Rabbit and man: genetic and historic approach

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Genet Sel Evol (1994) 26, Suppl 1, 167s-182s

Original article

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Summary – New data on mitochondrial DNA polymorphism in *Oryctolagus cuniculus* confirm the existence of 2 maternal lineages which are geographically well separated. They provide evidence in favour of northern Spain (and possibly southern France) as a refuge area for rabbit populations during the last major glaciation. Osteological analysis leads to the discrimination of populations and the recognition of discrete qualitative characters, which provide additional markers to describe population diversity. Characterization of different domains of mtDNA from ancient bones was used as a tool to resolve the general question of the origin of present populations. Results obtained from ancient and present rabbits living in Zembra (Tunisia) showed that the present-day population has descended from animals present on the island some 2 000 years ago. Archaeozoological data provide evidence for their introduction by Bronze Age, Punic or Roman people.

rabbit / mtDNA / osteology / ancient DNA / domestication

Résumé – Le lapin et l'homme : approche génétique et historique. *L'étude approfondie du polymorphisme de l'ADN mitochondrial chez le lapin,* Oryctolagus cuniculus, *confirme l'existence de 2 lignées maternelles bien séparées géographiquement: au Sud de l'Espagne pour l'une, dans le reste de l'Europe pour l'autre. Elle suggère que le Nord de l'Espagne, et éventuellement le Sud de la France, ont été une des zones refuge des populations de lapins lors des dernières importantes glaciations. Une analyse ostéométrique a été menée sur quelques populations. Elle a identifié des caractères discrets qui peuvent désormais être pris en compte dans la description de la diversité des populations. La caractérisation moléculaire de différentes régions de l'ADN mitochondrial extrait à partir d'os anciens a permis d'appréhender la question de l'origine, dans le temps, de populations de lapins site par site : les résultats obtenus à partir de matériel ancien et récent ont montré que les lapins actuellement présents sur l'île de Zembra (au large de Tunis) sont les descendants de ceux qui y vivaient il y a presque 2 000 ans. Les données archéozoologiques permettent*

de préciser que l'introduction du lapin sur cette île a pu se réaliser par les peuples de l'âge de bronze, les Puniques ou les Romains.

lapin / ADNmt / ostéologie / ADN ancien / domestication

INTRODUCTION

The rabbit, *Oryctolagus cuniculus*, has 2 interesting characteristics: on the one hand, wild populations still exhibit ample genetic diversity, on the other, this animal is one of the rare mammals originally domesticated in Western Europe. The means by which genetic diversity has been maintained remains to be explained since, in their ecosystem, rabbits may be a prey for numerous predators and are affected by epizootic diseases (such as haemolytic viruses or myxomatosis). In their natural environment, populations levels and equilibrium are also dependent on human activities; rabbits are often destroyed following major damage to fields and replanted forests. Moreover these animals are also used for hunting exploitation and consequently are often reintroduced in large numbers in places where game reserves are created.

The hypothesis for southern Spain as the area of origin for rabbits comes from the discovery of the oldest known rabbit fossil in Andalusia (6.5 million years, Lopez-Martinez, 1989). Later glacial events (until 12 000 years ago) probably strongly affected the biogeography of the rabbit in its original distribution area, *ie* Iberia and Mediterranean France. Recently human interference has affected the dispersion of this species through transportation within this area and outside, eventually accompanied by domestication and restocking.

Previous studies on some polymorphic markers (Ferrand *et al*, 1988; Arana *et al*, 1989; Biju-Duval *et al*, 1991; Van der Loo *et al*, 1991) have already provided first-hand information about the extent and the distribution of diversity in European rabbit populations. These preliminary results on populations from Spain and France are in agreement with the hypothetical view described above since they show that genetic diversity is consistently much larger in populations from southern Spain than in the other areas, but they do not prove it. A more exhaustive analysis of genetic diversity (including different markers), both in various populations and in relation to the geographical distribution of rabbits is necessary to support the proposed hypothesis conclusively. Moreover, the knowledge of the evolution of populations, through time, at given sites, is essential to understand the origin of the biogeography of genetic diversity. This can now be appraised through an archaeozoological approach complemented by a molecular study. Altogether, present and archeological data should help to define the role man has played in rabbit dissemination.

New data on mitochondrial polymorphism presented here confirm the existence of 2 maternal lineages, geographically well separated. They argue in favor of northern Spain (and southern France?) as a refuge area for rabbit populations during the last important glaciation. The osteological analysis leads to the recognition of

discrete qualitative characters which provide additional markers to describe population diversity. Characterization of different domains of mtDNA from ancient bones was used as a tool to resolve the general question on the origin of present populations. Results obtained from ancient and present rabbits living in Zembra (Tunisia) show that the present-day population is the progeny of animals introduced on the island at least 2 000 years ago.

MATERIALS AND METHODS

Animals

Wild *Oryctolagus cuniculus* are from 'natural populations' *ie* populations without evidence of recent import of animals from elsewhere. Domestic rabbits were obtained from INRA laboratories (New Zealand, California) and the Institut Pasteur (Burgundy, Tunisian and Portugese domestic types). Table I indicates the origins and numbers of all the animals studied; those with an asterisk have previously been described in Biju-Duval *et al* (1991).

Techniques

DNA extraction

Mitochondrial DNA for restriction analysis was extracted from soft tissues and purified according to Biju-Duval *et al*, 1991.

Total DNA was extracted from frozen tissues following Kocher *et al* (1989), and from bones as already described by Hardy *et al* (1994).

Restriction analysis and phylogenetic trees

For each mtDNA, the sites recognized by 14 restriction enzymes have been determined and mapped. In order to collate the results, the different mtDNA types have been named according to their maternal lineage (A or B see further in the results) and numbered following their description. This led us to rename the types already presented by Biju-Duval *et al* (1991). The correspondence is as follows: Lo 1 to 7 will now be referred to as A 1 to 7; Se = B 8; Tu = B10; Az = B9; Tv1 = B4; Tv2 = B5; Ce = B3; Fb1 = B1; Fb2 = B2; Ze1 = B6; and Ze2 = B7.

Phylogenetic analysis of restriction site data, based on parsimony, was performed using the phylogeny analysis using parsimony program (PAUP, Swofford, 1989). Nucleotide distances were calculated following Nei and Li (1979) and standard errors estimated through the jacknife method (Efron, 1979). Dendrograms were obtained through the neighbor-joining algorithm (Saitou and Nei, 1987) following the program 'restsite' (Miller, 1991)

Amplification and sequencing

The conditions of amplification within the 16s-rRNA gene, sequencing and restriction digests were previously described by Hardy *et al* (1994).

Table I. Origin of rabbits and mtDNA types.

Wild rabbits			
Origin	*Locality*	*Number of individuals*	*mtDNA types*
France			
Ile de France	Versailles	3*	B1
Pays de Loire	Cerizay	3*	B1, B3
Camargue	La Tour du Valat	33*	B4, B5
Landes	Arjuzanx	8	B1, B3
Drome	Donzère	2	B1
Spain			
Navarra	Arroniz	1*	B9
	Castejon	2	B9, B11
	Sesma	1*	B8
	Tudela	2* + 12	B9, B10
Andalusia	Las Lomas	30* + 31	A1–A8
Estramadur	Badajoz	16	A4, A9–A11, B3
Tunisia Tunis gulf	Zembra island	17*	B6, B7
Great Britain			
East Anglia	Norwich	1	B1

Domestic rabbits		
Origin	*Breed*	*Number of individuals*
Institut Pasteur	Fauve de Bourgogne	4*
INRA – Jouy-en-Josas	New Zealand	3*
INRA – Castanet-Tolosan	New Zealand	1
	California	1

The number of individuals sampled is given according to the locality. When 2 numbers are given they correspond to 2 different samplings. Individuals already studied are noted with *. The different types are given according to the nomenclature described in the text

Amplification within the *cytb* gene was performed using the primers *cytb3* (L15367, ATGAAACTGGCTCCAACAAC) and *thr3* (H15915, CTCCATTCCTG-GCTTACAAGAC) and accomplished by 2 min at 93°C, followed by: 1 min at 93°C; 1 min at 55°C; 30 s at 70°C through 40 cycles; followed by 2 min at 72°C. The PCR conditions were: 10 mM Tris HCl pH 9.0 at 25°C; 1.5 mM $MgCl_2$; 50 mM KCl; 0.1% Triton X100; 150 mM dNTPs; and 1 mM primers. An aliquot was then submitted to asymmetric amplification with only one primer as follows: 2 min at 93°C; 40 s at 93°C; 33 s at 55°C; 45 s at 72°C; 35 cycles. The products were purified through phenol chloroform extraction and ethanol precipitation (50%, 2.5 M ammonium acetate) and directly sequenced using the sequencing kit from Pharmacia and either the primer *cytb3* or an internal primer *cytb6* (L15644, GCTCTTGTCTTATCTAT

CCTTG). Internal primers specific for rabbit mtDNA were used for amplifying and sequencing ancient DNA: *cytb6*, *cytb11* (L15691, ATGT CTAAACAACGTAG-CATG), *cytb12* (H15835, CTTGCGAGGGGTATA AGAATA) and *cytb13* (H15765, GCGACTTGTCCAATGGTGATG.

Dating bones

The ages of the layers of the ancient bones of *Oryctolagus cuniculus* were obtained through Ly-4382 carbon dating of the charcoals (Hardy *et al*, 1994) and confirmed by the examination of potteries in the corresponding layers.

Osteometric analysis

The main dimensions of skull and the lengths of the limb segments were appraised using 12 distinct measurements and the discrete characters were analyzed as previously described (Vigne *et al*, in press).

RESULTS

Population analysis through RFLP (restriction fragment length polymorphism)

Reexamination of the population from Las Lomas (Andalusia, southern Spain)

A preliminary sampling of the population from Las Lomas had already revealed noticeable polymorphism (Biju-Duval *et al*, 1991). The aim of a new study of this population was to evaluate the significance of the sampling procedure and to more accurately describe the diversity. The study of 31 additional animals caught alive identifies 7 mtDNA types among which only one was new (A8). We can conclude that the diversity appraised from the first sampling, conducted 4 years earlier, reflects well that of the whole population.

Table II gives the distribution of mtDNA types according to the territory of the rabbits. The existence of several mtDNA types (at least 3 and sometimes 4) in each locality signifies that a noticeable diversity exists at the level of family grouping, *ie* the burrow.

Population from Badajoz (Estramadur, southern Spain)

Among 16 rabbit extracts analyzed, 5 mtDNA types were recognized. Four of them belong to the A lineage (see below): A4, 9, 10 and 11. A4, the only one previously described from Las Lomas, is represented in this sampling by one animal. This means that the diversity of this population, located more than 200 km north of Las Lomas, is also noticeable with little overlapping with the mtDNA types described there. Three animals exhibited the type B3 relevant to the other maternal lineage (see later).

Table II. MtDNA types present in rabbits from the second sampling of Las Lomas*.

Burrow	*mtDNA type*						
	A1	*A2*	*A3*	*A4*	*A5*	*A6*	*A8*
Boyar	2	3	1	1			
Ahijones				2		3	1
Varelo	1			4		1	
Mediana		1		4		1	
Malabrigo	1		2		3		

* The number of rabbits exhibiting a given mtDNA type is tabulated in terms of burrow they belong to.

Population from Navarra (northern Spain)

Four sites were sampled in Navarra, less than 70 km apart, with a total of 16 rabbits. Due to the low number of animals from 3 of these sites (Arroniz, Castejon and Sesma) it seemed more reasonable at present to consider them all together as representatives of one extended population. Four mtDNA types were detected: B8, 9, 10 and 11, all new, and again specific to this area.

Populations from France

Eight individuals from Arjuzanx, southwestern France (Landes) and 2 from Donzère (southeastern France) were studied. These last 2 rabbits, as well as 3 from Arjuznax, carried a mtDNA type B1, *ie* the type found in domestic breeds, whilst type B3 was found in the other 6 rabbits.

Rabbit from Norwich (United Kingdom)

The mtDNA type B1 was detected in the single individual analyzed.

Phylogenetic relationships inferred from RFLP analysis

The complete set of data is given in the appendix. Each mtDNA type is defined by the presence or absence of each of the 110 sites recognized and mapped. Two programs were used to establish phylogenetic relationships between the 22 mtDNA types actually described through RFLP analysis: Paup 30L, which is based on parsimony; and neighbor-joining, which is based on nucleotide distances. In both cases, results on mtDNA from *Lepus europaeus* and *Sylvilagus rufescens* were included as out groups. Figure 1a and b present the phylogeny obtained which confirms that proposed previously with less data (Biju-Duval *et al*, 1991). The rare differences are relevant to the shortness of some branches: they clearly show 2 groups of mtDNA types, referred to as A and B. The divergence between the 2 groups is high enough to consider them as representative of 2 well-separated maternal lineages. Each group is substantially diversified.

Fig 1. Phylogenetic relationships between *Oryctolagus cuniculus*, *Lepus europaeus* and *Sylvilagus floridanus* mtDNAs. The different mtDNA types of *Oryctolagus cuniculus* are named according to the maternal lineage to which they belong (A or B). Le1, Le2 and Le3 refer to the 3 types found in *Lepus europaeus*, the type described in *Sylvilagus floridanus* is named Sy. a) Parsimony method (PAUP) in which the 3 has been rooted taking *Bos taurus*, *Homo sapiens* and *Mus musculus* mtDNAs as outgroups. b) Neighbor-joining method in which the network has been folded in order to align the 2 most distant types: B5 and Sy. The significance values (more than 50%) for a bootstrap analysis of 200 iterations are noted on the branches as percentages.

Reexamination of mtDNA type B1 through sequencing

As mentioned above and in a previous work (Biju-Duval *et al*, 1991) the mtDNA type B1 was detected in animals of different origins: various domestic breeds (Burgundy, New Zealand, California, Portugal and Tunisia) as well as natural populations (Versailles, Cerisay, Arjuzanx, Donzère and Norwich). This result was surprising in that these animals have various geographical origins and, for the domestic breeds, are derived from very different sets of crosses (Arnold, 1981, and Standard officiel des lapins de race, 1984). In order to question the apparent homogeneity of this group, we sequenced the last part of the cytochrome b gene. No variation was detected among the 522 nucleotides sequenced from mtDNA extracted from different well-known domestic breeds: California; Burgundy; New Zealand; domestic animals from Tunisia and Portugal, the race of which was not known; and wild B1 animals (Cerisay, Donzère and Arjuzanx, see above).

Morphometry

Results given by multivariate data analysis (ACP and AFD) of Las Lomas populations have shown that factors of total length and breadth of the cranium may well characterize a rabbit population (Vigne *et al*, in press). Although such an analysis on other populations (Zembra, Domestic, Camargue) is not yet complete, a preliminary approach has validated them for population comparison. For example, individuals of southern Spain (Las Lomas) can be assigned as small type whereas rabbits of the Camargue are larger than average.

Eight original qualitative discrete characters were found (Vigne *et al*, in press) most of them being related to the posterior part of skull: the shapes of the 'scutellum' (DS1); the *crista nuchae* (DS2, DS3); and the *foramen magnum* (DS4). According to the recognition of their presence or absence, the animals from Las Lomas, Zembra and domestic breeds can be distinguished (table III). For example, DS4a is significantly absent in domestic rabbits. The combination of some characters may also be informative (table IV). DS1b-DS2a and DS1b-DS2b are the only ones observed in domestic rabbits, the first being in the majority, whereas rabbits from Zembra exhibit many others. Furthermore, the frequency of each discrete character within the population can also be taken into account. The use of discrete characters henceforth appears to be highly informative for studying rabbit populations.

Table III. Characterization of populations by the presence or absence of 3 discrete characters relating to 3 populations of rabbits.

Discrete character	*Population*		
	Las Lomas	*Zembra*	*Domestic*
DS4a	+	+	
DS4d		+	+
DS4e	+		+

Table IV. Characterization of populations *via* combinations of discrete characters.

First character	*Second character*	*Third character*		
		DS3a	*DS3b*	*DS3c*
DS1a	DS2a		Zembra	
	DS2b	Zembra	Zembra	Zembra
DS1b	DS2a	Domestic		
	DS2b	Domestic	Zembra	

Ancient DNA

Archaeozoological surveys on Zembra island (Tunisia) provided 30 fossiliferous sites. The oldest, which dates from the Upper Pleistocene when the island was linked to the mainland, testifies to the absence of rabbit in the north-eastern Maghreb during this period. Sediments dated from the end of the Neolithic or at the beginning of the Bronze Age, when Zembra was already isolated, also did not provide any rabbit remains. The oldest bones come from late Roman layers dated from the 3rd-4th centuries AD, and other more recent sites have confirmed the presence of rabbits on the island during the later periods (Vigne, 1988; Hardy *et al*, in press). Unfortunately, no Punic or early Roman desposits have been found yet. Present data indicate that rabbit was introduced to Zembra between the Late Neolithic and the 3rd century AD (*ie* by Bronze Age, Punic or Roman people). A preliminary analysis of mtDNA (part of the 16S-rRNA gene) from ancient bones (dated back to 130–390 AD) has demonstrated that the corresponding animals carried type B mtDNA (Hardy *et al*, 1994). A more detailed study has been conducted in order to more precisely show which B type was present in these animals. Preliminary sequencing of different domains of the cytochrome b gene have shown that the last part was one of the most variable ones (Howell, 1989, and present work) and revealed a clear distinction between mtDNAs of types B1 and B7. Two overlapping fragments were amplified, using *cytb6-cytb13* and *cytb11-cytb12*, and sequenced. Among the 190bp examined, 4 non-contiguous nucleotides, located at sites 926,1016, 1023 and 1038 (numbered from the beginning of the gene) appeared variable within type B mtDNAs. They are all different when B1 mtDNA (from domestic stock) is compared to B7 (from Zembra): T, A, A, G instead of C, G, G, A, respectively. mtDNA amplified from ancient bones (the same as in the previous study, see above) exhibits the pattern C, G, G, A, which is identical to the mtDNA from rabbits presently living on Zembra. Consequently, we believe that these animals are descended from those present on the island almost 2 000 years ago.

DISCUSSION

Origin of European rabbit populations

As shown in figure 2, the present diversity of mtDNA is organized as follows. All mtDNA types related to lineage A belong to animals from southern Spain,

whereas all the animals from northern Spain, France, Norwich and Zembra (Tunisia) carry mtDNAs of lineage B. Paleontologic data indicate the origin of *Oryctolagus* genus in southern Spain 6–6.5 million years ago (Lopez-Martinez, 1989). The value of the divergence between molecules from lineage A and lineage B (4.5%) and the geographical organization of diversity (fig 1) support the proposal that the diversification within each lineage was established independently about 2 million years ago (based on a divergence rate of 2%/million years, see discussion in Biju-Duval *et al*, 1991). This means that after the original dispersion of animals over Spain, the ancestors of populations actually carrying either type A or type B molecules must have been separated (geographically) some time before the age of the ancestor molecule of each lineage. Following this scheme, progeny of one sub-group remained in southern Spain (A) when the other was first restricted to northern Spain (B, where the maximum of diversity is found). More recently, the latter spread over Western Europe was possibly achieved by human interference (see below). Data on nuclear variability are in agreement with this description. The number of alleles at the b-locus of the immunoglobulin light chain constant region is far higher in southern Spain than in the population from Camargue, while rabbits from northern Spain exhibit an intermediate situation (Van der Loo *et al*, 1991; Van der Loo, personal communication). Although there is some discontinuity in rabbit samplings in central Spain and Portugal, the recognition of northern Spain as a region with specific properties is sustained by a recent work of Palomo *et al* (in press). Taking into account geographic, climatic and geological parameters, as well as present species diversity, these authors demonstrate that 2 domains can be defined in the Iberian peninsula: a northern part corresponding to about a quarter of the peninsula, and a central-meridional region roughly south of the 42nd parallel. Thus, the recognition of northern Spain as a plausible refuge area for maternal lineage B in rabbits can now be integrated in a more general understanding of species history.

The origin of the 3 rabbits from Badajoz with type B mtDNA deserves special attention. An hypothetical import from northern Spain or France by man seems unlikely. The high density of rabbits in southern Spain and Portugal precludes any need for massive reintroduction, but restricted introduction cannot be excluded. At present, another plausible explanation is that Los Quintos is part of the secondary contact zone along which rabbits from maternal lineages A and B meet again after some time of isolation.

Recent history, the role of man

From the late Pleistocene until Classic Antiquity, rabbits only occupied the Iberian peninsula and a narrow area in southern France (Donnard, 1982). As a consequence of transportation by man, the species is now represented in a large part of Europe, in South America, in Australia and many oceanic islands. The colonization of Europe is the only one preceeding the 17th century (Angerman, 1974). Archaeozoological data on the colonization process are incomplete and must sometimes be treated with caution. Dating may suffer some uncertainties due to the burrowing habits of rabbits. However, 3 major phases can be recognized.

Throughout antiquity, the Phenician, Greek and Roman people may have played a role in rabbit dissemination, although this is still obscure. Historical texts mention

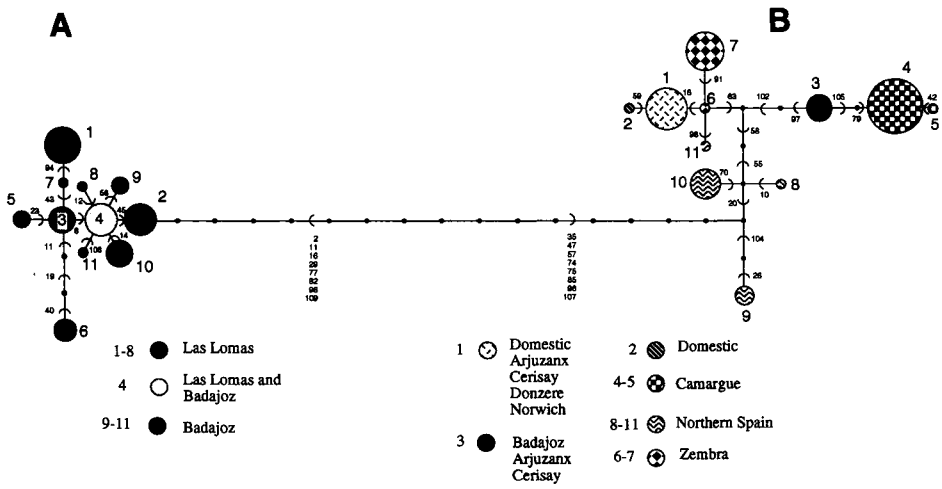

Fig 2. Geographical distribution and relationships of mtDNA types. Each circle of the parsimonious network represents one mtDNA type. Its size is proportional to the number of corresponding individuals, its design gives information on its geographical origin, and the related number corresponds to the code of the type within its maternal lineage (see table I). A and B refer to the 2 maternal lineages previously recognized. Black points represent potential intermediates. Numbers written between types indicate the restriction sites by which types differ (numbering is according to data given in the *Appendix*). They are present within the concavity and absent outside.

the introduction of rabbits in various regions of the western Mediterranean Basin by the establishment of *Leporaria*, ancestors of our Middle Age warrens (Bodson, 1978; Rougeot, 1981). Archaeozoological data are sometimes in agreement with this view but not always. For example, in Zembra, the absence of bones in a Late Neolithic layer, the age of the oldest rabbit bones, 3rd – 4th centuries AD (Vigne, 1988; Hardy *et al*, 1994) together with the evidence that Punic people occupied the island from the 6th to the 2nd centuries BC (Chelbi and Ghalia, personal communication) indicate that the introduction of the species must have been accomplished by Bronze age, Punic or Roman people. According to ancient mtDNA studies (Hardy *et al*, 1994 and present report) it can even be said that rabbits presently found on the island are the progeny of those living on the island at least 1 600 – 1 900 yr ago. On the other hand, in Corsica, the presence of rabbits in historical times was inferred (Bodson, 1978; Rougeot, 1981; Arthur, 1989) from the name '*cunicullariae*' given by Pliny the Old (*Nat hist* 3, 83) to the islands off Bonifacio and/or from Polybius' writings (*Nat hist* 12, 3) In fact, recent excavations have shown the absence of rabbits in Tyrrhenean islands at that time (Vigne, 1992). From this, it can be assumed that ancient authors confused *Oryctolagus cuniculus* with another Lagomorph species: *Prolagus sardus*, presently extinct, but abundant in Corsica and Sardinia at that time.

Most of the diffusion of rabbit in southern France and other western European countries was accomplished during the Middle Ages. Apart from a few descriptions

from the early Middle Ages which probably have to be related to the production of '*Laurices*' (Rougeot, 1981), texts indicate that the expansion was only important in the 9th – 12th centuries which is confirmed by osteoarchaeological data (Rougeot, 1981; Delort, 1984; Audoin, 1986). Around that time (10th century) in France, medieval warrens, previously restricted to nobles, were developed throughout the country (Gislain, 1980; Zadora-Rio, 1986). They remained open until mid-13th century, allowing some individuals to go back to the fields and found new colonies, completely independent of human interference and are probably the origin of present warren rabbits. Animals were also propagated through sales from one country to another and from gifts to foreign lords (Delort, 1984; Durliat, 1985). Domestication began with the 16th century (Rougeot, 1981; Audoin, 1986) while warrens were conserved (Zadora-Rio, 1986). After this period, 3 kinds of rabbits were living in Western Europe: wild rabbits (most of them coming from warrens), rabbits kept in warrens for hunting, and domestic stocks (Audoin, 1986).

Thus the most important diffusion through Western Europe took place between the 11th and 16th centuries AD, while the species was not domesticated but appropriated for hunting (Vigne, in press). Man managed to keep rabbit as a wild animal for the symbolic value of rabbit hunting (Houseman, 1990; Poplin, in press) and as a prestige and power instrument of a privileged social class (*ie* noble people). It seems clear that the absence of domestication of this species during the antiquity led to the dissemination of rabbits as wild animals. This human behavior is responsible for the foundation of wild populations, which can still be found all over Western Europe, because keeping them in a strict domestic status would have consequently limited their dissemination.

Origin of the rabbits taken for domestication

The analysis of restriction sites of mtDNA from a few races has revealed a surprising homogeneity: they all carry the B1 type. This type has also been detected in the rabbit from Norwich and in the population from Versailles, both being examples of animals transported by man at different times (the 16th century for Versailles and probably the 11th – 12th centuries for Norwich) and also in animals from natural populations (Cerisay, Arjuzanx and Donzère).

A more detailed examination by DNA sequencing confirms this result and no variation was detected in the 8 individuals analyzed in a variable region of the cytochrome b gene. Such an absence of variability may be interpreted in 2 ways. One may imagine that domestic breeds, some of them being more than 200 yr old (Arnold, 1981), as well as rabbits transported by man from the 11th to the 16th century were all issued from the same location. This is unlikely since it is known that some domestic stocks were established independently in different regions and even in different countries. Another, more plausible, explanation is that man has sampled individuals at different times and places from populations with a very low polymorphism. This would mean that by the 11th century, at the beginnning of the propagation of rabbits in non-Mediterranean areas, animals carrying mtDNA type B1 were the most frequent. A third possibility is that animals carrying maternal type B1 had some advantage (better breeder?) and therefore type B1 might have been selected for after domestication.

ACKNOWLEDGMENTS

Thanks are due to H De Rochambeau, INRA, Castanet-Tolosan, for providing domestic animals. We also wish to thank the Office National de la Chasse for the access to the natural populations it supervises, O Ceballos and D Bell for their help in getting samples from Navarra and Norwich, respectively. This work has been financially supported by Paris-Sud University and by a specific grant from Bureau des Ressources Génétiques.

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