THE EUROPEAN RABBIT : WILD POPULATION EVOLUTION AND DOMESTICATION

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Abstract - Populations of rabbits (*Orcytolagus cuniculus*) can be organized in two well differentiated groups (A and B) according to their characterization with molecular and osteological markers. Group A is restricted to S. and S.W. of the Iberic Peninsula while populations of group B are found in various places outside this area. Domestic rabbits belong to this last group. As evidenced from data on ancient bones (up to 12 000 years BP) mtDNA type B1 originated from Spain. Animals carrying this type were probably introduced in South of France at late Roman time and their dispersal was successful enough to have this mtDNA type the most frequent one in domestic races.

INTRODUCTION

In relation to the needs and the interests of society the European rabbit (*Oryctolagus cuniculus*) has acquired various considerations : a game reserve, a domestic animal, a pest for crops and true plantations. Contrary to most domesticated mammals this species is the only one of Western European origin. The earliest fossils, dated about 6 million years (Myr), were indeed found in Andalusia (LOPEZ-MARTINEZ, 1989). Both paleontological and archaeozoological data indicate that rabbits first intrusion in France dates back to less than 1 Myr (PAGES, 1980). From then onwards the range of rabbit dispersion fluctuated in relation with climatic changes : extensions and retrieval on refuge locations follow the cycle of warm or cold periods (DONNARD, 1982). Man interfered in the spontaneous dispersal of this species. He introduced it in places and regions which were not originally colonized with the aim of building up game reserves and later of developing domestication, breeding and productions (CALLOU, 1995). The latter goal became prevalent at the turn of the nineteenth century. Other factors also bear on the present extension of rabbits such as predators multiplication, man directed reductions of effectives to prevent crop destructions, viral infections (myxomatosis or haemolytical disease). We aim at ranking these various parameters that govern the presently observed geographical pattern of rabbit genetic diversity and at evaluating their reciprocal relations. Our approach is a two-step one : in a first phase a systematic survey of present wild and feral populations as well as domestic races has been launched. It is carried on with molecular genetic markers, namely mitochondrial DNA (mtDNA) genes and, more recently, nuclear DNA microsatellites, as well as osteological characters. This work is done in tight collaboration with the laboratories of W. van der LOO in Belgium, N. FERRAND in Portugal which respectively analyze under the same protocol immunoglobin genes, blood and liver proteins polymorphism.

A first set of data had evidenced the existence of two maternal lineages, labelled A and B (BIJU-DUVAL *et al.*, 1991, MONNEROT *et al.*, 1994), dating back to 2 Myr. Lineage A was described in Andalusia and Estramadur while lineage B was observed in Northern Spain, France, England, Australia and Kerguelen. Their limits in Iberic Peninsula had to be precised.

The second phase of our approach involves the examination of the processes that generated the present pattern of diversity organization (MONNEROT *et al.*, 1994) using ancient DNA studies and present population dynamics (HARDY *et al.*, 1995).

This paper presents data obtained along these two lines and discusses them in relation with information obtained by our colleagues.

MATERIAL AND METHODS

Animals

Additional wild animals were trapped in various localities specially in France, Spain and Portugal (Figure 1). On the whole more than 400 animals have now entered our collection. Domestic rabbits were from various breeders. Ninety three animals have been studied so far. They belong to 13 races.

Material

In some instances the whole animal was used; different tissues (lung, liver, kidney etc...) were kept at -20°C, blood collected and treated according to further studies (VAN DER LOO *et al.*, 1990, FERRAND, 1995). As often as possible, only blood was collected from ears and the animals stayed alive.

Ancient bones

Ancient bones were collected and processed as described in HARDY *et al.*, 1995. Eighteen additional bone samples were from Iberic Peninsula (Figure 2).

DNA extraction

Total DNA was extracted from soft tissues or ancient bones according to HARDY et al., 1995, and directly used for amplification.

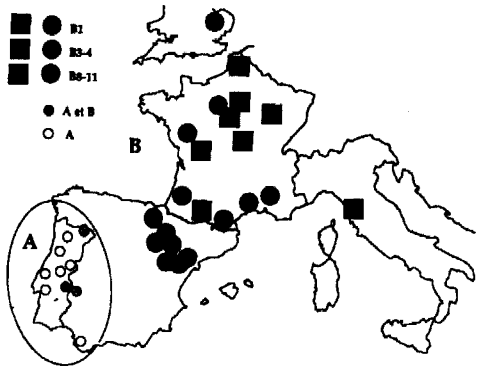
Amplification

Amplifications of mitochondrial DNA (233 bp from cytochrome b gene) were done following HARDY *et al.*, 1995. When necessary, amplifications involved a fragment (565 bp) from the 5' domain of the mt non-coding region using the following primers: Pro1 CCACCATCAGCACCCAAAGCT and NC4 ATGGCCCTGAGGTAAGAACC.

Analysis of amplified fragments

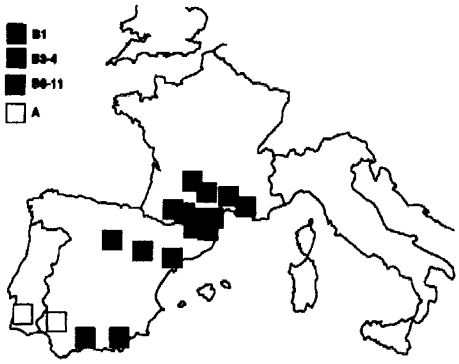
Depending on the resolution needed the amplified fragments were either directly sequenced (HARDY *et al.*, 1995) or anlysed through RFLP.

Figure 1 : Distribution of rabbit mtDNA types from Middle Ages to present times



Circles stand for present populations and squares for ancient populations.
Small figures represent mtDNA types from the maternal lineage A and large ones from the maternal lineage B.

Figure 2 : Distribution of rabbit mtDNA types from prehistoric to roman times



RESULTS AND DISCUSSION

A and B maternal lineages

The newly examined populations from Europe (8 from Portugal, 1 from Azorean Islands, 2 from France and 7 from northern Spain) provide a better geographical coverage of the distribution of wild rabbits in its original dispersion area (Figure 1). The occurrence of two maternal lineages initially discovered by an RFLP study of whole mt DNA (BIJU-DUVAL *et al.*, 1991) has been fully confirmed by sequencing or RFLP analysis of mtDNA fragments: each new mtDNA sample has been assigned to either A or B lineage (Figure 1). The maternal lineage A (polymorphic) is only localized in the Western and South-Western part of the Iberic Peninsula. Lineage B is present in Northern Spain (with rather polymorphic populations), in France (where population polymorphism is less extensive) and dispersed all over the world in the populations introduced by

man. Up to now all domestic races belong to the B lineage. Immunoglobin diversity data (VAN DER LOO *et al.*, 1990) as well as overall protein polymorphism (FERRAND, 1995) also point at the occurrence of two genetic groups that strictly coincide will the A and B maternal lineages. Properties of these two groups are very characteristic and suggestive of two subspecies.
Similarly some morphological characters (cranian and post-cranian) turn out to be distinctive of some significant differences between populations and allow to envisage an independent approach of the organization of genetic diversity of rabbits (VIGNE *et al.*, 1994).
Since the nuclear alleles found in the B group are only a part of the most frequent of the A group (FERRAND, 1995), B animals can be considered as a subset of the A ones. A conceivable scenario proposes that some animals moved from the South/South-West of the Iberic Peninsula to Northern Spain and Southern France, got separated: Climatic changes during the glaciation periods are acceptable candidates for this, and generated the B group (Figure 1).
Such an hypothesis could be tested in systematic analysis (with both nuclear and mitochndrial markers) of mixed populations such as Vila Viçosa (East Portugal) and Bragança (North-East Portugal). Only three cases where A and B maternal lineages coexist have been encountered up to now (Figure 1). Preliminary data indicate that in Vila Viçosa (East Portugal) and Badajoz (Estramadur) such a situation could reflect the original stock. On the contrary in Bragança (North-East Portugal) A and B animals on the site could be the consequence of a recent contact between two previously separated groups.

Mitochondrial DNA polymorphism within the B lineage; domestication

In a second phase we questioned the genetic organization of the B animals, characterized by a large geographical distribution and the connection of all domestic races.
The mt DNA polymorphism of B animals of wild populations (Figure 1) can be organized in three main groups (B1, B3-4, B8-11) with specific locations : B8-11 in northern Spain, B1 and B3-4 in northern Spain and France. The 93 domestic rabbits sampled constitute 29 independent maternal lineages : 3 of them are B3-4 and 26 B1.
For nuclear genes, domestication did not lead to any reduction of genetic diversity : the majority of alleles in wild animals from Navarra or France is also found in domestic races (FERRAND, 1995). This is consistent with the extensive morphological and color diversity of the 50 races catalogued in France. If some domestic morphotypes are rather similar to some wild animals from France or Navarra, most farm animals are easily distinguished from wild ones by their increased size or by the prevalence of some cranian characters (frontal line and frontal-nasal suture). It is difficult from such data to infer an acceptable scenario of the evolution of B rabbits. Clearly we observe the results of both natural processes of evolution and of changes directed by man. In the last ten years for instance wild animals from French populations have been moved to Navarra and we do not know how these events have contributed to the present genetic diversity.

Origin of domesticated rabbits

Two approaches have been devised to overcome the difficulties in knowing recent genetical pollutions in wild rabbit populations: 1) By the examination of nuclear microsatellites we intend to develop a finer genetic labeling of individuals and lines. 2) By the study of the DNA present in bones found in dated archeological sites we intend to reconstruct locally the genetic succession of animals.
Eleven microsattelite loci have been selected because of their polymorphism and their stable inheritance through meiosis. They double the number of available genetic markers and work is on the way to characterize wild populations and identify those where recent introductions occurred.
On the other hand a number of ancient bones are available for historical reconstructions (more than 150). However, as previously stated (HARDY *et al.*, 1995) great care is necessary in the consideration of this material : Bones must come from a well defined and unperturbed stratigraphic layer (rabbits dig holes...). They must be in a good state of conservation and precisely dated (radiometric datation when any doubt). At last their biochemical study needs to be carried in strict laboratory confinement conditions in order to exclude contaminations. DNA (although fragmented) can be recovered from bones as old as 10 000 years : mt DNA has been examined first. Results obtained with part of the cytochrome b gene are presented in figures 1 and 2. The absence of B1 animals in France until roman times (Figure 2) previously reported (HARDY *et al.*, 1995) contrasts with their presence in Eastern and Southern Spain in bones dating back to 7000 and 4 000 years respectively. From the Middle Ages the movement of rabbits above the 46 nd parallel in France and later in Europe is concomitant with the introduction of B1 individuals. The situation in present domestic races appears related to this process (26 B1 and 3 B3-4 out of 29 domestic lines studied). We suggest that B1 animals originated from East or South-East Spain were introduced in Southern France first (by the Romans ?) and kept

in the leporaria. Contacts between them and the local B3-4 animals created the populations where man sampled animals for transfers and domestication from the Middle Ages. This scenario may be put under test since it predicts that among B1 rabbits dispersed by man all over the world one should find nuclear alleles characteristic of the Spanish populations they come from. On the other hand this also suggests that a fragmented organization of genetic diversity has existed in Spain in ancient times between the era of rabbit appearance as a species and present times where Man has modified it. The examination of microsattellites polymorphism in the DNA extracted from ancient bones should help to clarify this point.

At last, turning to the future, we must reconsider the present strategy of reconstruction of game reserves and wild populations. Per example, if we persist in mating wild males and domestic females to produce progenies and transfer them back to nature, natural populations might be enriched in B1 type animals. Whether this presents an advantage or a danger needs some debate.

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Le lapin européen : évolution des populations sauvages et domestiques - L'utilisation de marqueurs moléculaires et ostéologiques a permis d'organiser les populations de lapins (*Orcytolagus cuniculus*) en deux groupes (A et B) bien différenciés. L'aire de répartition du groupe A est limitée au Sud-Sud-Ouest de la Péninsule Ibérique alors que des populations appartenant au groupe B sont rencontrées à peu près partout de par le monde. Les lapins domestiques appartiennent à ce dernier groupe. Une analyse d'ADN mitochondrial à partir d'os anciens (jusqu'à 12 000 ans) a montré que le type d'ADN mitochondrial le plus fréquemment observé chez les lapins domestiques (B1) est originaire d'Espagne où il est resté localisé jusqu'à la fin des temps romains. Des animaux porteurs du type B1 ont sans doute été d'abord introduits dans le Sud de la France puis dispersés avec des représentants des populations locales qu'il ont éventuellement supplantés au point que ce type moléculaire est majoritaire chez les lapins domestiques.