

# Which roe is in your backyard? Genetic make-up of two roe deer populations of central Italy

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## Introduction

In Italy, roe deer (*Capreolus capreolus*) populations declined during the last centuries, reaching a minimum at the beginning of the XX century. Therefore, reintroduction and restocking operations were carried out to restore its original range, by using stocks from northern Italy, central Europe and the Balkans. Such practices led to the contact between diverging gene pools, especially in central Italy where relict populations of native roe deer (classified as *C. c. italicus*) had survived. The resulting introgressive hybridization has raised concerns for the genetic integrity of the Italian endemic form.

We analyzed roe deer from the Massa-Carrara and the Arezzo provinces (Tuscany) on a combination of biparental (microsatellites) and uniparental (mtDNA) genetic markers, aiming to disentangle the genetic make-up of these populations and evaluate the possible influence of landscape features on it.

## Methods

Specimens from the two provinces were analyzed on 11 autosomal microsatellites and compared with reference populations from surrounding areas. Fine population structure and the effects of anthropogenic infrastructures on gene flow were investigated with Bayesian cluster analyses and analysis of variance. A portion of the mtDNA control region was also sequenced for a subset of samples and aligned with homologous sequences retrieved from GenBank. Haplotypes belonging to the *italicus* lineage were identified by a diagnostic nucleotide deletion and by phylogenetic analysis.

## Results

A total of 117 (Massa) and 196 (Arezzo) individuals were successfully genotyped. The two populations were sharply separated by the Bayesian analysis. The Massa population formed a single panmictic deme, while the Arezzo population was composed by two genetic clusters, one prevailing in the north and the other one in the south of the area, apparently separated by the Arno river and the A1 highway. The AMOVA and ANOVA analyses indicated that this separation was very significant, explaining 5% of the overall genetic variation in the sample and 51% of the cluster assignment in the Bayesian cluster analysis. A total of 32 and 74 individuals were successfully sequenced for the Massa and Arezzo provinces, bearing six and seven different haplotypes, respectively. The proportion of *italicus* haplotypes was very high (75%) for Massa and relatively low (20%) for Arezzo, where only one individual with such an haplotype was detected in the northern area.

## Discussion

We assessed the genetic composition of two roe deer populations of central Italy. In both areas, our analyses detected a contact between the *italicus* lineage and non-native roe deer. The Arezzo population was sharply structured, showing native roe deer in the southern area (likely spreading from the Siena province) and admixed individuals mainly in the central area, with a sharp genetic discontinuity explained by natural and anthropogenic barriers contrasting gene flow. Conversely, the Massa population was genetically homogeneous, diverging from the neighbouring populations of the Parma and Lucca provinces, and showing a surprisingly high proportion of *italicus* haplotypes. This pattern seems to indicate a possible role of genetic drift favored by genetic isolation due the barrier-effect of mountains or its origin from local introduction of native roe deer from southern populations.