Investigating geographic and temporal genetic variation in the black grouse (*Lyrurus tetrix*) in the Italian Alps

Alice Fraser[*,°], *Emiliano Trucchi*[°,§], *Francesco Maroso*[°], *Andrea Brunelli*[°], *Diego Micheletti*[**], *Giorgio Bertorelle*[°], *Heidi C. Hauffe*[*], *Barbara Crestanello*[*]

[*]Dept Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, Italy; [°]Dept Life Sciences and Biotechnology, Univ Ferrara, Italy; [§]Dept Life and Environmental Sciences, Marche Polytechnic University, Italy; **Computational Biology Unit, Research and Innovation Centre, Fondazione Edmund Mach, Italy

SCAN ME TO VIEW LATER

Weak geographical structuring across the Italian Alps

Isolation by Distance, altitude and urbanisation influence genetic structure

No significant changes over time

BACKGROUND

- Classified as Least Concern by the IUCN[1], the species suffers from habitat loss and shrinking populations in the southern part of its range.
- Found at altitudes ranging from 900 to 1800m in the Alps, the species prefers coniferous forests as well as areas with sparse vegetation[2].
- Main threats: habitat loss, hunting, climate change[1].

MAIN OBJECTIVES

- Investigate the geographic and temporal genetic variation for the black grouse in the Italian Alps using 628 samples collected from 1995-2017, with 10 STRs (445 individuals) and 2,442 SNPs (400 individuals).
- Compare the power of microsatellites (STR) and Single Nucleotide Polymorphisms (SNPs) to find the most efficient marker to examine genetic structure and demographic history of the species.

ISOLATION BY DISTANCE

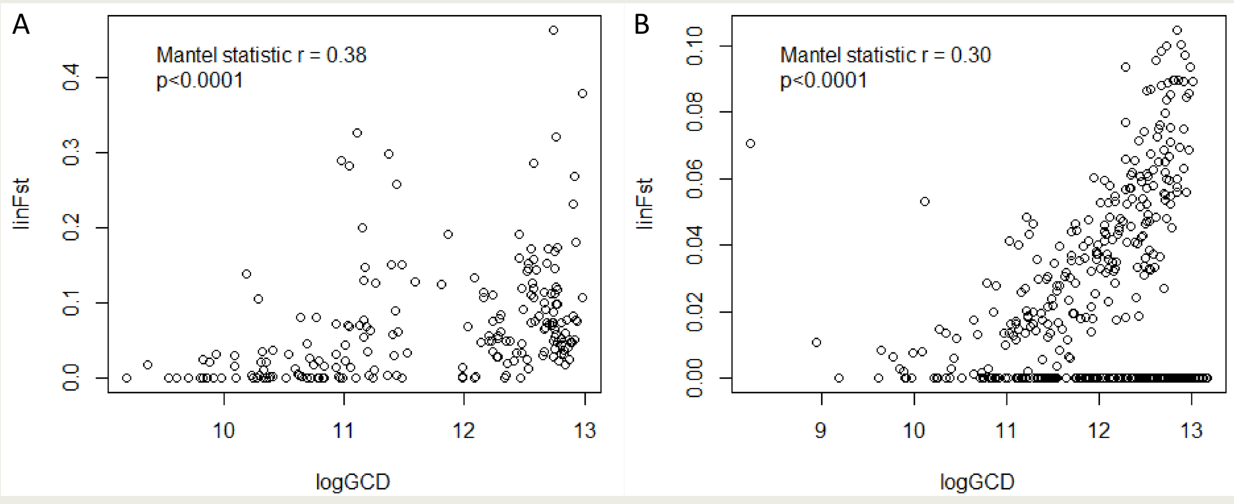

Figure 1: Isolation by Distance (IBD). Relationship between linearised F_{ST} and logarithm of the Euclidean distance between the different sampling locations, for the STR (A) and SNP (B) data. Any negative F_{ST} were changed to 0. Significant IBD patterns are evident for both datasets. Created in R[3].

Surface	SNP r	SNP p	STR r	STR p
Isolation by Distance	**0.239**	**0.003**	**0.638**	**0.001**
Altitude range, resistance:1 10 100	-0.0531	0.594	0.0565	0.366
Altitude range, resistance:1 100 1000	-0.0621	0.605	-0.0951	0.726
Altitude >2500m, resistance:10	**0.231**	**0.006**	**0.669**	**0.001**
Altitude >2500m, resistance:100	**0.216**	**0.039**	**0.661**	**0.001**
Altitude >2500m, resistance:1000	**0.207**	**0.09**	**0.639**	**0.001**
Altitude >3000m, resistance:10	**0.240**	**0.003**	**0.638**	**0.003**
Altitude >3000m, resistance:100	**0.240**	**0.002**	**0.639**	**0.002**
Altitude >3000m, resistance:1000	**0.240**	**0.003**	**0.639**	**0.003**
Urban areas, resistance:10	**0.496**	**0.001**	**0.644**	**0.001**
Urban areas, resistance:100	**0.436**	**0.019**	**0.636**	**0.002**
Urban areas, resistance:1000	0.427	0.078	**0.633**	**0.001**

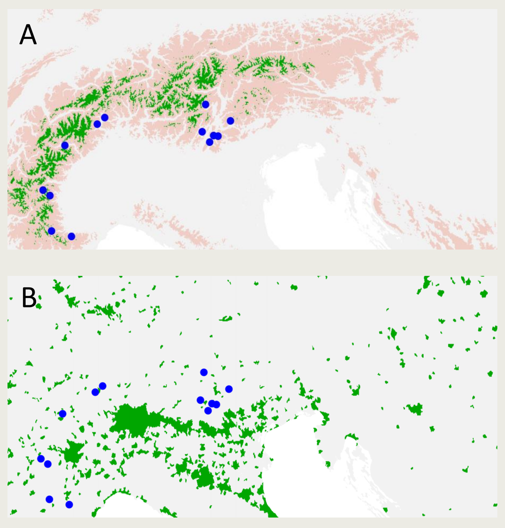

Table 1: Isolation by Resistance (IBR). Effect of altitude and urban areas as barriers for movement for the black grouse. IBR values (r) for a preliminary SNP and STR dataset and their corresponding probability (p) are shown in the table, significant results shown in bold. Calculated in Circuitscape[4] and R[3] using a mantel test comparing the F_{ST} and the 'all paths' distance between sampling locations (shown in blue in maps A and B). The altitude criteria used for the first test were <1000m, ≥1000-<2500m and ≥2500m, these altitudinal ranges were chosen based on the species' preferred range (map A). The resistance values at the end of each surface label correspond to the grade of resistance applied to each test. Neither combination of resistance values gave a significant result. The next two tests analyse higher altitudes as a barrier, these analyses were significant for both datasets. The final test was analysing the effect of urban areas (shown in map B), this was also found to be significant. This implies that both high altitudes and urban areas act as barriers to the movement of the species, and the STR dataset showed a stronger pattern of this.

GEOGRAPHIC STRUCTURE

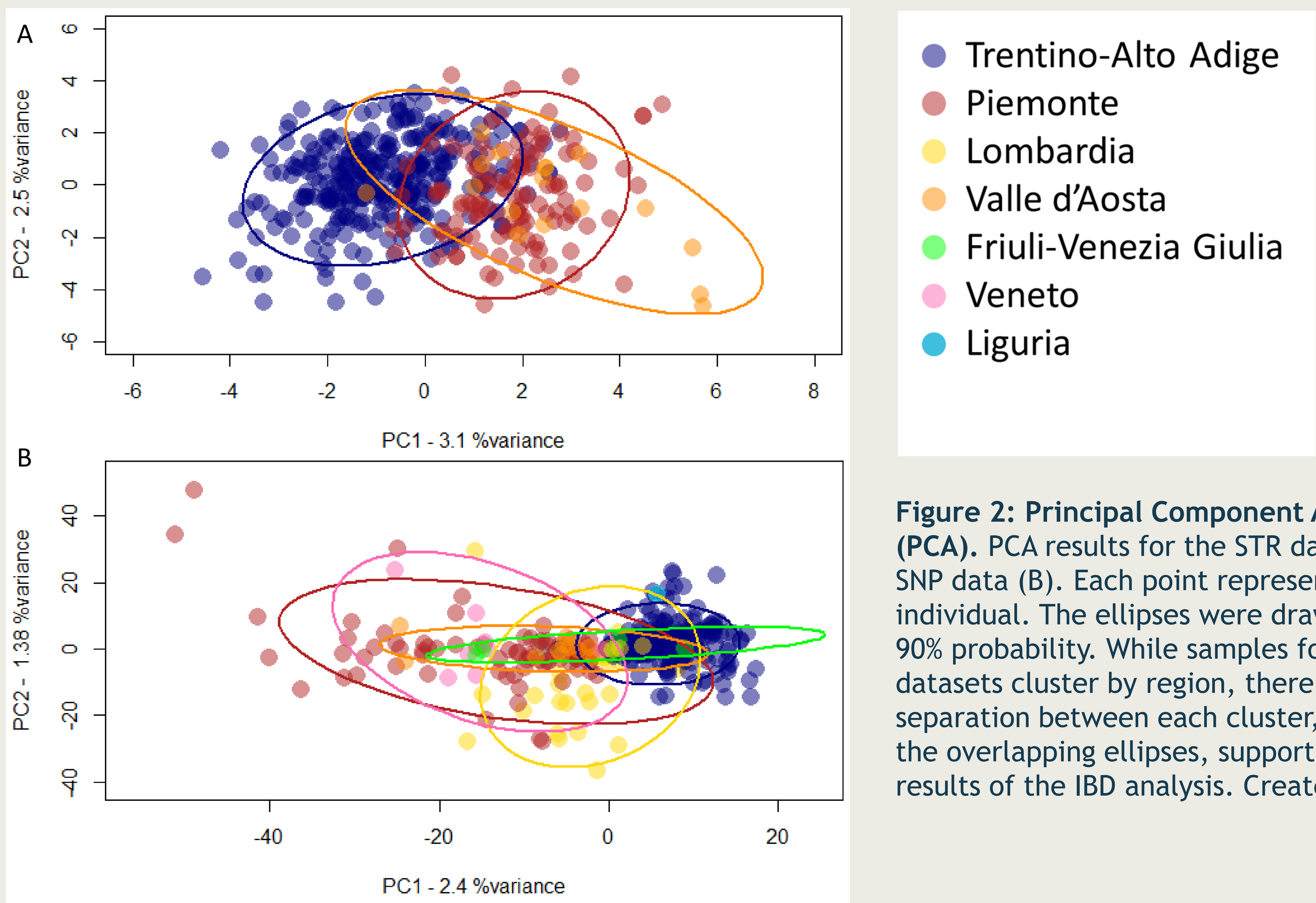

Figure 2: Principal Component Analysis (PCA). PCA results for the STR data (A) and SNP data (B). Each point represents an individual. The ellipses were drawn at a 90% probability. While samples for both datasets cluster by region, there is no separation between each cluster, shown by the overlapping ellipses, supporting the results of the IBD analysis. Created in R[3].

TEMPORAL ANALYSIS

A	NA	RA	Mean H_E
All data	12.1	14.5	0.741
1995-1999	7.6	7.9	0.719
2009-2010	7.9	9.1	0.731

B	H_E
All data	0.232
1995-1999	0.226
2009-2010	0.226
2015-2017	0.231

Table 2: Temporal comparison. Mean number of alleles (NA), mean allelic size range (RA), and mean expected heterozygosity (H_E) for the STR[5] (A) and SNP[6] (B) data for the Trentino-Alto Adige region only. No significant difference between time frames was detected (p>0.05, using a Wilcoxon test)[3].

IMPLICATIONS AND FUTURE DIRECTIONS

- Previous research on this species has been conducted with neutral genetic markers (mitochondrial DNA and STR); however, there is very little published work concerning genetic variation across the black grouse genome using SNPs[7,8]. Here, SNP analysis confirms and gives similar results to the STR.
- The PCA and IBD indicated a weak pattern of geographical structuring, and the significant IBD results suggest that the species is a continuous population in the Italian Alps, with high H_E confirming that there are no specific areas of conservation concern. However, restocking or reintroduction interventions should take this genetic continuum into account, since releasing birds from neighbouring areas is less likely to disrupt local adaptation.
- Both datasets showed that high altitudes (>2500m) and urban areas act as a barrier for the movement of the black grouse, and future work includes the application this technique to other factors. Shifting habitats caused by climate change may increase the importance of these barriers[9].

Acknowledgements

This project was supported by the Associazione Cacciatori Trentini (ACT) and the Unione Nazionale Cacciatori Zona Alpi (UNCZA).

References

[1]IUCN (2019). The IUCN Red List of Threatened Species. Version 2019-1. www.iucnredlist.org - accessed 16.05.2019; [2]Cattadori I & Hudson P (1999). *Ecography*, **22**: 374-383; [3]R Core Team (2018). R: A language and environment for statistical computing. www.R-project.org/; [4]McRae B *et al.* (2013). Circuitscape 4 User Guide. www.circuitscape.org; [5]Excoffier L & Lischer H (2010). *Molecular Ecology Resources.* **10**: 564-567; [6] Benazzo A *et al.* (2015). *Ecology and Evolution.* **5**:172-175; [7]Sittenthaler M *et al.* (2018). *Journal of Avian Biology*, **15:** e01681. [8]Wang B *et al.* (2014). *BMC Genomics*, **15:** 180-192; [9]Gehrig-Fasel J *et al.* (2007). *Journal of Vegetation Science. 18:571-582;* Image: RSPB black grouse www.rspb.org.uk.