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POPULATION GENOMIC ANALYSES OF SEVERAL COMMERCIAL AND FANCY RABBIT BREEDS

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ABSTRACT

The European rabbit (*Oryctolagus cuniculus*) is considered a multi-purpose species, mainly used as livestock for meat production in many different countries. Human driven artificial directional selection and breeding programmes have produced a lot of breeds and lines, adapted to a variety of environments and production systems, showing many phenotypic differences. This phenotypic diversity spans from economically relevant traits to morphological characteristics, which differentiate most fancy breeds. The genetic diversity within this species has not been extensively investigated at the genome level yet. In this study, we analysed genome-wide single nucleotide polymorphism (SNP) data obtained using the Affymetrix Axiom OrcunSNP array to characterize the population genomic structure of three meat rabbit lines developed by the Italian Rabbit Breeders Association (Italian Champagne d'Argent, Italian White and Italian Spotted) and ten fancy breeds (Belgian Hare, Burgundy Fawn, Checkered Giant, Coloured Dwarf, Dwarf Loop, Ermine, Giant, Giant White, Rhinelander and Thuringian) registered to their Italian rabbit herd books. A total of 380 rabbits were genotyped with this SNP array. Multidimensional scaling (MDS) analysis clearly separated Italian White and Italian Spotted breeds and clustered together all giant breeds. Admixture analysis carried out for the three commercial breeds showed several genomic structures mainly within the Italian White breed that could be derived by different within-breed lines. Four genomic inbreeding parameters were also calculated using SNP data. These measures could be useful to define conservation and breeding strategies in these breeds. This first exploratory analysis of population genomic parameters that included 13 rabbit breeds raised in Italy provided a general overview of the genetic diversity within and among breeds, complementing previous studies that characterized several candidate genes affecting breed-specific traits. Further studies are needed to complete the genomic characterization of the Italian rabbit genetic resources.

Key words: Admixture, Inbreeding, Livestock, Meat, *Oryctolagus cuniculus*.

INTRODUCTION

Among the domestic animals, the European rabbit (*Oryctolagus cuniculus*) is considered a multi-purpose species. In fact (i) it is used as livestock for meat production, (ii) it has relevant economic use for fiber and fur production, (iii) it is an important animal models and (iv) it is also considered a pet. Therefore, based on the variety of these uses, human driven artificial directional selection and breeding programmes have produced a lot of breeds and lines, adapted to a variety of different environments and production systems, showing many phenotypic differences. This phenotypic diversity spans from economically relevant traits to morphological characteristics, which describe many breed-specific traits. Most of the genetic diversity has been shaped by a combination of several genetic events (e.g. genetic drift, bottleneck, isolation, introgression, migration, crossbreeding) that contributed to define a lot of genetic resources, thus far mostly untapped and uncharacterized.

The genome of the European rabbit has been recently sequenced (Carneiro et al., 2014) and a few studies have started to evaluate, at the genome level, the extent of variability within rabbit populations (e.g. Bertolini et al., 2014). More recently the development of a commercial high throughput single

nucleotide polymorphism (SNP) genotyping tool has made it possible to detect genomic regions associated with economically relevant traits (Sosa-Madrid et al., 2020a, 2020b). Other studies have contributed to explain the variability of external traits, e.g. coat colour, that largely define breed-specific features in most traditional and fancy breeds (Demars et al., 2018; Fontanesi et al., 2006, 2010, 2014a, 2014b; Utzeri et al., 2014).

In this study, we analysed high density SNP genotyping data to characterize the genomic structure of three meat rabbit lines developed by the Italian Rabbit Breeders Association and to capture genomic information from other 10 rabbit fancy breeds registered to their Italian rabbit herd books.

MATERIALS AND METHODS

Animals and experimental design

Biological specimens (hair roots or buccal swaps) were collected from a total of 380 rabbits (137 males and 243 females) from 13 breeds, including three commercial rabbit lines [Argentata Italiana (Italian Champagne d'Argent), n. 17; Bianca Italiana (Italian White), n. 170; Macchiata Italiana (Italian Spotted), n. 43] and ten fancy breeds [Ariete Nano (Dwarf Loop), n. 19; Ermellino (Ermine), n. 11; Fulva di Borgogna (Burgundy Fawn), n. 6; Gigante (Giant), n. 21; Gigante Bianco (Giant White), n. 8; Gigante Pezzato (Checkered Giant), n. 42, Lepre (Belgian Hare), n. 18; Nani Colorati (Coloured Dwarf), n. 13; Pezzata Tricolore (Rhineland), n. 3; Turingia (Thuringian), n. 9]. Animals were selected in order to avoid highly related individuals (no full- or half-sibs). All animals had standard breed characteristics.

Genotyping

DNA was extracted using the Wizard Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA). Animals were then genotyped using the Affymetrix Axiom OrcunSNP Array (Affymetrix Inc., Santa Clara, CA, USA) which analyses 199692 DNA markers. PLINK v. 1.9 (Chang et al., 2015) was used to discard SNPs presenting a call rate < 0.90 , a minor allele frequency (MAF) < 0.01 and that were not in Hardy-Weinberg equilibrium ($P < 0.0001$). SNPs were mapped to the OryCun2.0 rabbit genome version. After filtering, a total of 131444 SNPs was used in the subsequent analyses.

Data analyses

Population structures were evaluated through multidimensional scaling (MDS) analysis as implemented in PLINK v1.9 (Chang et al., 2015). Admixture analysis was carried out for the three commercial breeds (Argentata Italiana, Bianca Italiana and Macchiata Italiana) using STRUCTURE software (Alexander et al., 2009). The number of cluster K was set to 3. Genomic inbreeding coefficients, i.e. $F_{\text{hat}1}$, $F_{\text{hat}2}$, and $F_{\text{hat}3}$, were computed in PLINK (function: --ibc). PLINK was also used to compute the method-of-moments (MoM) F coefficient (function: --het), F_{MoM} .

RESULTS AND DISCUSSION

An MDS-plot, reporting individuals of the analysed breeds, is shown in Figure 1A. Two out of three commercial breeds (Bianca Italiana and Macchiata Italiana) were clearly separated in a bidimensional scale (including components 1 and 2). Among the commercial breeds, Argentata Italiana was closest one to the main breed cluster including all other breeds. A few Argentata Italiana rabbits grouped within or in close contact with a heterogeneous mixed group, which formed a general continuous cluster constituted by several fancy breeds. Belgian Hare, even if clearly distinguishable, was the closest breed to Argentata Italiana. The bottom right end part of the continuous cluster included all three giant breeds, among which Checkered Giant was the most homogeneous group. Giant White rabbits were within the Giant group.

Results of the admixture analysis, that included the three commercial rabbit breeds, are reported in Figure 1B. Macchiata Italiana seems the most homogeneous breed. Bianca Italiana population could be described by at least four distinct patterns which might potentially identify a few within-breed lines. Argentata Italiana showed some patterns of potential admixture with the other breeds. Other breeds

might be included and other numbers of K could be tested to improve the interpretation of the observed population genomic structures.

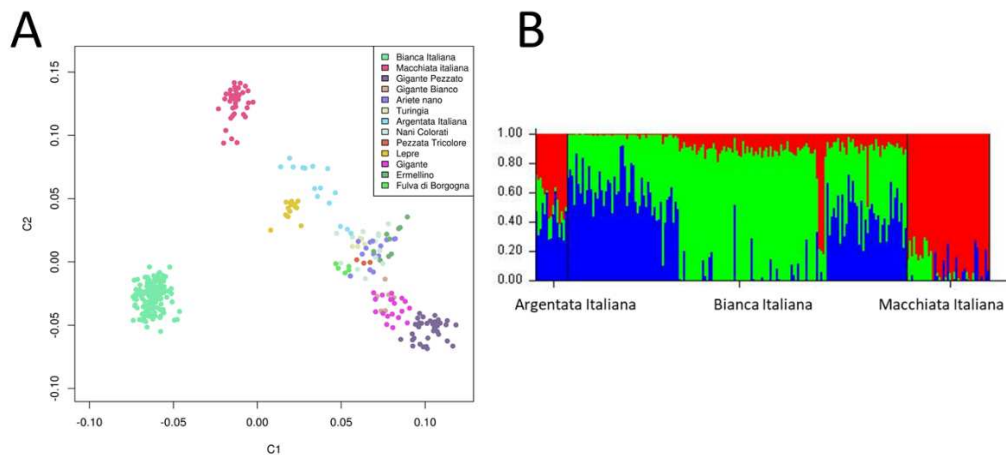


Figure 1. (A) Multidimensional scaling (MDS) plot of the analyzed breeds, considering components 1 (C1) and 2 (C2). Each point represents a genotyped rabbit. (B) Admixture plot of the three commercial breeds based on K = 3.

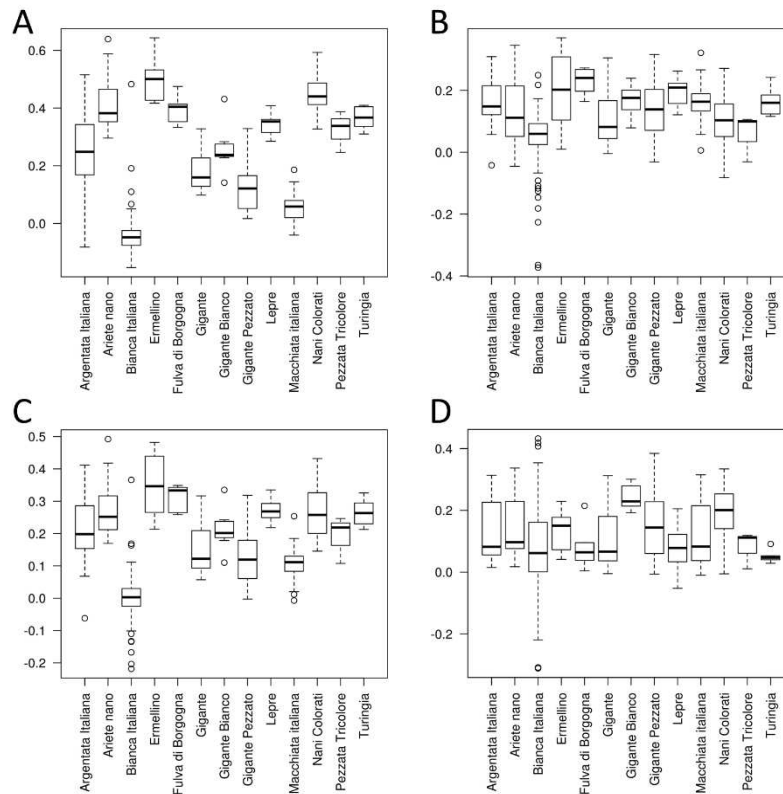


Figure 2. Box-plots of the estimated genomic inbreeding parameters in the analysed rabbit breeds: (A) F_{hat1} , (B) F_{hat2} , (C) F_{hat3} , and (D) method-of-moments F coefficient.

Several genomic inbreeding parameters were calculated using SNP data. Within-breed distribution and comparisons across breeds for these values are shown in Figure 2. Among the three commercial breeds, Bianca Italiana had the lowest mean value for all four estimated parameters, even if with quite large variability. Lepre (Belgian Hare) had a low level of within-breed variability for all four measures.

CONCLUSIONS

This first exploratory analysis of population genomic parameters that included 13 rabbit breeds raised in Italy provided a general overview of the genetic diversity and structure within and among breeds, complementing previous studies that characterized several candidate genes affecting breed-specific traits. Genomic inbreeding parameters could be useful to define conservation and breeding strategies in these breeds. Additional studies, including more animals and other lines and breeds, could complete the genomic characterization of the Italian rabbit genetic resources.

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