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SALIVARY GLAND CHROMOSOME AND GENE ARRANGEMENTS OF *DROSOPHILA KIKKAWAI* BURLA FROM THAILAND

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Summary

*Population samples of **D. kikkawai** from nine localities in Thailand were analysed for chromosomal polymorphisms. Only two inversions, 3LA and 3LB, have been detected in certain populations. Inversion 3LA seems to be adaptive in nature. Other chromosome arms are apparently monomorphic.*

Introduction

The genus *Drosophila* comprises some 1500 species known so far which occur throughout the world in a wide variety of habitats. *Drosophila kikkawai*, a subcosmopolitan species, is widely distributed from the Asian and Australian regions across the Pacific islands to the South American continent[1]. *D. kikkawai* was first described by Burla[2] from a strain in Brazil. However, because of its obscurity, it was identified in error as *D. montium* in earlier reports[3-5]. Recently, it has been discovered that "*D. kikkawai*" is in fact a cluster of at least 3 closely related species already known, viz. *D. kikkawai*, *D. leontia* and *D. bocki*[6-8]. Cytologically, *D. kikkawai* exhibits remarkably interesting metaphase karyotype variation which is entirely due to the different amount of heterochromatin, while its two sibling species show similar metaphase figures[9]. This fact warrants further extensive investigations of cytogenetic relationships within the *D. kikkawai* complex.

A salivary chromosome map of *D. kikkawai* has been recently prepared by Roy and Lakhotia[10] primarily for studying replication patterns in these chromosomes. Unfortunately, the original strain for reference as a standard gene sequence was no longer available. Thus a culture stock of the Thailand strain was chosen as a standard gene order to be used as a reference for naturally occurring inversion polymorphisms in this series of cytogenetic studies.

This report presents gene arrangements found in certain natural populations in Thailand.

Materials and Methods

The *D. kikkawai* strain from Samut Songkhram manifesting homologous gene arrangement in salivary gland chromosomes was arbitarily chosen as the standard culture stock. This stock has been maintained in the laboratory at 25 ± 1°C as the standard reference for current cytogenetic studies.

Preparation of the salivary gland chromosome photographic map was made. The designation and division into zones and areas of each chromosome banding pattern were primarily based on the photographic map prepared by Roy and Lakhotia[10].

TABLE I POPULATION SAMPLES OF *D. KIKKAWAI* FROM THE NINE LOCALITIES IN THAILAND

Collection no.	Locality	Collection date	No. of individuals		No. of chromosome tested	Chromosome 3L		
			Female	Male		+	A	B
A 15	Sam Phran, Nakhon Pathom	18.10.78	8	4	40	100	–	–
A 16	Bang Kruai Nonthaburi	18.11.78	11	8	60	98.3	1.7	–
A 17	Bang Khonti, Samut Songkhram	13.12.78	11	14	72	100	–	–
A 19	Tha Muang, Kanchanaburi	14.1.79	3	2	16	100	–	–
A 21	Ban Pong, Ratchaburi	25.1.79	10	7	54	87.0	13.0	–
A 22	Damrongraks Road, Bangkok (1)	4.2.79	16	13	90	77.8	17.8	4.4
A 23	Phan Thong, Chon Buri	11.2.79	8	12	56	100	–	–
A 24	Hat Yai, Songkhla	17.3.79	5	6	32	68.8	31.2	–
A 25	Saladaeng Road, Bangkok (2)	15.4 79	12	9	66	77.3	18.2	4.5

The localities are given as districts and provinces (except Bangkok). Inversion frsquencies are shown in percent. + = standard gene sequence. A and B = inversions 3LA and 3LB respectively.

Population samples of *D. kikkawai* were collected by one of us (SK) at several localities in Thailand (Fig. 1 and Table I). Flies were collected by sweep-netting over fermenting bananas placed as baits under trees overnight. Wild-caught females were isolated into individual medium vials and were allowed to lay eggs and produce F_1 larvae for salivary gland chromosome analysis. Each of the wild-caught males was crossed to the individual virgin female of the standard strain to obtain F_1 larvae for polytene study as well. At least 10 3rd instar larvae in each case were scored for gene arrangements in polytene chromosomes.

Photomicrographs were made with an Olympus microscope using 35 mm Kodak Plus -X Pan film with green filter.

Results and Discussion

The general metaphase karyotype of *D. kikkawai* consists of 1 pair of sex chromosomes, 2 pairs of V-shaped autosomes, and the 4th chromosome of variable configurations. The larval salivary gland chromosome complement consists of 5 long arms and one short arm (Fig. 2). The free end of each chromosome can be easily

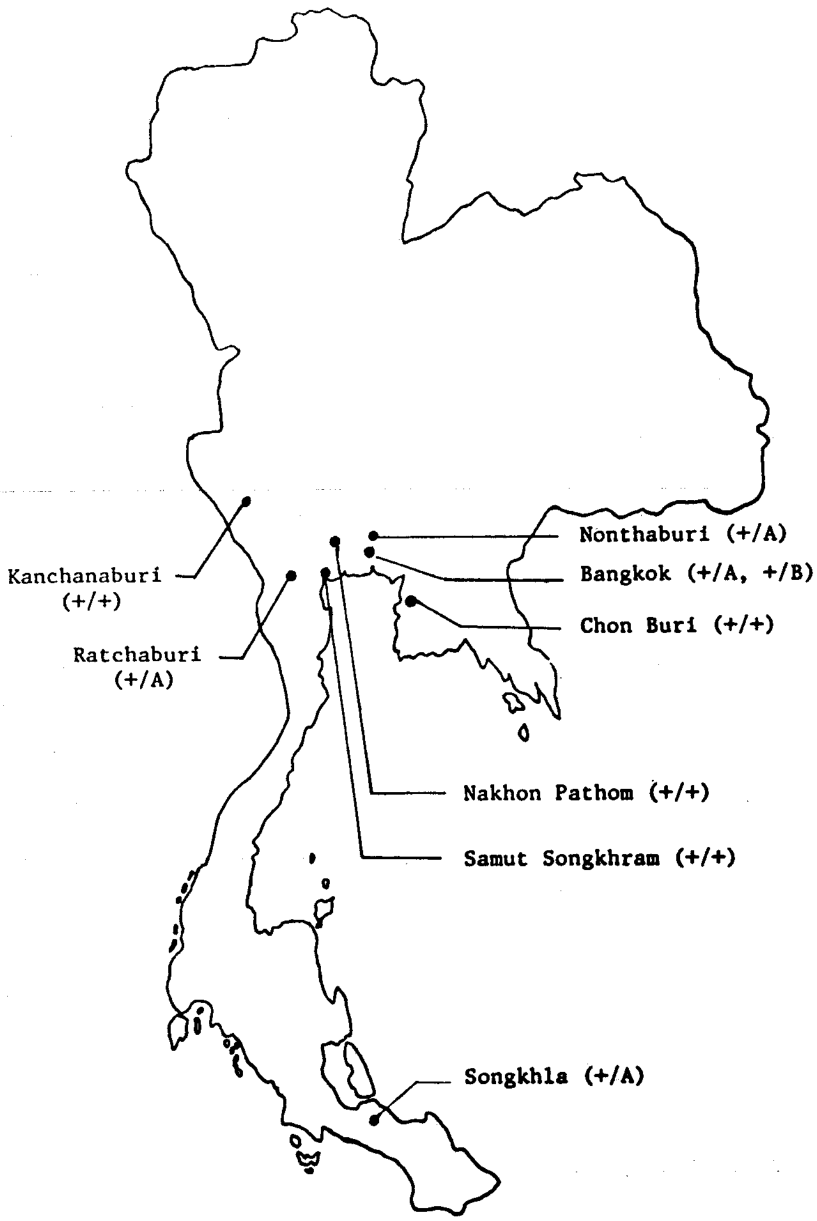

Fig. 1. Map of nine locations in Thailand where samples of *D. kikkawai* were studied. Gene arrangements in chromosome 3L are also indicated. + = standard gene sequence; A and B = inversions 3LA and 3LB, respectively.

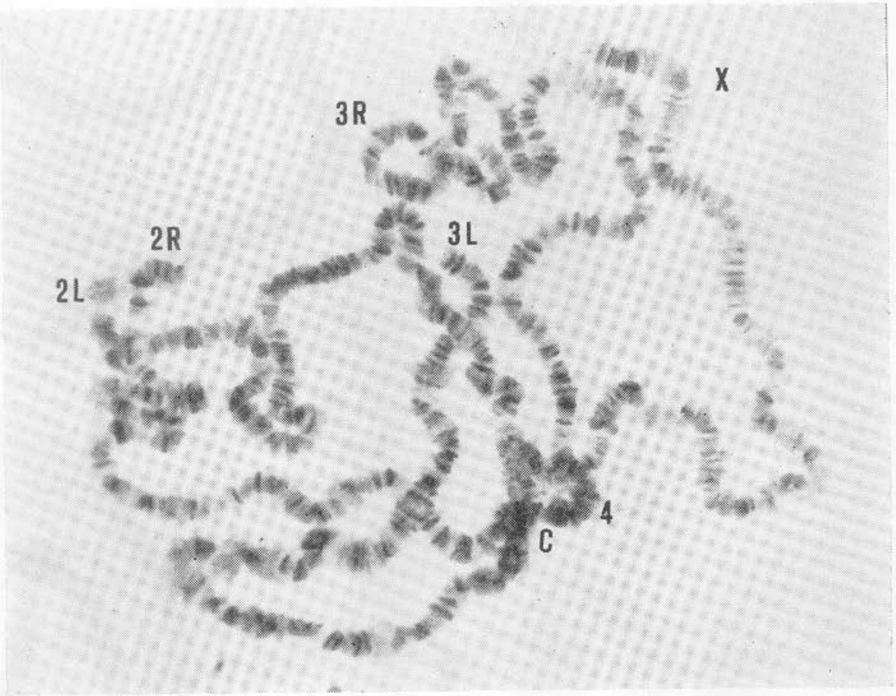

Fig. 2. Salivary gland chromosome complement of *D. kikkawai*. All the chromosome arms are associated at the centromeres, forming a chromocenter (C).

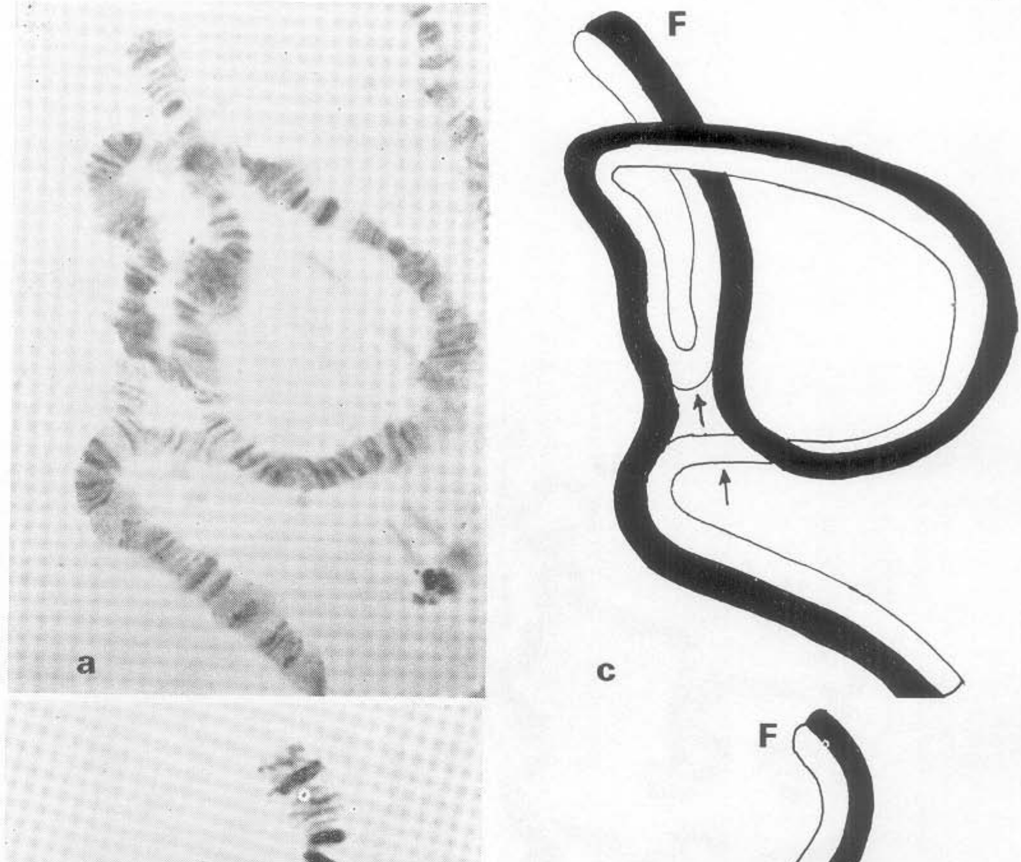

Fig. 3. Heterozygous inversions found in chromosome 3L of *D. kikkawai*. (a) and (b) are heterozygotes for inversions 3LA and 3LB respectively; (c) and (d) are schematic representation of the respective inversions. Break points for these inversions are indicated by arrows. The standard chromosome is shown by black line. F = free end of the chromosome arm.

recognised by its characteristic tip. The X chromosome is easily recognised in male larval polytene because of the light-staining banding patterns. Chromosome 3R is the longest arm.

Population samples of *D. kikkawai* from nine localities in Thailand were analysed for naturally occurring chromosomal gene arrangements (Table I). Among the 5 long arms, only chromosome 3L exhibits chromosomal polymorphism in gene arrangements. Two simple inversions have been detected in this chromosome arm (Fig. 3). Inversion 3LA has break points at 64D and 74C, and covers about 60 percent of the chromosome length. Inversion 3LB with break points at 64D and 70 covers about 32 percents of the chromosome arm. It is interesting to note that these two inversions have the same distal break point at the area of 64D. The data from this study indicate that standard gene sequence is apparently predominant in all populations. Inversion 3LA has been found in significantly different frequencies in four populations, i.e. Bangkok, Nonthaburi, Ratchaburi and Songkhla. It occurs in high frequency (31.2 percent) in the area of rubber plantations at Songkhla. However, this gene sequence occurs in considerably lower frequency in the Bangkok populations, 17.8 and 18.2 percent in locations 1 and 2 respectively (see Table I). Interestingly enough, the Nonthaburi population which is only some 20 km northeast of Bangkok yielded only one 3LA inversion among the 60 chromosomes tested. Such significant deviation in frequencies of inversion 3LA between the adjacent populations not too distant apart seems to reflect response to differences in environmental conditions. The Nonthaburi area is filled with tropical fruit orchards while the Bangkok surroundings are heavily populated urban or suburban areas. Moroever, inversion 3LA is apparently absent or very rare, if present at all, in other localities with orchard habitats similar to those in Nonthaburi. Thus our preliminary data seem to suggest that inversion 3LA is best adapted to human habitat.

In addition, inversion 3LB has been encountered in low frequency in the Bangkok populations (Table I). It has never been found in combination with inversion 3LA. Inversion 3LB has been detected in certain populations in Taiwan. There are some indications that inversion 3LA is widespread throughout the species range because it has been observed in certain localities in Taiwan and Brazil (unpublished data). Other polytene chromosome arms are seemingly monomorphic in natural populations throughout the species range.

The situation observed in *D. kikkawai* may be compared to other widespread species of *Drosophila* which have been extensively studied. *D. willistoni*, which is widely distributed in South America, is one of the most chromosomally polymorphic species, exhibiting some 50 different inversions[11-13]. Kastritsis[14] reported an extensive chromosomal gene arrangement in the incipient species of the *D. paulistorum* complex, another group of species widespread in South America. Recently Ashburner and Lemeunier[15] have demonstrated that *D. melanogaster*, a cosmopolitan species of worldwide distribution, is relatively more chromosomally polymorphic than the closely related *D. simulans*, which is apparently chromosomally monomorphic throughout its distribution. *D. kikkawai* apparently shows inversion polymorphisms only in chromosome 3L. The situation observed in *D- kikkawai* is probably different from that in *D. melanogaster*.

The 3LB inversion in chromosome 3L of *D. kikkawai* is also of particular interest. Data from salivary gland chromosome analysis of F_1 hybrids between *D. kikkawai* and *D. leontia*[16] reveal that the two sibling species share inversion 3LB. This fact clearly indicates the closely phylogenetic relationship between these sibling species. This phenomenon is not uncommon in Hawaiian *Drosophila*[17,18]. Further delailed investigation into this aspect in the *D. kikkawai* complex would throw some light on the process of speciation of this particularly closely related species group of the Oriental *Drosophila*.

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References

1. Bock, I.R. and Wheeler, M.R. (1972) *Univ. of Texas Publ.* **7213**, 1-102.
2. Burla, H. (1954) *Rev. Brazil Biol.* **14**, 41-54.
3. Ward (1949) *Univ. Texas Publ.* **4920**, 70-79.
4. Hsu, T.C. (1949) *Univ. Texas Publ.* **4920**, 80-142.
5. Baimai, V. (1969) *Dros. Inf. Serv.* **44**, 115-117.
6. Tsacas, L. and David, J. (1977) *Ann. Soc. Ent. Fr.* (N.S.) **13**, 675-693.
7. Baimai, V. (1978) *XIV International of Congress of Genetics.* Moscow, Part I, p 245.
8. Baimai, V. (1979) *Pacific Insects* **21**, 235-240.
9. Baimai, V. and Chumchong, C. (1980) *Genetica* (in press).
10. Roy, S. and Lakhotia, S.C. (1977) *Dros. Inf. Serv.* **52**, 118-119.
11. Da Cunba, A.B., Burla, H. and Dobzhansky, Th. (1950) *Evolution* **4**, 212-235.
12. Dobzhansky, Th. (1957) *Evolution* **11**, 280-293.
13. Dobzhansky, Th. (1970) *Genetics of the Evolutionary Process*, Columbia University Press, New York.
14. Kastritsis, C.D. (1966) *Chromosoma* **19**, 208-222.
15. Ashburner, M. and Lemeunier, F. (1976) *Proc. Roy. Soc. London B.* **193**, 137-157.
16. Baimai, V., Kitthawee, S. and Chumchong, C. (1980) *Jap. J. Genet.* (in press).
17. Carson, H.L. (1970) *Science* **168**, 1414-1418.
18. Carson, H.L.. Clayton, F.E. and Stalker, H.D. (1976) *Proc. Nat. Acad. Sci. U.S.A.* **57**, 1280-1285.

บทคัดย่อ

ตัวอย่างของแมลงหวี่ *D. kikkawai* จากประชากร 9 แห่งที่พบในประเทศไทย ได้รับการวิเคราะห์เพื่อหาการเปลี่ยนแปลงระดับโครโมโซม และพบว่ามีการเปลี่ยนแปลงแบบ inversion 2 แห่งในโครโมโซมสาย 3L ในจำนวนนี้ inversion 3LA ที่พบในบางประชากรแมลงหวี่สปีชีส์นี้ของเมืองไทยมีความสัมพันธ์กับสภาวะแวดล้อม ส่วนโครโมโซมสายอื่นนั้นไม่พบว่ามีการเปลี่ยนแปลงระดับโครโมโซมแต่อย่างใด