

## **Allegato 2b\_8**

### **Preliminary data on paternity in Alpine chamois**

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#### **Technical report**

By taking advantage of a sample of marked males, over the season 2011, 2012 and 2013, we collected faecal samples of Alpine chamois to investigate the feasibility of parentage analysis in the target species.

In 2011 we collected 33 faecal samples (28 samples of kids and five samples of females). The remaining DNA samples comprised 12 tissue samples of males. In 2012 we collected 47 faecal samples (32 samples of kids and 15 samples of females). The remaining DNA samples comprised 28 tissue samples of males and other females. In 2013 we collected 44 faecal samples (33 samples of kids and 10 samples of females).

Faecal samples were placed straight into silica gel, while tissue samples were stored in 96% ethanol. DNA from faecal and tissue samples was extracted using commercial kits (Qiagen) and the samples were genotyped using 17 microsatellite loci in three multiplex sets according to Zemanová et al. (2011). The faecal and tissue samples were analysed at least three and two times, respectively, for the reason of getting a reliable genotype.

Parentage was determined using the likelihood-based approach in CERVUS 3.0 (Kalinowski et al. 2007; available at <http://www.fieldgenetics.com>). The program calculates the likelihood that a particular male is the true father given the observed genotypes and their relative frequency in the population. The parentage analysis was conducted using 17 microsatellite loci. These markers exhibited little or no evidence of null alleles and showed sufficient levels of polymorphism (mean heterozygosity 0.65) making them suitable for paternity assessment in the studied population (combined exclusion probability: first parent 0.97, second parent 0.99). If alleles did not match at 1 or more loci, the putative father was excluded as a sire (in the attachment 1). Results were confirmed by the program ML-Relate (Kalinowski et al. 2006; available at <http://www.montana.edu/kalinowski/Software/MLRelate.htm>), a computer program for maximum likelihood estimation of relatedness and relationship. ML-Relate is useful for discriminating among four common pedigree relationships: unrelated (U), half-siblings (HS), full-siblings (FS), and parent-offspring (PO).

By the comparison of the genotypes we found that:

- in 2010 male M11 sired the kid LC3K, male M16 sired the kid LC7K and male M17 sired the kid LC18K. We did not find any other relationship father-offspring in the data;
- in 2011 male M11 sired the three kids k12\_13, k23\_13 and k26\_13, male M33\_13 sired the kid k20-13. Our laboratory confirmed the results of the previous year, the male M11 sired the kid LC3K, male M16 sired the kid LC7K and male M17 sired the kid LC18K. We also confirmed the relationship father-offspring in male M4 and kid LC13K. However, sample M4 was not working well and so was genotyped on only 12 loci. Use the extended data set, we found that male M7\_13 sired the kid LC21K and male M9\_13 sired LC24K. We did not find any other significant relationship father-offspring in the data
- in 2012 male M13\_13 sired the three kids k12\_14, k30\_14 and k3\_14, male M22\_13 sired the kid k25\_14. We did not find any other significant relationship father-offspring in the data.

These data, while being largely preliminary, confirm the opportunity offered by DNA analysis from indirect sampling of faeces to explore the pattern of paternity in an elusive mountain ungulate species. Further analyses are needed to investigate the distribution of paternity among individuals of different age and social status.

## References

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- Zemanová B, Hájková P, Bryja J, Zima J jr, Hájková A, Zima J (2011). Development of multiplex microsatellite sets for noninvasive population genetic study of the endangered Tatra chamois. *Folia Zoologica* 60:70-80.