

investigate further this abnormality, serum samples of animals with normal and abnormal TfD phenotype were treated as described by SPOONER<sup>5</sup>, with the enzyme neuraminidase (BDH, 500 units/ml), known to remove sialic acid from macromolecules<sup>6</sup>. Upon digestion for 24 h, abnormal phenotypes proved electrophoretically indistinguishable from treated normal phenotypes. These results remain very much the same as those obtained by SPOONER<sup>5</sup> and can be explained with a similar hypothesis, namely that in abnormal TfD phenotypes, the indicated bands B and D (Figure 3) which contain less sialic acid, are slowed down, thus coinciding partially with bands A and C that consequently stained more intensely. The possibility that the abnormal phenotype might be gene-controlled is being investigated and the data so far available are encouraging. The fact that treatment prolonged for more than 24 h tended to cause progressive disappearance of the fast moving bands at the advantage of the slower migrating ones (Figure 3), is in agreement with the above hypothesis<sup>7</sup>.

**Riassunto.** È descritto il polimorfismo delle albumine e delle transferrine nel bufalo allevato in Italia. Le tre varianti (AlbA, AlbAB, AlbB) dell'albumina sono controllate dai geni codominanti Alb<sup>A</sup> ed Alb<sup>B</sup> e le tre varianti (TfD, TfDE, TfE) delle transferrine dai due alleli dominanti Tf<sup>D</sup> e Tf<sup>E</sup>.

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<sup>5</sup> R. L. SPOONER, *Biochem. Genet.* 2, 371 (1969).

<sup>6</sup> A. GOTTSCHALK, *Biochim. biophys. Acta* 23, 945 (1957).

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## Chromosome Polymorphism in the Malayan House Shrew, *Suncus murinus* (Insectivora, Soricidae)

Insectivora cytogenetics has recently been reviewed by BORGAONKAR<sup>1</sup> and GROPP<sup>2</sup>. The chromosome number, fundamental number (number of major chromosome arms) and the sex chromosomes of 7 families (including Tupaiidae) comprising 61 species were tabularized and the available information summarized by BORGAONKAR<sup>1</sup>, while GROPP<sup>2</sup> discussed in greater detail 2 families, viz. Talpidae and Erinaceidae.

The house shrew, *Suncus murinus* (Linnaeus), belongs to the family Soricidae (cf. ELLERMAN and MORRISON-SCOTT<sup>3</sup>). According to CHASEN<sup>4</sup> and MEDWAY<sup>5</sup>, the Malayan form is *Suncus murinus murinus* (Linnaeus). The first report on the somatic chromosome number of *S. murinus* ( $\equiv$  '*Crocidura murina*') seems to be that of TATEISHI (1937 and 1938, cited by BORGAONKAR<sup>1</sup>). The diploid number of 40 was subsequently confirmed by MANNA and TALUKDAR<sup>6</sup> and RAY-CHAUDHURI et al.<sup>7</sup> for the Indian taxon, YOSIDA et al.<sup>8</sup> for the Japanese taxon, and DUNCAN et al.<sup>9</sup> for the South Vietnamese taxon.

The present paper deals with the Malayan house shrew. Chromosome studies on 15 specimens of *Suncus murinus* collected in Kuala Lumpur and Petaling Jaya, Selangor (West Malaysia), revealed intra-population variation in diploid number. 3 karyotypic classes were recognized with  $2n = 38, 39$  and  $40$  respectively (Figures 1, 2 and 3; Table I). Of the 15 specimens studied, 3 were found to possess a diploid number of 38, 9 with  $2n = 39$ , and 3 with  $2n = 40$ . The fundamental number, however, remained constant in all 3 karyotypes viz. N.F. = 56, and no variations or aberrations could be established within the same individual. Similarly, shrews of all 3 karyotypes

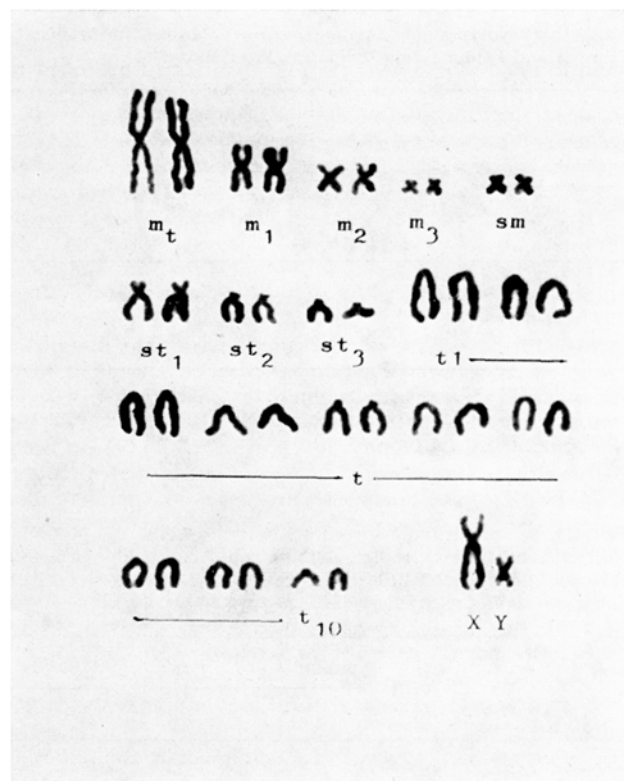


Fig. 1. Karyogram of a male *Suncus murinus* with a diploid number  $2n = 38$ . m, metacentric; sm, submetacentric; st, subtelocentric; t, acrocentric; m<sub>1</sub>, 'translocation' metacentric.

<sup>1</sup> D. S. BORGAONKAR, *Comparative Mammalian Cytogenetics* (Springer-Verlag, New York 1969), p. 218.

<sup>2</sup> A. GROPP, *Comparative Mammalian Cytogenetics* (Springer-Verlag, New York 1969), p. 247.

<sup>3</sup> J. R. ELLERMAN and T. C. S. MORRISON-SCOTT, *Checklist of Palearctic and Indian Mammals 1758-1946* (Brit. Mus. Nat. Hist., London 1951).

<sup>4</sup> F. N. CHASEN, *Bull. Raffles Mus.* 15 (1940).

<sup>5</sup> LORD MEDWAY, *The Wild Mammals of Malaya and Offshore Islands Including Singapore* (Oxford University Press, Kuala Lumpur 1969).

<sup>6</sup> G. K. MANNA and M. TALUKDAR, *Mammalia* 31, 288 (1967).

<sup>7</sup> S. P. RAY-CHAUDHURI, P. V. RANJINI and T. SHARMA, *Mamm. Chrom. Newsletter* 9, 82 (1968).

<sup>8</sup> T. H. YOSIDA, Y. MORIGUCHI and J. SONODA, *A. Rep. natn. Inst. Genet.*, Japan 18, 24 (1968).

<sup>9</sup> J. F. DUNCAN, P. F. D. VAN PEENEN and P. F. RYAN, *Caryologia* 23, 173 (1970).

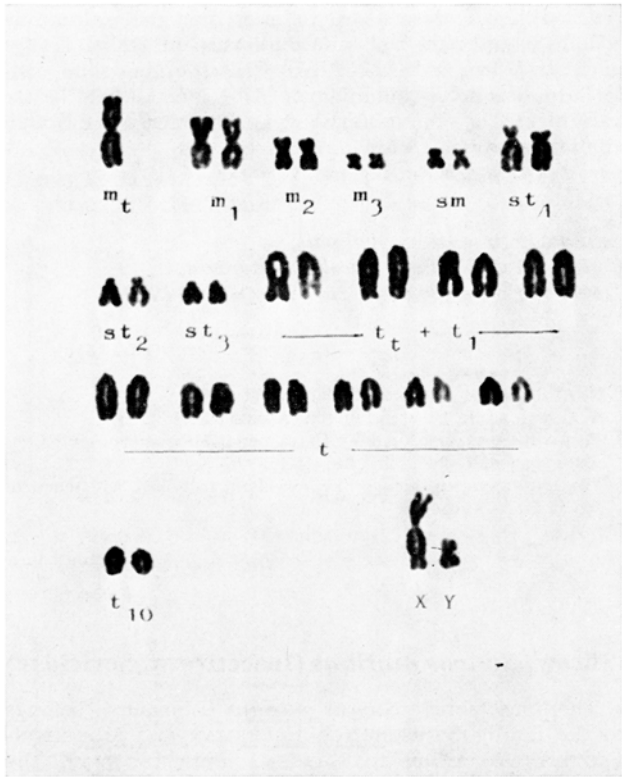


Fig. 2. Karyogram of a male *Suncus murinus* with a diploid number,  $2n = 39$ . t, 'translocation' acrocentric.

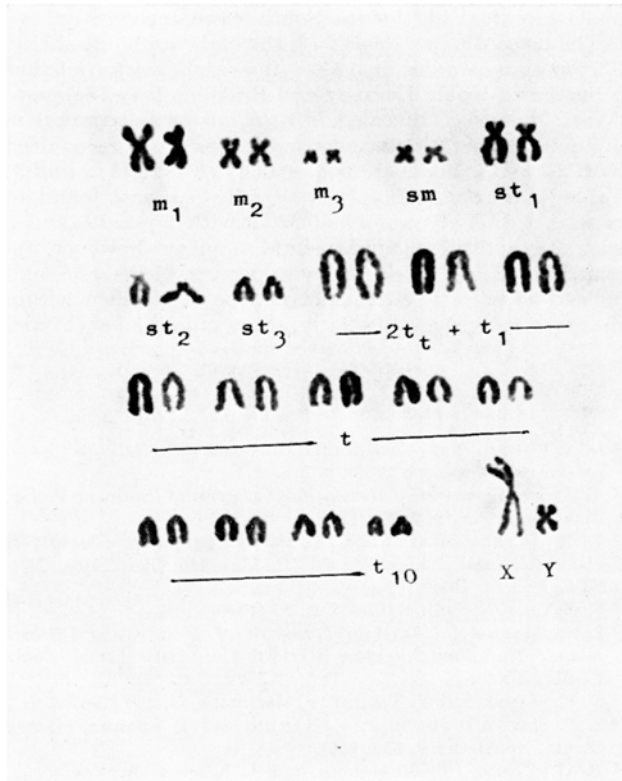


Fig. 3. Karyogram of a male *Suncus murinus* with a diploid number,  $2n = 40$ .

possessed submetacentric *X* and metacentric *Y* sex-chromosomes, the males being *XY* and the females *XX* (Figure 4), and the *X* chromosome was a distinct element in the complement.

The 3 karyotypic classes differed in the number of large bi-armed (metacentric) and large uni-armed (acro-

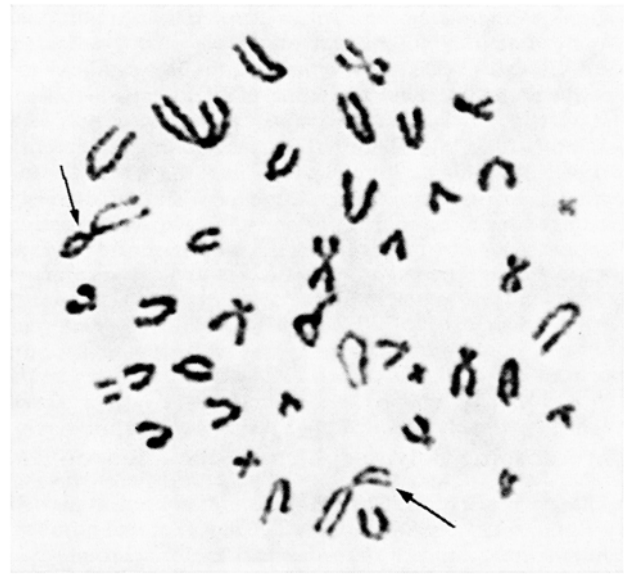


Fig. 4. Karyotype of a female *Suncus murinus* with a diploid number,  $2n = 39$ . *X*-chromosomes arrowed.

Table I. Karyotypes of *Suncus murinus* obtained from Kuala Lumpur and Petaling Jaya, Selangor (West Malaysia)

No. of specimens examined		2n	Autosomes *				Allosomes		NF
			m	sm	st	t	X	Y	
Male	Fe- male								
3	0	38	8	2	6	20	sm	m	56
6	3	39	7	2	6	22	sm	m	56
2	1	40	6	2	6	24	sm	m	56

<sup>a</sup> m, metacentric; sm, submetacentric; st, subtelocentric; t, acrocentric. <sup>b</sup> NF, fundamental number (number of major chromosome arms).

Table II. A comparison between the karyotypes of the Malayan (present investigation), Indian (MANNA and TALUKDAR<sup>6</sup>), Japanese (YOSIDA et al.<sup>8</sup>) and South Vietnamese (DUNCAN et al.<sup>9</sup>) taxa of *Suncus murinus*

Taxon	$2n$	Autosomes				Allosomes	
		m	sm	st	t	X	Y
Malayan	40	6	2	6	24	sm	m
Indian	40	4		4	30	st	t
Japanese	40	4	4	2	28	sm	sm
South Vietnamese	40	6	2	4	26	m	t

centric) autosomes. The  $2n = 38$  karyotype had a pair of distinctively large metacentric autosomes. This pair of extremely large metacentric autosomes was not present in the  $2n = 40$  karyotype. The  $2n = 40$  karyotype, however, possessed 2 extra pairs of large acrocentric autosomes. An intermediate condition was found in the  $2n = 39$  karyotype with only 1 extremely large metacentric autosome but with an extra pair of large acrocentric autosomes when compared with  $2n = 38$  karyotype (1 pair less than  $2n = 40$  karyotype). These differences in the 3 karyotypes could be ascribed to Robertsonian translocation.

In addition to numerical chromosomal polymorphism, the present results obtained from the Malayan species indicate that the karyotype of the Malayan house shrew differs from that of the Indian, Japanese and South Vietnamese taxa (Table II). Direct comparisons, however, would be fallacious due to the undefined nomenclature that were employed.

Chromosomal polymorphism in insectivores has been extensively reviewed (BORGONKAR<sup>1</sup>; GROPP<sup>2</sup>; FORD<sup>10</sup>). The family Soricidae, to which belong *Suncus murinus*, is one of the most extensively and widely studied. Robertsonian polymorphism had been found in *Sorex araneus* (SHARMAN<sup>11</sup>; FORD, HAMERTON, and SHARMAN<sup>12</sup>; MEYLAN<sup>13,14</sup>) and *Blarina brevicauda* (MEYLAN<sup>15</sup>; LEE and ZIMMERMAN<sup>16</sup>). The present finding is another concrete example of Robertsonian polymorphism, and it resembles that of *Blarina brevicauda*. Circumstantial evidence seems to favour centric fusion rather than fission as the mechanism giving rise to the observed numerical polymorphism.

Whether two or more pairs of autosomes are involved, however, cannot be resolved by the present data. Meiotic and population studies are now being conducted to unravel this and other related problems<sup>17</sup>.

**Zusammenfassung.** 15 Exemplare von *Suncus murinus* aus Kuala Lumpur und Petaling Jaya, Selangor (Malaysia) zeigten 3 Exemplare mit einer diploiden Zahl von 40, 9 weitere mit  $2n = 39$  und 3 Exemplare mit  $2n = 38$ . Der Unterschied der Chromosomenzahl innerhalb einer Intrapopulation wird mit der Robertson'schen Translokation in Zusammenhang gebracht.

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<sup>10</sup> C. E. FORD, *Human Population Cytogenetics* (Eds. P. A. JACOBS, W. H. PRICE and P. LAW; University of Edinburgh Press 1970).

<sup>11</sup> G. B. SHARMAN, *Nature*, Lond. 177, 941 (1956).

<sup>12</sup> C. E. FORD, J. L. HAMERTON and G. B. SHARMAN, *Nature*, Lond. 180, 392 (1957).

<sup>13</sup> A. MEYLAN, *Rev. Suisse Zool.* 71, 903 (1964).

<sup>14</sup> A. MEYLAN, *Rev. Suisse Zool.* 72, 636 (1965).

<sup>15</sup> A. MEYLAN, *Canad. J. Zool.* 45, 1119 (1967).

<sup>16</sup> M. R. LEE and E. G. ZIMMERMAN, *J. Mamm.* 50, 333 (1969).

<sup>17</sup> Acknowledgments. I wish to thank Dr. ANDRÉ MEYLAN for his generosity in sending me some of the above-cited literature. Thanks are also due to Mr. TEH KOK LENG for technical and Miss KUAN LAI WAH for clerical assistance.

## In vitro Induction of Vegetative Buds by Tobacco Smoke Condensate<sup>1</sup>

We reported earlier<sup>2</sup> that benz(a)anthracene (BaA), a tobacco smoke carcinogen, replaced the morphogenetic effect of 3-indoleacetic acid (IAA) and kinetin on the callus derived from the stem tissue of haploid tobacco plants. Recently, we have been able to induce vegetative buds on a similar callus grown in a nutrient medium supplemented with water-soluble extract of tobacco smoke condensate. Neither IAA nor kinetin was present in the medium.

Haploid plants were obtained by culturing immature surface sterilized anthers of *Nicotiana tabacum* (cv. 'Burley 21') on the nutrient medium used by NITSCH and NITSCH<sup>3</sup>. The callus was obtained by inoculating small pieces (4–5 mm long) of stems of these plants on modified MURASHIGE and SKOOG's medium<sup>4</sup> supplemented with 0.2 mg/l of  $\alpha$ -naphthaleneacetic acid (NAA) and 0.2 mg/l of IAA. This callus served as experimental material. Calli weighing about 300–350 mg were planted (Figure 1a) on modified MURASHIGE and SKOOG's medium supplemented with various concentrations of water-soluble extract of tobacco smoke condensate (TSC).

The TSC was prepared under the direction of Dr. J. F. BENNER, Department of Agronomy, University of Kentucky. The University of Kentucky Reference Cigarettes 1R1<sup>5,6</sup>, equilibrated at 20°C and 60% relative humidity, were smoked on a Borgwaldt smoking machine employing a standard smoking cycle (a 35 ml puff volume of 2 sec duration at 1 min intervals). The smoke was collected in a 3 l flask containing 100 ml of water cooled at 0°C. A specially designed pump, Chemap vibromixer,

was used to obtain maximum contact of the smoke with water. After 840 cigarettes were smoked, the water solution was transferred to a cooled graduated cylinder. The non-volatile residue weight was determined by evaporation of a 5 ml portion of the solution on a rotary evaporator at a pressure of 30 mm at 35°C with a 50 ml/min stream of nitrogen. The smoking flask was then rinsed with sufficient water in several portions to give a final concentration of 50 mg/ml of non-volatile residue. The TSC was subsequently diluted to have final concentrations of 5, 10, 15, 20, and 25 mg/l in the nutrient media. The pH of the medium was adjusted to 5.8. Sterilization was achieved by autoclaving at 18 lb  $\psi$  for 20 min. For each treatment 16 replicates were main-

<sup>1</sup> This study was carried out under contract No. 12-14-100-341(73) with the Agricultural Research Service, U.S. Department of Agriculture, administered by the Eastern Utilization Research Development Division, Philadelphia.

<sup>2</sup> T. S. KOCHHAR, P. R. BHALLA and P. S. SABHARWAL, *Planta* 94, 246 (1970).

<sup>3</sup> J. P. NITSCH and C. NITSCH, *Science* 163, 85 (1969).

<sup>4</sup> M. J. KASPERBAUER and R. A. REINERT, *Physiologia Pl.* 20, 977 (1967).

<sup>5</sup> W. O. ATKINSON, *Proceedings of the Tobacco and Health Conference*, University of Kentucky, Lexington, Kentucky (1970), p. 28.

<sup>6</sup> J. F. BENNER, *Proceedings of the Tobacco and Health Conference*, University of Kentucky, Lexington, Kentucky (1970), p. 30.