26th INTERNATIONAL CONFERENCE ON BEAR RESEARCH & MANAGEMENT
15th - 21st September 2018, Ljubljana, Slovenia

How to produce comparable data in conservation genetics for the Apennine brown bear

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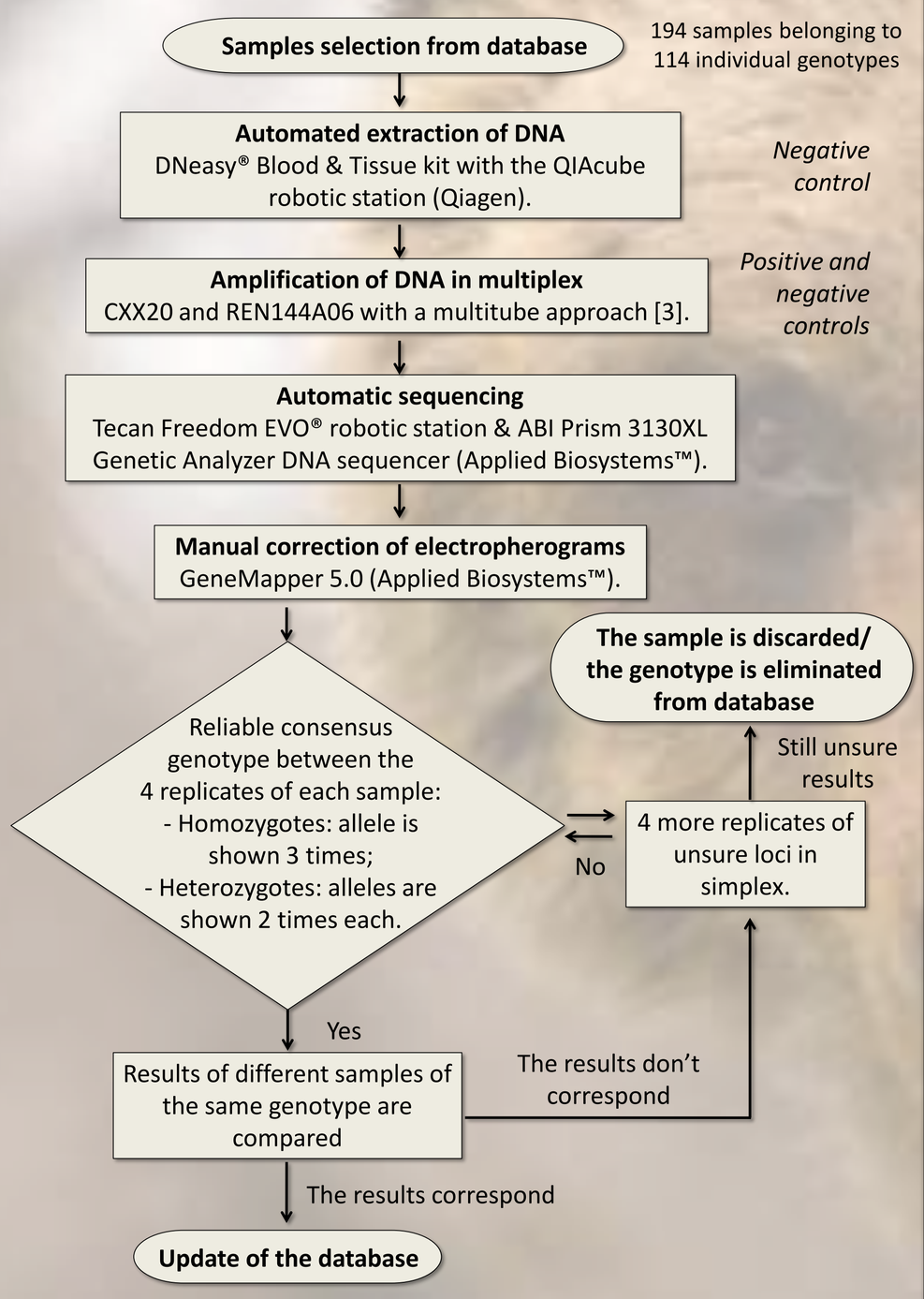
INTRODUCTION

The Apennine brown bear (*U. arctos marsicanus*) presents a low level of variability [1], therefore ISPRA conducted the individual identification on the basis of 11 Ursidae specific markers (STR) plus sex. In the last decade, two different labs (WGI, Wildlife Genetics International, B.C., Canada and ISPRA) conducted the genotyping of the Apennine brown bear. WGI added two markers designed on the domestic dog genome (CXX20 and REN144A06) and removed two ones that had been previously used. Thus, their total selection was of 11 markers, 9 of which in common with ISPRA (G1D, G10B, G10C, G10L, Mu05, Mu11, Mu50, Mu51, Mu59), with an additional marker in common to both labs for equivocal cases (G10P) [2]. For a population with a low variability it is important to select the optimal STR marker set for individual identification, in order to allow the correct identification of the individuals overtime and to reduce genotyping errors.

MATERIALS AND METHODS



The following software were used for data analysis:

- GenAlEx 6.4 for allelic patterns, H_o, H_e, HWE, P_{ID}, P_{IDsib}, number of MM.
- GIMLET 1.3.3 and MicroChecker 2.2.3 to estimate genotyping errors frequencies (ADO, FA, PCR+ and null alleles).
- R (*chisq.test* and *fisher.test*) for statistical significance among groups.

RESULTS

The comparison of the different marker sets (Tab. 1, Fig. 1-5) shows an improvement, albeit not significant, of the discrimination capacity using the complete set of 13 STRs + AMG compared to the other STRs marker sets. However canid loci show a higher occurrence of genotyping errors.

Allelic patterns (Fig. 7) show slight variation over time and PCA (Fig. 6) shows a substantial overlap of genetic diversity in the two considered periods.

Locus	Range (bp)	Conversion	P_{ID}	P_{IDsib}
CXX20	132-136	-3	0.22	0.50
REN144A06	110-130	+1	0.24	0.51
G1D	100-114	-72	0.25	0.53
Mu51	114-122	-92	0.26	0.53
G10B	112-128	-28	0.36	0.58
G10C	95-105	-102	0.37	0.59
Mu59	101-107	-128	0.38	0.60
Mu11	88-96	-100	0.39	0.62
Mu05	135-137	/	0.40	0.62
G10L	148-154	-9	0.41	0.63
Mu50	100-104	-32	0.41	0.63
G10P	152-164	+7	0.65	0.81
Mu15	117-121	Not used by WGI	0.71	0.84
Amelogenin	158-212	-46/-38	-	-

Table 1 - Range and conversion factors of the STRs.

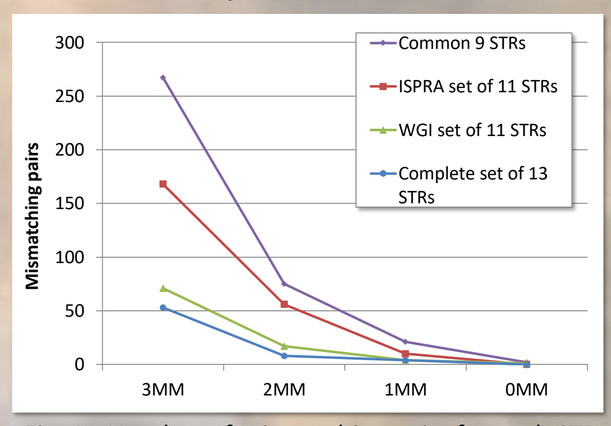


Fig. 1 - Number of mismatching pairs for each STR marker set.

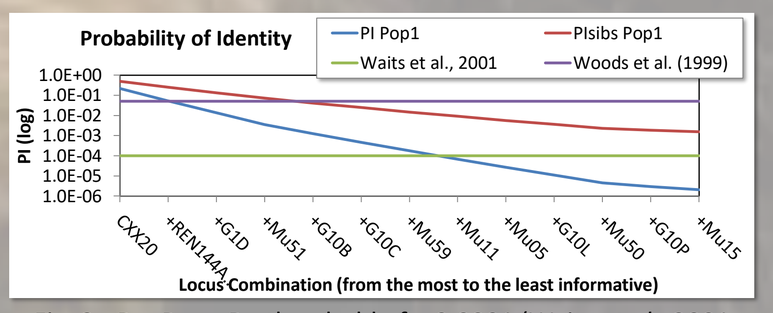


Fig. 2 - P_{ID}, P_{IDsib}, P_{ID} threshold of < 0.0001 (Waits *et al.*, 2001 [4]), P_{IDsib} threshold of < 0.05 (Woods *et al.*, 1999 [5]).

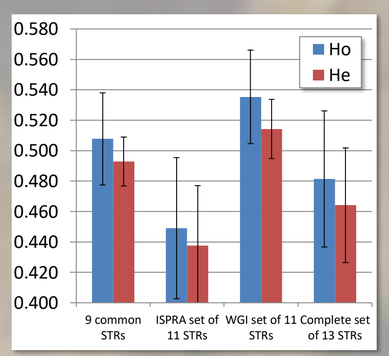


Fig. 3 - Mean H_e and H_o in the four STR marker sets.

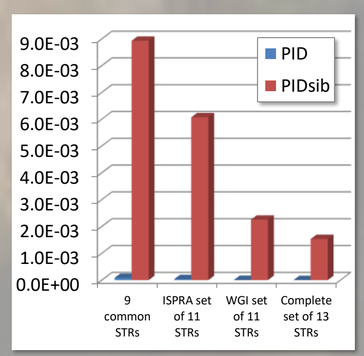


Fig. 4 - P_{ID} and P_{IDsib} in the four STR marker sets.

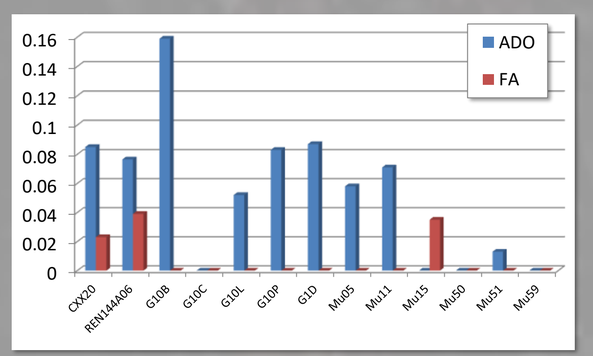


Fig. 5 - Genotyping errors at single locus.

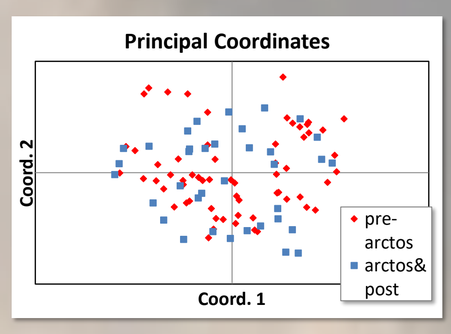


Fig. 6 - PCA of the variability from 2000-2010 to 2011-2017.

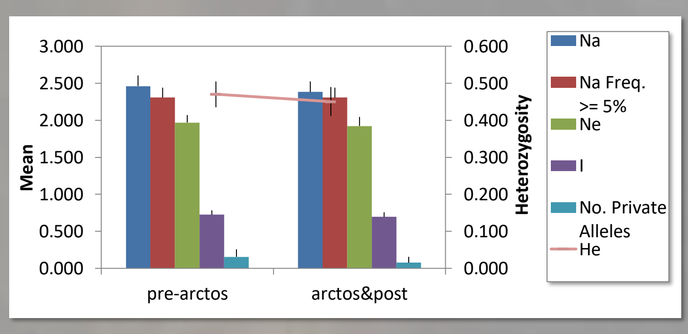


Fig. 7 - Variation of allelic patterns from 2000-2010 to 2011-2017.

CONCLUSIONS

- In order to avoid both underestimation (high values of P_{ID}) and overestimation (high levels of ADO and FA) in genotyping results, future monitoring will be conducted using the ISPRA set of 11 Ursidae-specific STRs with the addition of CXX20, that minimize the risk of shadow effect ($P_{ID} = 8.6 * 10^{-6}$; $P_{IDsib} = 3.0 * 10^{-3}$). In addition, marker REN144A06 will be used to improve the discriminatory capacity in uncertain cases.
- The population shows a slight and not significant loss of diversity due to genetic drift (Fig. 6, Fig. 7). Therefore the chosen STR panel is suitable for individual identification in the near future, but markers with higher discriminatory power will be needed for parentage analysis (eg. panel of SNPs).

ACKNOWLEDGMENTS

This project was supported by ISPRA (BIO-CGE), the National Park of Abruzzo, Lazio and Molise, the National Park of Majella and the Lazio Region.

A thank goes to Paolo Ciucci from The Sapienza University of Rome and to WGI for providing the biological samples.

I want to thank also the ISPRA lab staff for their support and advices.

Bibliography. ● Background picture from Società Cooperativa Sherpa. ● [1] Benazzo et al., 2017. Survival and divergence in a small group: The extraordinary genomic history of the endangered Apennine brown bear stragglers. *Proceedings of the National Academy of Sciences of the USA*, 114(45): E9589-E9597. ● [2] Ciucci P., Gervasi V., Boitani L., Boulanger J., Paetkau D., Prive R. & Tosoni E., 2015. Estimating abundance of the remnant Apennine brown bear population using multiple noninvasive genetic data sources. *Journal of Mammalogy*, 96(1): 206-220. ● [3] Taberlet P., Griffin S., Goossens B., Questiau B., Manceau V., Escaravage N., Waits L.P. & Bouvet J., 1996. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, 16: 3189-3194. ● [4] Waits L.P., Luikart G. & Taberlet P., 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology*, 10(1): 249-256. ● [5] Woods J.D., Paetkau D., Lewis D. *et al.*, 1999. Genetic tagging free-ranging black and brown bears. *Wildlife Society Bulletin*, 27: 616-627.