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Original paper

Y chromosome polymorphism found among house shrew populations in Myanmar

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Abstract: The house shrew (*Suncus murinus*) is a small mammalian species that is widespread in South Asia and Southeast Asia. Preceding analyses of mitochondrial DNA polymorphisms revealed two major groups among the wild populations of this species, continental and insular, and the division was inferred to lie in the central part of Myanmar. However, the wild house shrew populations in Myanmar are also known to comprise more than two groups. Considering this information, the present study attempted to further elucidate the wild population structure of house shrews in regions around Myanmar. We prepared chromosomal samples from the house shrews captured in several localities in Myanmar and compared the band patterns. The autosomal and X-chromosomal band patterns obtained from these animals resembled those of the standard suncus karyotype (continental type). In contrast, Y chromosomes revealed a considerable range of inter-individual variations. The absence of any common regionality in the distribution of Y chromosome polymorphisms suggests that the wild populations of house shrews in Myanmar are formed through a more complicated process than formerly postulated.

I. Introduction

The house shrew (*Suncus murinus*), a small mammal residing in human houses, is widespread in the tropical and subtropical regions of South Asia and Southeast Asia (Nowak 1999). Presumably, its habitat is expanded by human migration and commercial trade (Motokawa 2015, Ohdachi 2011). Its routes and processes of distributional expansion have been inferred from comparative studies of karyotypes, blood proteins and enzyme polymorphisms, and restriction fragment length polymorphisms (RFLPs) in mitochondrial DNA (Kurachi et al. 2007a, 2007b, Yamagata et al. 1995, Yamagata 2011, Yosida 1982). These studies suggested that this shrew species originally migrated from India to the Indochinese Peninsula. From the Indochinese Peninsula, the species presumably expanded its distribution to circum-East China Sea areas, including Kyushu (Japan), islands in Southeast Asia, West Asia and East African coasts. In South Asia and Southeast Asia, two main expansion routes have been suggested. One is the continental route from the east of South Asia to the Indochinese Peninsula, the Malay Peninsula, and to farther islands. The other is the maritime route from southern India and Sri Lanka, reaching the Malay Peninsula and Indonesia by sea.

Studies of mitochondrial DNA (mtDNA) RFLPs among the house shrew population have revealed that the shrew largely comprises two types of mtDNA, the continental and insular types. In Myanmar, the continental haplotype (which is common in South Asia) occurs in the northwest of the country, whereas the insular haplotype (which is common in Southeast Asia) occurs in the south, suggesting the border between the two lies somewhere in central Myanmar (Yamagata et al. 1995, Yamagata 2011). Recently, the sequence data of the mitochondrial cytochrome b gene from house shrews in Myanmar suggested the possibility that the population in Myanmar may consist of several lineages or species (Ohdachi et al. 2016). However, the number of capture sites was small and mtDNA provides only maternally inherited genetic information; therefore, strong conclusions could not be drawn. The house shrew population structure in Myanmar has not been fully elucidated. Thus, the present study aimed

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to provide a better understanding of the house shrew populations in Myanmar by comparing the karyotype specimens of wild house shrews from the known local populations.

II. Materials and methods

Two male house shrews were captured at different places (Pyin Oo Lwin and Mandalar Degree College) in Mandalay (northern Myanmar), one male captured at Nay Pyi Taw (Central Myanmar), and one male and female were captured at Bago (southern Myanmar). For comparison, animals of KAT and EDS laboratory suncus lines were used, which were derived from wild populations in Kathmandu, Nepal, and Mymensingh, Bangladesh, respectively (Ishikawa et al. 1987, Oda et al. 1992, Ohno et al. 2001, Yamagata et al. 1987) (Table 1). Metaphase chromosomes were prepared from primary cultures of fibroblasts and/or spleen lymphocyte cultures using conventional techniques (Miyashita et al. 1988, Rogatcheva et al. 1996). The karyotypes were then analyzed using conventional Giemsa staining. The banding pattern analyses were performed using G-band staining (Rogatcheva et al. 1997) and Q-band staining (Yosida 1975). Metaphase images were observed with a fluorescence microscope (Axio Photo 2; Carl Zeiss Co., Tokyo, Japan) and an optical microscope (OPTIPHOTO-2; Nikon Co., Tokyo, Japan). Karyotypes were analyzed on computers using the DS-L3 (Nikon CO.), Ilaros karyotyping system and Axio Photo 2 imaging system (Carl Zeiss Co.) by referring to the mouse standard karyotype (Levan et al. 1964) and the house shrew standard karyotype that was constructed from the KAT line (Rogatcheva et al. 1996).

III. Results

From a total of 138 metaphases sampled from four house shrews captured in the wild in Myanmar, and KAT and EDS suncus lines, the number of chromosomes was 2n = 40 in almost all cells observed (Table 1). The number of autosomal arms was 52 (FNa = 52), comprising four pairs of meta to submetacentric chromosomes (m to sm), three pairs of subtelocentric chromosomes (st), and 12 pairs of acrocentric chromosomes (a). All X chromosomes were of a large-sized submetacentric type, showing little variation between individuals, lines, or original localities. In contrast, Y chromosomes indicated immense variation in morphology between the individuals, lines, and localities. The house shrew captured in the wild at Pyin Oo Lwin in Mandalay had a subtelocentric type, one animal each captured at the Mandalar Degree College in Mandalay and at Nay Pyi Taw had a submetacentric type and the shrew from Bago had a metacentric type. Y chromosomes in the KAT and EDS suncus lines were subtelocentric and submetacentric, respectively, as reported previously (Ishikawa et al. 1989, Rogatcheva et al. 1996). For G- and/or Q-band patterns, which were revealed in the present study, each chromosome reported distinct band patterns (Figs. 1 and 2). However, for some chromosomes, due to size reduction, no high resolution chromosomal band patterns were available. Nevertheless, at least in comparison with the major chromosomes, autosomal and X-chromosomal band patterns in all samples resembled those of the standard KAT suncus line, whereas Y chromosomes clearly revealed variant band patterns between the individuals, lines, and localities. In particular, Q-band patterns revealed little homology.

IV. Discussion

Robertsonian chromosomal polymorphisms in house shrew populations in the wild have been reported (Aswathanarayana and Prakash 1976, Ishikawa et al. 1989, Sharma et al. 1969, Yosida 1982). In southern India and Sri Lanka, shrews with chromosomes with Robertsonian translocation (2n = 30 and 32) have been captured (Sam et al. 1979, Yong 1971, 1972). Furthermore, 2n = 35 - 39 karyotypes with Robertsonian translocation were reported from the Malay Peninsula (on the west coast between Pinang and Kuala Lumpur) (Yong 1971, 1972). The Malayan population with the Robertsonian chromosomes was thought to have arisen through hybridization between the animals with 40 chromosomes originally migrated there and those with reduced chromosome numbers which migrated through the Indian Ocean (Yosida 1982). The wild house shrews used in the present analysis were obtained from northern to southern Myanmar. They all were of 2n = 40 karyotype and no Robertsonian chromosomal polymorphisms were found (Table 1). Robertsonian polymorphisms have been reported for the SRI suncus line that originated from wild Sri Lankan shrews (Rogatcheva et al. 1997). In comparison with the published Y chromosome images of SRI animals, none of the Y chromosomes of the wild house shrew samples from Myanmar showed the same band pattern as SRI line. Furthermore, an insertion (or elongation) of heterochromatin on the chromosome 7 has also been reported in the SRI line (Rogatcheva et al. 1997). In the present study, none of the chromosomes 7 with such an additional heterochromatin was observed (Fig. 1). These indicate that the wild house shrew

Table 1. Localities where the house shrews were collected, and their karyotypes

Specimen #	Localities collected	Sex	Karyotypes*					
			2n	Autosomes			X	Y
				m to sm	st	a		
HH151112-1 (KUZ-M11697)	Mandalay, Myanmar (Pyin Oo Lwin)	Male	40	8	6	24	sm	st
HH151114-1 (KUZ-M11688)	Mandalay, Myanmar (Mandalar Degree College)	Male	40	8	6	24	sm	sm
HH151202-1 (SDO-15/12/2-1)	Nay Pyi Taw, Myanmar (Garden of Great Wall Hotel)	Male	40	8	6	24	sm	sm
HH151130-1 (SDO-15/11/30-1)	Bago, Myanmar (New Town near old Air Port)	Male	40	8	6	24	sm	m
HH161117-1	Kathmandu, Nepal (KAT wild-derived laboratory line)	Male	40	8	6	24	sm	st
HH160913-1	Mymensingh, Bangladesh (EDS wild-derived laboratory line)	Male	40	8	6	24	sm	sm

*m: metacentric type, sm: submetacentric type, st: subtelocentric type, a: acrocentric type

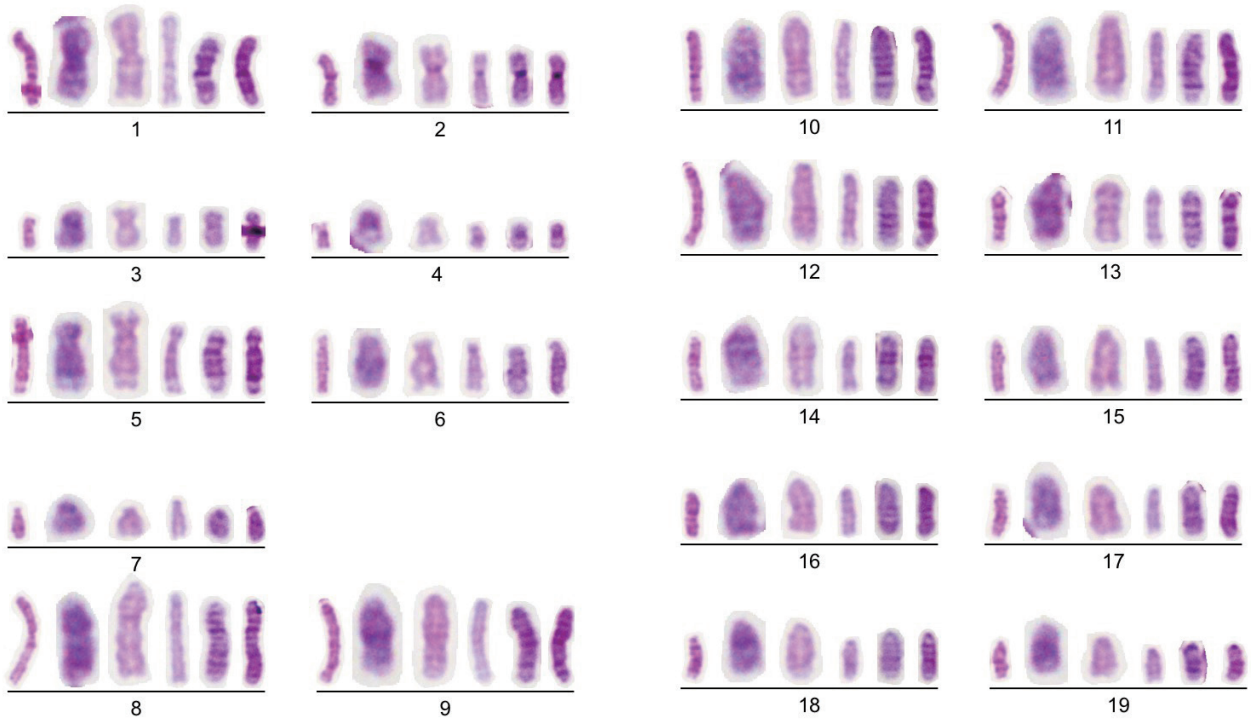

Fig. 1. G-banded autosomal chromosomes of the house shrew, *Suncus murinus* **used in this study.** From the left: Mandalay (Pyin Oo Lwin), Mandalay (Mandalar Degree College); Nay Pyi Taw; Bago; Kathmandu, Nepal (KAT line) and Bangladesh (EDS line).

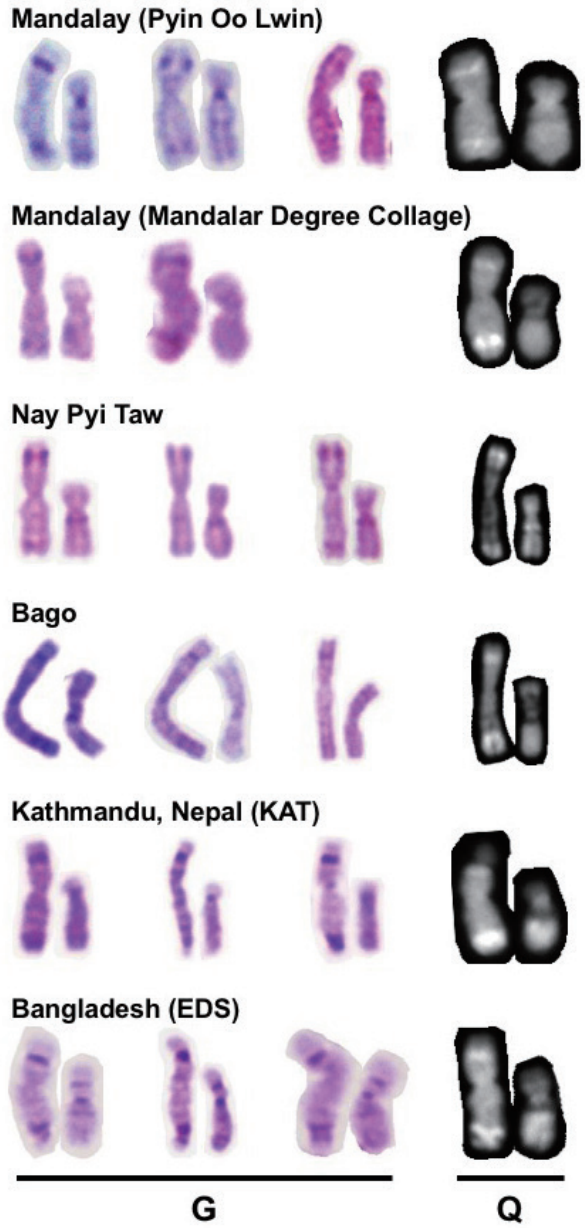

Fig. 2. G- and Q-band staining for the X- and Y-chromosomes of the house shrew, *Suncus murinus* used in this study, with the X chromosomes always on the left. Several G-banded chromosomes are also shown with different levels of staining. Q-banded chromosomes are shown in the rightmost column.

populations in Myanmar are neither the insular type represented by SRI nor any of the hybrid types reported from the Malay Peninsula, but contain chromosomes of the continental type.

From the wild house shrew populations, besides Robertsonian chromosomal polymorphisms, geographic variation in Y chromosome has been reported. Yosida (1982) reported five types of Y chromosome that differentiated in shape and size from India to Japan. Those differences were inferred to have resulted from reduction in length or partial loss of the chromosome and/or pericentromeric inversion. Although the Y chromosomes could not be measured statistically in the present study as the number of materials was restricted, all the wild house shrews sampled from Myanmar had medium-sized Y chromosomes about 2/3 the size of X chromosomes (Fig. 2). Unlike those reported by Yosida (1982), no reduction in the chromosome length was found. Moreover, in the Y chromosome band patterns, no trace of any inversion across the centromere was discernible by morphology. Therefore, the Y-chromosome polymorphisms observed in the wild house shrew populations in Myanmar are concluded not to have resulted from loss or inversion of

chromosomal fragments. Their variations must have resulted from more complicated processes, or have been formed due to other factors.

According to analyzes of mtDNA RFLPs, there is a dividing line between the north and south in the wild populations of house shrews in Myanmar. Those in the north have continental haplotypes, while those in the south have insular haplotypes (Yamagata et al. 1995, Yamagata 2011). In the present study, however, the chromosomal configuration revealed no difference between the north and south. In particular, Y-chromosomal polymorphisms showed little regionality in their distribution. Furthermore, in the Mandalay area, two types of Y chromosomes were found. The house shrew populations in Myanmar have no simple structure such as previously assumed from the two major groups in the north and south, but have likely resulted from unexpectedly complicated processes. This supports the results of the wide-ranging interspecific phylogeny analysis using sequence data of the mitochondrial cytochrome b gene of house shrews, and suggests that the wild populations of house shrews in Myanmar may comprise several lineages or species (Ohdachi et al. 2016). In the future, we need extensive studies covering a wide area aimed at further elucidation of the population structure of the wild house shrews in Myanmar. Diversified genetic analyses, such as use of more high-resolution karyotypic comparisons and studies of nuclear gene polymorphisms are required.

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蓬莱はるか・大舘智志・本川雅治・Kyaw San LIN・Thida OO・Khin Yu Yu SWE・Wynn THAN・目加田和之：ミャンマー産野生ジャコウネズミ集団で見出されたY染色体多型

摘要：ジャコウネズミ(*Suncus murinus*)は南アジアや東南アジアに広く分布する小型哺乳類である．ミトコンドリアの遺伝子の塩基配列に基づいた解析より，ジャコウネズミの野生集団には大陸型と島嶼型の２つのタイプがあり，その境界がミャンマーの中部であることが推察されている．そこで本研究では，ミャンマー地域におけるジャコウネズミの集団構造を理解するために，ミャンマー各地で捕獲されたジャコウネズミの複数のサンプルをもちいて染色体標本を作製し，分染バンドパターンよる比較を行った．解析に使用したミャンマー産ジャコウネズミの常染色体およびX染色体のバンドパターンは，どの個体も大陸型のものと類似していた．一方，Y染色体は個体により形態およびバンドパターンが異なっていた．Y染色体の多型の分布に明確な地域性がないことから，ミャンマーにおける野生ジャコウネズミの集団は予想以上に複雑な過程を経て形成されている可能性が考えられた．

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