Origin of rabbit (*Oryctolagus cuniculus*) in China: evidence from mitochondrial DNA control region sequence analysis

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Summary

A fragment of mitochondrial DNA (mtDNA) control region (~700 bp) was sequenced in 104 individuals from 20 breeds (three Chinese domestic breeds, five recently derived breeds and 12 introduced breeds) of domestic rabbits, *Oryctolagus cuniculus*. Nineteen sites were polymorphic, with 18 transitions and one insertion/deletion, and eight haplotypes (A1, A2, A3, A4, A5, A6, A7 and A8) were identified. Haplotype A1 was the most common and occurred in 89 individuals. In the 25 Chinese rabbits, only haplotype A1 was observed, while four haplotypes (A1, A3, A5 and A6) were found in 26 recently derived individuals. Haplotype A2 was shared by seven individuals among three introduced strains. The other six haplotypes accounted for 0.96–1.92% of the animals. Combined with the published sequences of European rabbits, a reduced median-joining network was constructed. The Chinese rabbit mtDNAs were scattered into two clusters of European rabbits. These results suggest that the (so-called) Chinese rabbits were introduced from Europe. Genetic diversity in Chinese rabbits was very low.

Keywords control region, genetic diversity, mtDNA, origin, rabbit.

Introduction

Knowledge of origin and evolution of domesticated species is not only an important academic question, but also has practical value for informed conservation of genetic diversity. Chinese rabbits have some desirable production traits, such as high reproductivity and disease resistance (Ban *et al.* 1996). However, the origin and genetic differentiation among the Chinese breeds is debated (Luo 1988; Chen & Wang 1991; Chen 1984). Some believe Chinese rabbits were introduced from Europe, perhaps through the Silk Road, because no fossils of domesticated or wild rabbits (*Oryctolagus cuniculus*) have been found in China so far (Luo 1988). Another hypothesis is that Chinese rabbit originated in China (Chen 1984; Chen & Wang 1991). Chen (1984 and references in) suggested that the lack of rabbit fossils was because the bones of rabbits were small, brittle and difficult to retain in fossils. This may be true for some of the bones, but not all, as rabbit remains were extremely abundant at many archaeological sites dating back over 10 000 years and even earlier in Europe (Hardy *et al.* 1995). However, both hypotheses were based on limited genetic evidence.

Analysis of mitochondrial DNA (mtDNA) is useful for investigating the genetic variations and evolutionary relationships of animals because of its specific characteristics such as high variability and maternal transmission. It has been widely used in the genus *Oryctolagus cuniculus* and suggests that modern European rabbits had the same roots as the European wild rabbits, *O. cuniculus*, in the Iberian Peninsula (Ennafaa *et al.* 1987; Mignotte *et al.* 1990; Hardy *et al.* 1995; Monnerot *et al.* 1994, 1996; Fuller *et al.* 1997).

At the present time, there are about 20 rabbit breeds (strains) in China. Some of them were European breeds imported into China, while other breeds are believed to be Chinese breeds or strains. Recently derived breeds have been developed in recent years using crossbreeding and genetic selection. In this study, we sequenced about 700 bp of mtDNA control region of 104 individuals from 20 breeds or

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strains collected in China, with the objective of elucidating the origin of Chinese rabbits.

Materials and methods

Sampling

Whole blood, white cells and muscle samples of 104 individuals from 20 breeds (three Chinese breeds, five recently derived breeds and 12 introduced breeds) were collected from seven provinces of China. The locations and the sample sizes of each breed or strain are listed in Table 1.

DNA amplification and sequencing

Total DNA was extracted by standard phenol/chloroform methods. A 700-bp fragment of mtDNA control region near the *tRNA-Pro* gene was amplified using primers L15438 (5′-GCTGATATTCTACTTAAACTA-3′) and H16138 (5′-AGGGTCTTCATTAGGTGCTCGTCT-3′). Nucleotide positions were numbered according to the complete rabbit mtDNA sequence (Gissi *et al.* 1998; GenBank accession number NC-001913). Polymerase chain reaction was performed using about 20 ng of DNA in a 25-μl reaction volume with 35 cycles (94 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min). The products were purified with a gel extraction kit (Watson Biomedical Inc., Shanghai, China) and sequenced on an Applied Biosystem ABI 377 Sequencer using the Bigdye™. Terminator Cycle Sequencing kit (P. E. Biosystems Inc., Foster City, CA, USA). Both strands were sequenced using L15438, H16138, and an inner primer L15863 (5′-CCATCCTCCGTGAAACCAACA-3′).

Data analyses

Sequences were aligned with DNASTAR software (DNASTAR Inc., Madison, WI, USA). All haplotypes, polymorphic sites and parsimony informative sites were identified with Mega 2.0 (Kumar *et al.* 2001). Published sequences and the haplotypes of European rabbits were used for comparison (Table 2). Relationships among the haplotypes were inferred in two ways. First, P-distances (proportional distance, proportion of nucleotide sites at which the two sequences are compared are different) between the sequences were used to construct a Neighbour-joining (NJ) tree with Mega 2.0 (Kumar *et al.* 2001). Bootstrap analyses (with 2000 replications) were used to assess the confidence in branching order. Some published sequences were excluded because they were shorter than our sequences. Secondly, a reduced median-joining network profile of the individuals was constructed according to Bandelt *et al.* (1995).

Results

Sequence variation

In the 104 individuals representing 20 breeds sequenced here (Table 1), 19 polymorphic sites were identified, with 18 transitions and one insertion/deletion. Eight haplotypes (A1, A2, A3, A4, A5, A6, A7 and A8) were resolved. Among the eight haplotypes, A1 was the most common and occurred in 89 individuals (Table 3). All other Chinese rabbits and the recently derived breeds except four individuals from strains O and Y shared haplotype A1. This

Table 1 Samples collected from 20 rabbit breeds in China.

Breed/strain (Abbreviation)	Locality	No. of samples	Origin
Qixing rabbit (A)	Chengdu, Sichuan	6	Derived
Haerbin white rabbit (H)	Chengdu, Sichuan	5	Derived
Zhenhai thick-hair Angora rabbit (O)	Ningbo, Zhejiang	5	Derived
Big-ear brown rabbit (S)	Baoding, Hebei	5	Derived
Yufeng brown rabbit (Y)	Puyang, Henan	5	Derived
Belgium (B)	Chengdu, Sichuan	5	Introduced
Californian (C)	Chengdu, Sichuan	4	Introduced
Dwarf rabbit (D)	Chengdu, Sichuan	4	Introduced
ELCO (E)	Nanjing, Jiangsu	5	Introduced
Germany great line of ZIKA rabbit (G)	Chengdu, Sichuan	5	Introduced
New Zealand rabbit (J)	Jiangsu Nanjing	5	Introduced
Rex from United States (M)	Jiangsu Nanjing	5	Introduced
Germany New Zealand of Zika rabbit (N)	Chengdu, Sichuan	4	Introduced
Japanese white rabbit (R)	Kunming, Yunnan	3	Introduced
Rex from Germany (T)	Chengdu, Sichuan	5	Introduced
Angora rabbit from Germany (W)	Chengdu, Sichuan	3	Introduced
Germany white of Zika rabbit (Z)	Sichuan Chengdu	5	Introduced
Fujian brown rabbit (F)	Fuzhou, Fujian	9	Chinese
Taihang Moutain rabbit (P)	Baoding, Hebei	6	Chinese
Sichuan white rabbit (X)	Chengdu, Sichuan	10	Chinese

Table 2 Sequence data used in the study.

Accession No.	Haplotype		Rabbit	References
	Original	This study		
AF003189	Aus-1	A1	European rabbit in Australia	Zenger *et al*. (unpublished data)
AF003190	Aus-2	A6	European rabbit in Australia	Zenger *et al*. (unpublished data)
AF003191	Aus-3		European rabbit in Australia	Zenger *et al*. (unpublished data)
AF003192	Aus-4	A2	European rabbit in Australia	Zenger *et al*. (unpublished data)
AF003193	Aus-5		European rabbit in Australia	Zenger *et al*. (unpublished data)
AF003194	Aus-6	A3	European rabbit in Australia	Zenger *et al*. (unpublished data)
AF003195	Aus-7		European rabbit in Australia	Zenger *et al*. (unpublished data)
AJ293831	B01Zsf		Fauve de Bourgogne	Bolet *et al*. (2000)
AJ293832	B01Zsh		Belgian hare	Bolet *et al*. (2000)
AJ293833	B01Zsg		Fauve de Bourgogne	Bolet *et al*. (2000)
AJ293834	B01Zsj		Argente de Champagne	Bolet *et al*. (2000)
AJ293835	B01Zsk		English	Bolet *et al*. (2000)
AJ293836	B01Zsl		Flemish giant	Bolet *et al*. (2000)
AJ293837	BZs14		Fauve de Bourgogne	Bolet *et al*. (2000)
AJ293838	BZs15		Hungarian Giant	Bolet *et al*. (2000)
AJ293839	BZs16		French Lop	Bolet *et al*. (2000)
AJ293840	BZs17		French Lop	Bolet *et al*. (2000)
AJ293841	BZs19		French Lop	Bolet *et al*. (2000)
AJ293842	BZs20	A6	Chinchilla	Bolet *et al*. (2000)
AJ293843	BZs21		Vienna White	Bolet *et al*. (2000)
AJ293844	Bzs22		Flemish Giant	Bolet *et al*. (2000)
NC-001913				Gissi *et al*. (1998)
U62924	A	A3	wild rabbit in Australia	Fuller *et al*. (1997)
U62925	B	A1	wild rabbit in Australia	Fuller *et al*. (1997)
U62926	C	A6	wild rabbit in Australia	Fuller *et al*. (1997)
U62927	D	A2	wild rabbit in Australia	Fuller *et al*. (1997)
X54172				Mignotte *et al*. (1990)
Z83340	A5Zsa		wild rabbit on Iberian Peninsula	van der loo et al. (1997)
Z83341	A2Zsa		wild rabbit on Iberian Peninsula	van der loo et al. (1997)
Z83342	A10Zsa		wild rabbit on Iberian Peninsula	van der loo et al. (1997)
Z83343	A1Zsa		wild rabbit on Iberian Peninsula	van der loo et al. (1997)
Z83344	A9Zsa		wild rabbit on Iberian Peninsula	van der loo et al. (1997)
Z83346	B10Zsa		rabbit in Spain	van der loo et al. (1997)
Z83349	B6Zsa		rabbit in Spain	van der loo et al. (1997)
Z83350	B4Zsa		rabbit in Spain	van der loo et al. (1997)
Z83351	B3Zsc		rabbit in Spain	van der loo et al. (1997)
Z83354	Bzs1		rabbit in Spain	van der loo et al. (1997)
Z83364	B2Zsa		rabbit in Spain	van der loo et al. (1997)
Z83365	B3zsa	A2	rabbit in Spain	van der loo et al. (1997)
Z83366	B2zsc	A3	rabbit in Spain	van der loo et al. (1997)
Z83367	B1zsb	A1	rabbit in Spain	van der loo et al. (1997)

haplotype was also observed in all introduced animals except strain G.

Alignment of these eight haplotypes and the reported sequences of European rabbit (Table 2) revealed 37 unique haplotypes. The current study resolved four new haplotypes (A4, A5, A7 and A8), and the other four haplotypes (A1, A3, A6, A2) were identical to previously published haplotypes (Table 2).

Phylogenetic analysis

The unrooted NJ tree of wild and domesticated rabbit mtDNA sequences is shown in Fig. 1. Two lineages (A and B) were clearly discerned, with lineage A composed of wild rabbits and lineage B including both domestic and wild animals. In lineage B, two clusters were discerned. Six haplotypes in the present study (A1, A4, A5, A7, A8 and A3) were scattered in the first cluster and the remaining two haplotypes (A2 and A6) belonged to the second cluster.

Table 3 Frequencies of eight mitochondrial DNA haplotypes in 20 rabbit breeds in China.

	Haplotype							
Breed/strain[1]	A1	A2	A3	A4	A5	A6	A7	A8
A	6 (1.00)	–	–	–	–	–	–	–
B	5 (1.00)	–	–	–	–	–	–	–
C	4 (1.00)	–	–	–	–	–	–	–
D	4 (1.00)	–	–	–	–	–	–	–
E	5 (1.00)	–	–	–	–	–	–	–
F	9 (1.00)	–	–	–	–	–	–	–
G	–	5 (1.00)	–	–	–	–	–	–
H	5 (1.00)	–	–	–	–	–	–	–
J	4 (0.80)	1 (0.20)	–	–	–	–	–	–
M	4 (0.80)	–	–	–	–	–	–	1 (0.20)
N	4 (1.00)	–	–	–	–	–	–	–
O	3 (0.60)	–	2 (0.40)	–	–	–	–	–
P	6 (1.00)	–	–	–	–	–	–	–
R	2 (0.67)	–	–	1 (0.33)	–	–	–	–
S	5 (1.00)	–	–	–	–	–	–	–
T	4 (0.80)	1 (0.20)	–	–	–	–	–	–
W	3 (1.00)	–	–	–	–	–	–	–
X	10 (1.00)	–	–	–	–	–	–	–
Y	3 (0.60)	–	–	–	1 (0.20)	1 (0.20)	–	–
Z	3 (0.60)	–	–	–	–	–	1 (0.20)	1 (0.20)
total	89 (0.85)	7 (0.07)	2 (0.02)	1 (0.01)	1 (0.01)	1 (0.01)	1 (0.01)	2 (0.02)

[1]Abbreviations for breeds are given in Table 1. Numbers of individuals that shared a haplotype are given outside the brackets; frequencies of the haplotypes are bracketed.

The sequences in this study have been submitted to GenBank and the accession numbers are AF534080–AF534108.

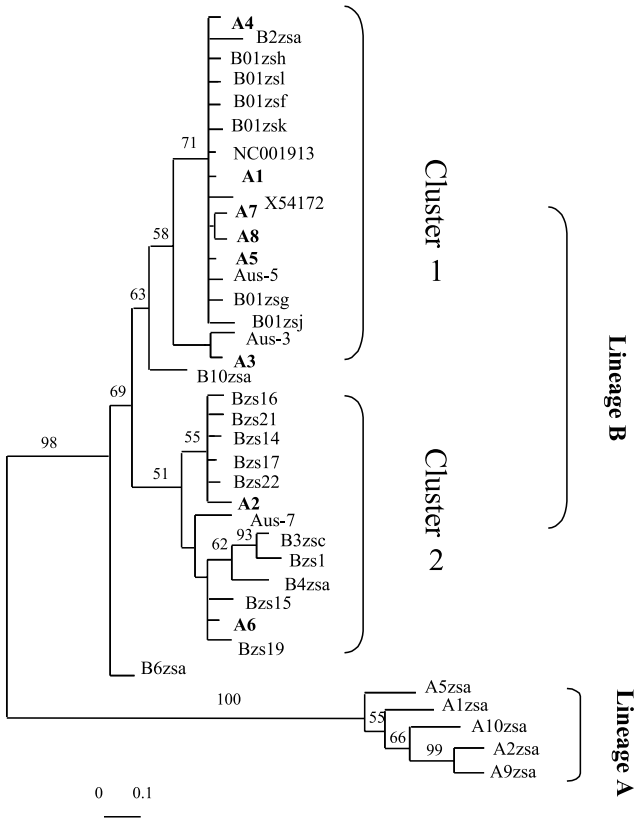

Figure 1 Unrooted Neighbour-joining (NJ) tree for 37 mtDNA sequences of rabbits using uncorrected P-distances. Numbers on the branches are percentage of bootstrap values from 2000 replications. The haplotypes identified in current study are in bold.

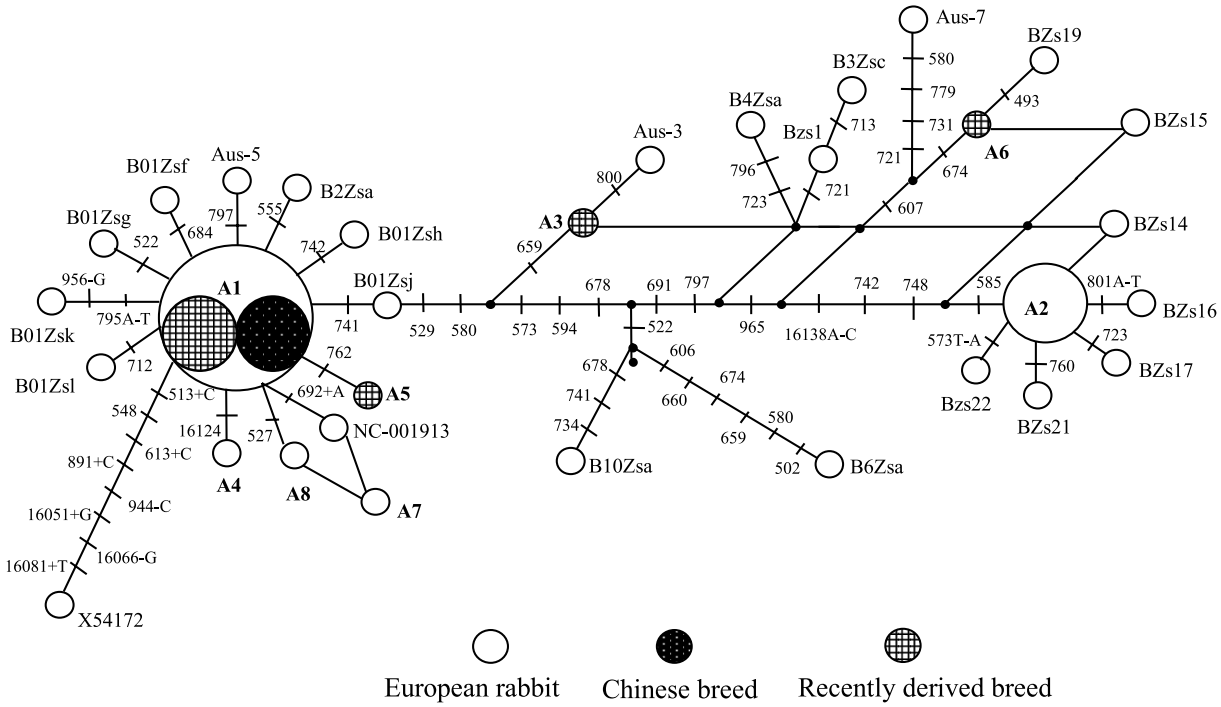

Figure 2 Network profiles of 32 mtDNA haplotypes. Each circle represents one haplotype with a size proportional to the number of individuals. The black points represent hypothetical sequences that were not observed. Branches between the haplotypes represent substitutions numbered according to Gissi *et al*. (1998). Site numbers below 16 000 are marked only the last three digits. The transversion and insertion/deletion are labelled. Recurrent mutations are underlined, designating putative distinct mutation events at the same position. The haplotypes identified in current study are in bold.

In the network connecting 32 haplotypes of lineage B (Fig. 2), the highly represented haplotypes A1 and A2 were centred with the other sequences rooted through these haplotypes. Most sequences fell within only a few substitutions of the central nodes. Haplotypes A4, A5 and A8 differed in only one site from haplotype A1. There were two and four substitutions between A7 and A1, and A3 and A1, respectively. In the present study, nine introduced strains (B, C, D, E, M, N, R, W and Z), all Chinese breeds (F, P and X), and four recently derived breeds (A, H, O and S) were scattered in the first cluster. The second cluster was centred on haplotype A2. All individuals from strain G belonged to this cluster. Two introduced strains (J and T) and one recently derived breed (Y) were intermingled in the two clusters.

Discussion

Differentiation between domesticated rabbit strains

In the present study, 19 strains shared haplotype A1 and three strains shared haplotype A2, respectively (Table 3). Thus mtDNA differences were not distinct among the 20 strains, although their morphological differences were large. The results suggested that genetic diversity was low both in the Chinese rabbits and the introduced strains. This low genetic diversity might be due to: (1) founder effects during domestication because of the limited introduction of genes from the wild population, and when imported from Europe (see Discussion below), (2) during cultivation of a strain, homogeneity mating occurred, resulting in genetic monotony, (3) in commercial animal production, selection pressure was high, which drastically decreased the effective number (N_e) and the inherent variability of the strain, and (4) crossbreeding decreased genetic diversity in some strains. As a result, the maternally inherited mtDNA haplotypes of breeds from which only bucks were used, will be lost.

Origin of rabbits in China

In general, it is thought that there are two maternal lineages in the European rabbit based on data of mtDNA and immunoglobulin genes (Biju-Duval *et al.* 1991; Monnerot *et al.* 1994; van der Loo *et al.* 1997; Branco *et al.* 2000). All domestic rabbits belong to the second lineage (Monnerot *et al.* 1996).

The origin of Chinese rabbits is controversial. Sequence comparisons and phylogenetic analyses indicated only one mtDNA haplotype (A1) was represented in Chinese rabbits (Table 3). Some European breeds were imported into China and they might introgress into Chinese animals, resulting in a decrease in genetic diversity of Chinese rabbits. However,

the number of imported rabbits was very small and the effects of European animals were limited. Thus, the founders of Chinese rabbit probably originated from a population that carried haplotype A1. Haplotype A1 was commonly found (about 70%) in many European domestic breeds, such as Fauve de Bourgogne, Argenté de Champagne, and Flemish giant (Bolet *et al.* 2000), and in wild populations from France (M Monnerot, personal communication) as well as Australia (K Zenger, unpublished data; Fuller *et al.* 1997). Chinese breeds, some of the recently derived breeds in China, and some European rabbits were intermingled in the cluster centred with haplotype A1 (Fig. 2). Therefore, a more plausible scenario is that the so-called Chinese rabbits were also introduced from European rabbits.

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