# POPULATION GENOMIC ANALYSES DESCRIBE UNIQUE GENETIC FEATURES OF SEVERAL RABBIT BREEDS AND LINES

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## ABSTRACT

The rabbit is globally bred for various purposes, including meat, fiber, and fur production and many breeds have been developed, including fancy breeds. The aim of this study was to analyse high density SNP genotyping data from 14 rabbit breeds to detect signatures of selection in the rabbit genome, which could explain the genetic diversity of these genetic resources. Combining a single marker and haplotype-based approaches such as $F_{ST}$, XP-EHH, and |iHS|, we identified signatures of selection that covered about half of the rabbit genome. Many regions consistently detected in several breeds and with the combination of more than one approach harbour genes associated with pigmentation processes and morphological traits, previously recognized in rabbits and other species (e.g., *ASIP*, *MC1R*, *TYR*, and *LIPH*). The results of this study contribute to a improve the knowledge on the genetic architecture of fancy and meat rabbit breeds and understand the genetic factors that contribute to the genetic diversity of these animal genetic resources.

**Key words**: Genetic resource, genetic variability, signature of selection, SNP.

## INTRODUCTION

The rabbit serves as a versatile livestock species raised for the production of meat, fiber, and fur. This species is also relevant as animal model and companion animal. Many breeds and lines have been therefore developed, having as main objectives the specialization and differentiation of the genetic stocks, which also defined their specific characteristics mainly based on exterior traits (such as coat color, hair structure, body size and shape, ear size and position, and skull structure) and/or performance and production traits. The genetic features of all these genetic resources have been shaped by various genetic events, including genetic drift, bottleneck, isolation, introgression, migration, and crossbreeding. Many of these breeds and lines remain largely untapped and uncharacterized. The sequencing of the rabbit genome, as well as the availability of a high-throughput single nucleotide polymorphism (SNP) genotyping platform, have facilitated genome-wide analyses in this species, including the identification of genomic regions influencing economically relevant traits in meat rabbits (Bovo et al., 2021) Other studies have contributed to explaining the variability of external traits (Fontanesi et al., 2014a). In this study, using single marker and a haplotype-based approaches, we analysed SNP array datasets obtained from several fancy and meat rabbit breeds or lines to identify signature of selection in the rabbit genome which can characterize the investigated rabbit genetic resources.

## MATERIALS AND METHODS

### Animals and genotyping

Biological specimens, comprising hair roots or buccal swaps, were collected from a cohort of 712 rabbits from Three meat breeds (Italian White, *n* = 256; Italian Spotted, *n* = 93; Italian Silver, *n* = 20) and all fancy breeds (Belgian Hare, *n* = 24; Burgundy Fawn, *n* = 6; Champagne d'Argent, *n* = 19; Checkered Giant, *n* = 79; Coloured Dwarf, *n* = 20; Dwarf Lop, *n* = 20; Ermine, *n* = 20; Giant Grey, *n* = 27; Giant White, *n* = 20; Rex, *n* = 19; Rhinelander, *n* = 28; and Thuringian, *n* = 9). These animals were officially registered by the National Rabbit Breeders Association (ANCI or were from a genetic nucleous under selection for production performances. Genomic DNA was extracted using the Wizard Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA). Subsequently, genotyping was

performed utilizing the Affymetrix Axiom OrcunSNP Array (Affymetrix Inc., Santa Clara, CA, USA), which encompasses 199,692 DNA markers. Data quality checks were conducted through the Axiom Analysis Suite and PLINK v.1.9 (Chang et al., 2015), resulting in the exclusion of samples and DNA markers with a call rate below 0.90. Following filtration, a dataset of 702 animals and 139,922 SNPs was used in the subsequent analyses. The SNP dataset for all investigated rabbits was then subjected to phasing using SHAPEIT2, with default parameters (Delaneau et al., 2012).

**Signatures of selection detection**

The identification of signatures of selection was performed using both single marker and haplotype-based approaches. In the single marker approach, $F_{ST}$ analyses were conducted using the Hudson estimator, chosen for its independence from sample size considerations (Bhatia et al., 2013). Signatures of selection were computed within 350 kb sliding genome windows, with a step size of 100 kb. A total of 7,951 genome windows were calculated, following the testing of windows with varying sizes as outlined by Rubin et al. (2010). Windows containing fewer than 3 SNPs were excluded from the analyses. Using information derived from the $F_{ST}$ approach, two distinct methods were applied to identify signatures of selection. The first method compared one breed against all others, while the second approach grouped certain breeds based on shared characteristics such as coat colour/colour patterns, body size, and use/specialization (fancy vs meat; Silver breeds vs all other breeds; Giant breeds vs all other breeds; Dwarf breeds vs all other breeds; Spotted breeds vs all other breeds; Albino breeds vs all other breeds). Following Rubin et al. (2010), putative signatures of selection were identified from genome windows situated at the extreme lower end of the distributions (99.98th percentile of the distribution), resulting in the identification of 14 genome windows for each comparison.

For the haplotypes-based approach, the rehh R package V 2.04 (Gautier et al., 2017) was used on the phased SNPs to derive integrated haplotype scores (iHS; Voight et al., 2006) for each breed and across-populations, along with extended haplotype homozygosity (XP-EHH; Sabeti et al., 2007) values for both breed comparisons and groups of breed comparisons. Signatures of selection were identified based on absolute values for haplotypes that passed the threshold of the top 98.00th percentile of the empirical distribution, requiring at least three consecutive SNPs within a 350 kb window. Genomic regions exhibiting signatures of selection from the $F_{ST}$, iHS, or XPEHH methods were annotated using *Bedtools v.2.17*(https://bedtools.readthedocs.io/) by retrieving annotated protein-coding genes from the OryCun2.0 NCBI's GFF file (https://www.ncbi.nlm.nih.gov/).

**Table 1**. Summary of methods and approaches used for signatures of selection detection.

| Approach | Methods | The first group of comparison | The second group of comparison |
|---|---|---|---|
| One breed approach | $F_{ST}$, XP-EHH | The analysed breed | The remaining rabbit breeds are considered a unique population |
| Groups of breeds | $F_{ST}$, XP-EHH | Rabbit breeds share the same features as a unique population | The remaining rabbit breeds are considered a unique population |
| One breed approach | \|iHS\| | The analysed breed | NA |
| Groups of breeds | \|iHS\| | Rabbit breeds share the same features as a unique population | NA |

## RESULTS AND DISCUSSION

In total, 309 distinct genome regions under signatures of selection were identified from the $F_{ST}$ analyses. Multiple genomic regions harboring genes associated with coat colour identified. These regions encompassed several genes already shown to affect coat coulor in rabbits or in other species, and including *ASIP, MC1R*, *MITF*, *OCA2* and *TYR.* Additional signatures of selection were detected in genomic regions harboring genes linked to (i) coat structure, such as *LIPH*, and (ii) body size, including (*COL2A1*, *LCORL*, and *GRK5*).

The top 20 |iHS| windows were distributed across the following rabbit chromosomes (OCU): OCU1, OCU2, OCU4, OCU7, OCU9, OCU12, OCU15, and OCU16. These windows encompass multiple genes involved in growth processes and body morphology. Notably, the highest |iHS| value, specifically identified in the commercial meat line and in the group of rabbit breeds/lines, highlighted a window on OCU2 containing the *LCORL* gene, which is well known to affect stature and body size in mammals (e.g. Pryce et al., 2011). Another significant genomic region, identified with the |iHS| method in the Giant breeds, was localised on OCU4 and encompassed the *HMGA2* gene, which is involved in several basic biological processes, including mesenchymal differentiation, adipogenesis, and post-natal myogenesis. The genomic region encompassing this gene was also evidenced in the Dwarf breeds.

The results from the XP-EHH analyses partially overlapped and complemented the |iHS| outcomes. For instance, the *HMGA2* gene region, previously identified in the |iHS| results, was detected also in the fancy Giant breeds. Other genomic regions, encompassing numerous genes associated with growth, body size, and development (e.g., *COL11A2*, *NCAPG2*, *SEMA4D*, *TGFBR2,* and *ZFAT*), emerged from within-breed comparisons and analyses based on groups of breeds using this method but were not evident with the |iHS| method.

Considering all breeds and all applied methods (single marker and haplotype-based approaches), signatures of selection were identified in approximately 1.8 Gb (65 %) of the rabbit genome (Figure 1). From this picture, it emerged that a large fraction of the rabbit genome experienced some relevant modifications in the process of breed constitution (considering that we analysed only a few breeds over the several tens or hundreds that have been constituted). Despite the interesting results obtained in our study, it is crucial to acknowledge certain limitations. The differentiation of genomic regions among rabbit breeds may not always directly imply selection due to the complex history of breed development. While annotated gene functions can aid interpretation, the incompleteness of our knowledge about certain genes introduces uncertainties, particularly in the context of complex traits influenced by multiple genes. Furthermore, the integration of whole-genome sequencing (WGS) data with other 'omics' datasets, such as transcriptomics and proteomics, holds promise for gaining a more profound understanding of the molecular mechanisms underlying selection signatures in the rabbit genome.

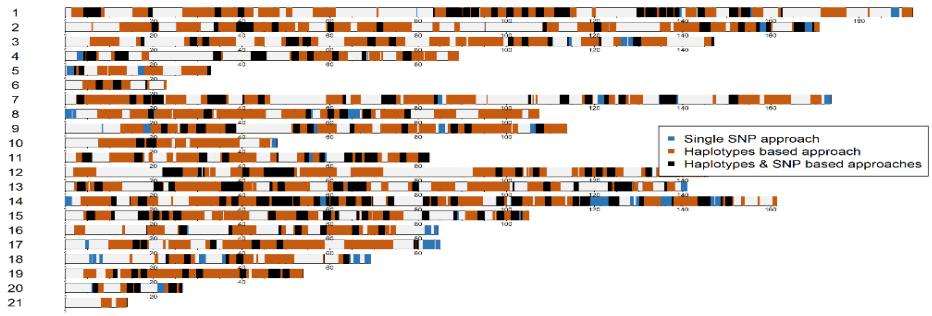


**Fig.1.** An overview of the genomic regions, distributed in all rabbit autosomes, covered by signatures of selection, identified in all analysed breeds by single marker and haplotype-based approaches.

## CONCLUSIONS

This study demonstrates the valuable utility of high-throughput genotyping data in unraveling the population genomic features of several rabbit breeds. Notably, the use of different methods to detect signatures of selection highlighted important genomic regions that could not emerge using just one or another approach. Including other approaches would be pontentially possible to identify additional signatures of selection. Many other rabbit breeds remain to be investigated at the genome level. Their full characterization may identify other important hints able to explain the large phenotypic diversity in this domesticated species.


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