

result from hydrophobic interactions of the Glt-( $\epsilon$ Ahx)<sub>1,2</sub>-residues with S<sub>2</sub> and/or other apolar sites<sup>9</sup> on the protein surface. However, this contact does not lead to an acceleration of catalysis due to the failure of the hydrogen bond between CO of P<sub>3</sub> and NH of Gly-216. In the series of Ac-, Glt-, Glt-( $\epsilon$ Ahx)<sub>1,2</sub>-Phe-Nan K<sub>M</sub> decreases, whereas k<sub>cat</sub> is nearly constant. Therefore, non-productive binding of the N-acyl residues should not be important, since such interactions lower both K<sub>M</sub> and k<sub>cat</sub><sup>17,18</sup>. In addition, non-productive binding of the aniline moiety could not be substantiated<sup>19</sup>.

The high affinity of Glt-( $\epsilon$ Ahx)<sub>1,2</sub>-Phe-Nan to the enzyme is reflected in the reaction rate under pseudo 1st-order conditions ([S]  $\ll$  K<sub>M</sub>). Thus, the k<sub>cat</sub>/K<sub>M</sub> value of Glt-( $\epsilon$ Ahx)<sub>2</sub>-Phe-Nan is 3 times higher than that of Glt-Ala-Phe-Nan. In contrast, the k<sub>cat</sub> values of Glt-Ala-Phe-Nan is 20 times higher than the k<sub>cat</sub> value of Glt-( $\epsilon$ Ahx)<sub>2</sub>-Phe-Nan. The k<sub>cat</sub> and k<sub>cat</sub>/K<sub>M</sub> values in the table show that Glt-Leu-Phe-Nan is more specific than the other substrates studied. Glt-Leu-Phe-Nan should also be of practical interest as a chromophoric chymotrypsin substrate because it is hydrolyzed much faster than the frequently used Glt-Phe-Nan.

- Abbreviations: Ac-, acetyl-;  $\epsilon$ Ahx-, 6-aminohexanoyl-; Boc-, tert-butyloxycarbonyl-; Glt-, glutaryl-; Nan-, 4-nitroanilide.
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## C-banding pattern on the chromosomes of the Japanese house shrew, *Suncus murinus riukiuanus*, and its implication

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**Summary.** The C-band on the chromosomes of the Japanese house shrew, *Suncus murinus riukiuanus* (Insectivora), was studied. Various types of C-banding pattern were found in the genome of this subspecies. Such banding patterns could be useful for an understanding of autosome and sex-chromosome polymorphisms within *S. murinus*.

Karyotypes of the house shrew, *Suncus murinus*, occurring in various areas of Asia have already been studied, and autosomal and sex chromosomal polymorphisms have been found in this species<sup>3-7</sup>, but information on the C-band of the house shrew has as yet only been obtained for the Indian taxon<sup>7</sup>. Nevertheless, C-band staining is a good tool for examining polymorphisms. In this paper, we report the C-banding pattern for the genome of the Japanese house shrew, *S. m. riukiuanus*, and also present its conventional data again here, because the karyotype figure has not been published in detail.

**Materials and methods.** 3 male specimens collected from Naha, Okinawa Is., Prefecture of Okinawa, were investigated karyologically<sup>8</sup>. The C-band treatment followed the method of Sumner<sup>9</sup>, and both conventional staining and C-band treatment were performed on the same preparation. For chromosomal classification the method of Patton was adopted<sup>10</sup>.

**Results.** The C-banding patterns on the autosomes of *S. m. riukiuanus* are as follows. Within the M · SM-elements (row 1, figure 1), a large pair has a centromeric C-band, which is difficult to detect (see also figure 2A); a small pair and a medium-sized one have a distinct centromeric heterochromatic region; and another small pair exhibits terminal C-bands on its short arm, but does not have centromeric C-band. 2 pairs of ST-autosomes are C-band negative. Of the A-autosomes (row 2 and 3, figure 1), only 5

pairs having a short arm were C-band positive; the remaining 8 pairs seem to be devoid of demonstrable C-band material. 4 pairs of the former A-autosomes have both centromeric C-bands and heterochromatic short arms, but a medium-sized pair (placed in the 2nd position of the 2nd row) is distinguished by a lesser amount of C-banded material than is present in the other 3 pairs. On the other hand, the smallest acrocentric pair (5th in the 2nd row), has a terminal C-band only.

The C-banding pattern on the sex chromosomes (figures 1 and 2, B and C) differs markedly from that of the autosomes. The SM-X chromosome has a broad C-band covering the distal one-third of the long arm and a small terminal C-band on the short arm. The SM-Y chromosome

Lengths of the X and Y chromosomes relative to the female haploid set in the Japanese house shrew, *Suncus murinus riukiuanus*, calculated from 20 metaphase plates

	X		Y	
	Mean	SD	Mean	SD
Relative length	10.49 (5.81)	0.72 (0.35)	5.02	0.60

Values in parentheses show the relative length of only the euchromatic portion of the X chromosome.

has a centromeric C-band and large blocks of interstitial heterochromatin on the long arm. The X and Y chromosomes occupy approximately 10.5% and 5.0% of the female haploid set respectively, and the relative length of the euchromatic portion of the X is about 5.8% of the female haploid set (table).

**Discussion.** It has been assumed that autosomal polymorphism found in Malaysian and Indian populations of the house shrew originated from a karyotype having  $2n=40$  such as that found in *S. m. riukiuanus*, and that its karyotypic alteration is caused by centric fusions<sup>3-7</sup>. The C-banding patterns observed on A-autosomes of the Japanese taxon, as shown in figure 1B, represent rather differentiated figures. Consequently, by their C-banding patterns, the A-autosomal complement can be classified into 2 groups, and some pairs in that can be clearly identified. It would be expected, therefore, that the C-band patterns characterized on A-autosomes of *S. m. riukiuanus* could serve as material for the discussion about which unarmed elements gave rise to the additional banded ones found in Malaysian and Indian populations. In fact, logical explanations referring to intraspecific karyotype evolution have been made in some rodents using the C-band<sup>11-13</sup>.

The C-banding patterns of the sex chromosomes in the Japanese taxon may explain the intraspecific sex chromosome polymorphism noted in the Asian *S. murinus*. It has been shown, from conventional data, that the banded X chromosome of the West Bengal (India) population<sup>14</sup> is evidently smaller than those of other populations in Asia<sup>5</sup>. As for the Y chromosome, in the Indian<sup>14</sup> and South Vietnamese populations<sup>15</sup> the Y has been regarded as the smallest A-element in each complement. On the other hand, in the West Malaysian population<sup>5</sup>, except for the Malacca one, the Y chromosome has been established as

M-element, being larger than those of the above 2 populations. In *S. m. riukiuanus* the Y is SM-element and appears larger in size than the M-Y of the West Malaysian population. In another Malaysian population (Malacca), the Y chromosome is 6.0-7.5% of the female haploid set<sup>5</sup>, though its morphology resembles that of the Japanese taxon. A larger Y (7.7-8.6% of the female haploid set) has been found in the Indian population from Delhi and Varanasi<sup>16,17</sup>. The large X chromosome in *S. murinus* is in agreement with the theory of the duplicate type X discussed by some workers<sup>18,19</sup>, because the X chromosomes of *S. m. riukiuanus* and the Indian taxon<sup>7</sup> possess a high amount of C-band material. This assumption is supported also by the facts that the euchromatic regions of the X chromosome in the Japanese taxon is approximately equal to the size of a typical eutherian X, and that autosomal Robertsonian translocation found in Malaysian and Indian has no effect on the formation of large X (no X-autosomal translocation). Thus, size variation of the X chromosome in the house shrew seems to be attributable to differences in the amount of C-band material.

Similarly, the long Y chromosome of the Japanese house shrew also contains a large amount of heterochromatin, although it is not C-band material alone. The amount and location of C-bands on the Y chromosomes of most mammals are variable<sup>13,19,20</sup>. Further, in Australian rodents, the Y chromosome polymorphism is due to the variation of constitutive heterochromatin<sup>21,22</sup>. Given the data presented in previous studies and the C-banding pattern on the Y chromosome, we believe that differences in the amount of constitutive heterochromatin are a chief factor in variation regarding shape and size of the Y chromosome in *S. murinus*. The prototypic Y in this species was probably a small acrocentric.

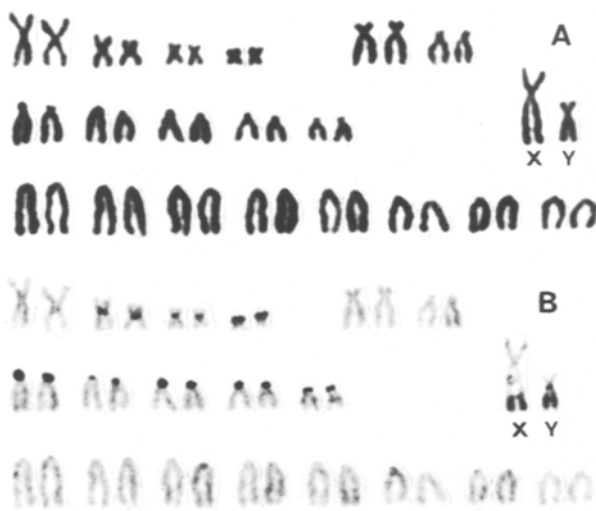


Fig. 1. Conventional (A) and C-banded (B) karyotype of the same cell in the Japanese house shrew, *Suncus murinus riukiuanus*.



Fig. 2. Standard conventional features (a) and their C-banding patterns (b) of a large metacentric or submetacentric autosome pair (A), the X chromosome (B) and the Y chromosome (C).

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