

Genetic relationships of the raccoon dog: A special reference to the individuals from the Imperial Palace and the Akasaka Detached Palace, Tokyo Metropolis, Japan

Masahiro A. Iwasa,¹ Taketo Takayama,¹ Tomoko Ogo,² and Shin-ichiro Kawada²

¹College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa Pref., Japan

²Department of Zoology, National Museum of Nature and Science, Tsukuba, Ibaraki Pref., Japan

E-mail: iwasa.masahiro@nihon-u.ac.jp

Abstract. Genetic relationships of the raccoon dog, *Nyctereutes procyonoides*, were researched in eastern Japan, focusing particularly on the populations of the Imperial Palace and the Akasaka Detached Palace, Tokyo Metropolis, Japan. We analyzed partial sequences of the mitochondrial D-loop region and obtained 17 haplotypes from all the samples of *N. procyonoides*. Most of the samples carried alternatively two haplotypes, A and B, and all the individuals from the two palaces had these haplotypes. Geographically, the two palaces are separated by approximately one km of high-traffic roads and busy commercial streets, yet young individuals of *N. procyonoides* may move among areas such as these palace grounds as they disperse. In other localities, there were haplotypes other than A and B that are relatively rare in the present samples. The gene distribution patterns have been influenced by the founder effect at the early period of foundations of the raccoon dog populations and dispersion, and home range patterns of *N. procyonoides*.

Key words: *Nyctereutes procyonoides*, Imperial Palace, Akasaka Detached Palace, genetic relationship

Introduction

Tokyo is one of the most urbanized cities in the world, and the center is constructed in a urban street and a residential area. Therefore, most of the environments are not appropriate for habitats of wild animals. On the other hand, there are the wild animals that have adapted to the urban environment, including indigenous and introduced species (Numata and Obara, 1982; Ecological Society of Japan, 2002; Ohdachi *et al.*, 2009). One of them, the raccoon dog, *Nyctereutes procyonoides*, is a popular, medium-sized mammal that has been observed in urban areas of Tokyo Metropolis and other prefectures (Numata and Obara, 1982; Ikeda, 1991a, b; Ohdachi *et al.*, 2009).

At the center of the urban areas of Tokyo Metropolis (23 wards), there are several larger forests consisting of native woods, for example, at the Imperial Palace and the Akasaka Detached Palace (Fig. 1). These palaces have been founded as natural forests since the Edo period, with artificial maintenance, and

have been surrounded by artificial structures during the era of Japanese economic progress after World War II. In such a situation, Endo *et al.* (2000) and Takeda *et al.* (2000) reported that the occurrence of the raccoon dog was confirmed. In addition, ecological research concerning the raccoon dog, such as feeding preference, has been performed in the palaces (Teduka and Endo, 2005; Sako *et al.*, 2008). However, it is unclear whether the raccoon dog populations in the palaces have been maintained as indigenous ones since the foundation of the palaces.

In this study, we collected samples of the raccoon dog mainly from eastern Japan and analyzed their mitochondrial DNA sequences. On the basis of current molecular data, we evaluated the originality of the raccoon dog population in Tokyo Metropolis, focusing particularly on the individuals from the Imperial Palace and the Akasaka Detached Palace.

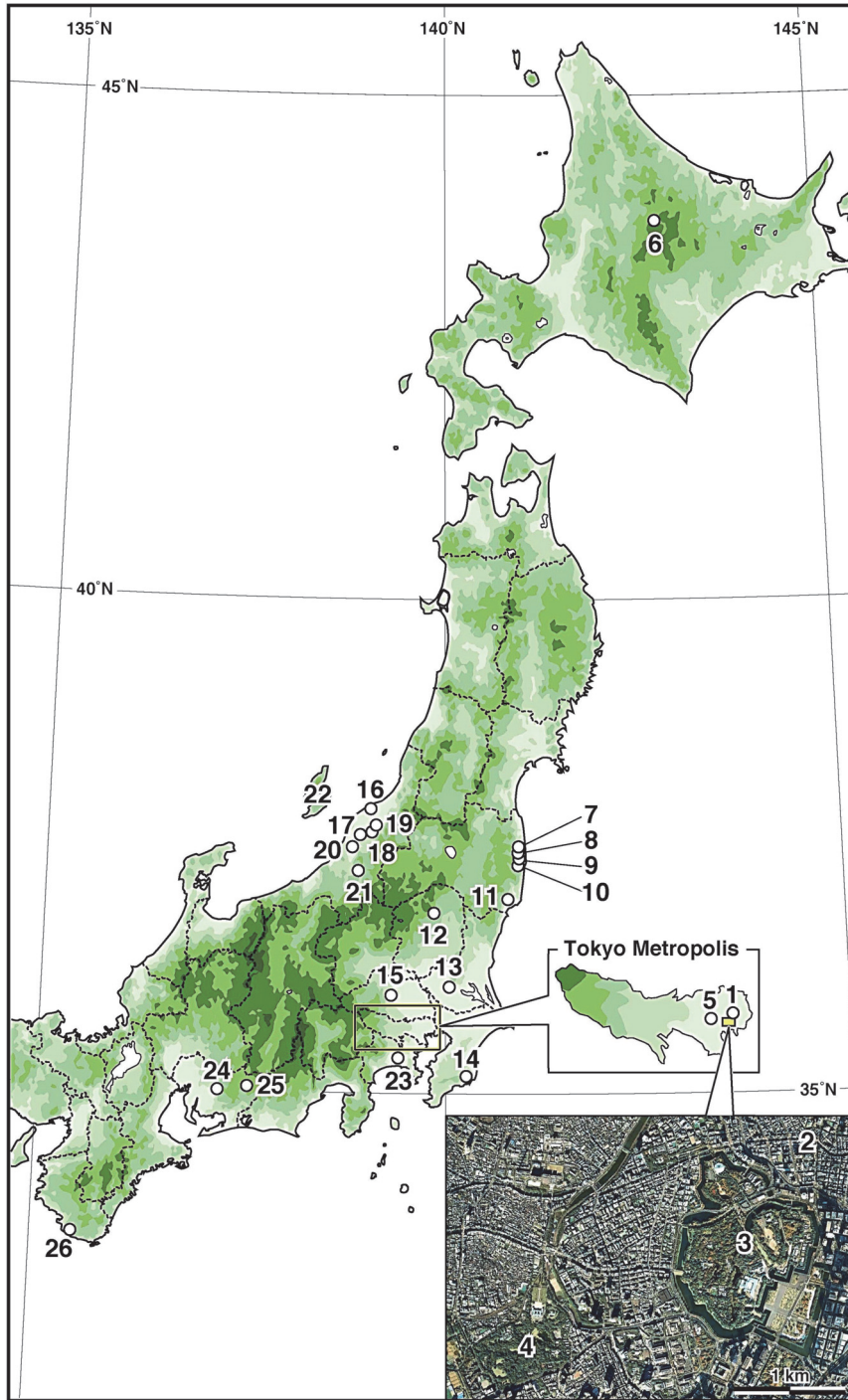


Fig. 1. Sampling localities of the raccoon dog examined in this study. Numbers are identical to those in Table 1. A land satellite photograph is used from Google Earth.

Table 1. Samples of *Nyctereutes procyonoides* examined in this study.

Locality	Individual No.*	Haplotype	Locality	Individual No.*	Haplotype
Tokyo Metropolis			Hokkaido		
Taito-ku Ward			Kamikawa (6)	NP53	F
Ueno (1)	NP03	N	Fukushima Pref.		
	NP09	G	Futaba (7)	NP38	O
Chiyoda-ku Ward			Ohkuma (8)	NP37	C
Hitotsubashi (2)	NP30	A	Tomioka (9)	NP36	C
Imperial Palace (3)	NP04	A		NP41	P
	NP10	B	Naraha (10)	NP47	C
	NP11	B	Iwaki (11)	NP39	O
	NP13	B	Tochigi Pref.		
	NP14	B	Yaita (12)	NP01	A
	NP15	B		NP02	H
	NP16	B	Ibaraki Pref.		
	NP17	B	Tsukuba (13)	NP54	N
	NP18	B	Chiba Pref.		
	NP19	B	Katsuura (14)	NP64	Q
	NP20	B	Saitama Pref.		
	NP21	B	Higashi-Matsuyama (15)	NP65	A
	NP22	B		NP66	A
	NP23	B		NP67	A
	NP26	B		NP68	A
	NP27	B		NP69	A
	NP28	B		NP70	A
	NP58	B	Niigata Pref.		
	NP59	B	Niigata (16)	NP43	A
	NP60	B	Sanjo (17)	NP40	A
	NP61	B	Kamo (18)	NP48	A
	NP75	B		NP49	A
	NP76	B		NP81	J
Minato-ku Ward			Kosudo (19)	NP80	A
Akasaka Detached Palace (4)	NP05	A	Nagaoka (20)	NP55	C
	NP06	A		NP56	A
	NP12	A	Uonuma (21)	NP42	A
	NP25	A		NP44	A
	NP29	A		NP45	A
	NP71	D		NP46	A
	NP74	A		NP50	A
Nerima-ku Ward (5)				NP51	A
Toyotama	NP79	A	Sado Is. (22)	NP52	E
Sakuradai	NP82	A	Kanagawa Pref.		
	NP83	A	Fujisawa (23)	MAI-1081	K
				MAI-1082	I
			Aichi Pref.		
			Toyota (24)	NP72	L
				NP73	L
			Shitara (25)	NP62	L
				NP63	L
			Wakayama Pref.		
			Susami (26)	NP57	M

Numbers in parentheses are locality codes that are indicated in Fig. 1.

*NP series are deposited in National Museum of Nature and Science. MAI series are preserved as Iwasa's private collection.

Haplotype A: ACCCTGCTCCACCATCAGCACCCRAAGCTGAAATTCCTTTAAACSTATTCCCTGGATACCTCACACCCTCTCTATTATATATATTACAGTACCTCCCTGTCGCA
 Haplotype B:T.....
 Haplotype C:C.....
 Haplotype D:
 Haplotype E:
 Haplotype F:
 Haplotype G:
 Haplotype H:
 Haplotype I:
 Haplotype J:C.....
 Haplotype K:A.....
 Haplotype L:
 Haplotype M:
 Haplotype N:C.....
 Haplotype O:G..ATC.....
 Haplotype P:C.....G..ATC.....
 Haplotype Q:C.....G..ATC.....

110 120 130 140 150 160 170 180 190 200
 Haplotype A: TGTCCGACGTCACCCCC--TATGTACGTCTGTCATTAATGGCTTGCCTCCCATGCAATATAAGCAGGTACATATCCCAATGTTATGTCACAATAGACATGAAC
 Haplotype B:C.....
 Haplotype C:
 Haplotype D:CC.....
 Haplotype E:
 Haplotype F:
 Haplotype G:G.....
 Haplotype H:G.....
 Haplotype I:G.....
 Haplotype J:
 Haplotype K:
 Haplotype L:G.....
 Haplotype M:
 Haplotype N:C.....T.....
 Haplotype O:A.....T.....
 Haplotype P:A.....T.....
 Haplotype Q:A.....T.....

210 220 230 240 250 260 270 280 290 300
 Haplotype A: TACTTAATCATACGGTATTTTACTTCRAGGACATGCTCAAGTTGCATATCCACCTAGTCCCAATAAGGGATTAATCCACCATGCTCCGAGAACCATCAATCC
 Haplotype B:
 Haplotype C:
 Haplotype D:
 Haplotype E:
 Haplotype F:
 Haplotype G:A.....C.....
 Haplotype H:A.....C.....
 Haplotype I:
 Haplotype J:
 Haplotype K:
 Haplotype L:A.....
 Haplotype M:A.....
 Haplotype N:A.....
 Haplotype O:C.....A.....
 Haplotype P:AA..CC..GTC.....A.T..AC.....
 Haplotype Q:AA..CC..TC.....A.....A.....

Materials and methods

A total of 84 individuals of *N. procyonoides* were examined in this study (Table 1 and Fig. 1). Total genomic DNA was extracted from the muscle tissues using a Wizard[®] Genomic DNA Purification Kit (Promega). Partial D-loop segments were amplified by PCR with the primer set L15411 (5'-TCCCTAAGACTCAAGGAAGAAGC-3')/H15841 (5'-GATGTCCCATTTGAGAGGATTAG-3') according to the complete mitochondrial DNA sequence of *N. procyonoides* with accession number GU256221 of the DNA databases (GenBank/EMBL/DDBJ). The PCR was carried out with the reaction mixtures (20 μ l) according to Iwasa and Nakata (2011). The PCR products were sequenced by an automated sequencer (model 310 or 3100, ABI) using a BigDye Terminator Cycle Sequencing Kit ver. 3.1 (ABI).

The present D-loop sequences were aligned using the Clustal X program (Thompson *et al.*, 1994). We constructed a neighbor-joining tree (Saitou and Nei, 1987) with the D-loop sequences on the basis of Tamura–Nei distances, which consider transitions and

transversions, and gaps at all sites (Tamura and Nei, 1993). Bootstrap analysis was performed (1,000 replicates) using the MEGA ver. 5.10 program (Tamura *et al.*, 2011). In addition, a network tree was also constructed by the Neighbor-Net method with D-loop sequences using the Splits Tree (Huson and Bryant, 2006).

Results and discussion

We determined the D-loop sequences to be 418–420 base pairs, which accorded with the nucleotide site 15416–15832 in the mitochondrial sequence of GU256221, and obtained 17 haplotypes of the D-loop sequences in the present samples (Table 1 and Figs. 2 and 3). The bootstrap values were entirely too low, and the topology of the tree may be unreliable in regard to the actual phylogenetic relationship for the present samples (Fig. 3). Thus, the tree indicates only the haplotype discrimination in the individuals examined. On the other hand, a network tree indicated a complicated relationship within all the haplotypes except for P and Q (Fig. 4).

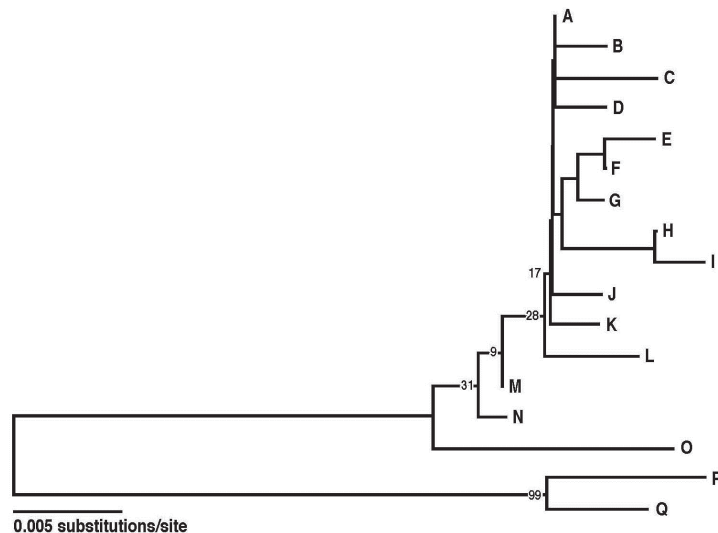


Fig. 3. A phylogenetic tree constructed using mitochondrial D-loop sequences of the present raccoon dog samples by the neighbor-joining method. Main bootstrap values are indicated at nodes. All the letters of the alphabet are indicated as the present haplotypes that are identical to those in Table 1.

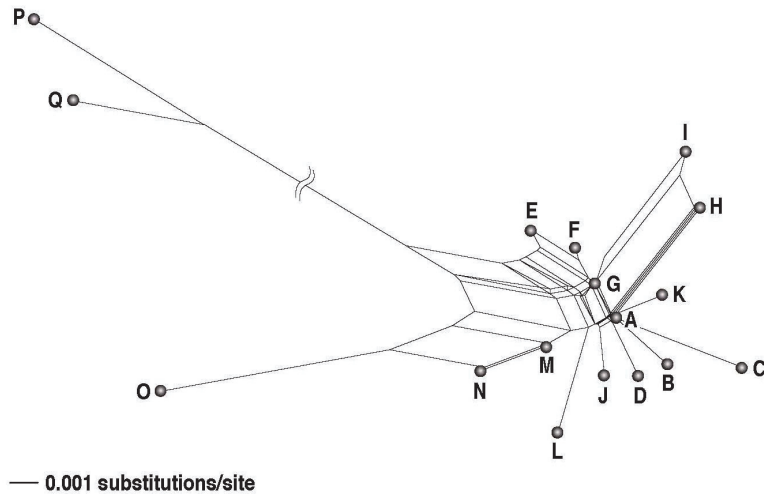


Fig. 4. A network tree constructed using mitochondrial D-loop sequences of the present raccoon dog samples by the Neighbor-Net method. All the letters of the alphabet are indicated as the present haplotypes that are identical to those in Table 1 and Fig. 3.

In the samples from the Imperial Palace, there were two haplotypes, A and B, and the former was observed in those from the Akasaka Detached Palace (Table 1 and Figs. 3 and 4). The Imperial Palace and the Akasaka Detached Palace are separated by approximately one km of high-traffic roads and busy commercial streets (Fig. 1). However, considering that the lifestyle of the raccoon dog shows usage of artificial structures (Numata and Obara, 1982; Ikeda, 1991b; Sako *et al.*, 2008; Ohdachi *et al.*, 2009), the present fact suggests that young individuals of *N. procyonoides* would possibly move areas including the Imperial Palace and the Akasaka Detached Palace. On the other hand, haplotypes G and N also were confirmed in Tokyo Metropolis. These haplotypes were rare, and haplotype N was also observed in individuals from Tsukuba, Ibaraki Pref., near Tokyo (Table 1 and Fig. 1). In addition, according to the current network tree, haplotype A would be considered a relatively ancestral type in haplotypes obtained from Tokyo Metropolis areas, including neighboring prefectures, because haplotype A was positioned at an inner node of the network showing complicated patterns (Table 1 and Figs. 1 and 4). Moreover, the network relationship

indicated that haplotype B seemed to be derived from haplotype A (Fig. 4). Thus, we concluded that haplotypes A and the derivative haplotype B have been mainly fixed in the Tokyo Metropolis areas, and the other haplotypes have been maintained to some extent though being lower frequencies, by slight founder effects and differentiations (Harrison, 1989).

Our current samples of *N. procyonoides* were collected mainly from eastern Japan (Fig. 1). There were some rare haplotypes, but haplotypes A and B occupied the greater part of all the individuals examined in this study (Table 1). Nevertheless, samples from Fukushima Pref. and Aichi Pref. showed haplotypes specific to each locality: haplotypes C, O, and P, and haplotype L, respectively (Table 1). On the other hand, most individuals from Niigata Pref. carried haplotype A, which is popular in Tokyo Metropolis (Table 1). Thus, the local fixation of the genetic constitutions is not always observed in *N. procyonoides* of Japan, and based on current results, the haplotypes are not characterized locally. Accordingly, we estimate that such situation considered with current haplotype distribution patterns would be ecologically related to the dispersion pattern of *N. procyonoides*. It is reported

that the home range of the raccoon dog varies from 10 to 600 ha, and dispersion of males is considered as greater than 1,000 ha (Yamamoto *et al.*, 1994; Ohdachi *et al.*, 2009). Therefore, these ecological tendencies of the raccoon dog would influence the gene distribution patterns, particularly in planes and lower altitudes (Saeki *et al.*, 2007). On the other hand, geographic barriers seem to influence the gene distribution patterns considering the haplotype patterns in Fukushima and Aichi prefectures. However, the sampling localities are insufficient at present. Thus, further research is needed to evaluate the geographic relationship of the haplotype patterns for the raccoon dog of Japan.

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タヌキの遺伝的關係 —特に皇居と赤坂御用地の個体について—

岩佐真宏・高山剛人・小郷智子・川田伸一郎

東日本産タヌキ *Nyctereutes procyonoides* の遺伝的変異について、特に皇居と赤坂御用地の集団に注目して調査した。ミトコンドリア D-loop の部分配列を分析した結果、合計17ハプロタイプ (A~Q) を検出した。多くの個体はハプロタイプ A もしくは B を有し、特に皇居と赤坂御用地の個体はすべてこれらのいずれかであった。皇居と赤坂御用地は距離的に約 1 km 離れており、その間に交通量の多い道路やオフィス街がある。しかし本結果は、タヌキがこの二地点間を移動している可能性を示唆した。またハプロタイプ A や B 以外の遺伝子型は、全体的に観察された頻度が低かった。したがって遺伝子型の分布様式は、タヌキの地域集団が確立された初期段階の創始者効果や、分散および行動圏パターンの影響を受けてきたものと推察された。

