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Salivary Gland Chromosomes of *Drosophila immigrans*

Ken-Ichi WAKAHAMA, Takaaki HAMAOKA and Machiko HATSUMI

Department of Biology, Faculty of Science, Shimane University, Matsue 690, Japan
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A total of 227 iso-female lines of *Drosophila immigrans* collected at three populations near Matsue were investigated to make the standard photo-maps of the salivary gland chromosomes and to analyze the pattern of the chromosomal polymorphism of this species in this district.

Some characteristic descriptions of each chromosome were given.

Three types of chromosomal inversions were detected in this study and two of them were identical to Brncic's inversions A and B found in the Chilean populations of *D. immigrans*.

The mean frequency of heterozygous inversion was very low and was 0.053 per larva.

Introduction

The salivary gland chromosomes are very important and useful tool in studying phylogeny and divergence of *Drosophila* species. According to Ashburner and Carson (1983), the standard maps of the salivary gland chromosomes have been constructed, up to the present time, for 103 species of *Drosophila* representing about 5 per cent of the total number of species described for the world.

Drosophila immigrans is one of the dominant species in Matsue and its adjacent localities and it has been said that this species is a nearly cosmopolitan species which occurs mostly in association with man and is also found in natural habitats (Parsons and Stanley, 1981).

The standard maps of the salivary gland chromosomes of *D. immigrans* have been constructed by five authors (Singh and Gupta, 1979 and others). All of them, however, were schematic ones and were not enough to be useable for comparative studies of chromosomal polymorphism between populations of this species.

Since Freire-Maia *et al.* (1953) reported the chromosomal polymorphism in the Brazilian populations of this species, Brncic (1955), Toyofuku (1957, 1961), Hirumi (1961), Richmond and Dobzhansky (1968) and Singh and Gupta (1979) have been analyzed the chromosomal polymorphisms in the Chilean, Japanese, Hawaiian and Indian populations of this species, and a very few types of chromosomal aberrations has been recognized in all of these populations.

The purpose of this study is to show the standard photo-maps of the salivary gland chromosomes of *D. immigrans* and to analyze the pattern of the chromosomal polymorphism occurring in three populations near Matsue of this species.

MATERIALS AND METHODS

Collections of flies were carried out in three localities near Matsue (Kumaino-Taki, Ryuzuno-Taki and Ichibata-Yakushi; Fig. 1) during the period from June to November in 1986. Flies were collected by exposing fermented bananas as baits.

A total of 227 iso-female lines of *D. immigrans* (106 individuals in Kumaino-Taki, 80 in Ryuzuno-Taki and 41 in Ichibata-Yakushi; Table 1) were established for this study.

One well grown-up larva was selected from each iso-female line and was examined for chromosomal polymorphism. For the preparations of the salivary gland chromosomes, the usual method of squashing glands in acetic-orcein was employed. Well spread chromosomes having no chromosomal variation were prepared to make the standard photo-maps of this species. The slides were observed under Olympus Optophoto Microscope, using 40× or 100× object lenses and 10× occular lens.

RESULTS AND DISCUSSION

Two karyotypes have been reported for *D. immigrans*. Patterson and Stone (1952) distinguished the strains with these two karyotypes as *immigrans* I and *immigrans* II. The majority of reports concerning *immigrans* karyotypes were *immigrans* I consisting one pair of median length telocentrics (X chromosome), one pair of metacentrics (Chromosome 2), one pair of long telocentrics (Chromosome 3) and one pair of short telocentrics (Chromosome 4). The Y chromosome was submetacentric. *Immigrans* II was found only by Emmens (1937) and Wharton (1948); their karyotypes consist of a pair of metacentrics, two pairs of median-length telocentrics and one pair of submetacentrics.

Two of the present authors (K. I. W. and M. H.) and their colleagues reported the karyotypes of *D. immigrans* collected in Japan and China and recognized them as *immigrans* I (Wakahama *et al.* 1983). The salivary gland chromosomes have four long euchromatic strands and one euchromatic dot in the present study, as can be expected from the configurations in the metaphasic plates.

Singh and Gupta (1979) have already reported the diagnostic features of the salivary gland chromosomes of *D. immigrans* collected in India. They also divided each chromosomal arm into several sections. Although their descriptions were hand-writing ones, we recognized the banding patterns of our *D. immigrans* were identical with those of India, judging from the arrangements of distinct bands and remarkable puffs (Fig. 3). The notations of sections on each chromosomal arm were decided to consent to those of their descriptions as much as possible.

In the following, some characteristic descriptions of each chromosome are given.

X chromosome: This chromosome is one of the moderately long arms and is distinguishable by thinner structure and paler coloration in male than the autosomes.

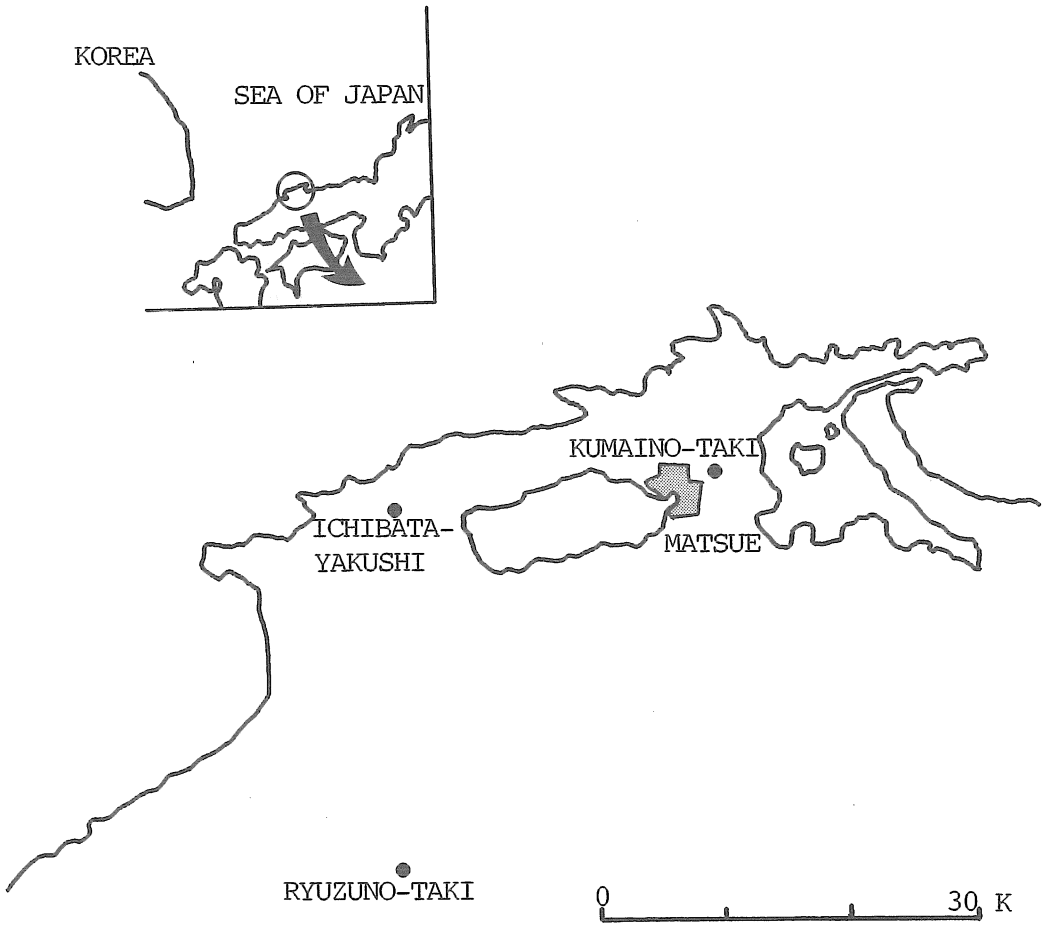

Fig. 1. Maps showing location of Matsue and localities where collections made.

Most characteristic features of this chromosome are represented by swollen free tip and a very conspicuous big puff in regions 4A–B. Two consecutive puffs with three or four dark thick bands placed both proximally and distally on sections 7D–8C are also the diagnostic features of this chromosome.

Left arm of Chromosome 2: Among the four long euchromatic strands, this chromosome is the shortest one and is characterized by a rectangular puff containing two thick bands proximally and four deeply stained bands placed distally in 1A–B. Some spindle-like structures in 1C, 3A, 4C, 5A, 5D and 6B are representative features of this chromosome. In addition more than ten deeply stained bands placed consecutively in 4B–4D are also diagnostic features of this chromosome.

Right arm of Chromosome 2: This chromosome is easily distinguished from the other chromosomal arms by its fan shaped free tip and by a constriction placed

immediately free tip in 6D. A big bulb-like structure seen in region 2D is a representative features of this chromosome. In addition four spindle-like structures placed in regions 1D, 4C–D, 5C and 6B are also remarkable traits of this chromosome.

Chromosome 3: This chromosome is the longest chromosome and all species belong to the *immigrans* species group, as far as we know, have this double length chromosome which caused by tandem fusion between two original autosomal telocentrics of *Drosophila* karyotype and/ or by a pericentric inversion of a two-element metacentric or a total translocation of one telocentric chromosome to the end of another (Wilson *et al.*, 1969 and Wakahama *et al.*, 1983). Easily distinguishable spindle-like features are seen in regions 2B with five darkly stained bands proximally, in 4C–D with two dark bands distally and in 10A–11A with two dark stained bands distally.

Chromosome 4: This is a short telocentric chromosome having several distinct bands.

Concerning to the chromosomal variations of *D. immigrans,* Brncic (1955) surveyed ten populations of this species in Chile and he reported three types of inversions designated as inversions A, B and C. Inversion A was found in the middle part of left arm of Chromosome 2 while inversions B and C were observed on the right arm of Chromosome 2. No inversion was detected from Chromosomes X, 3 and 4. He also summarized the distribution and incidence of inversion heterozygotes and pointed out that inversion B distributed through out Chile together with the standard gene arrangement, while inversion A was restricted to the southern Chile and inversion C was endemic in only one population. Mean frequency of heterozygous inversion per individual in the Chilean populations was about 0.20 and the widely distributed inversion B showed a frequency of 0.13 per larva.

Richmond and Dobzhansky (1968) studied the chromosomal polymorphism in four populations of *D. immigrans* on the island Maui, Hawaii and they found three types of inversions and recognized these three inversions were apparently identical to those described by Brncic from the Chilean populations. They also reported the frequencies of three inversions. According to them, the mean frequency of heterozygosity per individual was 0.35, substantially higher than value of the Chilean populations.

Singh and Gupta (1979) investigated the populations of Indian *D. immigrans* and found one inversion and one deletion. This inversion was observed on left arm of Chromosome 2 and was seemed to be identical to Brncic's inversion A judging from their description on the breaking points of this inversion.

The same inversion was also detected in the Brazilian populations of *D. immigrans* (Freire-Maia *et al.*, 1953).This was only one inversion found in Brazil.

As regards the chromosomal polymorphisms of the Japanese *D. immigrans,* Toyofuku (1957, 1961) examined three populations in Hokkaido and reported eight types of chromosomal aberrations from Chromosomes 2 and 3. Two of them were identical to Brncic's inversions A and B found in the Chilean populations. Others were complex inversions A and B in right arm of Chromosome 2, complex inversion C in left

Table 1. Numbers and percent of inversions found in heterozygous condition in *Drosophila immigrans*.

Inversion	Localities			
	Kumaino-Taki	Ryuzuno-Taki	Ichibata-Yakushi	Total
	106*	80*	41*	227*
A	2 (1.89)	4 (5.00)	0 (0.00)	6 (2.64)
B	1 (0.94)	2 (2.50)	2 (4.88)	5 (2.20)
2L-complex	1 (0.94)	0 (0.00)	0 (0.00)	1 (0.44)
Total	4 (3.77)	6 (7.50)	2 (4.88)	12 (5.28)

*: Numbers of individuals studied.
( ): Percent of inversion.

arm of Chromosome 2 and complex inversion D, submedian inversion and translocation in Chromosome 3. And she also reported frequency of chromosomal aberrations of this species was 0.05 per larva.

In addition, Hirumi (1961) surveyed the salivary gland chromosomes of 263 larvae of *D. immigrans* collected from several natural populations in the southern-central districts of Japan. And he observed three inversions which were identical to Brncic's inversions A, B and C. He also found the mean frequency of inversions was 0.19 per larva.

In the present study, we investigated 227 larvae and found three kinds of inversions. One inversion was detected in the middle part of left arm of Chromosome 2 which corresponded to Brncic's inversion A. Breaking-points of this inversion were in regions 2B and 4C (Figs. 2 and 3). One more inversion was observed in the same chromosome and named 2L-complex which covered almost total length of this chromosome. Breaking-points of this complex inversion were proximal end and 5B (Figs. 2 and 3). The third inversion was the subterminal inversion found in right arm of Chromosome 2 and this inversion was apparently identical to Brncic's inversion B whose breaking-points occurred in regions 5C and 6C (Figs. 2 and 3). Numbers of larvae examind and numbers and frequencies of three inversions found in this study were summarized in Table 1. Frequency of each inversion was 0.026 per larva for inversion A, 0.022 per larva for inversion B and only 0.004 per larva for 2L-complex. And the mean frequency of heterozygous inversions was 0.053 per larva. This value was almost similar to that of Toyofuku in Hokkaido but was extraordinary low value than those of Hirumi in the southern-central Japan, of Brncic in Chile and of Richmond and Dobzhansky in Hawaii. These differences might be occurred by reflection of the climatic and ecological conditions. Dobzhansky *et al.* (1950) and Da Cunha *et al.* (1955) have shown that in several species of *Drosophila,* there exists a correlation between the amount of the chromosomal polymorphism and the diversity of habitats in which population lives. Brncic (1955) reported that *D. immigrans* tolerates low

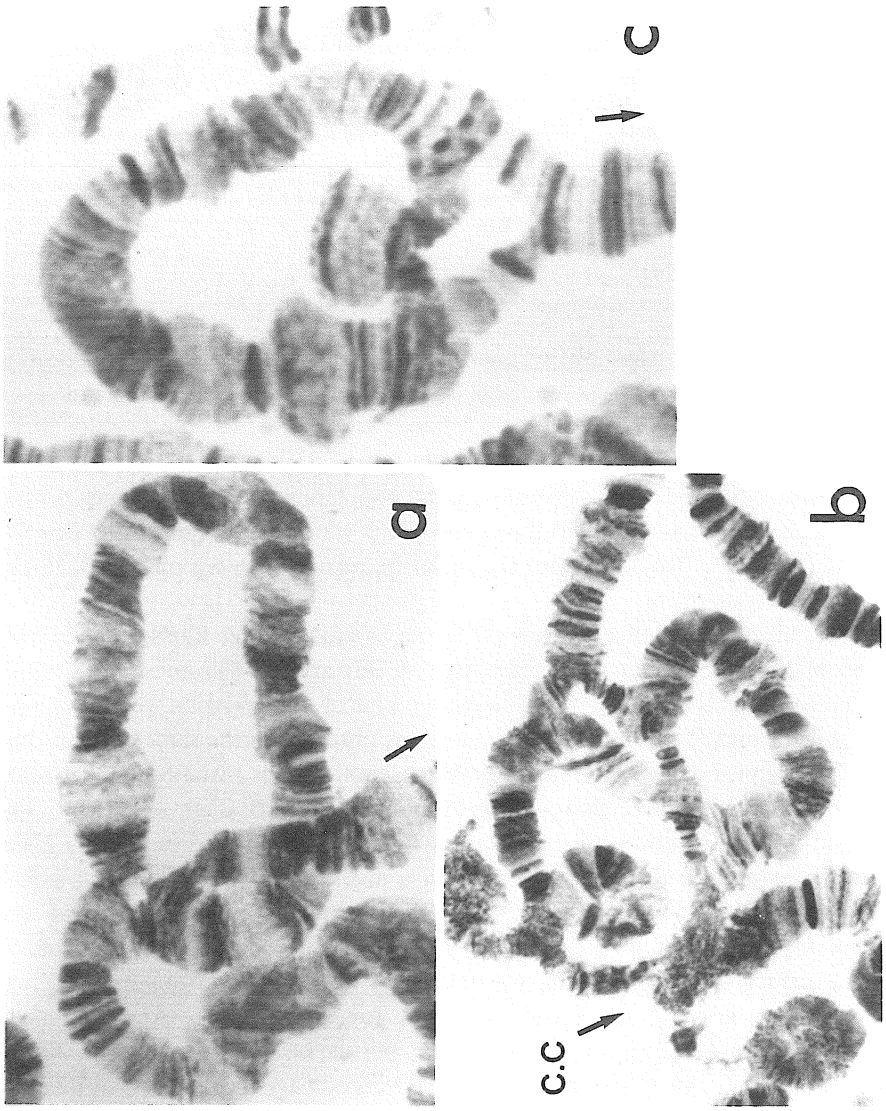

Fig. 2. Inversions of *Drosophila immigrans*. a: Inversion A. b: Complex inversion in left arm of Chromosome 2. c: Inversion B. Arrow indicates direction of chromocenter.

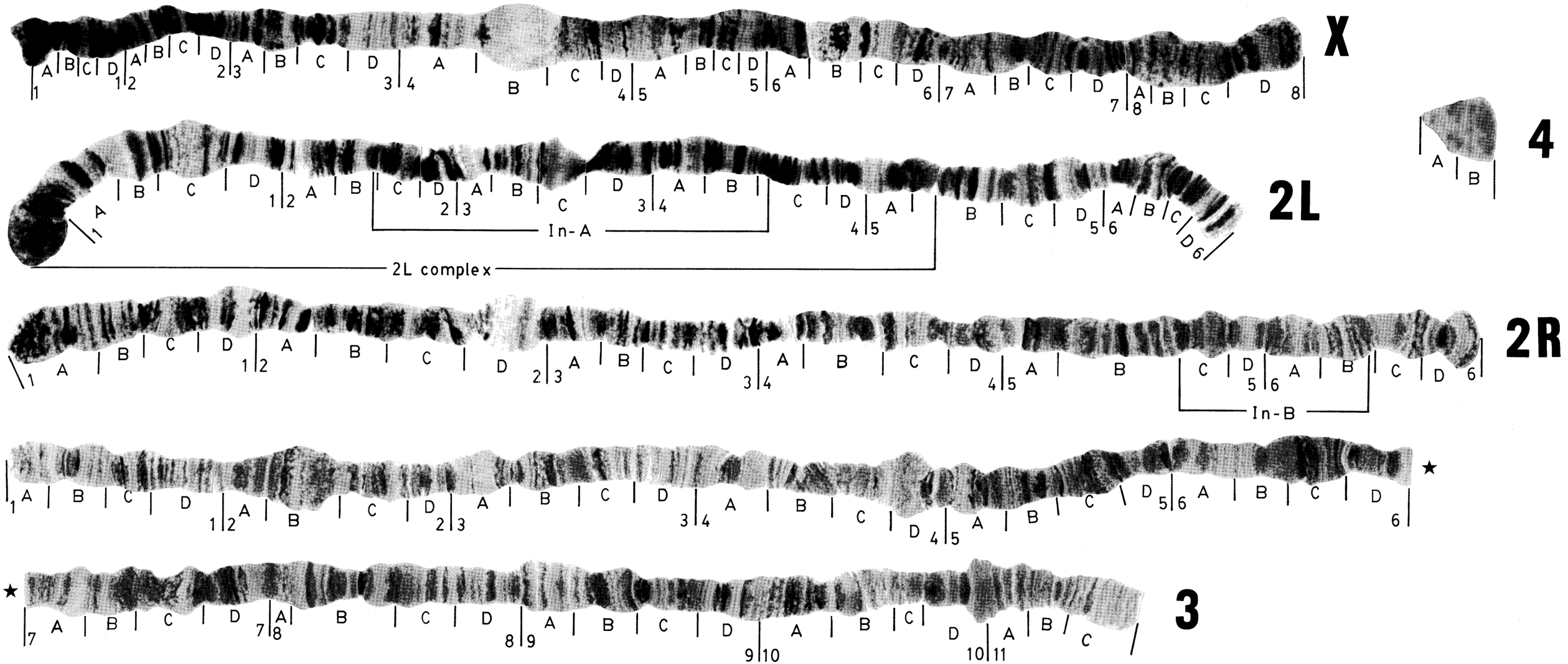

Fig. 3. Standard photo-map of the salivary gland chromosomes of *Drosophila immigrans* and breaking-points of inversions.
X: X chromosome. 2L: Left arm of Chromosome 2. 2R: Right arm of Chromosome 2. 3: Chromosome 3. 4: Chromosome 4.
Chromocenter locates on left side of each chromosome.

temperature and was one of the commonest species in spring and autumn collections, but rare during the summer in Chile. Hirumi (1961) also observed the seasonal behavior of this species and reported that this species showed a bimodal seasonal activity, showing dominancy in the end of June to the beginning of July and next peak of seasonal activity in October and November, and very rare in the mid summer in low land populations of the southern-central districts of Japan. The data of Richmond and Dobzhansky (1968) in Hawaiian *D. immigrans* were based on the collections made in June and July. According to our present data, however, this species showed the seasonal dominancy in the mid summer.

As to quality of the inversion polymorphisms of *D. immigrans,* the same and only three types of inversions, inversions A, B and C, were found commonly in the populations of Chile, Hawaii and Japan. Inversion A was also detected in the Indian and Brazilian populations. As far as we know, such world wide distribution of the same inversion is not so oftenly found in the species of *Drosophila,* except inversion like as *In(2L)t* of *D. melanogaster.* Although several complex inversions were observed in Hokkaido by Toyofuku (1961), it is said that these inversions occurred incidentally in the specific season and disappeared soon.

According to the ecological niche hypothesis of Dobzhansky *et al.* (1950), the *Drosophila* species with wide geographical distribution are more polymorphic than those restricted to a narrow range of ecological opportunities. Although *D. immigrans* is a cosmopolitan species and distributes both in domestic and wild areas, this species exhibits only a few types of chromosomal inversions in all populations studied and is not in accord with the working hypothesis of Dobzhansky, together with the other domestic species.

On the other hand, Carson (1965) has classified the eight cosmopolitan *Drosophila* species according to degrees of chromosomal morphism: 1. Polymorphism essentially ubiquitous; a few rare local aberrations–*ananassae, busckii, hydei, immigrans,* and *melanogaster,* 2. Polymorphism frequent and extensive in some populations but reduced in others–*funebris,* and 3. Monomorphism throughout–*repleta, simulans.* Three populations of *D. immigrans* investigated in the present study, are apparently belonging to Carson's category 1. As mentioned above, in spite of the world wide distribution, *D. immigrans* showed very low degree of chromosomal polymorphism in all populations studied. This might indicate that gene groups within the inversion loops are not so effective for adaptation to environments in this species.

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