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The Salivary Gland Chromosomes of *Drosophila nasuta* Collected from Seychelles

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Abstract

The characteristic features of the salivary gland chromosomes of *D. nasuta* collected from Seychelles were described. The banding patterns of four long arms of this species somewhat differ from those of the Indian *nasuta* which is closely related species to the Seychelles *nasuta*.

Chromosomal polymorphisms of this species in five populations, Sri Lanka, Seychelles, Kenya, Madagascar and Mauritius, were investigated. 6 heterozygous inversions of the X chromosome, 7 of 2L chromosome, 3 of 2R chromosome and 14 of 3 chromosome were found in each arm. The most of inversions showed very low frequency, while 4 inversions of 3–C, 3–F, 3–G and 3–J were found abundantly in Seychelles. More than 80% individuals showed these four inversions simultaneously or independently. It was known that individuals which had these inversions were always at advantage and a rigid polymorphism concerning these four inversions is established in the Seychelles *nasuta* population.

Drosophila nasuta is a speices belongs to the *Drosophila nasuta* subgroup of the *Drosophila immigrans* species group, and was first described by Lamb (1914) from the Seychelles Islands in the Indian Ocean.

Since Wilson et al. (1968) made an epoch making publication on the *nasuta* subgroup, the *D. nasuta* subgroup has been watched with keen interests by many investigators as one of the most suitable species group for studies of evolution and genetics, and many publications have been reported (Wakahama, Kitagawa & Yamaguchi, 1971 for example). But among them no, but one, work concerrned with the original Seychelles *nasuta* has been published.

Recently, some studies on the so called as the Indian *nasuta* which seems to very close to the original *nasuta* have been made by the Indian Geneticists (Sajjan & Krishnamurthy, 1973 and others), but there is no evidence to decide that the original Seychelles *nasuta* and the Indian *nasuta* are the same species or not.

We fortunately had opportunity to visit Seychelles and some other localities (1971) with the financial aid from the Ministry of Education, Japan, and we could collect a lot of cultures of *D. nasuta.*

In this paper, we describe the standard map of the salivary chromosomes of the

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Seychelles *nasuta* and also note a preliminary report on the chromosomal polymorphisms of *D. nasuta* found in Kandy, Sri Lanka; Mahé, Seychelles; Mombasa, Kenya; Tananarive, Madagascar and Mauritius.

Materials and Methods

For making the standard map of the salivary chromosomes, 19 iso-female lines of *D. nasuta* collected from Seychelles in 1971 were used. And for analyzing the chromosomal polymorphisms, all of the Seychelles lines, 4 lines collected from Kandy, Sri Lanka, 3 lines from Mombasa, Kenya and 2 lines from Tananarive, Madagascar were investigated. Eight to twelve larvae, at least, were selected at random from each line, and their salivary glands were prepared. Preparations of the salivary chromosomes were made by dissecting the third instar larvae in 45% acetic acid and, fixing in 1N HCl, staining and mounting were made in lactic-acetic orcein.

The salivary chromosomes of the hybrid larvae between Seychelles and Mauritius lines were also investigated in the same way to compare the chromosomal structures of two isolated populations.

Observations

1). The standard map of the salivary chromosomes of *D. nasuta*.

The metaphase karyotypes of all iso-female lines were already investigated (Wakahama & Kitagawa, 1972). It consists of a pair of sex chromosomes, the X was rod shaped, while the Y was J-shaped, a pair of V's (Chromosome 2), a pair of rods (Chromosome 3) and a pair of dots (Chromosome 4).

The salivary gland chromosomes show 4 long arms and a short arm (Fig. 1). Four long arms are the X, 2L, 2R and 3. 2L and 2R designate the left and right arms of the V of the metaphase configuration and 3 is the double length rod chromosome. The short arm is Chromosome 4.

The 4 long arms and a short arm of the salivary chromosomes were devided into 138 sections, following the report of Ranganath and Krishnamurthy (1974). Number of sections in each arm are as follows:

X=1 to 27
2L=28 to 52
2R=53 to 83
3=84 to 137
4=138.

Comparing with the photo-map of the Indian species, section numbers of the salivary chromosomes of the Seychelles *nasuta* were decided to concent to those of the Indian species, as much as possible (Figs. 2–5). But banding patterns of the Seychelles

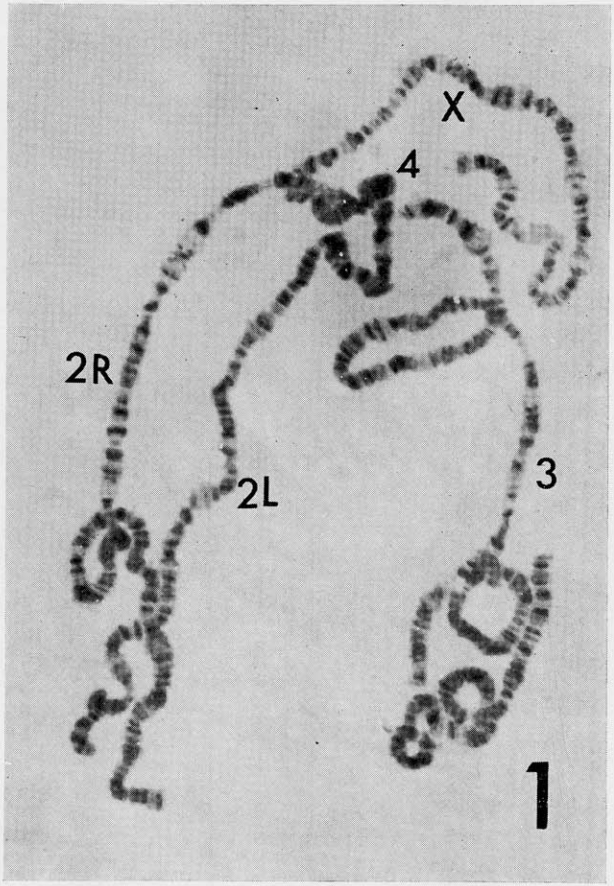
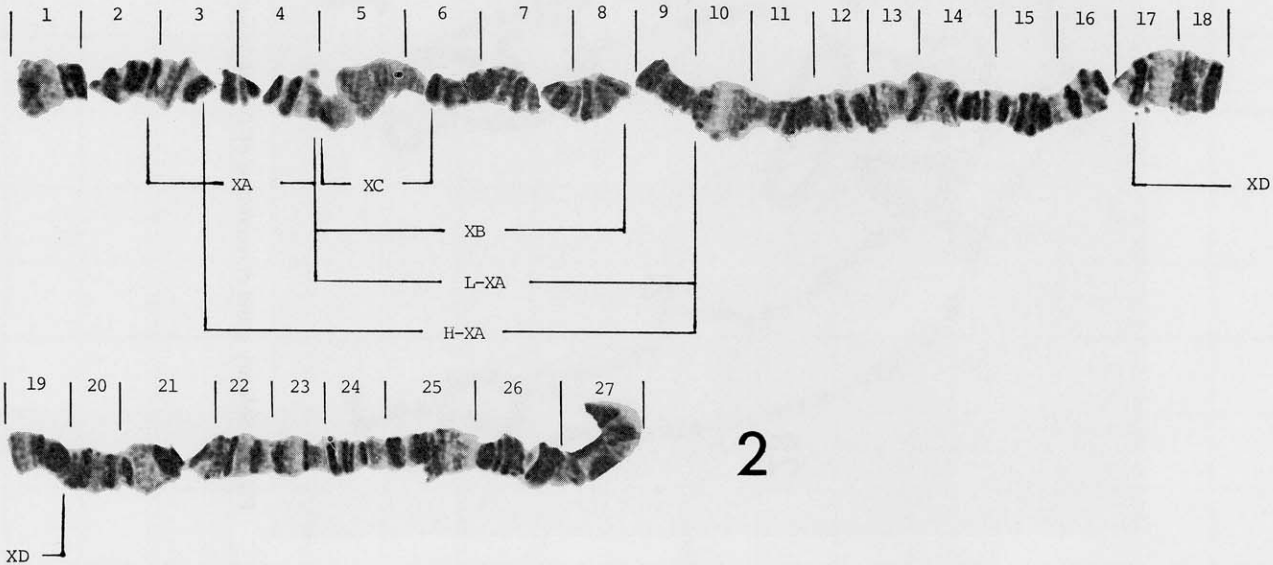
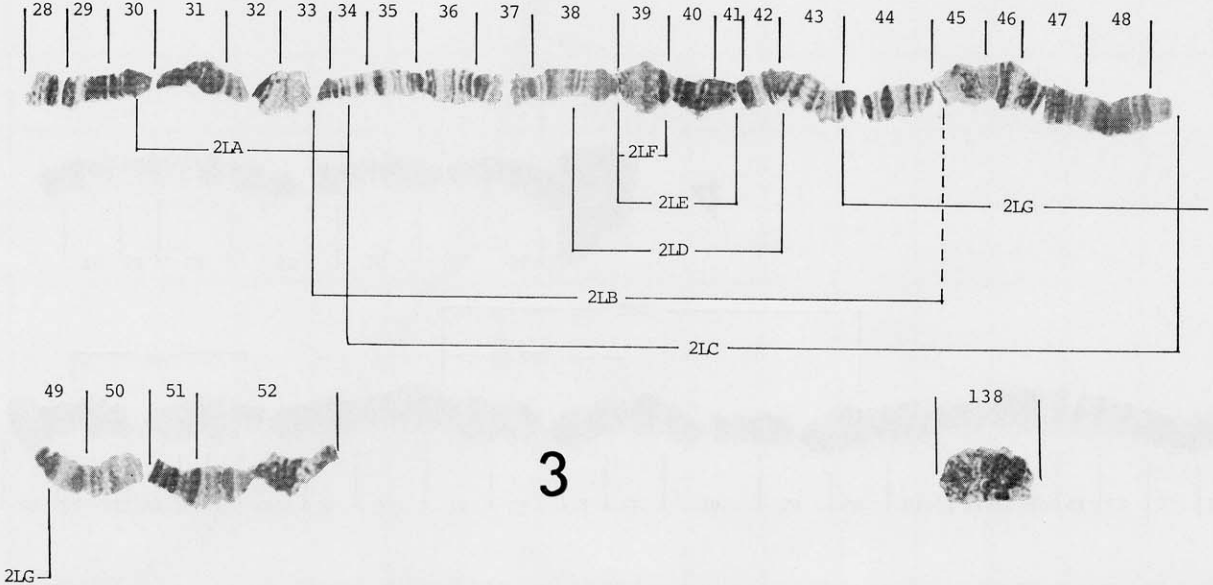


Fig. 1. Salivary gland chromosome of *Drosphila nasuta*.

Figs. 2–5. Photomaps of the standard salivary gland chromosomes of *Drosophila nasuta* and breakingpoints of inversions. 2: X chromosome. 3: Left arm of Chromosome 2(2L) and Chromosome 4 (right below). 4: Right arm of Chromosome 2 (2R). 5: Chromosome 3.

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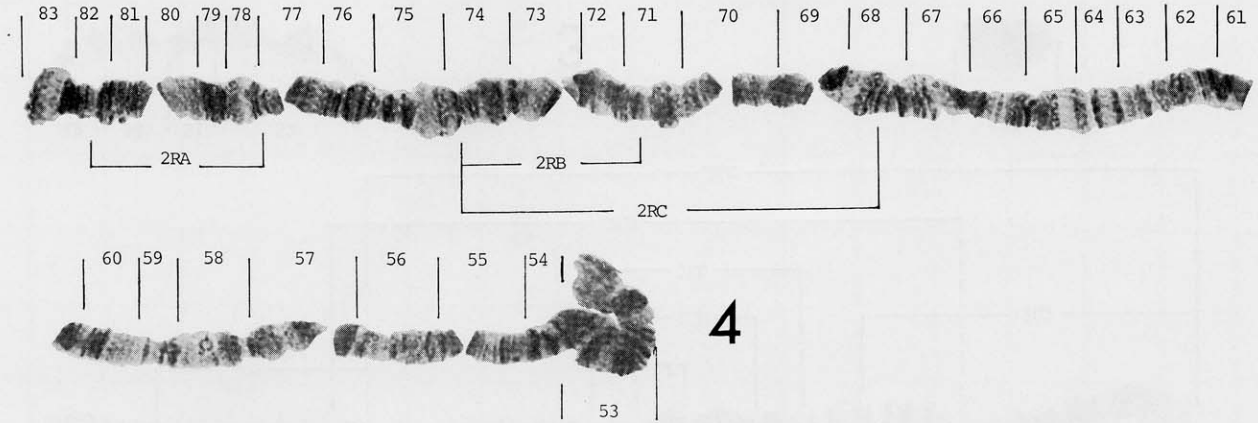
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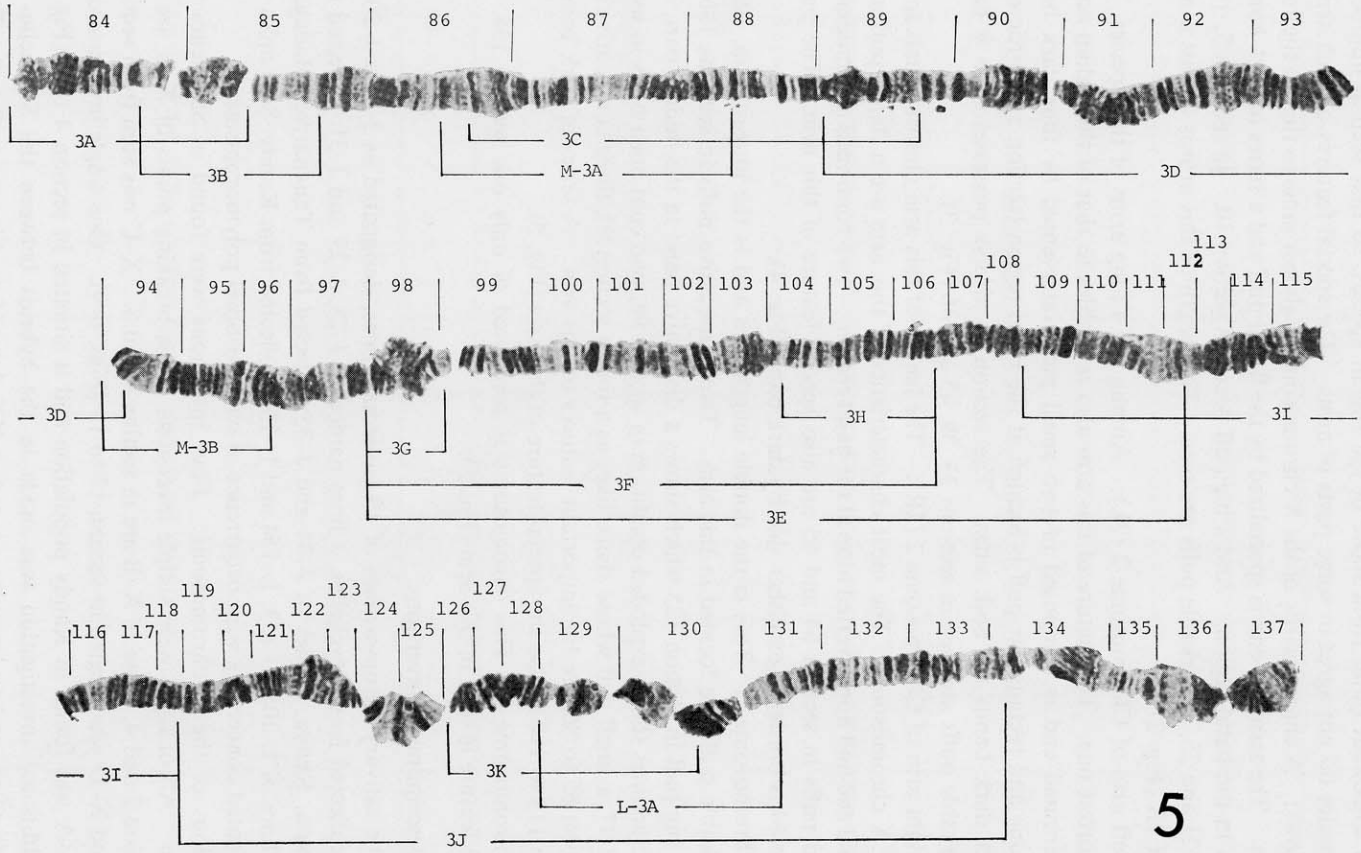
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nasuta somewhat differ from those of the Indian species, so that section numbers of two species do not agree in some parts of arms. The special features of each arm are as follows: A characteristic of the X chromosome is almost same to that of the Indian species. The terminal end is specialized by the flared puff and a series of dark bands is shown in the basal region. And a big puff occupies section 10. In sections 5, 14, 17 to 18, 21 and 25, remarkable puffs are seen. The length of this arm is almost same as that of 2R (Fig. 2).

Left arm of Chromosome 2 (2L): Among the 4 long arms of this species, 2L is the shortest one. The feature of this arm also resembles to that of the Indian *nasuta*. The terminal end is composed of two small puffs intervented by four dark bands. A unique and rectangular puff is located at the basal region (section 51) bordered by distinct dark bands on both sides. The section 48 always possesses 8 to 9 bands. Remarkable puffs are seen in sections 33, 39, 45 and 46 (Fig. 3).

Right arm of Chromosome 2 (2R): The length of this arm almost equals to that of the X chromosome. The most characteristics of this arm are a flared puff at the terminal end and non inverted loop at the basal region. Two continued and moderately stained puffs in section 64 and 65 are also special feature of this arm. The puff in section 64 is bordered on either side by dark band (Fig. 4).

Chromosome 3: This is the double length arm and is the longest arm. Many remarkable puffs are located in this arm. Two consecutive puffs in sections 136 and 137, a big puff in section 125 which shows a distinctive band in the middle part, a big puff sometimes shows exploded condition in section 98, two continued puffs in section 96 and 97, a small puff whose shape likes as a top in section 89, three consecutive puffs in section 85 to 86, are the important features of this arm. A series of dark bands in section 113 to 118 is also the special feature of this arm (Fig. 5).

Chromosome 4: This chromosome is composed of only one section 138. No special feature is seen in this arm (Fig. 3).

2). Description of inversions.

The salivary chromosomes of 19 iso-female lines, designated as J–3 to J–20 and J–60, collected from Seychelles, 3 lines named as J–28, J–29 and J–31, collected from Mombasa, Kenya, 2 lines of J–34 and J–35 collected from Tananarive, Madagascar and 4 lines of L–105, L–153, L–154 and L–156, collected from Kandy, Sri Lanka were investigated concerning with occurrence of chromosomal polymorphisms.

Inversions of the X chromosome: Four inversions were found in Seychelles population. All of them were simple inversions. The breaking points of X–A are seen in section 2 and 4, those of X–B are in section 4 and 8. X–C was seen from section 4 to 6 and X–D was located in section 17 to 19 (Figs. 6–9). One single inversion called as LX–A was found in Kandy population and it situated in section 4 to 9 (Fig. 30). An additional investigation was made in the hybrids between the Seychelles and Mauritius lines and an inversion named as HX–A was found in section 3 to 9. Except

X–D, all inversions were concentrated in the terminal region.

Inversions of the left arm of Chromosome 2 (2L): Seven inversions were found in this arm of Seychelles population and they were designated as 2L–A to 2L–G. All of them were simple and paracentric inversions. Location of each inversion is as follows: 2L–A; section 30–34, 2L–B; section 33–45, 2L–C; section 34–39, 2L–D; section 38–43, 2L–E; section 39–43, 2L–F; section 39, and 2L–G; section 40–49 (Figs. 10–16). Inversions of this arm were occurred along the total length of this chromosome. 2L–C and 2L–E were also found in Kandy population of Sri Lanka (Figs. 12 & 14). No inversion was seen in this arm of the other populations.

Inversions of right arm of Chromosome 2 (2R): Three inverions of 2R–A, 2R–B and 2R–C were found in Seychelles poulation. 2R–A was located in section 77 to 82, 2R–B was in section 71 to 74 and 2R–C was in section 68 to 74. All of them were simple and paracentric inversions (Figs. 17 & 18). No inversion was detected in the other populations.

Inversions of Chromosome 3: This is the longest chromosome in *D. nasuta*. There are many breaking points along the total length of this chromosome and a lot of inversions is concentrated in this arm. In Seychelles population, 11 inversions were found and named as 3–A to 3–K (Figs. 19–29). Breaking points of each inversion are as follows: 3–A was seen in section 84 to 85, 3–B is also in 84 to 85, 3–C in 86 to 89, 3–D in 89 to 94, 3–E in 97 to 112, 3–F in 97 to 106, 3–G in 97 to 99, 3–H in 104 to 106, 3–I in 109 to 118, 3–J in 118 to 134, and 3–K in 126 to 130. All of them, except 3–G, were simple inversion. 3–F is occurred as simple inversion in many cases but sometimes found as an included inversion with 3–G (Fig. 25). And 3–A was situated in the terminal region and made a ring inversion (Fig. 19).

In Mombasa, no inversion which was commomn to those of Seychelles was found, but 2 of M3–A (section 86–88) and M3–B (section 94–96) were detected. These two inversions were unique inversions in Mombasa population (Figs. 32 & 33).

3–J and 3–K inversions which were seen in Seychelles, were also found in Madagascar population. But these inversions appeared as an included inversion in Madagascar, while in Seychelles, these were simple inversions.

In Chromosome 3 of Sri Lanka population, 3–A, 3–C, 3–F, 3–G and 3–J were also found. Besides them, L3–A of a unique inversion in this population was found and this inversion always occurred as an included inversion with 3–J and this inversion was located in section 129 to 131 (Fig. 34).

In Mauritius population, 5 iso-female lines were investigated. But no inversion was detected.

Among 5 populations studied, Seychelles and Mauritius are island populations far aparted from the continents. So that to compare the chromosomal structures of two island populations, the hybrid chromosomes between two populations were studied (Figs. 35 & 36). In the hybrids, an inversion named as HX–A was found in the X chromosome. This inversion was located in section 3 to 9 (Figs. 2 & 31). And

three inversions of 3–C, 3–F and 3–J were detected in Chromosome 3 (Figs. 35–39 and Table 1). These three inversions were found in Seychelles, but were not observed in the Mauritius population, so that these inversions were seemed to be originated from Seychelles. As HX–A was not found in Seychelles, this inversion was a unique one in Mauritius and this gene arrangement was only difference between the two populations. No inversion was seen in two arms of Chromosome 2 (Figs. 35 & 37).

Table 1. Inversions found in Chromosome 3 of hybrids between Mauritius (M–3) and Seychelles (J–8), and Seychelles (J–8).

M–3 ♀ × J–8 ♂	#2			3 J		
	#3	3 C	3 F			
	#4	3 C	3 F			
	#6	3 C		3 J		
	#9	3 C	3 F			
	#10	3 C				
J–8 × M–3	#21			3 J		
	#22			3 J		
	#23			3 J		
	#29			3 J		
J–8	#1	3 C	3 F	3 J	3 G	
	#2	3 C	3 F	3 J		
	#3	3 C				
	#4					3 I

Discussion

Chromosomal polymorphisms of the *nasuta* subgroup have been studied by Wilson et al. (1968), Wakahama et al. (1971), Mather et al. (1975a, b), Lin (1977), Lambart (1976 & 1978) and Rangnath and Krishnamurthy (1974a, 1975a, b & 1978b). Among them, we have special interests to the studies of Rangnath and Krishnamurthy. Because, they used the so called Indian *nasuta* which seem to be closely related species to the Seychelles *nasuta* and the former inhibits in the continent and the latter lives in the islands far aparted from the continent (distance between two localities is about 3000 km).

They reported 3 inversions in the X chromosome, one is an overlapping inversion in the terminal region and the others are small and simple inverions in the median region. In our study, 4 inversions were detected in this chromosome and 3 were concentrated in the terminal region. Only one was found in the submedian region. No overlapping inversion was found in Seychelles. In the left arm of Chromosome 2, 4 inversions were reported in Indian populations, while 7 inversions were detected in Seychelles. 2L chromosome of Seychelles is more polymorphic than that of India.

Contrastly, the Indian *nasuta* showed 7 inverions in the right arm of Chromosome 2, but we found only 3 inversions in 2R of Seychelles. And all of them were simple ones. In India, they reported one pericentric inversion in the basal regions of 2L and 2R, no such inversion was seen in Seychelles. In Chromosome 3, they reported 23 inversions including both of the simple and complex inversions. In Seychelles, we detected 11 inversions and among them, only one was an including inversion. Chromosome 3 of both populations were highly polymorphic. Of 23 inversions, 19 were concentrated in the basal region of this chromosome in India, but at random distribution of inversions was seen in this chromosome of Seychelles.

Table 2. Frequeny of the inversion pattern concerning 4 inversions of 3–C, 3–F, 3–G and 3–J.

Type	Inversion pattern	Frequency (%)	Type	Inversion pattern	Frequency (%)
I	C F J	42.68	VII	C	3.66
II	non	14.63	VIII	F J	2.44
III	C F	13.41	IX	C J	2.44
IV	C F G J	9.76	X	F G J	1.22
V	C F G	3.66	XI	F G	1.22
VI	J	3.66	XII	F	1.22

In Seychelles, a total of 25 heterozygous inversions were found. Of 25 inversions, most of inversions were observed only in one or two strains. While inversions 3–C, 3–F, 3–G and 3–J showed high frequency. Concerning 4 inversions of 3–C, 3–F, 3–G and 3–J, all individuals in this population were devided into 12 types by the inversion pattern shown by each individual (Table 2.) Frequency of individuals which showed 3–C independently, or 3–C and other inversion simultaneously, was 76.61%, that of 3–F was 76.82%, that of 3–G was 15.85% and that of 3–J was 62.20%. Frequency of individuals which did not show any of these four inversions was 14.63%. And further, individuals which had 3–C, 3–F and 3–J simulataneously was 42.68%. Frequency of individuals which had 3–C and 3–F concurrently was 13.41% and that of individuals which showed 3–C, 3–F, 3–G and 3–J simulataneously was 9.76%. Individuals which had no inversions in Chromosome 3 was only 8.54% of a total specimens investigated (Table 2).

Ranganath and Krishnamurthy (1978a) reported 23 inversions in Chromosome 3 of Indian populations and among them 6 inversions were found as common and widespread inversions. And they said that population structures could be discussed either with reference to a peculiar inversion or to general heterozygosity of the individual due to some inversions, and also discussed the flexibility or the rigidity of inversion polymorphism either with reference to the frequencies of a particular inversion or types

or mean number of inversion, in different populations. In Indian populations, more than 50% individuals showed any heterozygous inversions. In Seychelles, more than 90% individuals had any heterozygous inversions.

Carson (1959) proposed the word of "heteroselection" and he (1965) reported that the chromosomal polymorphism in geographically widespread species of *Drosophila* had indicated that most of the polymorphisms described among widespread species are rigid polymorphisms maintained by a process of heteroselection. More than 90% of the Seychelles *nasuta* showed heterozygous inversions, so that, it will be possible to say that individuals which have 3-C, 3-F, 3-G and 3-J simulataneously or independently are always at an advantage and a rigid polymorphism concerning these four inversions is established in the Seychelles *nasuta* population.

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Explanation of Figures

Figs. 6–29. Inversions found in the Seychelles Islands. Arrow indicates chromocenter.

Figs. 6–9. Inversions of the X chromosome. 6: X–A. 7: X–B. 8: X–C. 9: X–D.

Figs. 10–16. Inversions of 2L. 10: 2L–A. 11: 2L–B. 12: 2L–C. 13: 2L–D. 14: 2L–E. 15: 2L–F. 16: 2L–G.

Figs. 17 and 18. Inversions of 2R. 17: 2R–A and 2R–B. 18: 2R–C.

Figs. 19–29. Inversions in Chromosome 3. 19: 3–A. 20: 3–B. 21: 3–C. 22: 3–D. 23: 3–E. 24: 3–F. 25: 3F+3G. 26: 3–H. 27: 3–I 28: 3–J. 29: 3–K.

Fig. 30. Inversion LX–A found in Kandy of Sri Lanka.

Fig. 31. Inversion HX–A found in the hybrid between Seychelles and Mauritius.

Figs. 32 and 33. Inversions found in Mombasa of Kenya.

32: M3–A. 33: M3–B.

Fig. 34. Inversion L3–A found in Kandy.

Figs. 35–39. Salivary gland chromosomes of hybrids between Seychelles and Mauritius. 35 and 37: Total figures. 36: Inversion 3–J. 38: Inversion 3–F. 39: Inversion 3–C.


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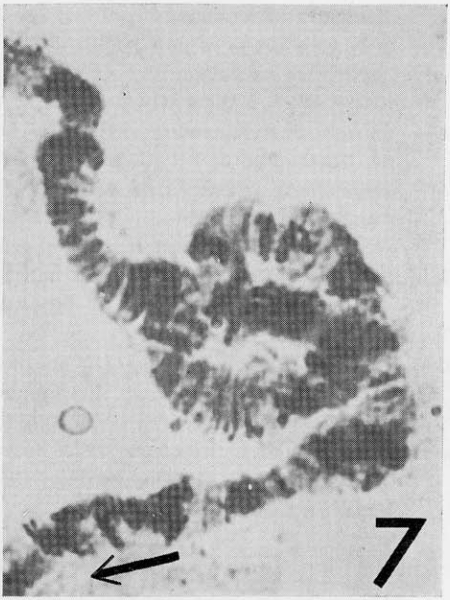
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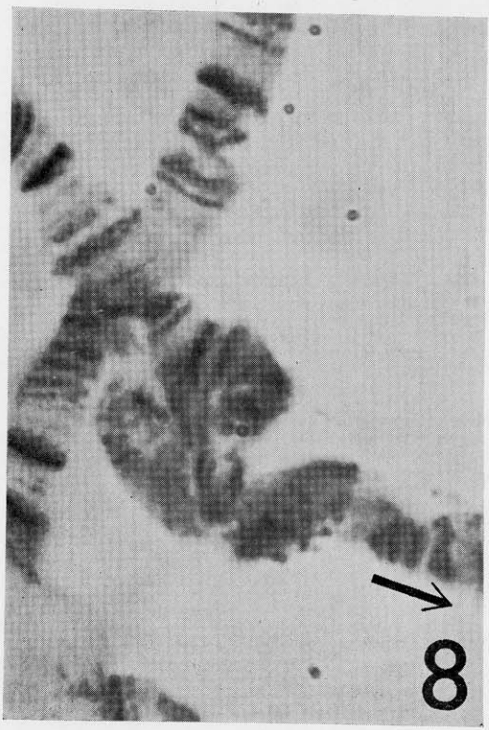
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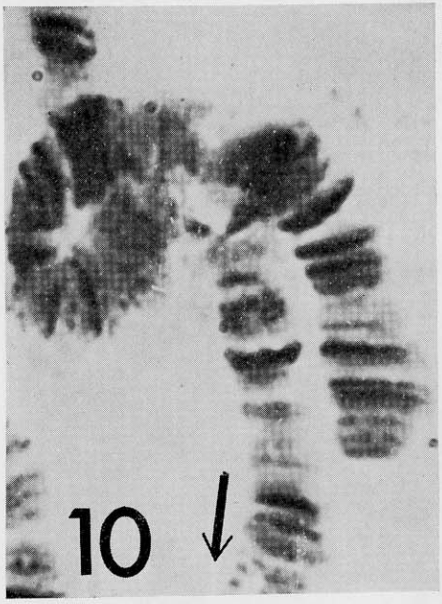
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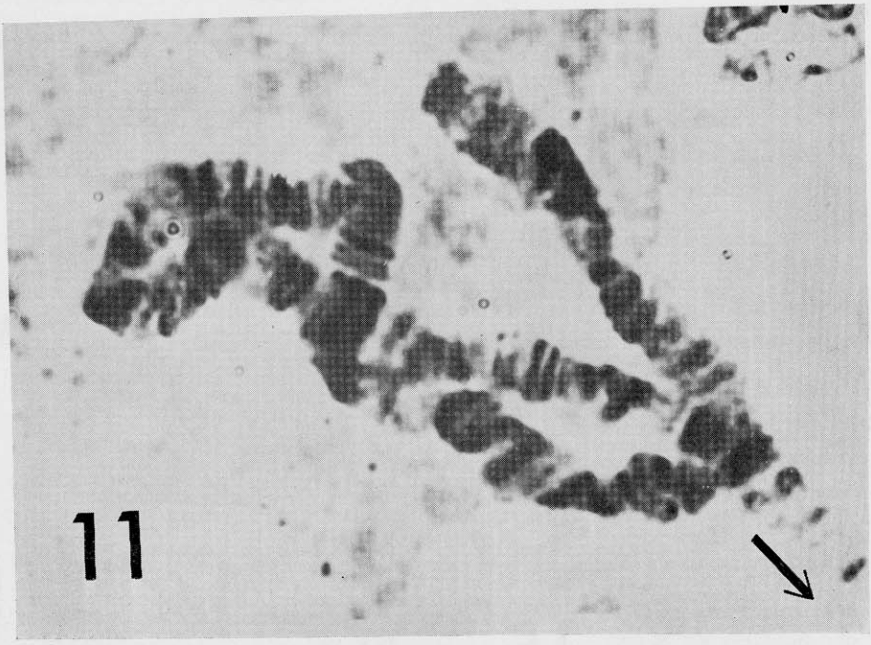
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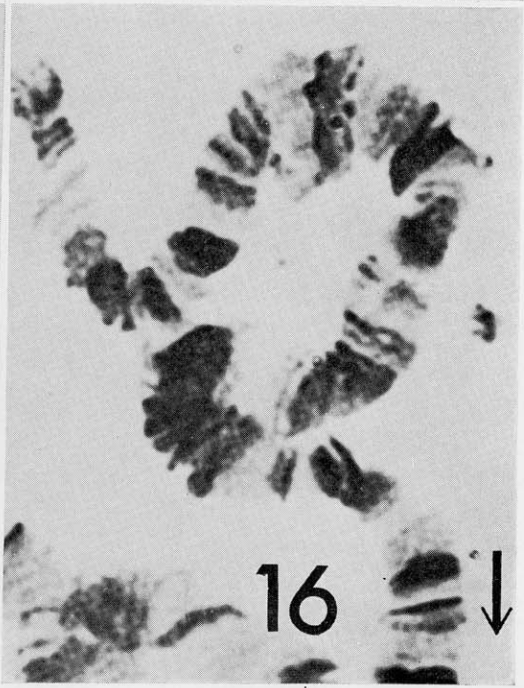
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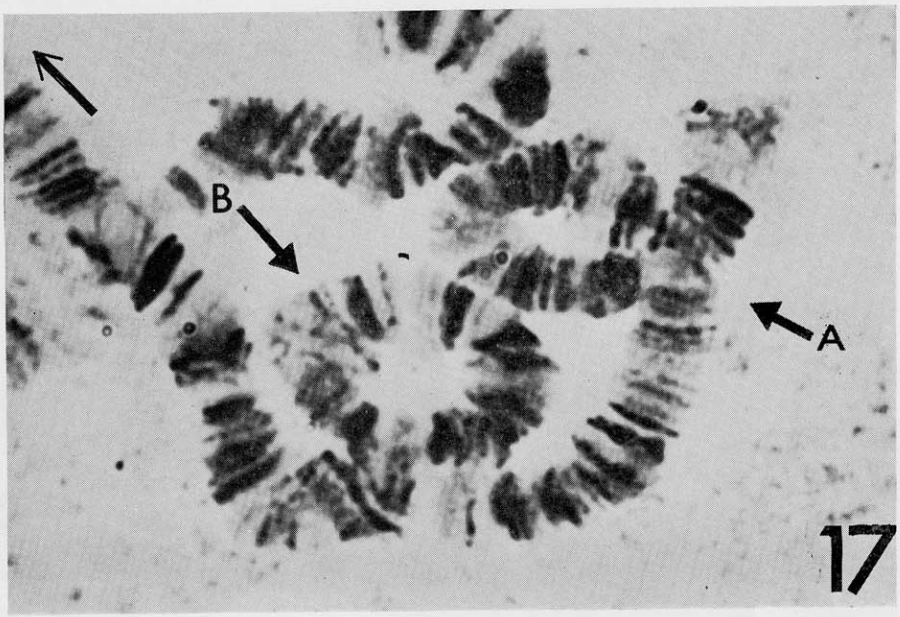
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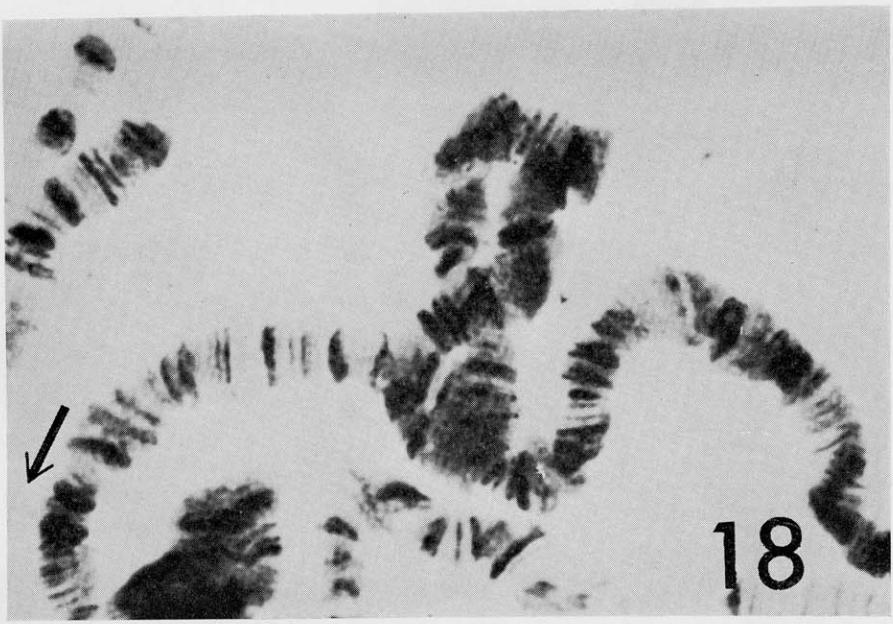
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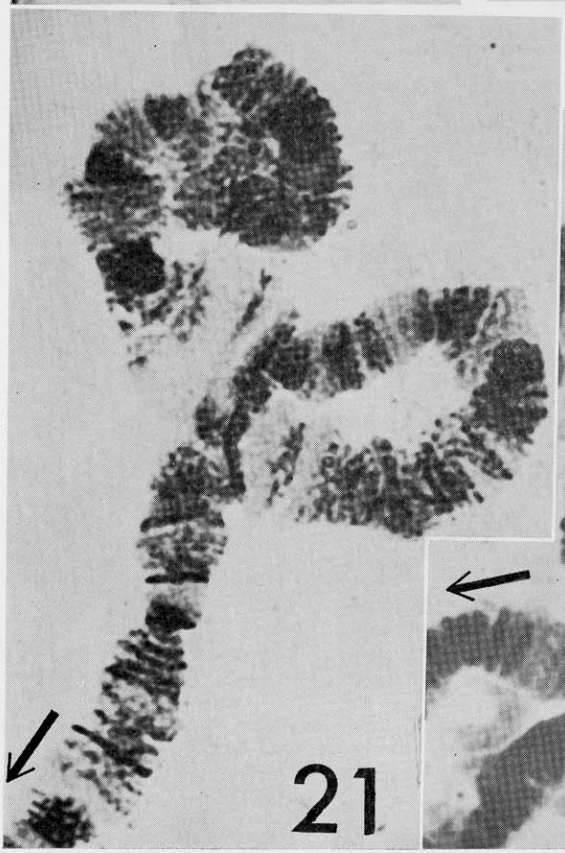

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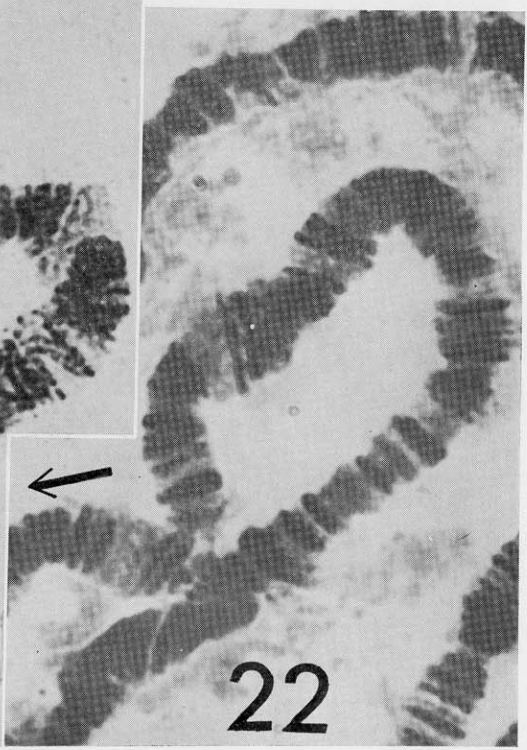

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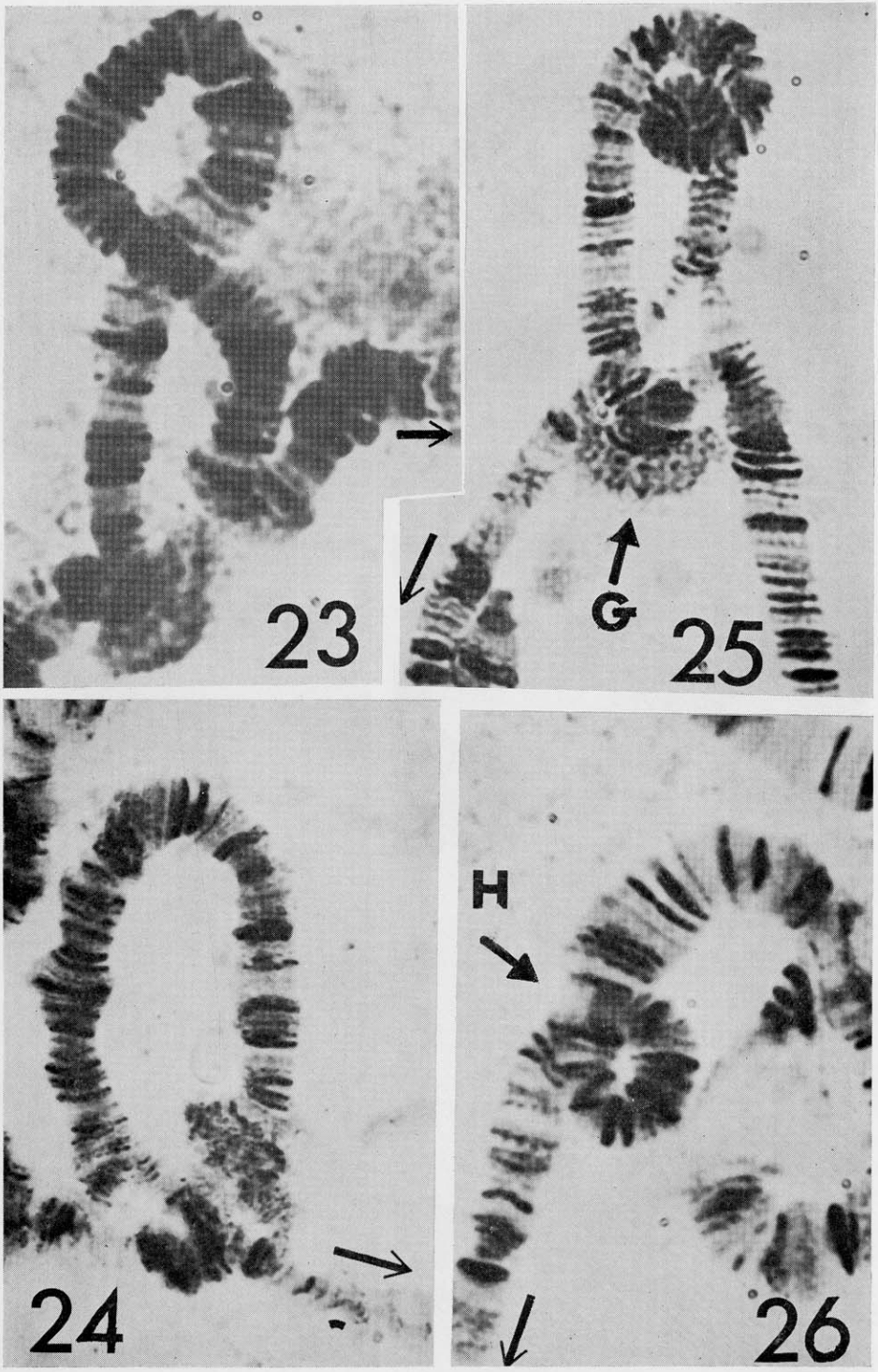
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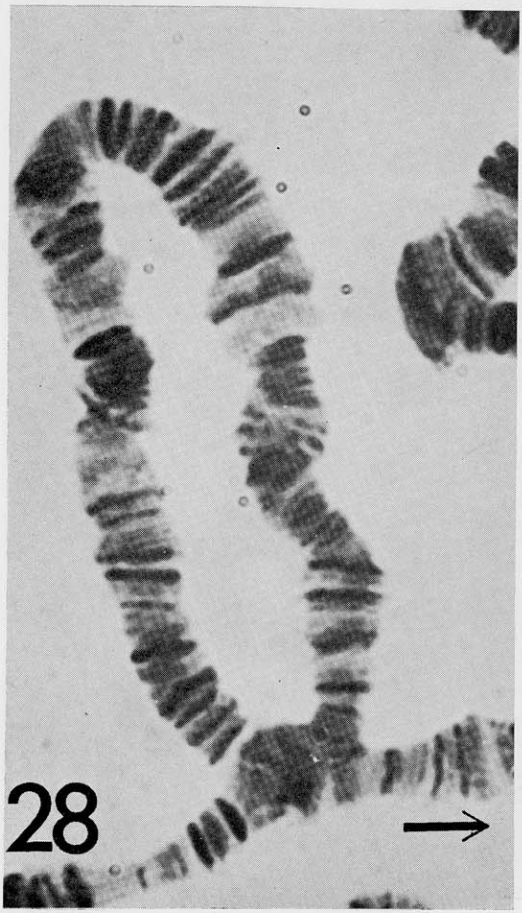
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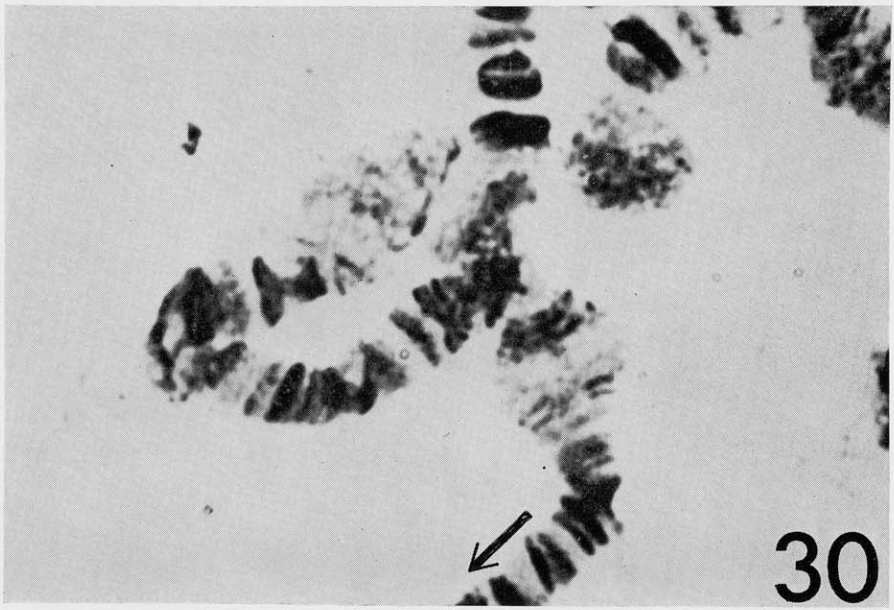
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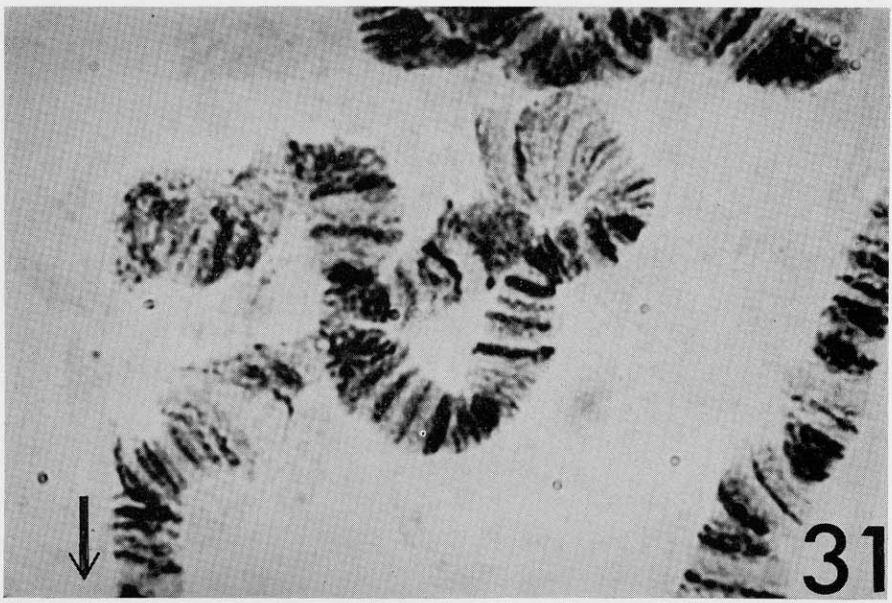
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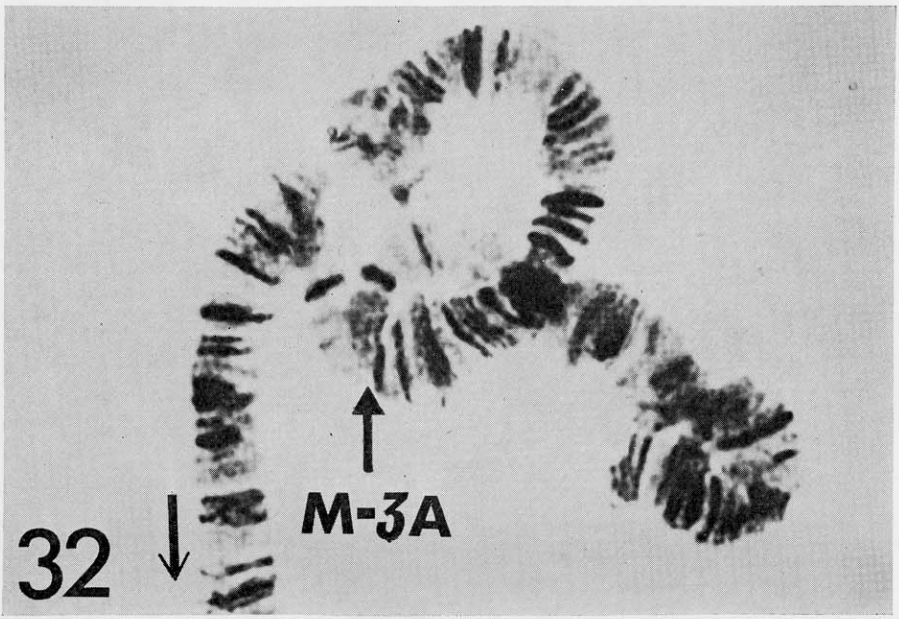
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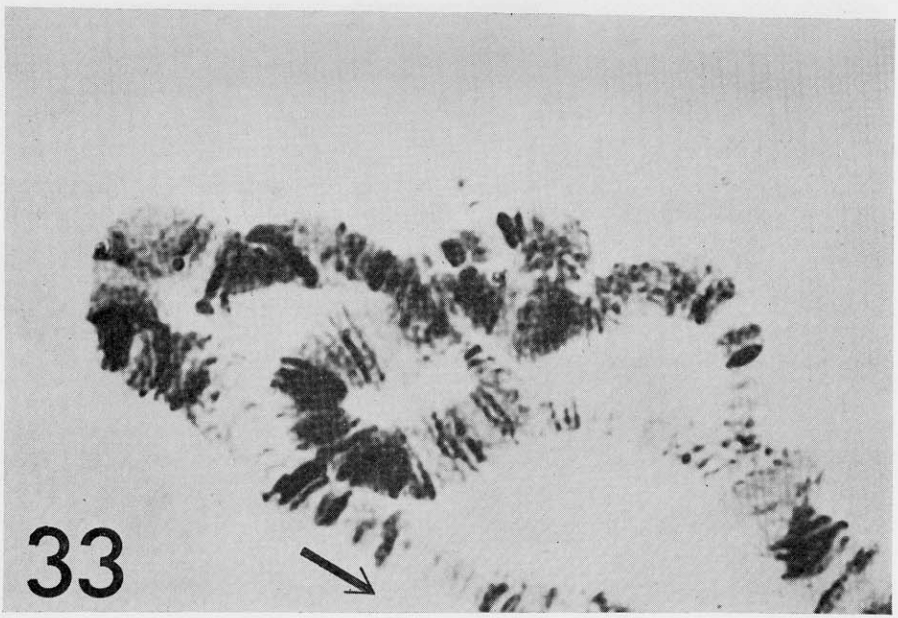
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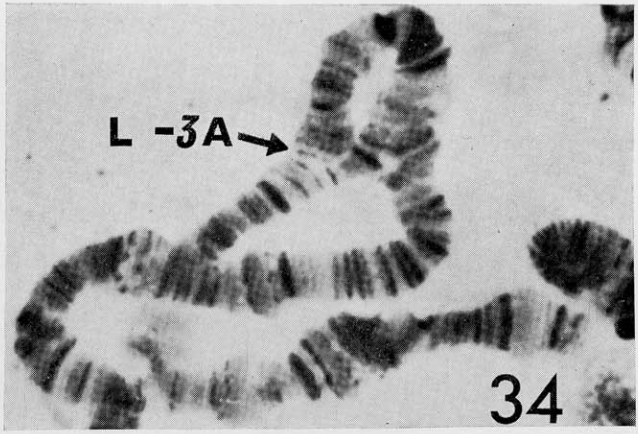
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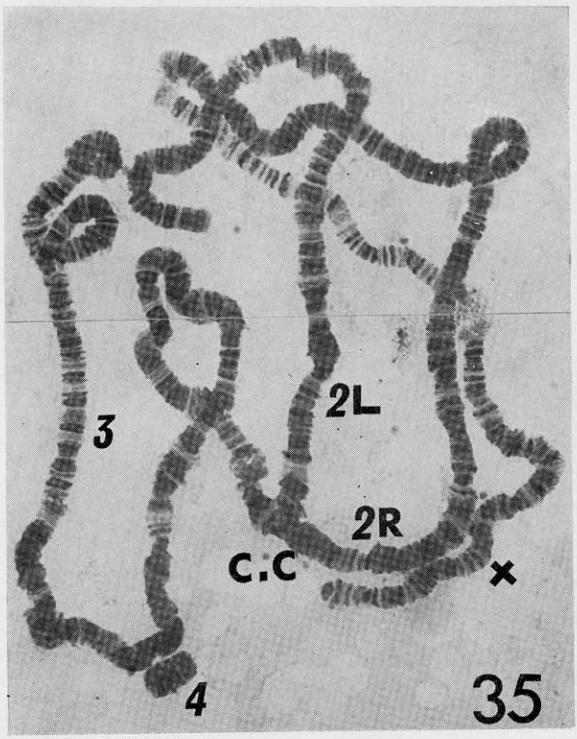
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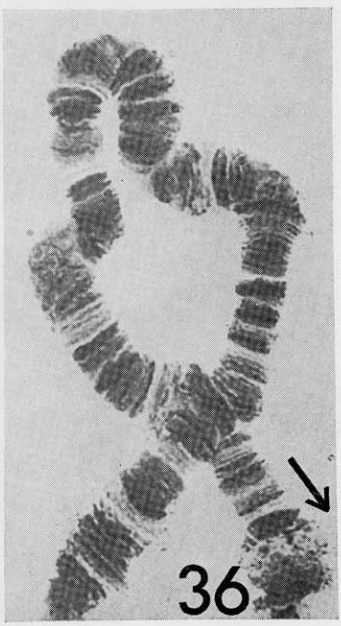
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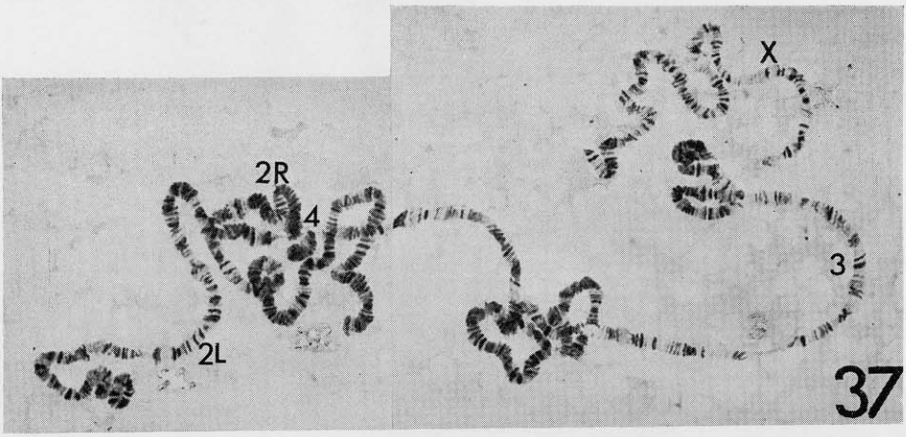
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2L
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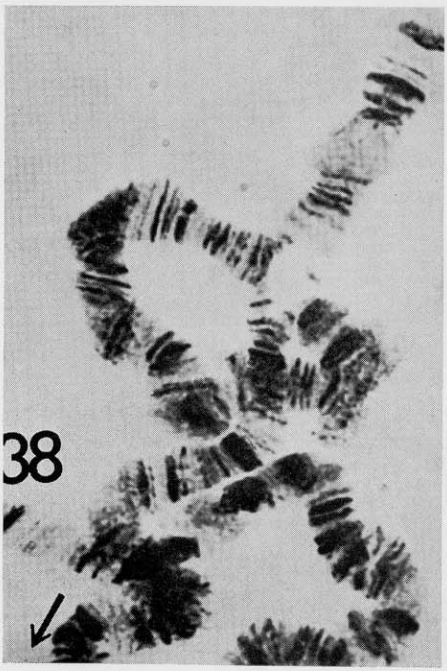
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39