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Source: Zoological Science, 18(1) : 107-114

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.18.107

ZOOLOGICAL SCIENCE **18**: 107–114 (2001)

Phylogeography of the Japanese Giant Flying Squirrel, *Petaurista leucogenys*, Based on Mitochondrial DNA Control Region Sequences

Tatsuo Oshida[1†], Koichi Ikeda[2], Kazuhiko Yamada[3] and Ryuichi Masuda[1*]

[1]*Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan*
[2]*Fukuoka Prefecture Forest Research and Extension Center, Kurume 839-0827, Japan*
[3]*Laboratory of Cytogenetics, Division of Biosciences, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan*

ABSTRACT—To investigate genetic diversity among populations of the Japanese giant flying squirrel *Petaurista leucogenys*, the mitochondrial DNA control region sequences (1,052–1,054 bases) were determined in 37 specimens from 17 localities on the Honshu, Shikoku, and Kyushu Islands of Japan. Of the 37 animals examined, 24 haplotypes were identified. All haplotypes from Kyushu consisted of 1,052 bases, whereas those from Honshu and Shikoku consisted of 1,054 bases including two insertions, except for three haplotypes (which had 1,052 or 1,053 bases). Phylogenetic relationships reconstructed using neighbor-joining and maximum parsimony methods indicated that *P. leucogenys* is essentially separated into three major lineages: Group A consisting of a single haplotype from Kyushu, Group B consisting of some haplotypes from Kyushu and one haplotype from Honshu, and Group C consisting mostly of haplotypes from Honshu and Shikoku. Animals with the Kyushu haplotypes were split into two lineages (Groups A and B), suggesting that Group A diverged at an earlier point from the other groups. Genetic distances in Group C were not related to geographic distances between sampling localities, indicating that ancestral populations of this group recently expanded their distribution in a short time, possibly after the last glacial stage.

INTRODUCTION

The Japanese giant flying squirrel *Petaurista leucogenys* is a mammalian species indigenous to the Kyushu, Shikoku, and Honshu Islands of Japan (Corbet and Hill, 1991; Nowak, 1991). Although Corbet and Hill (1980) explained that this species is distributed throughout Japan and central China, they more recently treated the central Chinese population as a distinct species *Petaurista xanthotis* (Corbet and Hill, 1991; 1992). Based on characteristics of their pelage, Imaizumi (1960) classified *P. leucogenys* into three subspecies: *leucogenys*, *nikkonis*, and *oreas*. At present, however, the validity of his classification is uncertain, as there are variations of pelage in *P. leucogenys*.

The ecology of *P. leucogenys* has been studied in detail (Baba *et al.*, 1982; Ando and Imaizumi, 1982; Ando and Shiraishi, 1983; Kawamichi, 1997a; 1997b; 1998), and cytogenetic information on this species has also been reported by Oshida and Obara (1991; 1993) and Oshida and Yoshida (1999a; 1999b). However, little information about the phylogeography of *P. leucogenys* has been known heretofore. Oshida and Obara (1993) reported the variation of constitutive heterochromatin of chromosomes in *P. leucogenys*, but they did not find any geographically specific features.

In the present study, in order to study the phylogeography and subspecies classification of *P. leucogenys*, we analyzed the mitochondrial DNA (mtDNA) control region sequences. Since the control region contains variable blocks which evolve about 4–5 times faster than the other regions of mtDNA molecules (Greenberg *et al.*, 1983; Horai and Hayasaka, 1990; Brown *et al.*, 1993), this region is a very valuable molecular marker for investigating relationships among closely related species or conspecific populations (e.g., Baker *et al.*, 1993; Arctander *et al.*, 1996; Nagata *et al.*, 1998; 1999; Barratt *et al.*, 1999; Kurose *et al.*, 1999; Matsuhashi *et al.*, 1999). Based

* Corresponding author: Tel. +81-11-706-3541;
FAX. +81-11-736-6304.
E-mail: masudary@ees.hokudai.ac.jp
† Present address: Laboratory of Molecular Ecology, Department of Biology, Tunghai University, Taichung Taiwan 407, R. O. C.

on the control region data, we here discuss phylogeographic relationships within Japanese populations of *Petaurista leucogenys*.

MATERIALS AND METHODS

Animals

A profile of the Japanese giant flying squirrel *Petaurista leucogenys* examined in the present study is shown in Table 1. Thirty seven specimens of *P. leucogenys* were collected from 17 localities in Japan (Fig. 1). A female red giant flying squirrel *Petaurista petaurista melanotus* (PPM) imported from Hong-Kong to Japan in 1990 was used as an out-group.

DNA preparation and sequencing

Total DNAs were extracted from muscle or liver tissues using the phenol/proteinase K/sodium dodecyl sulfate method of Sambrook *et al.* (1989). The whole control region was amplified using polymerase chain reaction (PCR), with a set of newly designed primers: L15933 5'-CTCTGGTCTTGTAAACCAAAAATG-3' and H637 5'-AGGACCAAACCTTTGTGTTTATG-3'. Primer names correspond to the light (L) or heavy (H) strand and the 3'end-position of the primers in the human mtDNA sequences (Anderson *et al.*, 1981). The reaction mixture of 50μl contained 100 ng of genomic DNA, 25 picomoles of each primer, 200 μM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 2.5 units of r*Taq* DNA polymerase (Takara). Amplification was carried out for 35 cycles as follows: 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, and then the extension reaction was performed at 72°C for 10 min. PCR products were purified with the Qia-quick PCR purification kit (QIAGEN) and directly sequenced using an automated DNA sequencer (SQ5500L, Hitachi). For sequencing, two PCR primers and another new primer (5'-CCTAATGGATATCCCCTTCCAACG-3') were used.

Phylogenetic analysis

All sequences were aligned using the computer software GeneWorks (Intelligenetics). The phylogenetic tree was constructed via the neighbor-joining (NJ) method (Saitou and Nei, 1987) in Clustal W (Thompson *et al.*, 1994) and via the maximum parsimony (MP) method using a heuristic search algorithm with the 50% majority-rule consensus in PAUP (Swofford, 1993). In the NJ tree, the numbers of

Table 1. Specimen profiles of *Petaurista leucogenys* examined in the present study

Sample name	Sex#	Sampling locality (Supplier)	No.** of locality	Haplotype	Accession No. of sepuence***
AM1*	M	Sannohe-gun, Aomori Pref.	1	H6	AB043805
AM2*	F	Sannohe-gun, Aomori Pref.	1	H5	AB043804
ITI	M	Koromogawa, Iwate Pref. (Morioka Zoo)	2	H14	AB043813
IT2	F	Koromogawa, Iwate Pref. (Morioka Zoo)	2	H12	AB043811
NN1*	M	Shiga-Height, Nagano Pref.	3	H11	AB043810
NN2*	M	Shiga-Height, Nagano Pref.	3	H4	AB043803
NN3*	M	Shiga-Height, Nagano Pref.	3	H13	AB043812
TY1*	F	Nakaniikawa-gun, Toyama Pref.	4	H13	AB043812
TY2*	M	Nakaniikawa-gun, Toyama Pref.	4	H4	AB043803
YN1*	M	Nirazaki, Yamanashi Pref.	5	H4	AB043803
YN2*	F	Nirazaki, Yamanashi Pref.	5	H10	AB043809
TG1	M	Nikko, Tochigi Pref. (Tochigi Prefectual Museum)	6	H7	AB043806
TG2	M	Shioya, Tochigi Pref. (Tochigi Prefectual Museum)	7	H8	AB043807
KN1	M	Hakone, Kanagawa Pref. (Kanagawa Prefecture Natural Environment Conservation Center)	8	H15	AB043814
KN2	F	Aikawa, Kanagawa Pref. (Kanagawa Prefecture Natural Environment Conservation Center)	9	H16	AB043815
WK1*	M	Hashimoto, Wakayama Pref.	10	H9	AB043808
WK2*	F	Hashimoto, Wakayama Pref.	10	H4	AB043803
GF1*	M	Kamioka, Gifu Pref.	11	H4	AB043803
GF2*	F	Kamioka, Gifu Pref.	11	H5	AB043804
KT1	M	Kyoto, Kyoto Pref. (Mr. M. Kishioki)	12	H2	AB043801
HS1	F	Hiroshima Pref. (Asa Zoological Park)	13	H1	AB043800
KG1*	M	Takamatsu, Kagawa, Pref	14	H4	AB043803
EH1	?	Omgo, Ehime Pref. (Omogo Mountain Museum)	15	H3	AB043802
FO1	F	Hirokawa, Fukuoka Pref.	16	K1	AB043792
FO2	M	Hirokawa, Fukuoka Pref.	16	K1	AB043792
FO3	M	Hirokawa, Fukuoka Pref.	16	K1	AB043792
FO4	F	Joyo, Fukuoka Pref.	17	K5	AB043796
FO6	F	Joyo, Fukuoka Pref.	17	K1	AB043792
FO7	F	Joyo, Fukuoka Pref.	17	K3	AB043794
FO8	F	Joyo, Fukuoka Pref.	17	K8	AB043799
FO9	M	Joyo, Fukuoka Pref.	17	K2	AB043793
FO10	M	Joyo, Fukuoka Pref.	17	K4	AB043795
FO11	M	Joyo, Fukuoka Pref.	17	K2	AB043793
FO15	M	Joyo, Fukuoka Pref.	17	K5	AB043796
FO16	M	Joyo, Fukuoka Pref.	17	K1	AB043792
FO17	M	Joyo, Fukuoka Pref.	17	K6	AB043797
FO18	F	Joyo, Fukuoka Pref.	17	K7	AB043798

* Specimens obtained commercially from pet stores in Japan: Takita Store, Sannohe-gun and Saitama Sougou Pet, Koshigaya.

** Locality Nos. correspond to those in FIg.1.

*** Sequence data will appear in the DDBJ nucleotide sequence databases with these accession numbers.

M, male; F, female.

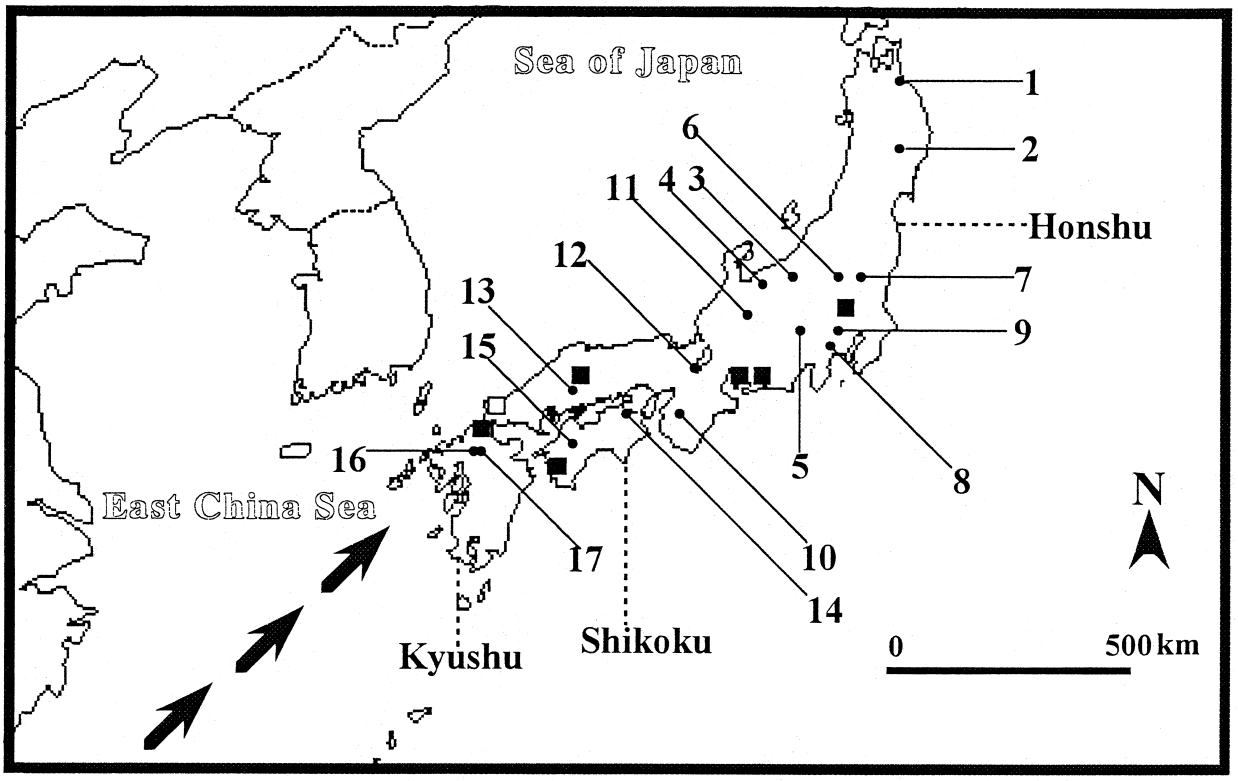

Fig. 1. Sampling localities in the present study and fossil localities of *Petaurista leucogenys*. Closed circles, sampling localities; open square, Middle Pleistocene fossil localities; closed squares, Late Pleistocene fossil localities. Sampling locality numbers correspond to those of Table 1 and Fig. 2. Arrow indicates the presumed migration route of *P. leucogenys* from southern China to Japan.

nucleotide substitutions per site were estimated for multiple substitutions by Kimura's (1980) two-parameter method. Using sequences without gap-sites, the MP tree was produced by unweighted parsimony. To assess the branching confidence, bootstrap values (Felsenstein, 1985) were derived from 1,000 replications of the NJ method and 100 replications of the MP tree.

RESULTS

Sequence Divergence of mtDNA Control Region

All mtDNA control regions (1,052–1,054 bases) of *P. leucogenys* from 17 localities in Japan were successfully sequenced. All sequences of animals from Kyushu had 1,052 bases, while those of populations from Honshu and Shikoku had 1,054 bases with insertions except for three specimens: EH1 (1,052 bases), KT1 (1,052 bases), and HS1 (1,053 bases) (Table 2). Of all sequences obtained, 145 sites were variable: transitions at 117, transversions at 14 sites, and both transitions and transversions at 12 sites (Table 2). In the 37 animals, 24 haplotypes were identified (Tables 1 and 2). The sequence divergence among haplotypes was 0.7–4.8% (Table 3).

The control region of *P. leucogenys* was divided into three domains: two variable domains (the 5' and 3' ends) and one conserved central domain (Table 2). The 5' end domain was more variable than the 3' end domain and contained one gap-site (site number 281) in all haplotypes from Kyushu, in H1 and H2 from Honshu, and in H3 from Shikoku. The 3' end domain contained an additional gap-site (site number 1,052) in all haplotypes from Kyushu, in H2 from Honshu, and in H3 from Shikoku. The sequence of *P. petaurista* used as an outgroup has 1,051 bases (accession number in DDBJ: AB043816).

Molecular phylogeny based on the mtDNA control region

Phylogenetic relationships reconstructed via NJ and MP methods were similar to each other. In the NJ tree, the Japanese population of *P. leucogenys* was separated into three major lineages: Group A consisting of K1, Group B consisting of K2, K3, K4, K5, K6, K7, K8, and H1 (95% bootstrap value), and Group C consisting of H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, and H15 (62% bootstrap value) (Fig. 2a). In the MP analysis, only one most-parsimonious phylogenetic tree was obtained by unweighted parsimony, and it had a consistency index of 0.668. The three major groups were also recognized in the MP tree: Group A (K1), Group B consisting of K2, K3, K4, K5, K6, K7, K8, and H1 (88% bootstrap value), and Group C consisting of H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, and H15 (57% bootstrap value) (Fig. 2b). In both trees, H1 from Honshu was clustered with the Kyushu population consisting of K2, K3, K4, K5, K6, K7, and K8 with high bootstrap values (95% in the NJ tree and 88% in the MP tree), and H2, H3, and H16 were not included in the major three lineages. In addition, in both trees, genetic differences between haplotypes of Group C did not correspond to geographic distances. The sequences of Groups A and B had

Table 2. Sequence variation of the mt DNA control region (1,052–1,054 bases) in *Petaurista leucogenys*. Dots indicate identical nucleotides or

Variable

Haplotype	1 2 2 3 5 6 6 6 6 7 8 9 9 1 1 2 2 2 3 3 4 4 4 5 5 5 6 6 6 6 7 7 7 7 8 8 1 2 3 3 3 3 3 3 3 3 4 4 4 4 4 4 5 5 5 5 6 6 6 7 7 7 7 8 2 9 1 3 5 1 4 0 1 2 5 7 8 4 6 4 5 3 4 5 2 4 4 7 9 0 8 9 0 3 7 9 4 6 7 8 0 6 6 9 0 1 3 4 5 6 8 9 0 5 6 7 8 9 1 2 6 7 1 5 9 0 2 4 5 8 0
K1	T T C C T T T T C A C T A T G A T T A T A C G A T C A G T A A C C T C T A A C C C C G C T T C T T T G C C C G A C T T G T T T G T A A
K2	C . . . C . . . T T . . . . . T . . . T . A C . . T . C . C . . T . . T . . . . . C C . . . . . A G . . C . . . C . C . T
K3	C . . . C . . . T T . . . . . T . . . T . A C . . T . C T C . . T . . T . . . . . C C . . . . . A G . . . . . C . C . .
K4	C . . . C . . . T T . . . . A . . . . . T . . . T . A C . . T . C . C . . T . . T . . . . T C C . . . . . A G . . . . . C C . C . G
K5	C . . . C C . . T T . . . . A . . . . . T . . . T . A C . . T . . . C . . T . . T . . . . . C C . . . . . A G . . . . . C C . C . .
K6	C . . . C . . . T T . . . . A . . . . . T . . . T . A C . . T . . . C . . . . A T . . . . . C . . . . . A G . . . . . C C C C . .
K7	C . . . C . . . T T . . . . A . . . . . T . . . T . A C . . T . . . C . . . . A T . . . . . C . . . . . A G . . . . . C C C C . .
K8	C . . . C . . . T T . . . . A . . . . . T . . . . . A C . . T . . T C . . T T . T . . . . . C C . . . . T A G . . . . . C C . C . .
H1	C . . . C . . . T T . C . . A . C . . . . T . . . . . A C . . T . . . C . . T . . T . . C . . C C A . . . . A G . . . . . C C . C . .
H2	C . . . C . . . . C . . . . A C . . T . C . C . . . . . T A T . . . C . . . . . T . . T . . . . C C . . . .
H3	C . . . C . . . . A . . . . . T . . . . . A C . . . . C . . . G . . . T A . . . . C . . . . . . G . . . A . C . . C . .
H4	C . . . C . . . . C A . . . . . T . . C . . A . . . T T C . C . . . . C . . . . . A G . . . A . C . . . . T
H5	C . . . C . . . . C A . . . . . T . . . . . A . . . T T C . C . . . . C . . . T . . A G T . . A . C . . . . T
H6	C . . . C . . . . . T . . C A . . . . . T . . . . . A . . . T T C . C . . . . . A . . . . C . . . . . A G T . . A . C . . . . T
H7	C . . . C . . . . G . . . C . . A . . . T T C . C . . . . C . . . . T T A G . . . . . C . . . . T
H8	C . . . C . . . . A . . . . . T . . . . . A . . . T T C . C . . . . . T . . . . T . T A G . . . A . C . . . . T
H9	C . . . C . . . . C A G . . . . G T . G C . . A . G G T T C . C G . . . . C . . . . T . A G T C . A . C . . . G T
H10	C . . . C . . . . G C . T . . . . . A . . . T T C . C G . . . . . T C . . . . . A G . . . . . C C
H11	C . . . C . C . . . . T . . . . G A . . . . T C . C . . . . C . . . . . T A G . C . . . C . . . . T
H12	C . . . C . C . . . . A . . . . . T . . . . G A . . . T . C . C . . . . C . . . . . T A G . C . . . C . . . . T
H13	C . . . C . C . . . . A . . . . A . . . T T C . C . . . . C . . . . . A G T C . . . C . . . . T
H14	C . . . C . C G . . . . . . A . . . . C . T . . . . . A . . . T T C . C . . . . C T C . . A . . T A G . C . . . C . . . . T
H15	C C . . C . . . . . . C G . A . . . . . . T . . . . . A . . . T T C . C . . . . . . A . C . . C . . . . . T . G . . . . . C . . C . T
H16	C . T T C . . . . A . . . . . . T A . . . . A . . . . . C T C . G . . . . A . . . . C . . . . G . . . . C C . . C . T

Table 3. Pairwise comparison of mt DNA control region sequences without gap-sites (1,052 bases) between 24 haplotypes from *Petaurista leucogenys*

	K1	K2	K3	K4	K5	K6	K7	K8	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16
K1		3.7	3.7	4.4	4.3	4.1	3.9	4.3	4.2	3.2	3.4	3.0	3.2	3.7	3.7	3.1	4.1	3.1	3.4	3.7	3.4	4.0	3.6	3.8
K2	35/4		0.9	1.6	1.8	2.0	1.8	2.0	2.1	3.0	3.3	3.2	3.3	3.6	3.4	3.2	4.4	3.3	3.3	3.4	3.3	3.7	3.5	3.6
K3	38/1	6/3		1.2	1.3	1.7	1.5	1.3	2.0	3.1	3.0	3.4	3.5	3.8	3.6	3.4	4.6	3.4	3.5	3.5	3.5	3.9	3.7	3.5
K4	44/2	13/4	12/1		0.9	1.1	0.9	0.9	2.1	3.6	3.2	3.4	3.7	3.6	3.9	3.4	4.6	3.4	3.9	3.7	3.7	3.9	3.7	3.5
K5	43/2	15/4	13/1	9/0		1.1	1.0	1.0	2.2	3.7	3.1	3.6	3.5	4.0	3.9	3.6	4.8	3.8	4.1	4.1	3.9	4.3	3.9	3.7
K6	39/4	15/6	15/3	9/2	10/2		0.2	1.0	2.2	3.5	3.3	3.4	3.7	3.8	3.9	3.4	4.6	3.6	3.9	3.7	3.7	4.1	3.7	3.7
K7	37/4	13/6	13/3	7/2	8//2	2/0		1.0	2.0	3.3	3.1	3.2	3.5	3.6	3.7	3.2	4.4	3.4	3.7	3.5	3.5	3.9	3.5	3.5
K8	43/2	17/4	13/1	9/0	10/0	8/2	8/2		2.0	3.5	3.3	3.6	3.9	4.0	4.0	3.4	4.8	3.8	3.9	3.7	3.9	4.1	3.7	3.5
H1	41/3	17/5	19/2	19/3	20/3	18/5	16/5	18/3		3.8	3.8	3.3	3.6	3.6	4.3	3.3	4.5	3.3	3.4	3.7	3.4	4.0	3.2	3.8
H2	34/0	28/4	32/1	36/2	37/2	33/4	31/4	35/2	37/3		2.9	3.3	3.2	3.5	3.6	3.3	4.3	3.5	3.2	3.6	3.2	3.8	3.2	3.3
H3	34/2	29/6	28/3	30/4	29/4	29/6	27/6	31/4	35/5	28/2		3.1	3.4	3.5	4.1	3.5	4.3	3.5	3.4	3.9	3.6	4.0	3.2	2.8
H4	30/1	31/3	34/2	33/3	35/3	31/5	29/5	35/3	31/4	34/1	30/3		0.7	1.0	2.2	1.1	1.1	1.4	1.6	2.4	1.4	1.8	1.8	3.0
H5	33/1	32/3	35/2	36/3	34/3	34/5	32/5	38/3	34/4	33/1	33/3	7/0		1.1	2.3	1.1	1.6	1.7	1.9	2.7	1.3	2.1	2.1	3.2
H6	36/3	33/5	36/4	33/5	37/5	33/7	31/7	37/5	32/6	34/3	32/5	8/2	9/2		2.8	1.5	1.9	1.7	2.0	2.8	1.6	2.2	2.0	3.1
H7	37/2	32/4	35/3	37/4	37/4	35/6	33/6	37/4	40/5	36/2	39/4	22/1	23/1	26/3		1.9	3.0	2.3	2.5	3.0	2.1	2.7	2.9	3.7
H8	32/1	31/3	34/2	33/3	35/3	31/5	29/5	33/3	31/4	34/1	34/3	12/0	11/0	14/2	19/1		2.3	1.4	1.6	2.4	1.2	1.6	1.8	3.0
H9	42/1	43/3	46/2	45/3	47/3	43/5	41/5	47/3	43/4	44/1	42/3	12/0	17/0	18/2	30/1	24/0		2.4	2.6	3.3	2.2	2.8	3.0	4.1
H10	32/1	32/3	34/2	33/3	37/3	33/5	31/5	37/3	31/4	36/1	34/3	15/0	18/0	16/2	23/1	15/0	25/0		1.7	2.7	1.5	1.5	2.1	3.2
H11	34/2	31/4	34/3	37/4	39/4	35/6	33/6	37/4	31/5	32/2	32/4	16/0	19/1	18/3	24/2	16/1	26/1	17/1		1.7	1.1	1.5	2.1	3.1
H12	31/8	26/10	28/9	29/10	33/10	27/12	25/12	29/10	28/11	30/8	31/10	18/7	21/7	20/9	24/8	18/7	28/7	21/7	10/8		1.9	2.1	2.9	3.8
H13	35/1	32/3	35/2	36/3	38/3	34/5	32/5	38/3	32/4	33/1	35/3	15/0	14/0	15/2	21/1	13/0	23/0	16/0	11/1	13/7		1.3	2.1	3.2
H14	38/4	33/6	36/5	35/6	39/6	35/8	33/8	37/6	35/7	36/4	36/6	16/3	19/3	18/5	24/4	14/3	26/3	13/3	12/4	14/8	11/3		2.3	3.6
H15	36/2	33/4	36/3	35/4	37/4	33/6	31/6	35/4	29/5	32/2	30/4	18/1	21/1	18/3	28/2	18/1	30/1	21/1	20/2	22/8	21/1	20/4		2.5
H16	38/2	34/4	34/3	33/4	35/4	33/6	31/6	33/4	33/5	33/2	25/4	30/1	33/1	29/3	37/2	30/1	42/1	33/1	31/2	32/8	33/1	34/4	24/2	

Data above the diagonal represent percentage differences between haplotypes. Data below the diagonal are the numbers of nucleotide substitutions (transitions/transversions).

indels with those of haplotype K1

site	
	1111111111
	2222222223333333333333344444555555555566677777777778888899999999990000000000
	8888888900111122555589011682222234577782344555567148891123456788901123344455
	1234567993501230501571490120234562625789013042348763946236177385349058039238 12
H1 (13)	–CATATACAATGTTCGCTTCCCATGACTATCTCGCAA TACGCGTGATGAGACAATCACTGTAGAGCGTTCCTCGTAT–
H2 (12)	.AT.G.....T...TT.....G..CTA..G.....C.....T.T..C...A.....AC...
H3 (15)	.T..G...GG.....T...TT....T.G..CTA..G.....T.T..C...A.....AC...
H4 (3, 4, 5, 10, 11, 14)	.T..GC..G.....T...T.....T.G..CTA..G.TT..A.....TGT..C...AT.....AC...
H5 (1, 11)	.T..G...G..A....T...T.....TCG..CTA..G.TT.....TGT..C...AT..C....AC...
H6 (1)	.T..G.....T...T.....T.G..CTA..G.TTA.A.....CTGT..C...AT.....AC...
H7 (6)	.T..G.....T...T.....T.G..CTA..G.TT..A.....TGT..C...AT.....AC...
H8 (7)	.T..G...G.....T...T.....T.G..CTA..G.TTA.A.....TGT..C...AT.....AC...
H9 (10)	.T.CG..T....C...T...T.....G..CTA..G.....A.A.....G....T.T.....A.....ACT.T
H10 (5)	..G.....CT.T...TT.....CTA..G.....G...A.T...T.T...G...T.....C...
H11 (3)	....G.C.G.C..CT.....TTG...TC....TA..G.....G.....T.T.....ATA.....C...
H12 (2)	C.....C....C.T.....C...C.A..G.....A.A.....T.T....AG.....A...T
H13 (3, 4)	T.....A.C.AT.C.T.....C...C.A..G.....A.....T.T....AG.....A...T
H14 (2)	C...GC.....AC..T.C.T.....C...C.A..G....TA.A.....T.T....AG.....A..AT
H15 (8)	C...G.....A.ATC..T.....C...CTAT.G.G....C...A.....T.T..C.AG.....T.C.A...T
H16 (9)	C...G.....AT...T.....C...C.AT.G.....A.A.....T.T....AG.....A...T
K1 (16, 17)	C.....C....C.T.....C...C.A..G.....A.A.....GG.T.T....AG.....A...T
K2 (17)	C...G.....CC..T...T.....C.....AT.G.....A.A.....T.T....AG.....A...T
K3 (17)	T..CG..T.....CT.T...TT.....C...C.A.TG.....A.A.....T.T....AG.....A...T
K4 (17)	C...G..T.....C..T...TT.G..A.TAACTC...G....A.....T.T....AG.....T..A...T
K5 (17)	C...G..T.....C.AT...TT.....C...C.AT.G.....A.A.....T.T.A..AG.....A...T
K6 (17)	C...G.....C..T...TT.GTG.C...C.AT.G.....A.A.....T.T....AG.....A...T
K7 (17)	C...G.....C..T...T.....C...C.A..G.....A.A...C.....T.TC...AG.....T.C..T
K8 (17)	C.G.G...G.....A.T..TT.....C...CTA..G.....A.A.....T.T.....T.C...CT.C..T

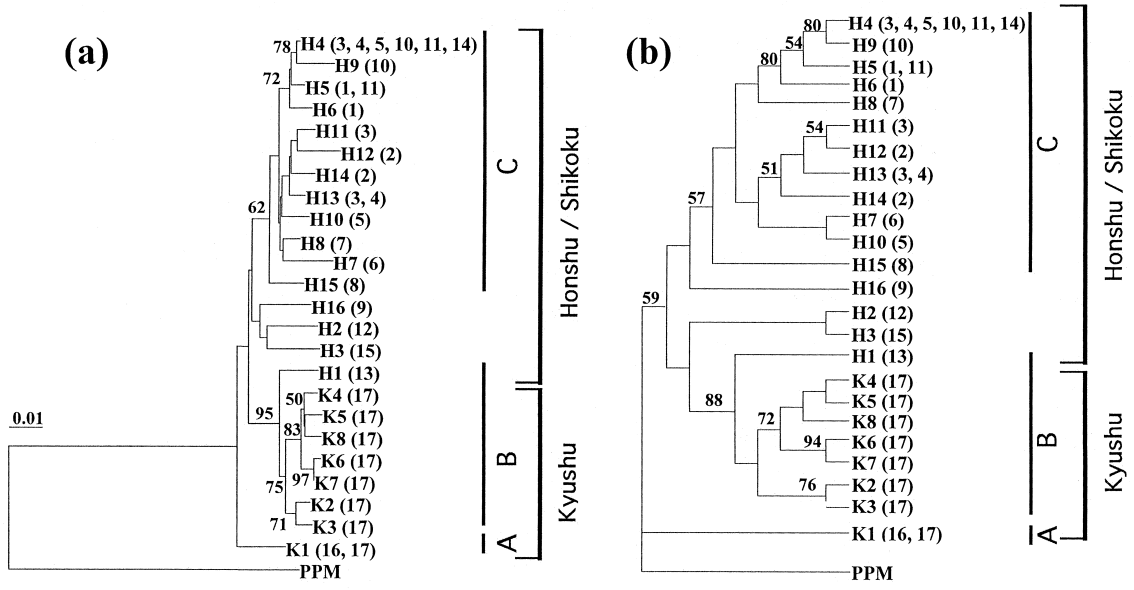

Fig. 2. Phylogenetic relationships reconstructed by (a) the neighbor-joining (NJ) and (b) the maximum parsimony (MP) methods based on the control region sequences of *Petaurista leucogenys*. The scale bar for NJ tree represents branch length in terms of nucleotide substitution per site. Numbers at nodes indicate bootstrap values higher than 50% derived from 1,000 replications for NJ tree and 100 replications for MP tree. Numbers in parentheses, which correspond to those of Table 1 and Fig. 1, indicate sampling localities.

1,052 bases and those of Group C had 1,054 bases, although there was an exception (H1).

DISCUSSION

Characterization of the mtDNA control region in *Petaurista leucogenys*

In vertebrates, it has been reported that the control region consists of two variable domains (5' and 3' ends) and a conserved central domain (Brown *et al*., 1986; Southern *et al*., 1988; Saccone *et al*., 1991). In the same way, the control region of *P. leucogenys* examined in the present study was also divided into three domains (Table 2). In particular, the 5' end domain was more variable than the 3' end domain and contained one gap-site (site number 281) in all haplotypes from Kyushu, in H1 and H2 from Honshu, and in H3 from Shikoku. Another gap-site (site number 1,052) was recognized in the 3' end domain in all haplotypes from Kyushu and in H2 and H3. The two gap-sites were specific to the control region of the Kyushu population. In addition, repetitive sequences, which were reported in the control region of some mammals (e.g., Hoelzel *et al*., 1994; Nagata *et al*., 1998; Kurose *et al*., 1999; Matsuhashi *et al*., 1999), were not found in *P. leucogenys* and *P. petaurista*.

Phylogeography of *Petaurista leucogenys*

According to pelage characteristics, Imaizumi (1960) classified *P. leucogenys* into three subspecies (*leucogenys*, *nikkonis*, and *oreas*), and demonstrated that *P. l. leucogenys* is distributed throughout the Kyushu and Shikoku islands, that *P. l. nikkonis* occurs in the eastern part of the Honshu Island, and that *P. l. oreas* occurs in the western part of the Honshu Island. However, in the present study, phylogenetic relationships among haplotypes did not correspond to subspecies classification and distribution.

Despite of the small number of specimens collected (Fig. 1), the Kyushu population was divided into two lineages: K1 lineage (Group A) and another lineage consisting of K2, K3, K4, K5, K6, K7, and K8 in Group B (Fig. 2). In the NJ and MP trees, K1 was likely to have been isolated from the other haplotypes by the first dichotomy, although the bootstrap values were not so high (< 50% in NJ tree; 59% in MP tree), suggesting that K1 diverged from the other haplotypes at an earlier point. On the other hand, although H1 was closely related to K2, K3, K4, K5, K6, K7, and K8 in both trees, the phylogenetic positions of H2 and H3 in the NJ tree were different from those in the MP tree (Fig. 2). The three haplotypes (H1, H2, and H3) shared the two gap-sites specific to the Kyushu population: site number 281 in H1, H2, and H3 and site number 1,052 in H2 and H3 (Table 2). This indicated that H2 and H3 are more closely related to K2, K3, K4, K5, K6, K7, and K8 or that they are intermediate haplotypes between the Kyushu population and Honshu/Shikoku population. The phylogenetic position of H16 that was not clustered with the three major lineages was not clear in the present study.

In terms of the Honshu/Shikoku population in the NJ and MP trees, it is noteworthy that genetic distances between haplotypes do not always correspond to geographic distances of sampling localities, and exhibit low bootstrap values (Fig. 2). Moreover, six specimens from different localities (Gifu, Kagawa, Nagano, Toyama, Wakayama, and Yamanashi) shared the H4 haplotype, and two specimens from Aomori and Gifu had the H5 haplotype (Fig. 1 and Table 1). In the Honshu/Shikoku population, except for H1, H2, H3, and H16, haplotype H4 seemed to be most common.

Based on some fossil records, Kawamura (1988) reported that *P. leucogenys* had presumably migrated from southern China to Japan through the land bridge (Fig. 1) which was formed around the area of the present East China Sea in the Early Middle Pleistocene. It was not known that *Petaurista* had existed in the Korean Peninsula at that time. Accepting Kawamura's hypothesis (1988), inevitably, the first place where *P. leucogenys* had migrated from southern China could have been the Kyushu Island in Japan, and then it could have extended its distribution toward the Honshu and Shikoku Islands (see Fig. 1). Fossils of *Petaurista* before the Holocene period are very rare in the Japanese islands. The Middle Pleistocene fossils of *P. leucogenys* were found in two localities of Japan (Hasegawa, 1966; 1972; Kowalski and Hasegawa, 1976; Kawamura, 1988) which are very close to each other as shown in Fig. 1. Moreover, the Late Pleistocene fossils of *P. leucogenys* were also recognized in six localities of Japan (Shikama, 1949; Hasegawa, 1966; Kowalski and Hasegawa, 1976; Kawamura, 1980; 1981; 1982; 1988; Kawamura and Sotsuka, 1984; Kawamura *et al*., 1986), as shown in Fig. 1. Judging from these fossil records, by the Middle or Late Pleistocene, *P. leucogenys* could already have been distributed in the Honshu and Shikoku Islands. *Petaurista leucogenys* is an arboreal animal and inhabits the temperate forests (Nowak, 1991). Therefore, during glacial stages in the Pleistocene, the habitats of this animal may have been reduced due to the southward shifting of temperate forests in Japan. The results of the present study show that the genetic distances in the Honshu/Shikoku population were not related to geographic distances of sampling localities. This suggests that *P. leucogenys* rapidly extended its distribution in a short time during the northward expansion of temperate forests in Japan after the last glacial stage of the Pleistocene.

Although the evolutionary rate of humans may not always correspond to that of the giant flying squirrel because of the differences of generation time between humans and giant flying squirrels, applying the evolutionary rate (approximately 8.4% per million years, Myr) of the human control region reported by Vigilant *et al*. (1989), the divergence times between Groups A and B, between Groups A and C, and between Groups B and C were estimated to be approximately 0.4–0.5, 0.4–0.5, and 0.4–1.0 Myrs ago, respectively. On the other hand, the divergence times in Group B and in Group C were approximately 0.1–0.3 and 0.1–0.5 Myrs ago, respectively. Accordingly, the divergences among haplotypes of *P. leucogenys* may have occurred rapidly from the Middle to Late Pleistocene.

ACKNOWLEDGMENTS

We would like to thank Dr. T. Kawamichi and Dr. S. Uni (Osaka City University), Dr. M. Baba (Kitakyushu Museum of Natural History), Mr. H. Kuribayashi, Dr. C. Katoh, and Dr. M. Itoh (Kanagawa Prefecture Natural Environment Conservation Center), Mr. T. Tsujimoto (Morioka Zoo), Mr. T. Okayama (Omogo Mountain Museum), Dr. S. Yachimori (Tochigi Prefectural Museum), Asa Zoological Park, Mr. M. Kishioki, and Ms. M. Shigeta, for supplying specimens. This study was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

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(Received June 8, 2000 / Accepted September 11, 2000)