GENETIC DIVERSITY OF RABBIT POPULATIONS IN TUNISIA USING MICROSATELLITES MARKERS

Ben Larbi M.[1]*, San-Cristobal M.[2], Chantry-Darmon C.[3], Bolet G[3].[4]

[1]Laboratoire des ressources animales et alimentaires, Institut National Agronomique de Tunisie, 43 Avenue Charles Nicole, 1082 Tunis, Tunisia
[2]INRA UMR 444 Laboratoire de Génétique Cellulaire, chemin de Borde-Rouge 31326 Castanet Tolosan, France
[3]LABOGENA, INRA, Domaine de Vilvert – 78352 Jouy en Josas, France.
[4]INRA UR 631 Station d'Amélioration Génétique des Animaux, 31326 Castanet Tolosan, France
*Manel BEN LARBI: arbi_mana@yahoo.fr

ABSTRACT

This study attempts to provide a comprehensive insight into the prevailing genetic status of Indigenous Tunisian rabbit population using microsatellite markers. Fifteen rabbit populations from villages of Tozeur and Gafsa area were analysed using a panel of 36 microsatellite markers. 294 individuals from these populations were genotyped. In general, high genetic diversity (observed heterozygosity ranging from 0.3 to 0.53) and large population differentiation (F_{ST} = 0.11) were observed. The current study is the first detailed analysis of the genetic diversity of the Tunisian indigenous rabbit populations. The data generated here provides valuable information about the genetic structure of the 15 rabbit populations and this can be used for designating priorities for their conservation.

Key words: Rabbit, Tunisia, local population, genetic diversity, microsatellites

INTRODUCTION

Understanding the genetic structure of domestic species provides a window into the process of domestication and motivates the design of studies aimed at making links between genotype and phenotype. The European rabbit (Oryctolagus cuniculus), progenitor of all domestic rabbits, offers an excellent opportunity for testing intraspecific population genetics models through the different molecular markers. Domestic rabbits have a single and recent origin of domestication, and the wild populations from which they originated are still abundant today and easily accessible. Rabbits exhibit exceptional phenotypic diversity, they are of great commercial value, and serve as important animal models in biomedical research (Carneiro *et al.*, 2011). Genetic diversity and phylogeography of European rabbit are well documented through different kind of markers as mitochondrial DNA (Biju-Duval *et al.*, 1991) Immunoglobulins (Van der Loo *et al.*, 1999) proteins (Ferrand, 1995) and microsatellites (Mougel, 1997; Queney, 2000; Alves *et al.*, (Submitted).

In Tunisia, village rabbit kept under smallholder-low input systems are considered important genetic resources that should be preserved against production threats. Indigenous rabbit are raised by smallholder farmers with little resources (Ben Larbi *et al.*2008). Characterization of these genetic resources will serve as essential prerequisite for the identification, effective management and utilization of Tunisian indigenous rabbit, which will facilitate their conservation.

In the present study, we used 36 microsatellite markers, to elucidate the degree and pattern of genetic variability in 15 local rabbit populations in the south of Tunisia explain their genetic relationship, and assess their integrity and degree of admixture.

MATERIALS AND METHODS

Sampling and genotyping of microsatellite markers

A random sample of local rabbit populations from 15 villages of Tozeur and Gafsa area with the support of the Office of Livestock and Pastures regional agencies was conducted. Morphological characteristics and geographical distribution of the sampled populations have been detailed by Ben Larbi (2006).

Blood samples were collected from 294 animals from the marginal vein of ear into in 5 ml vacutainer tubes containing EDTA as anticoagulant and stored at 4°C until analyses were performed. Considering the samples from the same village as a population.

Genomic DNA samples were extracted from white blood cells. Blood samples obtained from Tunisian rabbits were transported to LABOGENA laboratory (Jouy-en-Josas). DNA extraction was performed with a QiASymphony DNA kit (Qiagen).

For this study, a panel of 36 microsatellite markers well distributed over the chromosomes was selected for genotyping (Chantry-Darmon, 2005; Mougel *et al.*, 1997). The PCR amplifications were carried out by the GIE LABOGENA. After amplification using fluorescent primers, PCR products were migrated on capillary sequencer (3730xl DNA Analyzer d'Applied Biosystems).

Statistical analyses

Genetic diversity parameters within each population were calculated using the GENETIX 4.05.2 software package (Belkhir *et al.*, 1998) and specific custom-made programmes. The calculated parameters were mean number of effective alleles (A_e), observed (H_o) and expected heterozygosity (H_e) from Hardy–Weinberg proportions. Tests for deviation from Hardy–Weinberg equilibrium were performed by the GENEPOP 3.4 software (Raymond and Rousset, 1995), using the exact test of Guo and Thompson (1992). Genetic differentiation among and within the populations was estimated based on F-statistics (F_{IS}, F_{IT} and F_{ST}) according to Weir and Cockerham (1984), using the GENETIX software packages. Significance of non-zero F_{IS} values per population was established by permutation (1000 permutations per population) and computed using the GENETIX program.

The Reynolds genetic distance (D_R) was calculated for each pair of populations based on allele frequencies (Reynolds *et al.*1983) using the GENETIX software. Pairwise genetic distance between individuals was estimated from the proportion of shared alleles, according to Bowcock *et al.* (1994). In this manner, a 294 * 294 interindividual genetic distance matrix was generated. From this allele-sharing distance matrix, a dendrogram was then build using the hierarchical cluster (hclust) function and Ward's method (Ward 1963) implemented in the standard statistical software, R (http://www.r-project.org).

RESULTS AND DISCUSSION

Genetic diversity within populations

In total, 119 alleles were observed for the 36 loci surveyed across the 15 populations. The number of alleles per locus per population ranged between two (INRA0105, INRA0143 and INRA0274) and 18 (INRA0172) with an average of 3.30 (Table 1). The highest effective number of alleles (A_e) was computed. Ne population showed the highest mean effective allele number (4.55) while Gh remained the population with the lowest locus variability (2.88).

Table 1 shows also the values of observed and expected heterozygosity. Expected heterozygosity across all the populations varied between 0.39 and 0.58. Dg population showed the highest genetic variability (as measured by observed heterozygosity), while Gh population showed the lowest. The difference between expected and observed homozygoty (F_{IS}) was also calculated for each population.

Table 1: Within-population summary statistics: number of data (N), of effective alleles (Ao), observed heterozygosity (Ho) and expected heterozygosity (He) of local Tunisian rabbit populations

Population	N	H_e	H_o	A_o	F_{is}
Je	17	0.48	0.43	3.11	0.14024
Ai	26	0.45	0.4	3.16	0.13896
Dk	25	0.54	0.56	4.47	0.14818
Dg	24	0.58	0.46	4.25	0.22514
Ec	11	0.42	0.37	3	0.15532
Gh	13	0.39	0.3	2.86	0.26252
Ha	20	0.52	0.53	3.58	0.018 (NS)
Li	41	0.54	0.47	4.11	0.14711
Ne	39	0.58	0.44	4.55	0.26278
No	17	0.56	0.42	3.77	0.27370
Si	11	0.46	0.35	3.33	0.29767
So	10	0.51	0.51	3.52	0.05530
Tz	25	0.57	0.51	4.08	0.12503
To	18	0.57	0.47	4.19	0.20546

Genetic variation and relationship between populations

The global deficit of heterozygotes across populations (F_{IT}) amounted to 9.1%. No overall significant deficit of heterozygotes was found in the analysed loci ($F_{IS} = 0$). The overall genetic differentiation among populations (F_{ST}) was low (1.1%).
This genetic differentiation (F_{ST}) among populations (1.1%) implies that 98.9% of the total genetic variation was explained by individual variability. This level of differentiation, although rather low, is within the range reported in the literature for F_{ST} values in rabbit European populations (Alves *et al.*, In press)

Genetic distances between populations were measured by pair-wise F_{ST} .The Ai population was the most differentiated one. It's a population from a remote village and difficult access. The largest similarity was detected between Ne and Ha (populations from tow near villages) animals ($F_{ST} = 0.057$). The degree of genetic differentiation among the studied populations and the low levels of significance for the between population F_{ST} estimates indicate a relatively large gene flow between these populations. Principal components analysis was performed including all animals and loci using allele frequencies to summarize population relationships (Figure 1). The analysis indicated the very clear separation between three groupings representing Ai population, the Dg population and other population. A total of approximately 12% of the variance accounted for the first three dimensions of the PCA (Figure 1). Axis 1 (approximately 4.4% of total variance explained) separated Dg population from the other populations, while Axis 2 (approximately 4.6%) further separated Ai population from all populations.

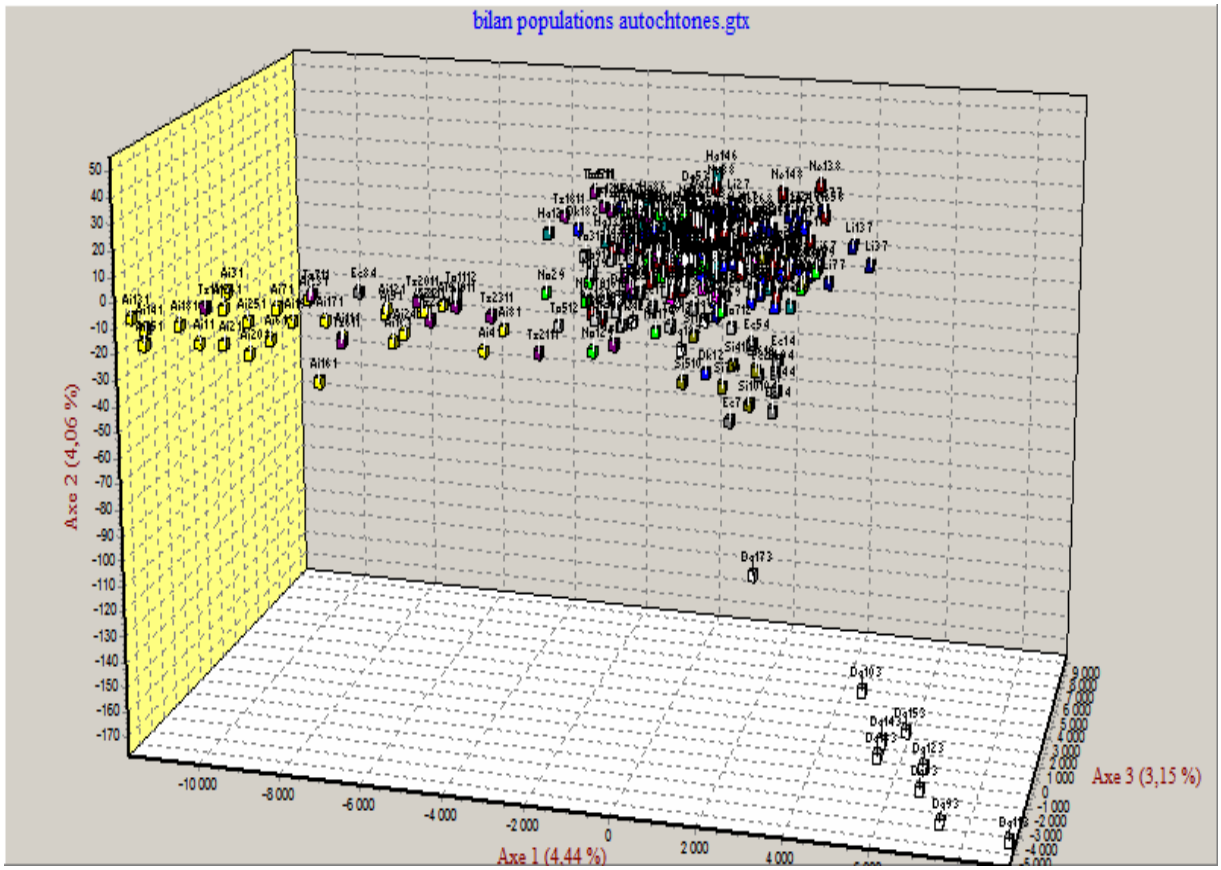

Figure 1: Plot of multivariate correspondence analysis showing the distribution of individual animals of the 15 populations. Per cent value in each axis indicates contribution to the total genetic variation.

Allele frequencies were also used to estimate Reynolds genetic distances for each pair of populations. Figure 2 shows the dendrogram constructed using allele-sharing distances between individuals. The largest branch length seen for Ai population reflects its difference with the other populations, it is an isolated population it does not show a great variability, it is a population that has evolved in its place. Dg is a population that had migrated, Tz is a population with high heterozygosis, and it is near to Ai (view genetic distance).

The Ne population shows a high heterozygosis. Ha is intermediary population between the population of the governorate of Tozeur and Kebili. Dk is an isolated population with large variability.

CONCLUSIONS

The current study is the first detailed analysis of the genetic diversity of the Tunisian indigenous rabbit populations. The knowledge of genetic diversity is paramount to the conservation of these populations but also for the identification of loci involved in economically important traits.
The knowledge thus generated would enable prioritization and monitoring of the indigenous Tunisian rabbit biodiversity for its efficient management, improvement and conservation.

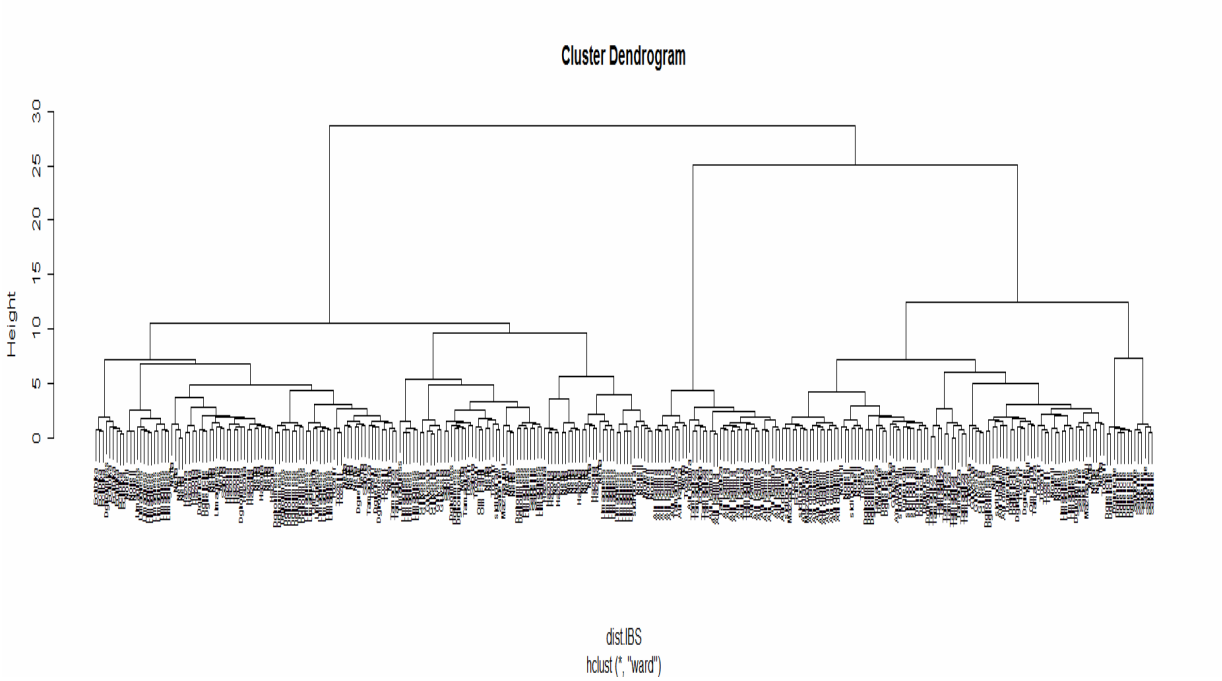

Figure 2: Dendrogram based on the microsatellite data. The tree branch sizes are proportional to the allele-sharing distances among the studied animals.

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