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# Investigate the Population Organization and Genetic Variation of Various Domestic Rabbit Breeds

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**Abstract**
This research intends to offer insight into the variety of domestic rabbit breeds and their consequences for breeding and conservation efforts by examining the population structure and genetic variation among them. The domestic rabbit (Oryctolagus burrow (O.c)) has been bred and reared all over the globe for a variety of purposes, including companionship, meat production, lab research, and display. Both breeders and academics have been interested in the distinctive qualities and attributes of various rabbit breeds. To do this research, a vast dataset of rabbit populations from various geographic regions was gathered, including a broad variety of breeds and lineages. Genetic materials taken from individuals of each breed were subjected to genome analyses to assess the genetic diversity (GD) and population structure.

**Keywords**: Genetic diversity (GD), Oryctolagus Cuniculus(O.c), population, Hardy-weinberg equilibrium(H-W)

## Introduction

The domestic varieties of the European rabbit (O.c), which is the only source of domestic rabbits, are found all over the globe[1]. Its geographical origins may be linked to the Hibriain(IP), where two sub-species live together: O.c[2]. Burrow, which is found in the region's northeastern region, and O. c. algirus, which is found in the region's southwest. The O.c crossed the Pyrenees and extended to the South of France, wheliumre it naturally occurs after last glacial maximum, some 18,000 years ago. The subsequent global spread was aided by humans, with the Middle Ages serving as its most significant era.Rabbits have been hunted by humans for thousands of years, but they were not domesticated until relatively recently [3,4]. Historical documentation contains conflicting information on the genesis of domestic rabbits. Sources claim that Romans who bred rabbits in large enclosed colonies for meat and fur in the IP in the first century BC were responsible for the first steps. The likelihood of this behavior occurring without selective breeding is, nevertheless, high. The actual process of domestication, which included selective breeding and taming, is thought to have taken place in French monasteries over the preceding 1,500 years, according to a plethora of historical data. Pope Gregory I perhaps unwittingly began this when helium prohibited eating Laurices, a treat prepared from newborn or not born rabbits, circa 600 AD.The genomic evidence favors a French origin for domestication and demonstrates that domestic rabbits exhibit a portion of

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GD present in the O. c[5-7]. Burrow French wild populations. The majority of rabbit breeds were just recently established; this process started in Western Europe ending in the 18th century. The method of diversification, however, maybe started sooner, as shown by the 16th-century reports of breeds in a variety of sizes and colors. More than 200 breeds of rabbits and strains are now available, and they have been chosen for a variety of uses, including medicinal nutrients, wool, companion animals, meat, and fur. These breeds show a startling amount of phenotypic variability for a wide range of attributes during a very short period.

Five different haplotypes of the DNA D-loop region were found in the study [8]. A population genetic structure distinguishing the European group from Kenyan rabbit farms was found after integrating 32 known haplotypes. Inferring a substantial genetic variance in the tested people and the contain variants, "Analysis of molecular variance (AMOVA)" across and within the populations showed a significant genetic differential among and within the analyzed areas (0.05).

Study [9] evaluated rabbit farming methods and problems, as well as the animals' morphometric and growth characteristics, GD, and carcass attributes of crosses produced in the North-Rift and Western Kenya. There was adequate room for the rabbit breeds that met the requirements. The findings showed that farmers may improve their local types by using the improved New Zealand White rabbit breed that was developed.

Study [10] investigated diversity and variations in the expression patterns of the genes FGF5, PGAM2, TLR2, and IL10 in Baladi Black and Red O.c, V-line. The discovered SNPs, together with variations in FGF5, PGAM2, TLR2, and IL10 mRNA levels, may serve as a biomarker for accurate breed classification and may aid in the development of marker-assisted growth selection and immunological features in O.c.

The Yangzhou University Animal Care and Use Committee's suggestions were followed while conducting the study [11]. "The Laboratory Animal Requirements of Environment and Housing Facilities (GB14925-2001)" were strictly followed while conducting operational operations. The average of each measure of GD utilizing 22 ideal loci and 43 unique loci for the F0 and F1 generations, as well as New Zealand white rabbits, did not vary significantly ($p > 0.05$) when applying the genetic assessment method developed in this work. This supports the viability of employing the 22 best locus mixtures for the first genetic assessment of rabbits.

Using data from high-density single nucleotide polymorphisms in study [12], they examined the genomes of "12 fancy rabbit breeds", as well as three breeds of commercial meat rabbits from Italy the Italian White, Italian Spotted, and Italian Silver. They provide a genome-wide analysis of the genetic regions underlying the major pheliumnotypic variations in the examined O.c reproduce that may able to comprehend the change in O. C.burrow from the development of breeds via the process of domestication through research.

A population of 200 Poltavske sriblo breed rabbits was used in the study[13]. After weaning at 45 days, the young were kept in cages with three to four other animals apiece. Males that were 3 months old were kept in separate cages until they were 150–160 days old when they could be used for breeding. Allelic variants' distribution of the myostatin gene and progesterone receptor in the context of genealogical lines was used to analyze the peculiarities of the genetic structure of Poltavske sriblo rabbits, and the findings are reported.

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The impact of five polymorphisms in the IGFBP genes was examined by study [14] in several rabbit breeds. For carcass, meat, and growth quality parameters, statistical significances were discovered; however, not for all examined breeds. IGFBP5's g.158093018A > T polymorphism seems to be the most promising one. For g.4988G > A, we removed those SNPs from statistical analysis because of C282Y FG and a near absence of minor alleles.

Study [15] found candidate genes associated with rabbit skeletal muscle development and investigated their possible regulatory mechanisms. A total of 25 individuals were chosen to examine the variations in the skeletal muscle transcriptome in Fujian white rabbits at various developmental stages (days 20 and 26 of the embryo and birth 1, 30, and 60 days old). By illuminating the molecular regulation mechanism of muscle growth and development in Fujian white rabbits, results should serve as a crucial theoretical basis for improving the meat performance and growth rate of Chinese native meat rabbit breeds.

By analyzing their population structure and genetic diversity, this study tries to provide insight on the range of domestic rabbit breeds and how it affects breeding and conservation efforts.

## Materials and Methods

### Sample Choice

We used three criteria to get a fair representation of the genetic variety of domesticated rabbits captured. First, as breeds with a documented ancient history are anticipated to act as genetic variety archives, and because historical records suggest that the majority of extant breeds are descended through crosses between ancient breeds, we took samples of breeds that have a long history. Second, we selected breeds from several geographic locations to better capture the wide various genetic variation existing among tamed people. Considering that greater pheliumnotypic divergence may, in theory, indicate superior GD, we next concentrated on a group of breeds that represented the pheliumnotypic features that differ the most. The 16 distinct breeds listed below were utilized: Belgian Hare, English Silver, Fauve de, Champagne Silver, English Spot, and Chinchilla (Standard). Based on genetic and historical data indicating the most likely path leading in favor of domesticating the European rabbit, wild rabbit samples were chosen. We conducted surveys of people who belonged to the O. c. burrow subspecies in nine locations in France and four locations in the IP's northeast. We separated them into three main categories, including domestic O.c (n = 320), wild O.c from Iberia (n = 34), and wild O.c from France (n = 82), which included both wild and domestic animals. In several of the research listed below, breeds or strains were further divided into the domestic category. While standard techniques such as phenol-chloroform or high salt were used to extract genomic DNA from blood.

### Molecular Marker Selection

To examine levels and patterns of GD, 45 microsatellites (MS) dispersed over the whole rabbit genome were used. We selected 7 MS on the X chromosome and 38 MS on the autosomes. Twelve extra MS was created to enhance the number of loci and offer a more uniform illustration throughout the genetic code. The bulk of The MS utilized in this analysis (n = 33) was collected from earlier studies. The chromosomal position of these new MS was determined

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utilizing the reference genome sequence for the rabbit. All other MS were made up of dinucleotide patterns except for STR06, which had a tetranucleotide structure.

**Microsatellite Genotyping**

Pairs of primers created using Primer3plus' web-based interface and the AUTODIMER program was used to check for primer-dimer interactions. These interactions as well as the anticipated lengths of the various amplicons were taken into consideration while designing multiplex panels. Using fluorescently labeled primers, nine separate multiplex reactions were conducted. The primer mix, which is a solution comprising all the dyes and primers in varying water, genomic DNA, and concentrations were used to create PCRs in a 5 L overall reaction volume. An automated sequencer, Genescan-500 LIZ, and ABI Prism 3130xl as size typical were used to separate PCR products by capillary electrophoresis. Genotypes were evaluated using visual inspection and the program GENEMAPPER 4.0.

The programs “ARLEQUIN version 3.5.1.2” and “MICRO-CHELIUMCKER version 2.2.3” to search for any probable blank allelomorph and variations from the “Hardy-Weinberg equilibrium (H-W)”, were utilized to investigate each microsatellite. Since groups of more than 5 wild each are more likely to reflect people generated by random mating, both experiments were carried out there. We could not discover any systematic proof for the existence of null alleles or departures from H-W for the remaining loci, except for STR22 and STR25. The studies reported below employ the whole dataset of 45 MS since the findings were qualitatively the same with or without these MS.

**Data evaluation**

**Brief GD and differentiation statistics**

The software GENALEX 6.4.1 was used to determine the sample size for each Fixation Index (FIS), anticipated heterozygosity (Helium), number of alleles (Na), and locus (n). The software HP-RARE 1.0 was used to estimate private “allelic richness (PAr)” and “allelic richness (Ar)”, using a rarefaction technique to account for variations in sample sizes across groups. Domestic breed genetic differentiation was computed using ARLEQUIN and approximated using pairwise and global FST.

The division of genetic variability was also investigated using the same software using a hierarchical “Analysis of molecular variance (AMOVA)”. While treating a three-level arrangement of genetic variation, both domestic breeds and wild populations were studied independently for this investigation. The significance of statistics.

**Phylogenetic analysis**

Using the computer software POPULATIONS version 1.2.31, phylogenetic trees of people were created utilizing the allele-sharing distance and chord distances. The study of domesticated species has often used both distances. We utilized the neighbor-joining approach because of its quick computing performance and the vast number of people sampled. The cluster re-rooted the tree that was created by the People as a whole for purposes of a display created by IP untamed rabbits. Instead of collecting genetic distances between individuals to analyze genetic links between breeds, distances were created between breeds and the

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population of wild France. 1000 genetic distance matrices were replicated using version 4.05 of the program "MICROSATELLITE ANALYSER (MSA)".

**Population structure**

Two different methods were used to examine the genetic structure of the domesticated population. We started by using in "Bayesian clustering" method included in STRUCTURE, version (2.3.3). For a dataset made up of all domestic rabbits, five different runs with K (the possible number of genetic clusters) values between 1 and 20 were tested.

We selected a wide range of K to i) explore patterns of clustering arising from lower values of K to infer probable ancestral links across breeds, and ii) inspect the presence of possible breed-specific substructure by concentrating on greater values of K. Following a "burn-in" phase of 50,000 iterations, each run was completed with 100,000 iterations. The admixture model was used to conduct the study, and it was assumed the correlation between allele frequencies. The Discriminant Analysis of Principal Components, or DAPC, was done second. DAPC optimizes the division of the groupings while limiting variance, resulting in a superior prejudice towards established genetic groupings than other popular multivariate techniques (such as "Principal Component Analysis-PCA or Factorial Correspondence Analysis-FCA)". Additionally, it enables the creation of a visual depiction of the connections between the detected clusters.

The benefit of this approach is that it does not depend on a specific model using population genetics, such as the linkage disequilibrium assumptions and H-W, which are fundamental tenets of the algorithm used in STRUCTURE. Non-random mating causes numerous breaches of the H-W in domesticated people, therefore this is very important to them. We carried out the DAPC investigation with several breeds. Finally, we used GENALEX to perform a 16-domestic breed population assignment test.

**Results and discussion**

**A comparison of the genetics of wild and domesticated animals**

We used all 471 individuals in a study of phylogeny to examine the connections between wild and domestic animals. The three main demographic groupings taken into account in this study can be distinguished by their ensuing topology. According to earlier research that implies WR from Iberia is the source of French WR, WR from Spain was found to be organized within a genetic database existing in the Iberian Peninsula. Within the genetic reservoir of French WR, every farmed individual was eventually grouped. This proximity is further corroborated by FST values, which showed less divergence between these two groups than between rabbits at home and rabbits that originate from the Hibriain(FST = 14%).

These values were summed across all markers. With one portion of the Alicante population, which did not cluster with the other Iberian groups, an alternate phylogenetic using a different genetic separation generated essentially identical results.

**Microsatellite GD and differentiation levels and patterns**

Table 1 provides an overview of the information on genetic variation. Comparing the global patterns of diversity between all three categories demonstrated an absence of genetic variety that moved from Hibriain WR to France, then to domesticated O.c. This decrease in biological

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variation was consistent over a majority of the loci investigated. The WR from the Hispania had the highest median amount of GD across loci (Helium = 0.786), afterward those from Spain (Helium = 0.734), and finally domesticated rabbits (Helium = 0.567). Private and public allelic wealth displayed similar trends. All summary data for these GD declines were statistically significant. This pattern fits the incidence rather well.

**Table 1** Genetic diversity measurements for breeds and groups

| Pressure | Nitrogen | Sodium | argon | Par | Helium |
|---|---|---|---|---|---|
| category | | | | | |
| Domestic | 322 | 6.234 | 4.34 | 0.11 | 0.567 |
| Wild (France) | 99 | 8.657 | 6.97 | 0.63 | 0.734 |
| Wild (Iberia) | 45 | 10.429 | 9.89 | 5.01 | 0.786 |
| Breeds | | | | | |
| English Silver | 7 | 2.897 | 3.19 | 0.05 | 0.490 |
| Netherland Dwarf | 22 | 4.234 | 2.45 | 0.06 | 0.455 |
| Champagne Silver | 23 | 2.567 | 2.56 | 0.05 | 0.478 |
| Himalayan | 21 | 3.234 | 2.23 | 0.05 | 0.460 |
| French Lop | 29 | 3.983 | 1.38 | 0.14 | 0.530 |
| English Spot | 23 | 4.003 | 2.34 | 0.03 | 0.467 |
| New Zealand | 34 | 3.634 | 3.98 | 0.09 | 0.498 |
| Giant Rabbit | 22 | 3.212 | 2.46 | 0.04 | 1.501 |
| French Ankara | 23 | 3.765 | 2.39 | 1.08 | 0.493 |
| Belgian Hare | 20 | 3.023 | 3.14 | 0.02 | 1.346 |
| Hungarian | 5 | 2.236 | 3.01 | 0.02 | 0.429 |
| Chinchilla | 27 | 3.854 | 2.89 | 0.11 | 0.534 |
| Rex | 22 | 3.621 | 2.36 | 0.05 | 0.595 |
| INRA 1077 | 54 | 2.734 | 2.13 | 0.01 | 0.367 |
| INRA 9077 | 11 | 2.245 | 2.78 | 0.09 | 0.428 |
| Thuringer | 11 | 2.629 | 2.30 | 0.05 | 0.419 |
| Castor | 8 | 2.639 | 2.06 | 0.00 | 0.320 |
| Chinchilla | 5 | 2.128 | 2.34 | 0.00 | 0.386 |
| White | 7 | 2.345 | 2.29 | 0.03 | 0.478 |
| Mean* | | 3.934 | 2.39 | 0.05 | 0.589 |
| SE* | | 0.123 | 0.024 | 0.003 | 0.002 |
| Vienna White | 20 | 2.285 | 2.39 | 0.06 | 0.430 |

The GD observed among the many breeds is estimated similarly, and most of the time modestly, which suggests additional reproduce formation bottlenecks. The typical anticipated inbreeding across mate was 0.477, and the median number of alleles was 2.39, according to the 8-gene minimum sample size.

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Values for individual number of alleles were also low, averaging 0.05. AMOVA was performed for three types: (Table 2 figure 1**(**A) Sum of squares of wild Iberian (B) Variance component of wild Iberian, figure 2 (A) Sum of squares of domestic (B) Variance compounents of domestic figure 3 (A) Sum of squares of wild french (B) Varience components of wild french) lists further sampling areas as well as four sample points from the Hibriain, a sample of nine locations from France, and 16 domestic breeds. The populations with the highest levels of variety were domestic rabbits (20%) and WR from Spain (13%), followed by WR from the Hibriain (6%).

**Table 2** Molecular variation analysis for domestic breeds and wild regions

| | source of variation | d.f. | total squares | variational elements | Variation percentage |
|---|---|---|---|---|---|
| Wild Iberian | Within individuals | 39 | 530.5 | 13.60256 | 80.6 |
| | Among localities | 3 | 120.019 | 1.14629 | 7.79 |
| | Among individuals within localities | 35 | 627.994 | 2.17006 | 12.72 |
| Total | 77 | 1278.513 | 16.91891 | | |
| Domestic | Within individuals | 340 | 2518 | 7.40588 | 68.95 |
| | Among individuals within breeds | 324 | 3010.446 | 0.94281 | 8.90 |
| | Among breeds | 15 | 1651.728 | 2.39515 | 20.6 |
| Sum | 701 | 7456.235 | 11.84235 | | |
| ferus French | Among people in small communities | 83 | 892.053 | -0.13162 | -1.06 |
| | Among localities | 8 | 358.409 | 1.79126 | 14.16 |
| | Within individuals | 92 | 1013 | 11.01087 | 86.08 |
| Total | 183 | 2263.462 | 12.67051 | | |

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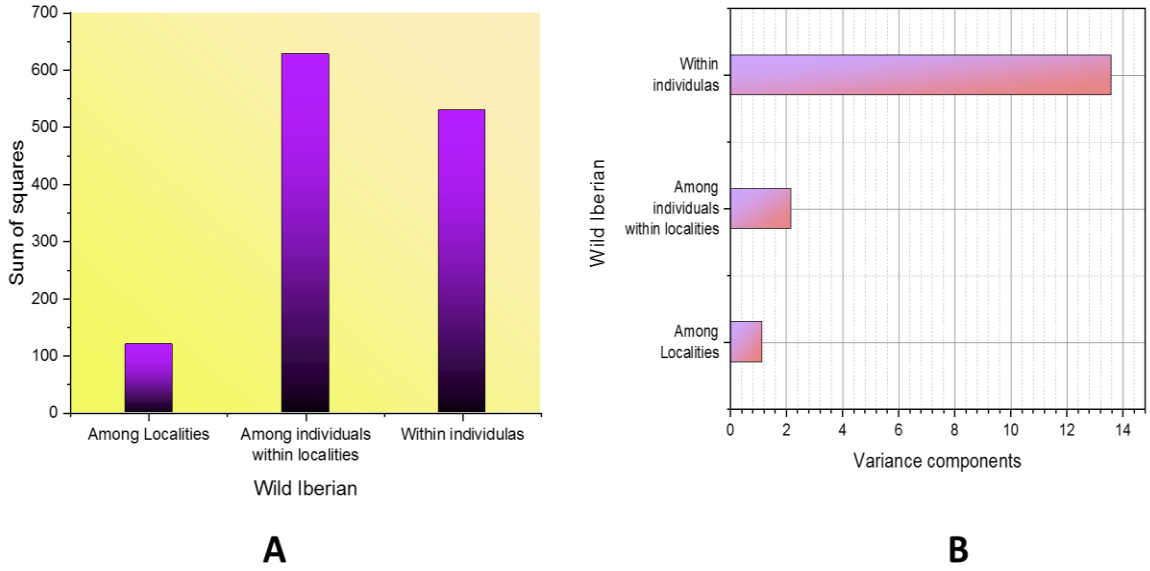


**Figure 1 (A)** Sum of squares of wild Iberian **(B)** Variance component of wild Iberian

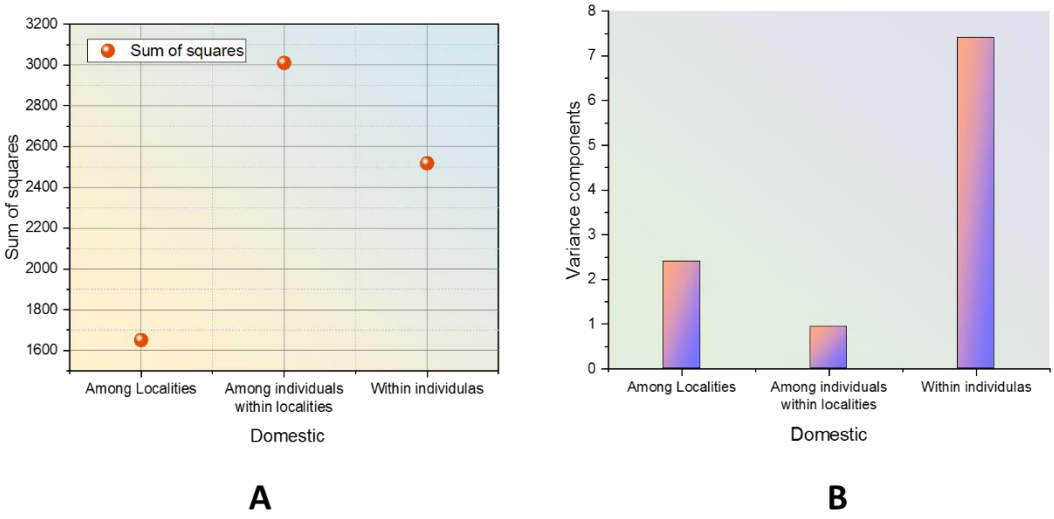


**Figure 2 (A)** Sum of squares of domestic **(B)** Variance compounents of domestic

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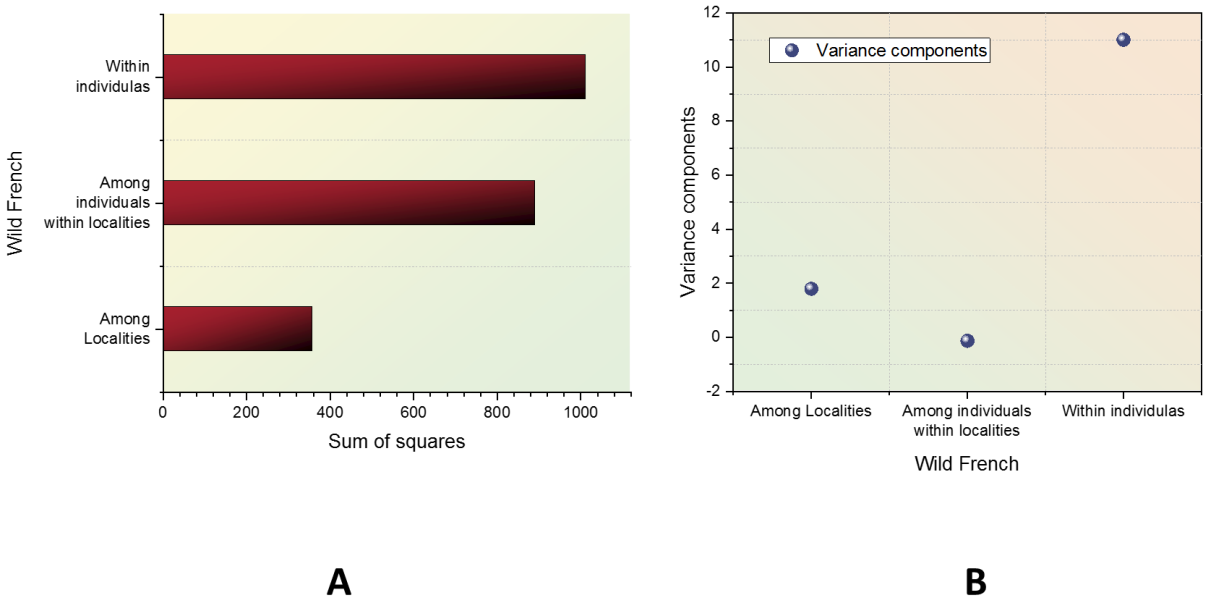


**Figure 3 (A)** Sum of squares of wild french **(B)** Varience components of wild french

**Genetic variety is lost during domestication and breed development**

We used a resampling approach to further explicit quantification of the degree to which the initial domesticated period reduced GD, the establishment of Spain as a colony, and the method of developing breeds. The benefit of this method is that it lessens biases brought on by inbreeding, sampling closely related people, or using different sample sizes across groups. Helium served as our summary of the GD reduction. By taking a single chromosome from every single one of the 4 wild Iberian populations, and 9 wild French groupings, and dividing the results by 1, researchers were able to determine how much variety was lost when Spain was colonized. Sampling one provided an estimate of the diversity loss brought on by the first domestication phase.

We estimated that the native range's migration of Spain in Hiberia resulted in an 11% reduction in genetic variation, a 20% drop at the beginning of the domesticated procedure, and a 21% reduction in the method of breed development when aggregated among breeds. These findings are consistent with serial population contractions. For specific breeds, the GD loss varied from 13% for Rex to 37 for Belgian Hare.

**Breed-specific genetic linkages and population-level trends in rabbits at home**

We created two distinct trees of people using solely the domestic dataset, utilizing lengths between chords and allele-sharing, respectively. Similar findings were obtained from the inferred trees, which also demonstrated that domestic rabbits had a well-defined population structure. Individuals of a particular breed tended to congregate in most instances, demonstrating that domestic rabbits have a higher genetic affinity for their breed than for other breeds.

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Instead of using individual distances, we generated add acceptance trees using chords and allele-sharing similarities across species to more extensively investigate the relationships between species and obtain supporting values. In compliance with we solely took into consideration the majority-rule requirement correlations to be statistically confirmed if over fifty percent of the duplicates produced a similar arrangement.

The produced trees continually demonstrated that all breeds were placed collectively and that, with good quantitative support, all domesticated breeds originated from wild French rabbits. Additionally, several breed ties matched up with knowledge of their historical ancestry. For instance, historical documents show the Flemish Giant is the breed that gave rise to the Hungarian Giant and the breeds Flemish Giant. Using the clustering program STRUCTURE, we next looked at population structure trends among domestic rabbits. 340 samples from 16 domestic breeds were examined using this technique without any previous knowledge about the breeds' origins. There were between 2 and 30 pre-defined clusters (plots from all experiments are displayed. Researcheliumrs examined future intricacy within varieties by utilizing measurements of K that were greater than the total number of breeds, in addition to ancestral ties across organisms (inferred by the cluster that developed with a small value of K). Considering that there was minimal stability in the beginning values of K overruns, it appears to be a sign that domestic rabbits lack strong organizational structures. Further supporting this finding included the coefficient's substantial similarities all through the many runs, so we were forced to limit to a level of K about 9 to accommodate processing constraints. The layout of the plot shows a clear differentiation between a large proportion of the examined varieties when the overall population is assumed to be identical to the number of investigated breeds (K = 16), and successive runs start developing a pattern corresponding to higher amounts of K (assessed visually).

Only two outliers were found: The Castor and the Flemish Giant, which are grouped (perhaps due to the similarities they shared in the past). The placement of individuals belonging to the same breed into various clusters was driven by additional levels of substructure that we discovered in addition to the considerable breed difference. Higheliumr values of K (K > 19) marked the beginning of this extra substructure's consistent emergence, however in some instances, it came before the division of all breeds (for example, the New Zealand and Rex Strains. Wheliumn performed the Structural assessment separately for each breed, and these findings were verified. The Hardy-Weinberg equilibrium (HW) of the genes across the entire population is one of the fundamental hypotheses. To examine departures from HW across all markers for all breeds, we employed the technique of chi-square testing along with a search for small satellites that demonstrated significant variations across all specimens in naturally occurring populations.

Researcheliumrs discovered that just a tiny percentage of variables substantially departed from HW equilibrium after using a Bonferroni correction to account for further investigation. We discovered that, on an average basis, every breed's marker had this deviation, except for the New Zealand rabbits, which showed 19.05%, and four breeds (Thuringer, English Spot, and Vienna White, Hungarian Giant) displaying 0%. Breeds have traits that frequently deviate from the Hardy-Weinberg equilibrium. The DAPC also calculates each person's chances of participation in a particular cluster. In line with STRUCTURE, affiliations to groups identified

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through this non-model-focused technique indicated a distinct, well-established community pattern in the size of the domesticated population, with over 90% of those assessed being correctly allocated to the breeds of derivation.

The lack of further levels of organization beyond the level of genetics was also supported by the visual evaluation of between-population variance using dispersal plots, and almost all of the predicted groups were clustered in the plot's center. The English Spot, Belgian Hare, New Zealand, and Angora breeds were discovered to differ from the other breeds to a significantly high helium level.

## Conclusion

The population structure and GD of different domestic rabbit breeds show a wide range. Domestic rabbit breeds are categorized according to certain qualities and characteristics listed by various organizations and associations for rabbit breeding, which vary from nation to country. These breeds were carefully cultivated to have certain characteristics including size, fur type, color, and temperament. As a result, we exhibit a broad variety of genetic variability. Thoughts regarding inbreeding and its effects on genetic health are raised by certain breeds' limited breeding processes. Breeders, conservation groups, and geneticists have joined forces in conservation efforts to conserve uncommon and endangered rabbit breeds to solve these problems.

Additionally, selective breeding and the identification of distinctive genetic traits may lead to new breeds gaining acceptance over time. In the future, additionally, recent selection pressures, such as artificial selection for certain features, may have an impact on the degree of GD within each breed and may not fully represent the genuine natural genetic variety of the original wild rabbit populations. Determining the GD of wild rabbit species, which may be important for conservation efforts and comprehending wider evolutionary processes, may be difficult given that this inquiry largely focuses on domestic rabbit varieties.

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