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Received February 11, 2002

Accepted March 5, 2002

Corresponding Editor: Muriel Davisson

## Different Levels of Human Intervention in Domestic Rabbits: Effects on Genetic Diversity

G. Queney, A.-M. Vachot, J.-M. Brun, N. Dennebouy, P. Mulsant, and M. Monnerot

The effects of human interaction on domestic rabbits were evaluated through the analysis of animals (up to 267) belonging to fancy breeds (22), a commercial breed (1), and selected strains (2). Microsatellite loci and mtDNA polymorphism revealed that the genetic pool of domestic rabbits studied only originated from that available in France. The good conservation of the original diversity was probably ensured through the multiplicity of samplings from wild populations. Selected strains, be-

cause of the breeding strategy, keep a fairly high level of diversity compared to other breeds.

Domestic rabbits as well as wild rabbits belong to the species *Oryctolagus cuniculus* (European rabbit), which is the only domesticated mammal of western European origin. Domestic rabbit populations comprise local populations, breeds, and strains (de Rochambeau 1989, 1998; Lauvergne 1982). Local populations, used in traditional backyard farming, are not surveyed and are currently disappearing. According to Arnold (1994), the present establishment of rabbit breeds is extremely recent: unlike other domestic animals, it only began at the end of the 18th century in western Europe (Helmer 1992). Breeds established from the previously mentioned local populations are presently defined by a standard based on external appearance (body shape and size, pattern of fur coloration, etc.).

Breeding associations were set up at the end of the 19th century. They organized agricultural meetings and competitions and the number of rabbit pure breeds increased significantly. For example, French breeders' associations now manage more than 60 pure breeds (as described in the "Standard officiel des lapins de race," 2000), but fancy breeders usually rear a small number of animals. However, one of these breeds (Normand) is commercialized by a private owner for its meat quality. Rabbit strains developed for commercial purposes started in the middle of the 20th century. A strain corresponds to a fairly homogeneous collection of individuals subjected to artificial selection for a performance trait (size at birth, fur, etc.). Most of the strains descend (eventually with mixture) from a few breeds (e.g., New-Zealand White, Californian, Fauve de Bourgogne). Fancy rabbits, commercial breeds, and strains thus represent different states of the domestication process.

The wild rabbit population's history is well documented through genetic and archaeological studies (Biju-Duval *et al.* 1991; Branco *et al.* 2000; Callou 1995; Ennafaa *et al.* 1987; Ferrand 1995; Hardy *et al.* 1994, 1995; Loreille *et al.* 1997; Monnerot *et al.* 1994, 1996; Mougél 1997; Queney *et al.* 2001; van der Loo *et al.* 1991; Vigne 1988). The European rabbit originates from the southern Iberian Peninsula (Andalusia) and dates back to the middle Pleistocene (Lopez-Martinez 1989). From the upper Pleistocene to the Neolithic, the geographic distribution of the species increased to

**Table 1. Origins of the studied rabbits and mitochondrial DNA typing**

	Number of breeders	Number of individuals analyzed	Mitochondrial DNA RFLP typing	
			B1 lineage	B3–4 lineage
Fancy breeds	33	144	126	18
Argenté de champagne	3	14	10	4
Belgian Hare	2	10	9	1
Big Chinchilla	1	2	2	
Black and Tan	2	11	11	
Black Dwarf Lop	1	3	3	
Blanc de Hotot	1	1	1	
Brun Marron de Lorraine	2	5	5	
Californian	1	2	2	
Castor rex	1	5	4	1
Chinchilla	1	4	4	
Chinchilla rex	1	4	4	
Dalmatian rex	1	3	3	
Fauve de Bourgogne	1	6	6	
Flemish Giant	2	10	9	1
Géant blanc du Bouscat	1	8	8	
Gris du Bourbonnais	1	3	3	
Havana	1	4	4	
Himalayan	4	20	16	4
New Zealand	1	14	14	
Thuringer	2	4	2	2
Vienna Blue	1	5	5	
Vienna White	2	6	1	5
Commercial breed				
Normand	1	43	43	0
Strains		80	80	0
Strain 1601	INRA	43	43	
Strain 2066	INRA	37	37	
Total (breeds and strains)		267	249	18

the south of France. Since late Roman times, the dispersal of the species has been closely related to human activities. The species was first dispersed in northern Europe in the Middle Ages and then to the other parts of the world, especially during the 18th and 19th century (North and South America, Australia, New Zealand, and a great number of Pacific islands). Today, the domestic rabbit coexists with its wild form in western Europe (see review in Callou [1995]).

To better understand the relationship between domestic and wild rabbits, we initiated the genetic characterization of fancy breeds several years ago. We further intended to evaluate, on both the breed and strain levels, the effects of the domestication process on genetic diversity. Two complementary markers were chosen: mitochondrial DNA (mtDNA), able to characterize female lineages, and highly variable nuclear markers (microsatellites), which allowed access to the genetic structure of the populations. These analyses should provide information on the effects of human interaction at the different levels of the domestication process.

## Material and Methods

### Breeds, Strains, and Populations

Table 1 provides the characteristics of the individuals analyzed and gathered within

three categories: (1) fancy breeds: 144 rabbits belonging to 22 breeds were reared for competitions by private breeders [names and geographical locations of breeders can be found in Vachot (1996)]; (2) commercial breeds: 44 rabbits from the Normand breed which was under selection for a label in meat quality (Bergamelli J-M, personal communication); (3) strains: 80 rabbits came from two strains, A1601 and A2066, from INRA (National Agronomical Research Institute, France). Strain A2066 was formed in the 1970s from animals of the Russian giant and Californian breeds and selected for litter size in a closed selection nucleus. Strain A1601 descended by duplication from the "Verde" strain, which was founded in 1981 from two hybrids of four strains and selected for litter size at INIA (Research Institute in Valencia, Spain).

Previously analyzed wild populations (Queney *et al.* 2001) were used to reflect the potential diversity of wild rabbits. Populations from the Iberian Peninsula and France were gathered as two subsets, labeled IP and FR, respectively.

### Genetic Characterization

Blood samples were collected from the marginal vein of the ear on visits to fancy breeders or breed exhibitions and by cardiac punctures for synthetic strains. Total DNA was extracted following the method

described by Rico *et al.* (1992) and used directly for amplification. Rapid discrimination between mtDNA types B1 and B3–4 (2% nucleotide divergence in the non-coding domain), the most frequently described in France (Mougel 1997), and other lineages (more divergent) was done using diagnostic sites in two mtDNA fragments (565 bp from the cytochrome *b* gene, digested with *AluI* and 586 bp from the noncoding region, digested with *RsaI*) according to Mougel (1997). The analysis also used six microsatellite loci: *sat 2*, *sat 4*, *sat 5*, *sat 7*, *sat 8*, *sat 12* (Mougel *et al.* 1997).

### Statistical Analysis

Intrapopulation structure was investigated using the  $F_{IS}$  parameter and genetic differentiation between populations within groups (domestic or wild) was estimated from the  $F_{ST}$  parameter. Estimators of  $F_{IS}$  ( $f$ ) and  $F_{ST}$  ( $\theta$ ) and their 95% confidence intervals (CIs) were calculated using FSTAT (Goudet 1995). A Wilcoxon–Mann–Whitney test was used to test for significant differences in allelic diversity (STATVIEW; Abacus Concepts Inc., Berkeley, CA, 1996). Genetic distances between populations based on allelic frequencies (Nei's standard distance; Nei 1987) were calculated and a neighbor-joining network of populations was constructed with the help of the PHYLIP package (Felsenstein 1993).

## Results

### Mitochondrial DNA

The results of mtDNA typing are given in Table 1: only the B1 and B3–4 lineages were detected, the B1 lineage being widespread and the only one in the strains.

### Microsatellite Loci

Table 2 summarizes the data on allelic diversity for domestic breeds and strains and the two groups of wild populations considered (FR and IP). The difference in allelic diversity between domestic and wild populations from France (FR) was only significant at  $P = .05$ , but not at  $P = .01$ , when  $P < .0001$  for the difference between FR and IP. The mean number of alleles per locus for all domestic individuals was 3.6, a probably underestimated value due to the low sampling size of fancy breeds. Domestic animals did not exhibit private alleles; furthermore, all the alleles of domesticated animals but two were present in the wild populations studied from France.

Checking for heterozygote proportions

**Table 2. Allelic diversity at microsatellite loci**

	Number of individuals analyzed	Mean number of alleles per locus per population
Fancy breeds	129	ND
Commercial breed: Normand	41	3.2
Strains		
Strain A1601	38	4.0
Strain A2066	34	3.5
All domestic individuals	242	ND
Wild populations		
From France: FR (13 populations)	508	5.1 (3.3 to 6.5)
From the Iberian Peninsula: IP (11 populations)	321	8.9 (6.7 to 12.2)

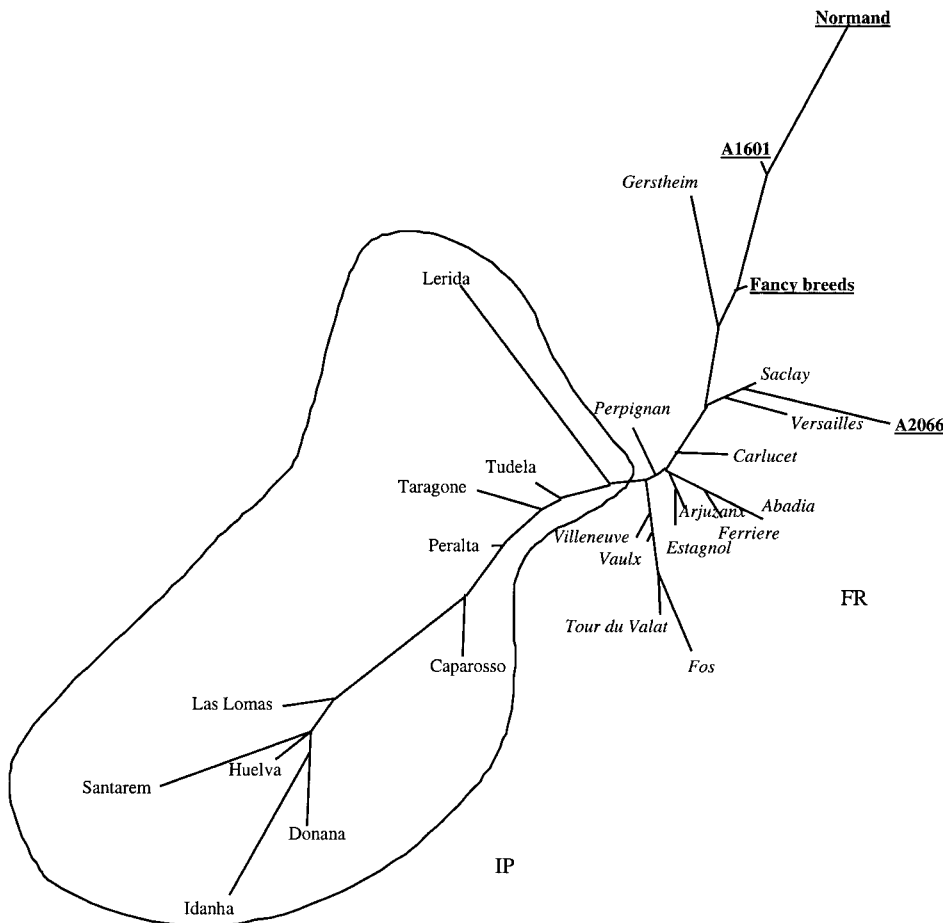
ND = not determined owing to the small sample size of each fancy breed.

could only be performed for the Normand breed and the two strains. Observed heterozygosity was relatively high for each one ( $H_o = 0.504, 0.477, \text{ and } 0.486$ ), but lower than that of wild populations ( $H_o = 0.596 \text{ and } 0.693$  for groups FR and IP, respectively). The two strains showed a deficit for heterozygotes ( $H_e = 0.520 \text{ and } 0.491$ ), but  $F_{IS}$  was not significantly different from zero (95% CI not shown), when the Normand breed exhibited a significant excess of heterozygotes ( $F_{IS} = -0.090$ ).

The differentiation between the Normand breed and the two strains was significantly (95% CI not shown) higher ( $F_{ST} = 0.283$ ) than that between the wild populations ( $F_{ST} = 0.144$  within FR and  $0.062$  within IP).

**Relationships Between Domestic and Wild Populations**

The network presented in Figure 1, based on Nei's genetic distances, includes the two strains from INRA (A1601 and A2066), the Normand breed, all the animals from



**Figure 1.** Neighbor-joining network based on microsatellite data using Nei's standard distance. Domestic populations (in bold and underlined characters) fall among wild populations from France (FR, in italics) and far away from populations from the Iberian Peninsula (IP).

the fancy breeds pooled together, and the two groups of wild populations considered (FR and IP). All domestic populations (bold and underlined characters in Figure 1) fell within wild populations from France (FR) and further from the other wild populations (IP). The Normand breed, fancy breeds, and one strain fell along the same line.

**Discussion**

For most domesticated species, the biological and geographical origins are on the way to being understood. Multiple origins are frequently evidenced, for example, for cattle (Mannen *et al.* 1998 and references therein), dogs [for a review see Vilà *et al.* (1999)], goats (Luikart *et al.* 2001), horses (Vilà *et al.* 2001), pigs (Giuffra *et al.* 2000), and sheep (Heindleder *et al.* 1998). The situation for domestic rabbits is theoretically simple, since both wild and domestic animals belong to the same species, *Oryctolagus cuniculus*, originating from Europe, and domestication is recent compared to that of other species. However, knowledge of the diversity of breeds and strains, a prerequisite for a comparison of domestic animals with wild populations in an attempt to accurately define the effect of domestication processes, is needed. A first set of data (Biju-Duval *et al.* 1991; Ennaffa *et al.* 1987; Monnerot *et al.* 1994) has shown the existence, in wild populations, of two maternal lineages, labeled A and B, separated for about 2 million years and restricted, for the first lineage, to the southwestern part of the Iberian Peninsula, while the second was observed in the north of Spain, in the rest of Europe, and in most populations spread all over the world. The very few domestic animals (about 20) analyzed at this time belonged to the B lineage, and more precisely to mtDNA type B1. Present data confirm that domestic breeds and strains are only related to the B maternal lineage. These data reveal that the mtDNA polymorphism is slightly higher than what was first believed, but that the mtDNA type B1 remains predominant (72% of individuals from breeds, 100% of individuals from strains) more so than in wild populations from France (52%, unpublished results). None of the types solely described in wild populations from the Iberian Peninsula (Mougel 1997) were found.

When nuclear markers were considered, domestic individuals were a pretty good image of the diversity described in wild populations from France, but contrasted

with the characteristics of wild populations from the Iberian Peninsula (Queney *et al.* 2001). This is illustrated by the location of domestic populations on the network presented in Figure 1 (as well as from genetic distances; data not shown).

All results thus converge toward the idea that domestic breeds (at least the ones involved in this study) only originated from the genetic pool available in France. These results are congruent with data on the polymorphism of immunoglobulins (van der Loo *et al.* 1991) and proteins (Ferrand 1995), but on a less representative sampling.

At this point, the effects of initial human intervention may be summarized as follows: (1) a slight decrease in allelic diversity (the mean number of alleles per locus per population ranging from 3.2 to 4 instead of 3.3 to 6.5), (2) a strong differentiation between domestic populations as attested by the high  $F_{ST}$  value. The latter could simply be a consequence of the low level of allelic diversity, but both probably reflect the existence of some founder effect at the origin of each domestic population, followed by genetic drift. In other words, breeds (at least at their origin) could represent various independent samplings within the original genetic pool.

The next step in human intervention can be appraised through the characteristics of the Normand breed, which has been commercialized for meat quality. The relatively low nuclear diversity (3.2 alleles/locus) probably reflects the low number of individuals at the origin of the sampled population. However, there is no inbreeding ( $F_{IS}$  not significantly different from zero) and the negative value of  $F_{IS}$  probably has to be correlated with the habits of the breeder, which introduces, from time to time, males from other breeds to precisely escape from inbreeding.

The last step leads to the establishment of strains. Occurring within one breed, strain breeding is expected to cause the loss of alleles through founder effects and genetic drift. However, when the strain formation involves several breeds (as in the synthetic strain), an initial increase in genetic diversity occurs through initial crossbreeding. Our study did not allow the evaluation of the effects of strain breeding, since initial breeds were not individually known. In both strains studied, the total number of alleles was not much different from that in the pool of fancy breeds. Two facts account for the fairly high genetic diversity of these strains:

they are both synthetics and the selection strategy (within sire family selection) is aimed at keeping the number of paternal lineages constant. When comparing the strains A2066 and A1601, however, the higher number of alleles in strain A1601 was consistent with its larger genetic base and its shorter selection history.

The differentiation of microsatellite loci between strains A2066 and A1601 ( $F_{ST} = 0.327$ ) was associated with that of quantitative trait loci as revealed by the high level of heterosis in the cross between the two strains (Brun *et al.* 1999): about 20% of the parental average on litter size, 14% on fertility rate, and 5% on doe body weight.

The present work was not designed to link a given breed to an original wild population: more individuals per breed, with a broader sampling, are required. The RESGEN project, supported by the European Community (Bolet *et al.* 1999), which involves 12 breeds (some endangered ones, with as many as 30 individuals per breed) will help solve this problem.

In conclusion, one may consider that the relatively recent domestication process in the European rabbit only involved rabbits issued from the genetic pool available in France. In that respect, the loss of genetic diversity through rabbit domestication is real, but not dramatic, when breeds are globally considered. When a breeding strategy is well applied, strains grown for commercial purposes keep a fairly high level of diversity compared to pure breeds.

From the Centre de Génétique Moléculaire, CNRS, 91198 Gif sur Yvette Cédex, France (Queney, Vachot, Dennebouy, and Monnerot), Station d'Amélioration Génétique des Animaux, INRA, BP27, 31326 Castanet-Tolosan, France (Brun), and Laboratoire de Génétique Cellulaire, INRA, BP27, 31326 Castanet-Tolosan, France (Mulsant). We thank J. Arnold for his constant interest in our work, J.-C. Mounolou for helpful discussions and W. Brand-Williams for English revision of the manuscript. The authors are also indebted to Messrs. Baty, Beltzung, Bergamelli, Bocquet, Bohrer, Boucher, Cabrespine, Charlemaine, Forn, Ginfray, Hebert, Holzhaus, Koegele, Kurtz, Lehner, Liger, Meioni, A. Meyer, C. Meyer, R. Meyer, Munch, Neyer, Pichard, Roger, Soileilhac, and Thomas for their welcome and allowing (and even for some of them actively participating in) the collection of blood samples from very precious individuals. A.-M. Vachot is currently at the Department of Obstetrics and Gynaecology, Westmead Hospital, Westmead, Sydney, NSW 2145, Australia. Address correspondence to Monique Monnerot at the address above or e-mail: monnerot@cgm.cnrs-gif.fr.

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Received October 14, 2000

Accepted March 5, 2002

Corresponding Editor: Robert Wayne

## A Blond Coat Color Variation in Meadow Vole (*Microtus pennsylvanicus*)

J. T. Curtis

Color mutations occur frequently among rodents. Here we describe a blond coat color mutation in the meadow vole (*Microtus pennsylvanicus*) that arose in a captive

breeding colony established from wild-caught animals from southern Illinois. The blond coat coloration results from changes in the color and distribution of pigments in the hair. The mutation is monogenic autosomal recessive.

Voies are small (~35-60 g) mouse-like mammals that occupy a variety of habitats throughout the Holarctic. Many species of voles can be reliably bred and housed in captivity, and over the past two decades, voles increasingly have become common subjects for laboratory studies of social behaviors and reproductive physiology.

Color mutations have been described in many animal species and are common in rodents (Gaines 1985; Silvers 1979). These mutations often involve the loss or replacement of melanin pigmentation (cf., Roth and Dawson 1996). Here we describe a blond color mutation that arose in a captive breeding colony of meadow voles (*Microtus pennsylvanicus*).

The voles used in this study were individuals of the F<sub>3</sub>, F<sub>4</sub>, and F<sub>5</sub> generations of a laboratory breeding colony of meadow voles originating from a population in southern Illinois. Animals were maintained on a 14 h light:10 h dark photoperiod (lights on at 0700) to enhance reproductive success. Breeder pairs were housed in plastic, shoebox-style cages (47 cm × 25 cm × 20 cm) with pine chips as bedding, and straw was provided for nesting material. Food (Purina rabbit chow) and water were provided ad libitum. Breeding pairs were checked daily and the phenotypes of any neonates were noted. Pups were weaned at 21 days and housed in same-sex sibling pairs in 29 cm × 18 cm × 13 cm cages. Test crosses were made after the animals had reached sexual maturity (60-70 days of age).

An inadvertent mating between an F<sub>3</sub> male and his daughter produced a litter containing two females with a blond coloration. All individuals from this litter were retained for subsequent breeding. As both of the blond voles from the original litter were female, these individuals were bred back to the original F<sub>3</sub> male to provide further breeder stock. After sufficient breeding stock was available, a series of test crosses was performed to assess the genetics of the mutation. In crosses involving suspected homozygous dominant individuals, two litters with a minimum total of eight pups, none of which displayed the blond phenotype, were assumed to verify the suspected genetic status. To minimize disturbance to the breeder pairs,

pups were not weighed at birth, nor was sex ascertained until weaning. Since some pups were lost prior to weaning, this resulted in the loss of data on sex for those pups. At the end of the experiments, specimens were placed with the Harvard Museum of Comparative Zoology (Cambridge, MA; catalogue numbers MCZ 63146-63167).

Four test crosses between the original, wild-type (B) F<sub>3</sub> male and female descendants displaying the blond (b) phenotype produced a total of 17 pups with a phenotype ratio of 9 B:8 b. This ratio is consistent with our original hypothesis that the father was heterozygous while the blond individuals were homozygous recessive. In addition to the two blond females, the initial father-daughter mating also produced three wild-type males. These individuals were mated with blond females. Two of the three males sired litters containing both wild-type and blond pups (three litters, 3 B:7 b). The remaining male from the original litter sired two litters, neither of which contained a blond pup (8 B:0 b). Thus the phenotype ratio of the original father × daughter litter was 1 BB:2 Bb:2 bb. Blond males were crossed with blond females in four testcrosses. In all cases, only blond pups were produced ( $n = 16$ ). When four blond females were crossed with unrelated wild-type males ( $n = 8$  litters), no blond pups were produced (33 B:0 b).

The mean ( $\pm$ SE) litter size for females displaying the blond phenotype ( $n = 20$ ) was  $3.9 \pm 0.3$  pups. The mean litter size for an equal number of wild-type females chosen at random from the breeding colony as a whole was  $4.0 \pm 0.5$  pups. The sex ratio was 0.8 male:1 female ( $n = 33$ ).

### Phenotype

Wild-type voles of the common North American species are very similar in coloration (Carlton 1985). The dorsal and lateral fur is a uniform dark brown color, while the fur on the ventral surface is generally lighter. Both the ears and tail are covered with fur. Blond voles display a cream-colored fur, although the underside was still slightly lighter in color, as displayed in wild-type voles. Differences in eye color are commonly seen in other rodents displaying blond color mutations (cf., Owen and Shackelford 1942; Roth and Dawson 1996). Eye color in the blond voles was similar to that seen in wild-type voles. Pups with the blond phenotype were recognizable at birth. At postnatal