Skull Morphology and Mitochondrial DNA Sequence Analysis in the Lesser Japanese Mole (*Mogera imaizumii*) from the Imperial Palace (Tokyo, Japan)

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ABSTRACT. Since about 1630, the Imperial Palace has been biologically isolated from other habitats by the development and urbanization of Tokyo. We morphologically examined the skulls of the lesser Japanese mole (*Mogera imaizumii*) from the Imperial Palace and compared them with those from Kanto District, while the sequences of the cytochrome *b* and 12S rRNA genes were also analyzed to clarify the genetic status of this isolated population. The skulls from the Imperial Palace were much larger than those from Kanto District in the length items. We suggest that the Imperial Palace skulls morphologically may compose a cluster as a large body-sized type in Kanto District within the dots of *Mogera imaizumii* in charts of principal component analysis. The mitochondrial DNA sequences of the Imperial Palace population were highly homologous to those of other Tokyo population at the level of 98.5% in cytochrome *b* and 98.7% in 12S rRNA genes.—**KEY WORDS:** cytochrome *b*, Imperial palace, Japanese mole, 12S rRNA, skull.

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The lesser Japanese mole (*Mogera imaizumii*) is mainly distributed in eastern part of Honshu Island in Japan. Although the locality includes Tokyo Metropolitan Area, and Kanagawa and Saitama Prefectures in Kanto District, the species has been extinct in the center of Tokyo Metropolitan Area and some regions of Kanto District because of urbanization. In the Imperial Palace (Chiyoda Ward, Tokyo) including Fukiage Area, however, woods and forests have been artificially maintained, and the lesser Japanese mole has been separated from outside habitats and conserved within the orchard. Fukiage Area, a part of Edo-Jo (Fortress of Edo) during Edo Era, has been biologically isolated since about 1630 under the reign of Iemitsu, the third Tokugawa Shōgun. So, the lesser Japanese mole in this area is suggested to consist of a long-term isolated population, and we expect to morphologically and genetically examine the present status of the population.

MATERIALS AND METHODS

Osteometry: We used totally 92 skulls of the lesser Japanese mole (*Mogera imaizumii*) from Imperial Palace, Tokyo Metropolitan Area, and Kanagawa and Saitama Prefectures. The skulls of Tokuda's mole (*Mogera tokudae*) from Sado Island (Niigata Prefecture) were also examined as outgroup materials. The fauna and flora research program in the Imperial Palace by the National Science Museum, Tokyo, has enabled us to collect some lesser Japanese moles in the orchard of Fukiage Area within the Imperial Palace and we have stored the skull specimens in National Science Museum, Tokyo, since 1996. The specimens from other localities have been also maintained in National Science Museum, Tokyo.

The locality and sex of the specimens were dependent on the biological data of the collection and on the observation of urogenital organ in the carcasses. The composition of locality and sex are arranged in Table 1. We selected and used the adult specimens according to the age estimation standard using molar attrition and capture date [4, 10, 11].

The skull measurement was carried out in 18 items with a vernier caliper to the nearest 0.05 mm. The nomenclature in measurements were fundamentally based on the textbook of osteometry [3] and the item abbreviations were arranged in Table 2.

The mean values of items were calculated in each sex and locality to elucidate the size and shape variation. The mean values were compared by Student's *t*-test between the populations to clarify the size specificity of the Imperial Palace population. We also applied the whole data of 18 measurements for the principal component analysis to discuss the long-term isolation effect in the Imperial Palace.

Mitochondrial DNA sequence analysis: We used 5 individuals of the lesser Japanese mole from Fukiage Area

Table 1. Locality and sex composition of skull specimens

Locality symbol	Origin of specimens	Male	Female
I	Imperial Palace	3	2
T	Tokyo Metropolitan Area	9	8
K	Kanagawa Prefecture	19	21
S	Saitama Prefecture	11	9
D	Sado Island*	5	5
Total		47	45

* This population belongs to Tokuda's mole.

Table 2. List of the measurements and their abbreviations

1. Cranium		
Profile length		PL
Condylbasal length		CL
Short lateral facial length		SL
Length from Infradentale to the caudal end of pterygoid hamulus		LPT
Zygomatic width		ZW
Least breadth between the orbits		LBO
Greatest neurocranium breadth		GNB
Median palatal length		MPL
Length from Basion to Staphylion		LBS
Dental length		DL
Greatest palatal breadth		GPB
Greatest mastoid breadth		GMB
Height from Akrokranium to Basion		HAB
2. Mandible		
Length from the condyle		LC
Length from the angle		LA
Length from the Infradentale to aboral border of the alveolus		LIA
Height of the vertical ramus		HR
Height of the mandible at M1		HM

The measurements were based on Driesch [3].

of the Imperial Palace, and 2 animals from Hino City, the eastern part of Tokyo Metropolitan Area. We also examined 2 Tokuda's moles from Sado Island.

The skeletal muscles were excised from each individual and stored in -20°C freezer or in 70% ethanol. DNA was extracted from 50 mg per animal skeletal muscle by digestion in 100 mM Tris-HCl (pH8.0)/10 mM EDTA/100 mM NaCl/0.1% SDS/proteinase K (5 µg/ml) for 2 hr at 50°C. Total DNA was purified by adding an equal volume of water-saturated phenol, phenol/chloroform (1:1), and chloroform, and then precipitated with ethanol.

Primers for the cytochrome *b* genes (L14724; CGAAGCTTGATATGAAAAACCATCGTTG and H15149; GCCCTCAGAATGATATTTGTCCTCA) and the 12S rRNA (L1091; CAAACTGGGATTAGATACCCC ACTAT and H1478; GAGGGTGACGGGCGGTGTGT) were prepared. Polymerase chain reaction (PCR) was performed with a PCR reagent kit including *Ex Taq* polymerase (Takara, Japan). Each cycle of the PCR consisted of denaturation for 1 min at 94°C, hybridization for 1 min at 50°C, and extension for 1 min at 72°C. This cycle was repeated 35 times. PCR products were run on 1.5% agarose gel and stained with ethidium bromide to check amplification, and then treated with the spin-column to remove the single stranded DNA and the remaining dNTPs.

After the spin-column treatment, the secondary PCR was performed with a cy5-labelled primer and a cycle sequencing kit (Amersham Pharmacia Biotech, Uppsala, Sweden). The PCR products were sequenced by ALF DNA sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden).

RESULTS

Osteometry: The mean value and standard deviation of 18 measurements in both sexes are given in Table 3, with the results of Student's *t*-test between the populations. The principal component charts from all measurement data are drawn in male and female in Figs. 1 and 2, respectively.

The skulls from the Imperial Palace are much larger than those from Kanto District in the length items of PL and LC. We can conclude from various measurement data that the Imperial Palace population is relatively larger in skull size among populations of Kanto District (Table 3).

The three populations of Tokyo Metropolitan Area, and Kanagawa and Saitama Prefectures are not separated in the principal component chart in both sexes. The Imperial Palace dots are plotted in the positive area of the second principal component, and indicate the relatively large size among the lesser Japanese mole populations in the first principal component.

Mitochondrial DNA sequence analysis: The nucleotide sequences were selected in the surely-obtained region and are shown in cytochrome *b* and 12S rRNA genes (Figs. 3 and 4). The Imperial Palace data showed 98.5% of homology to the sequence of Hino City, and 90.3% to that of Tokuda's mole in cytochrome *b*, while 98.7% to Hino population and 96.2% to Tokuda's mole in 12S rRNA. In cytochrome *b*, the differences at the level of amino acid sequence are also shown in Fig. 3.

DISCUSSION

The variation of skull size in lesser Japanese mole has been recorded in some districts [1, 2]. The skull from the Imperial Palace is smaller than that from some regions in the Tohoku District, and we can recognize the large variation in size among the lesser Japanese mole populations. We suggest that the Imperial Palace skulls may morphologically compose a cluster within the dots of *Mogera imaizumii* in charts of principal component analysis.

Since the lesser Japanese mole has been already extinct in some specimen localities of the Tokyo Metropolitan Area, the present morphological data is noteworthy. The Tokuda's mole is significantly much larger in most items than the lesser Japanese mole [12]. The dots of Tokuda's mole are also obviously separated from the lesser Japanese mole populations in the principal component analysis.

The mitochondrial DNA sequences are being analyzed to elucidate the phylogenetical status of moles and other Japanese insectivores [5, 6], while the restriction fragment length polymorphism was indicated in some species of Japanese mole [7]. The chromosome types have been also clarified in almost mole species of Japan [8, 9]. We can conclude that the Imperial Palace population has higher homology in both nucleotide sequences to Hino city population than to Tokuda's mole. It is consistent with the results of Okamoto [6] demonstrating that Tokuda's mole is genetically distinct from each population of *Mogera*

Table 3. The mean value (mm) and standard deviation of skull measurements and the significant differences between localities in both sexes

		PL	CL	SL	LPT	ZW	LBO	GNB	MPL	LBS	DL	GPB	GMB	HAB	LC	LA	LIA	HR	HM
1. male																			
Imperial Palace	mean value	36.42	31.12	13.47	21.33	12.95	8.03	17.35	15.15	16.10	15.03	9.20	11.18	6.27	23.47	23.27	13.27	10.57	2.02
	standard deviation	0.71	0.62	0.18	0.55	0.18	0.38	0.23	0.45	0.43	0.18	0.17	0.38	0.08	0.85	0.66	0.28	0.46	0.10
Tokyo Metropolitan Area	mean value	35.06	30.17	13.01	20.67	12.59	7.83	16.89	14.30	16.04	14.37	9.01	10.96	5.88	22.59	22.36	13.12	9.92	2.22
	standard deviation	0.73	0.77	0.35	0.63	0.27	0.26	0.29	0.44	0.37	0.33	0.16	0.33	0.24	0.53	0.57	0.21	0.46	0.24
Kanagawa Prefecture	sig. dif.*	+	+	+	—	+	—	+	+	—	+	—	—	+	+	+	—	+	—
	mean value	34.94	30.00	12.76	20.44	12.38	7.73	16.59	14.39	15.67	14.42	8.81	10.63	5.81	22.57	22.07	12.95	9.73	2.11
Saitama Prefecture	standard deviation	0.69	0.67	0.33	0.42	0.44	0.23	0.36	0.34	0.53	0.46	0.31	0.35	0.33	0.51	0.42	0.40	0.36	0.27
	sig. dif.*	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+	—	+	—
Sado Island	mean value	35.12	30.34	12.97	20.71	12.41	7.80	16.66	14.45	15.98	14.43	8.95	10.57	5.56	22.64	22.39	12.94	10.00	2.20
	standard deviation	0.96	1.04	0.48	0.74	0.53	0.20	0.46	0.60	0.49	0.59	0.48	0.49	0.29	0.64	0.62	0.32	0.44	0.22
Sado Island	sig. dif.*	+	—	—	—	—	—	+	+	—	—	—	+	+	+	+	—	+	—
	mean value	39.53	34.13	15.52	24.13	14.60	8.54	18.60	17.50	16.77	17.57	10.39	12.18	6.19	26.20	25.95	15.70	11.19	2.80
	standard deviation	0.65	0.52	0.34	0.44	0.46	0.25	0.34	0.26	0.42	0.24	0.19	0.24	0.20	0.74	0.58	0.42	0.35	0.18
	sig. dif.*	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+
2. female																			
Imperial Palace	mean value	36.65	31.78	13.63	21.83	13.03	7.88	17.60	15.53	16.33	15.15	9.30	11.53	6.23	23.53	23.03	13.40	10.50	2.25
	standard deviation	0.42	0.18	0.53	0.46	0.67	0.25	0.04	0.25	0.07	0.35	0.39	0.25	0.18	0.11	0.42	0.35	0.21	0.21
Tokyo Metropolitan Area	mean value	34.79	29.74	12.90	20.53	12.55	7.86	16.90	14.23	15.61	14.40	8.98	10.86	5.66	22.35	22.13	12.96	9.84	2.17
	standard deviation	0.78	0.74	0.32	0.49	0.30	0.25	0.59	0.27	0.61	0.44	0.48	0.29	0.27	0.57	0.55	0.25	0.35	0.21
Kanagawa Prefecture	sig. dif.*	+	+	+	+	—	—	—	+	—	+	—	+	+	+	+	+	+	—
	mean value	34.41	29.50	12.53	20.15	12.18	7.79	16.52	14.15	15.46	14.29	8.79	10.56	5.81	22.04	21.70	12.80	9.60	2.12
Sado Island	standard deviation	0.45	0.53	0.32	0.42	0.22	0.31	0.24	0.32	0.41	0.30	0.21	0.26	0.34	0.39	0.43	0.33	0.24	0.26
	sig. dif.*	+	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	—
Sado Island	mean value	38.75	33.39	15.10	23.63	14.14	8.40	18.22	17.11	16.38	17.29	10.08	12.08	6.11	25.54	25.36	15.55	10.72	2.60
	standard deviation	0.85	0.97	0.39	0.65	0.17	0.25	0.50	0.60	0.51	0.53	0.30	0.61	0.20	0.65	0.69	0.33	0.27	0.19
	sig. dif.*	+	+	+	+	+	—	+	—	+	+	—	—	+	+	+	+	—	+

The mean values are shown in the upper rows, and the standard deviation in the lower rows. * significant difference between data of Imperial Palace and those of each locality: * $P < 0.05$. — No significant difference.

Fig. 1. The principal component chart of male skulls between the first and second transformed variables from 18 measurements. Alphabets indicate the locality (Table 1). PC1, the first principal component. PC2, the second principal component. The percentage of the variation explained by PC1 is 79.1, and that by PC2 is 5.9. The items, which largely contribute to the second principal component, are HAB, GPB and LBS in higher ranking.

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Fig. 2. The principal component chart of female skulls between the first and second transformed variables from 18 measurements. Alphabets indicate the locality (Table 1). PC1, the first principal component. PC2, the second principal component. The percentage of the variation explained by PC1 is 76.9, and that by PC2 is 6.1. The items, which largely contribute to the second principal component, are HAB, HM and LBS in higher ranking.

Imperial Palace	ATGACAAACA	TTGAAAGAC	ACACCCACTA	ATAAAAATTG	TTAATAACTC	ATTGATTGAC
Hino City	-----	-----	-----	-----	<u>T</u> -----	-----
Tokuda's mole	-----	-----A--	-----T--	-----	-----C-----	-----T-----
Imperial Palace	CTACCAGCAC	CATCAAACAT	CTCTTCATGA	TGAAATTCCG	GATCACTACT	TGGAATCTGC
Hino City	-----	-----	-----	-----	-----	-----
Tokuda's mole	-----	-----	---A--G--	-----	-----T-----	G-----
Imperial Palace	CTAATCCTAC	AAATCCTAAC	AGGACTATTT	CTAGCAATAC	ACTACACCTC	GGACACAATA
Hino City	-----	-----	-----	-----	-----	-----
Tokuda's mole	T-----	-----	-----C	T-----	-----	A-----
Imperial Palace	ACAGCCTTCT	CATCTGTAAC	CCACATCTGC	CGAGACGTTA	ATTACGGTTG	ACTTATCCGA
Hino City	-----	-----	-----	-----	-----	-----
Tokuda's mole	-----	-----C-----	---T-----	-----A-	-----C--	-----T----
Imperial Palace	TATTTACACG	CAAACGGCGC	ATCAATATTG	TTTATTTGTT	TATTCCTACA	CGTAGGACGA
Hino City	-----	-----	-----	-----	-----	-----
Tokuda's mole	--C-----	-----	-----T	-----C-	----TT----	-----
Imperial Palace	GGCCTGTATT	ATGGATCCTA	TATGTTTATA	GAAACCTGAA	ACATTGGAGT	TATCTTACTT
Hino City	-----	-----	-T-----	-----	-----	-----
Tokuda's mole	---T-A--C-	-C-----	---A--C--G	-----G-	-----	<u>AC-TC</u> -----
Imperial Palace	TTGCGCGTAA	TAGCCACCGC	ATTCATAGGG	TACGTCCTAC	CA	
Hino City	-----	-----	-----C--	-----C--C--	--	
Tokuda's mole	---A-----	-----T--	-----	--T--TT--	--	

Fig. 3. Composition of nucleotide sequences of cytochrome *b* gene of the lesser Japanese mole from the Imperial Palace and Hino City, and of the Tokuda's mole from Sado Island. Underline indicates the mutations at the level of amino acid sequences.

Imperial Palace	AAGATTGTTG	GCCAGAGAAC	TACTAGCAAC	AGCTTAAAAC	TCAAAGGACT	TGGCGGTGCT
Hino City	-----	-----	-----	-----G--	-----	-----
Tokuda's mole	-----T--	-----	-T--T--	-----	-----	-----
Imperial Palace	TTATATCCCT	CTAGAGGAGC	CTGTTCTGTA	ATCGATAAAC	CCCGATAAAC	CTCACCAAGC
Hino City	-----	-----	-----	-----	-----	-----
Tokuda's mole	-----	-----	-----	-----	-----	-----
Imperial Palace	CTTGCTAATT	CAGCCTATAT	ACCGCCATCT	TCAGCAAACC	CTTAAAAGGA	ATCATAGTAA
Hino City	-----G--	-----	-----	-----	-----	-----
Tokuda's mole	-----	-----	-----	-----	-----	G-A-----
Imperial Palace	GCACAAGTAT	ATACATAAAA	ACGTTAGGTC	AAGGTGTAGC	TAATGAGTTG	GGAAGAAATG
Hino City	-----	-----	-----	-----	-----	-----A-----
Tokuda's mole	-----	T-----	-----	-----	-----C--	-----
Imperial Palace	GGCTACATTT	TCTATTTCTA	GAATATAA T	TT AACGAA	AACCTTTATG	AAATGAAAGG
Hino City	-----	-----	-----	-----	-----	-----T-----
Tokuda's mole	-----	-----	---C---T-	C--A-----	-----C--	-----
Imperial Palace	TTAAAGGAGG	ATTTAGTA				
Hino City	-----	-----				
Tokuda's mole	-----	-----				

Fig. 4. Composition of nucleotide sequences of 12S rRNA gene of the lesser Japanese mole from the Imperial Palace and Hino City, and of the Tokuda's mole from Sado Island.

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