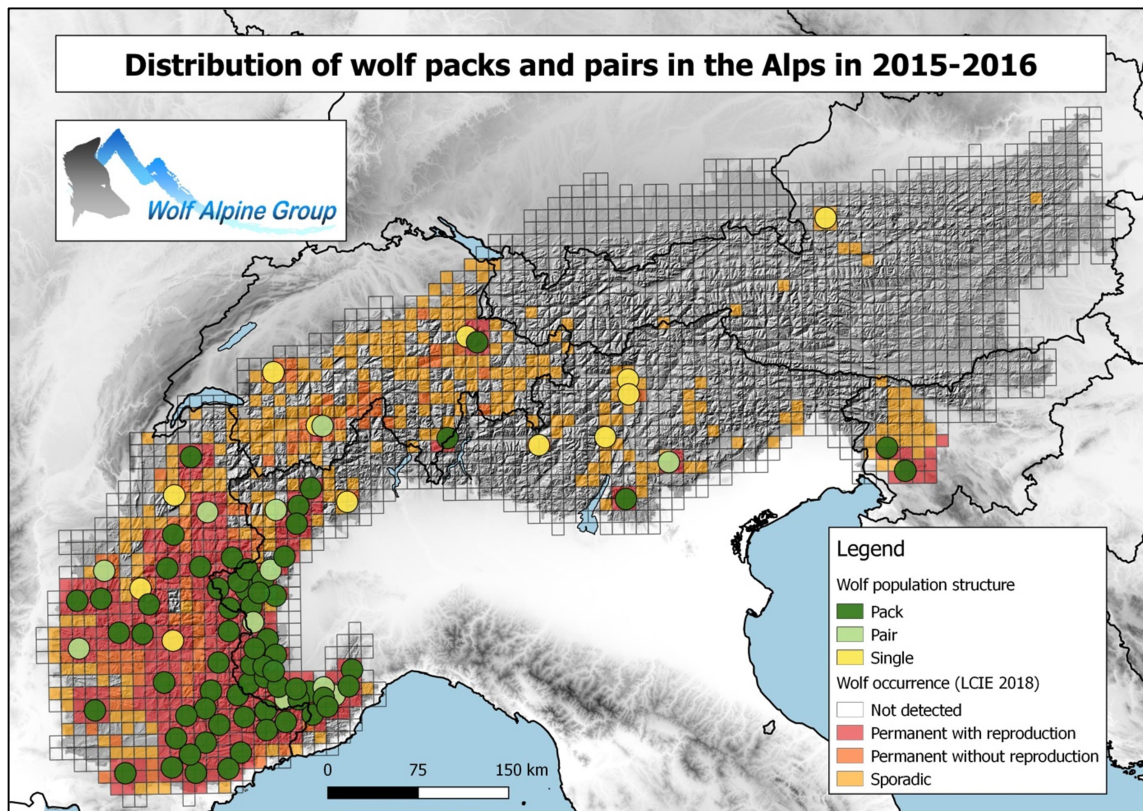

Figure 1: Distribution of packs, pairs and single resident wolves in 2015-2016 (1st May 2015 - 30th April 2016) over the Alpine range. Packs are documented either by previous summer reproduction records or by records of at least 3 individuals travelling together in the same area; Pairs are defined as one male and one female holding a territory for 2 or more consecutive winters (e.g. potentially reproductive units) as recorded by non-invasive tracking.

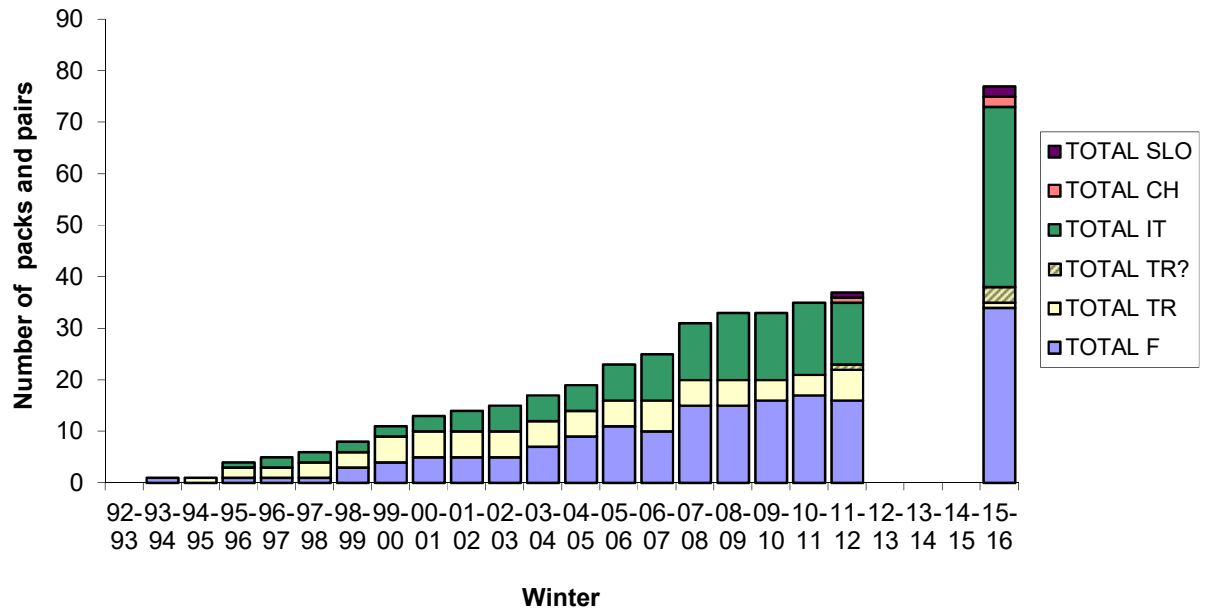**NB/ packs and pairs = at least 1M + 1 F for two consecutive winter or breeding evidence next summer**

Figure 2 : Temporal trend of the number of wolf packs and pairs across the Alpine range. F : France; IT : Italie, CH: Switzerland; SLO: Slovenia, Tr: transboundary documented with genetic proof, Tr?: likely transboundary regarding spatial distribution of presence signs but not genetic proof. For years 2012-2013 and 2013-2014 data is missing for the Italian Alps therefore a complete figure cannot be given.

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ANNEX 1.

Wednesday 28th October 2015 - Visitor Centre of Stelvio N.P. – Valfurva, Bormio, Italy

WOLF MONITORING OVER THE ALPS

– TOWARDS A UNIQUE GENETIC APPROACH –

8th Wolf Alpine Group Workshop in Bormio, Italy

Summary of the Genetic Workshop

Chairman : M. Schwartz

(National Genomics Center for Wildlife and Fish Conservation, US, responsible for Italian Alpine genetics analysis)

The Wolf Alpine Group brought together geneticists from multiple countries to discuss the latest findings related to alpine wolf populations and to examine the newest developments in genetics and genomics that can be applied to developing a better understanding of wolves. The dominant uses of molecular genetics in wolf management currently are to identify unique genotypes (e.g., unique individuals) from non-invasive samples (e.g., scat and hair), to describe introgression of dogs and wolves, and to quantify migration from one region or population into another. Each research group has been attacking these issues independently, with cooperation facilitated by the Wolf Alpine Group. This meeting provided the ideal opportunity to discuss how new genomic tools can address each of the issues.

> **Objective – to monitor alpine wolf populations found within different counties using comparable molecular genetic tools.**

Given these objectives we assessed:

1) the importance of collaboration,

2) how new genomic technologies will change the molecular approaches used, and

3) how we can standardize our approaches to use these new technologies in the long term for i. detecting unique individuals (genotypes) and ii. monitoring introgression and identifying hybrids.

Presentations

The first presentation was from Dr. Luca Fumagalli, University of Lausanne, Switzerland, on the standardization of the genetic analyses among the different laboratories involved in the Wolf Alpine Project. Dr. Fumagalli reviewed the progress that has been made on standardization of a set of microsatellites (STRs) since the Wolf Alpine Project organized in Lausanne University in June 2008. At this meeting Italian, French, American, Spanish, and Swiss geneticists met to discuss a standardization of genetic tools and to encourage collaboration among teams. From this meeting a set of 8 common microsatellite markers has been established and run on a set of 15 tissues to capture the range of observed microsatellite variation and to standardize scoring. The loci in this panel are as follows: FH2054, FH2140, FH2161, FH2096, FH2137, PEZ17, FH2088, and CPH5.

Dr. Marta De Barba, from the Universite Joseph Fourier, Laboratoire d'Ecologie Alpine, next presented data on a new high throughput sequencer (HTS) approach to genotyping individuals from non-invasive genetic samples. She first noted the technical limitations of standard microsatellite analysis with PCR / gel electrophoresis. The standard approach is easy to implement in most genetic laboratories, but poses limitations on the number of samples and markers than can be processed simultaneously and in automation, presents technical challenges to genotyping that can lead to errors, especially with low quality samples, and produces genotype data that cannot be directly compared among different platforms. Dr. De Barba described the new HTS-genotyping approach, which LECA lab (Grenoble univ. FR) is implementing in brown bears. This approach is electrophoresis-free and highly automated, allows access to the DNA sequences directly (as opposed to the somewhat arbitrary scores assigned from traditional microsatellite approaches), and can be conducted in parallel with the evaluation of other regions of the genome. The HTS-genotyping thus can allow the processing of a large number of samples and markers, can be

easily standardized, can reduce time and costs of analysis, is platform and laboratory independent, and provides non-ambiguous allele calling.   Work with brown bear non-invasive samples suggests approximately an 80% genotyping success rate, with an allelic dropout rate between 11%-14%. A wolf HTS-genotyping panel currently being developed at the Laboratoire d'Ecologie Alpine consists of 13 tetranucleotides (with 5-10 alleles per locus), a ZFX/ZFY sexing gene, and a 153 bp mtDNA region. While this approach holds much promise there are still some technical challenges and limitations. Primarily it is recognized that the HTS-genotyping method works very well for large-scale projects, but at the moment is not efficient or cost-effective for small scale (e.g., forensic) cases as a minimum unit for the analysis is a 96-well PCR plate. There are also limitations associated with bioinformatics, which is needed to analyze the data, concerns about tag jumping, and the concern that this approach requires each lab to essentially start over and re-genotype historical samples. Currently Taberlet's lab is relying on a commercial laboratory for the library preparation and sequencing (protocol MetaFast, Fasteris Switzerland, http://www.fasteris.com), but libraries can also be prepared using Illumina kits that do not include PCR cycles.   There was lively discussion and excitement about this new approach. Dr. Pierre Taberlet and Dr. De Barba suggested that they would be willing to have other members of the Wolf Alpine Group visit the Laboratoire d'Ecologie Alpine to share this new approach.

Dr. Carsten Nowak from the Senckenberg Research Institute and the Natural History Museum Frankfurt, subsequently described a single nucleotide polymorphism (SNP) based approach (see also Kraus et al. 2015 *Molecular Ecology*). This approach is able to use Fluidigm's nanofluidic SNP typing platform to assay 96 SNPs simultaneously. His research group initially worked with 192 SNPs selected from the Affymetrix v2 Canine SNP Array. They optimized a set of 96 SNPs based on repeatability and ease of scoring. Based on matched samples of non-invasive and tissue samples they determined a genotyping error rate of ~1%. This is one of the first uses of Fluidigm's integrated fluidic circuit for non-invasive samples.   Currently Nowak's lab optimizes the technology further and tests the application in comparison to traditional microsatellites.

Dr. Ettore Randi, ISPRA (Bologna, Italy), presented information on genetic approaches to detect hybrids and his experience in the Apennine wolf population. He summarized several of his recent publications and described the tools and databases used in his laboratory. Specifically, he described his use of 39 microsatellites applied to a large database of Italian wolves and domestic dogs (606 samples of Italian wolves, 273 samples of dogs from >20 breeds, 102 wild wolf x domestic dog hybrids, 81 saarloos and Czechoslovakian wolf dogs, and 64 American wolf dogs). Using this panel he was able to identify hybrids up to the 2nd and 3rd backcross. Dr. Randi also described a second Italian wolf haplotype (W16) that his team discovered in addition to the well-known W14 haplotype. This haplotype has never been described in domestic dogs. Dr. Randi noted that of the 11 packs analyzed hybrids occurred at a rate of 4-8%; domestic dog mtDNA was absent in these packs, but there were some dog Y haplotypes present. Interestingly, many of the hybrid packs had the Kb (3bp)

deletion (β-defensin, K-locus) which creates melanistic animals. Dr. Randi also described the chromosomal pattern of introgression in Italian wolves based on the 170,000 Illumina SNP panel, an extensive look of introgression which is in progress.

Discussion

The session was concluded with a discussion by Dr. Michael Schwartz (National Genomics Center for Wildlife and Fish Conservation) working with the Centro Conservazione e Gestione Grandi Carnivori at the Parco Naturale Alpi Marittime. The discussion first reviewed the new technologies available to genotype non-invasive samples and tissue samples, noting that some approaches (e.g., commercial SNP chips such as the the Affymetrix v2 Canine SNP Array) may not perform well on non-invasive samples, while being very powerful with tissue samples. Other approaches such as the HTS-genotyping are promising and if funding is available to re-genotype the extensive, existing samples from the past decades. The Fluidigm 96-SNP platform is also an effective new technology, but requires an expensive startup to purchase the SNP assays, and would also require re-running historical samples. However, this approach is proven with non-invasive samples.

Next the discussion turned to the pros and cons of all groups across Europe working together and what would be necessary to facilitate collaboration. The largest advantage of working together is that large scale analyses regarding gene flow and landscape genetics would be easily conducted. Furthermore, forensic cases and cases of dispersal could be detected if there was one central database, or multiple databases created with the same marker system. The disadvantage was that groups such as the French and Slovenian group (led by Dr. Tomaž Skrbinšek) were already invested in the HTP-genotyping approach, the German were invested in the Fluidigm approach, and the Italian groups had large numbers of historical samples already analyzed making the existing microsatellite approaches appealing (i.e., most new approaches require re-running historical samples).

Final agreements among genetic labs in the framework of the WAG

Through the discussion we agreed to:

- keep communicating about the new technologies
- exchanging samples of previously genotyped individuals
- continue to exchange microsats genotypes, based on the already standardized genotyping approach presented by L. Fumagalli (for the labs who did this)
- test the new HTS-genotyping method. Dr. Taberlet was amenable to scientists from the various research groups visiting his laboratory once the HTS-genotyping method was completed to be trained in the technique
- Dr. Nowak offered to test samples for anyone interested in the Fluidigm SNP technology

Participants

Luca Fumagalli (University of Lausanne, Switzerland)

Carsten Nowak (Senckenberg Research Institute and Natural History Museum Frankfurt, Germany)

Michael Schwartz (National Genomics Center for Wildlife and Fish Conservation, USA; working with the Centro Conservazione e Gestione Grandi Carnivori - Parco Naturale Alpi Marittime, Italy)

Tomaž Skrbinšek (University of Lubjiana, Slovenia)

Ettore Randi (Istituto Superiore per la Protezione e la Ricerca Ambientale - ISPRA, Bologna, Italy)

Pierre Taberlet, Marta De Barba, Christian Miquel (Universite Joseph Fourier, Laboratoire d'Ecologie Alpine, France)