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CYTOGENETIC STUDIES OF *CHIRONOMUS PLUMATISETIGERUS* (DIPTERA : CHIRONOMIDAE) IN THAILAND

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Abstract

The larval salivary gland of Chironomus plumatisetigerus *shows polytene chromosome arm combinations: AE, BF, CD and G, which are typical of the pseudothummi cytological group. The Bangkok populations of* Ch. plumatisetigerus *are polymorphic for six paracentric inversions distributed in five chromosome arms. The limits of these inversions are given. The gene sequences* B_1, F_1, D_2, C_1 E_1, A_2 *and* G_1 *are common in all populations studied. Most of the inversions conform to Hardy-Weinberg expectations.*

Introduction

Chironomids are suitable for population cytogenetic studies. They have a low chromosome number and like other larvae of the insect Order Diptera (especially the genera Drosophila, Simulium and Anopheles) have large and well-staining salivary gland polytene chromosomes. The larvae of some species are red in color due to the presence of hemoglobin in the blood known as bloodworms[1]. Excellent polytene chromosome preparations can be obtained from many species of chironomid larvae[2]. These allow a study of chromosomal polymorphisms[3-7], cytotaxonomy and phylogeny[8,9]. Keyl[8] constructed a phylogenic tree of twenty European *Chironomus* species on the basis of chromosomal inversions and translocations. Similar studies have been carried out with a group of Australian *Chironomus* species[9]. However, little is known on chromosomal polymorphism of the Asian chironomid species. Recently, chromosome maps of five Indian chironomid species, including *Ch. plumatisetigerus,* were made under Keyl's system and chromosomal polymorphisms have been studied[10].

Chironomus plumatisetigerus is one of the common chironomid midges found in shallow or deep waters in the Bangkok populations. This species exhibits good polytene chromosomes suitable for population cytogenetic studies. This report presents the chromosome map under Martin's system and the chromosomal polymorphism of *Ch. plumatisetigerus* of the Bangkok populations.

Materials and Methods

Larval specimens in this study were collected from three suburban areas of Bangkok : Chitralada, Yomaraj and Din-Daeng. All living late fourth instar larvae were sexed based on the invagination of the 12th abdominal segment in the ventral surface [5,11].

Salivary gland polytene chromosome preparations were made using 1.6% orcein in lactic acid: propionic acid: water (2 : 2 : 1)[12]. The system of cataloguing the salivary gland chromosomes was based on the method proposed by Martin (1969)[13]. The standard sequence is denoted by the letter designating the chromosome arm with the number 1 and higher numbers are used for alternative sequences in the same arm[14,15].

Results and Discussion

I. *Observations of chromosome inversions*

The karyotype of *Ch. plumatisetigenus* (2n = 8) consists of 3 pairs of long metacentric chromosomes and one pair of short acrocentric chromosomes forming seven polytene chromosome arms in larval salivary gland cells.

Chromosome I, the longest chromosome consists of arms B and F (Fig. 1). Two chromosome inversions were found in arm B (B_2 and B_4) but none in arm F among the samples examined. The B_2 sequence is apparently derived from the standard sequence (B_1) with breakpoints at about 2A1 and 3A3 (Fig. 1). The B_2 was found in the heterozygous condition with B_1 (Fig. 2a) in some larvae. The B_3 found in the Indian populations[10] has not been detected in the present study. The sequence B_4 is also presumed to be derived from the standard sequence with breakpoints at 1A6 and 1D6 (Fig. 1). Inversion B_4 was found in heterozygous condition with B_1 (Fig. 2b) in one larva from Chitralada. Thus it is likely that these three gene rearrangements of chromosome I arose independently.

Chromosome II, the second longest of the genome comprises arms D and C. Each chromosome arm exhibits one inversion. The D_2 sequence differs from the standard by a simple inversion covering the region 8B1 to 9B5 (Fig. 1). Inversion D_2 was found in the homozygous condition in the present study. The C_2 is derived from the standard C_1 sequence with the breakpoints at 11B6 and 11D8 (Fig. 1). It was also found in the heterozygous condition with C_1 (Fig. 2c).

Chromosome III, third in length among the four chromosomes, consists of arms E and A. Inversion A_2 has breakpoints at 14E5 and 15D1 (Fig. 1) and was found in heterozygous condition with A_1 (Fig, 2d) in some samples from all localities except at Din-Daeng II. There were no inversions observed in arm E.

Chromosome IV, the shortest (arcrocentric) chromosome, consists of one inversion, G_2, with breakpoints at 17A1 and 17D10 (Fig. 1). Inversion G_2 has been found in heterozygous condition with G_1 (Fig. 2e) in all populations studied.

II. *The frequencies of inversions*

The frequency of the various inversion sequences is summarized in Tables 1-3. Since there is no difference in the frequencies of inversions between sexes in all populations studied, the tests for goodness of fit could be carried then out on the total data. The figures in brackets of these tables represent Hardy-Weinberg expected values.

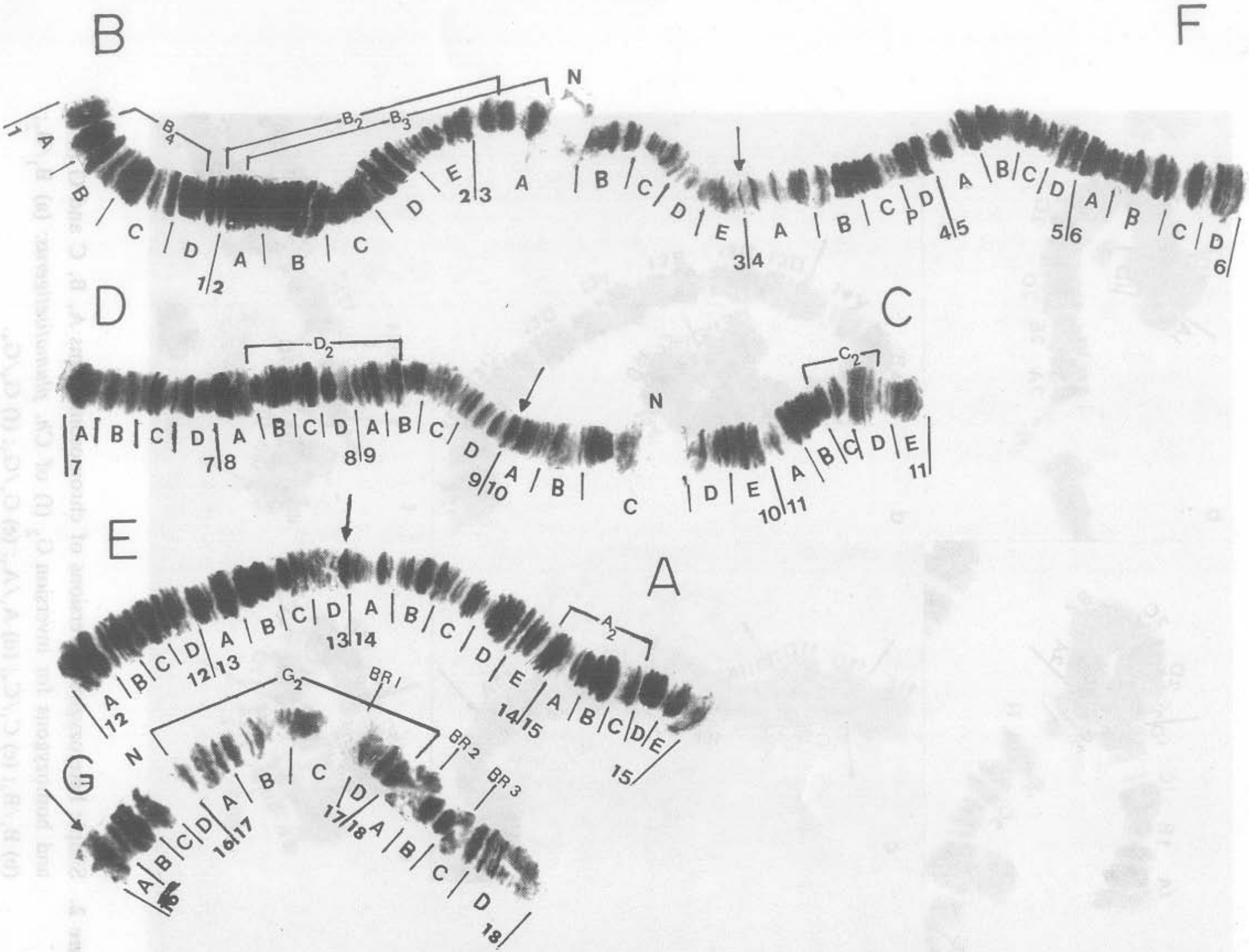

Figure 1. Photographic map of *Ch. plumatisetigerus* (under Martin's system). Brackets above regions indicate the limits of inversions. Centromere is indicated by arrows; "N" indicates nucleolus and "BR" indicates Balbiani ring.

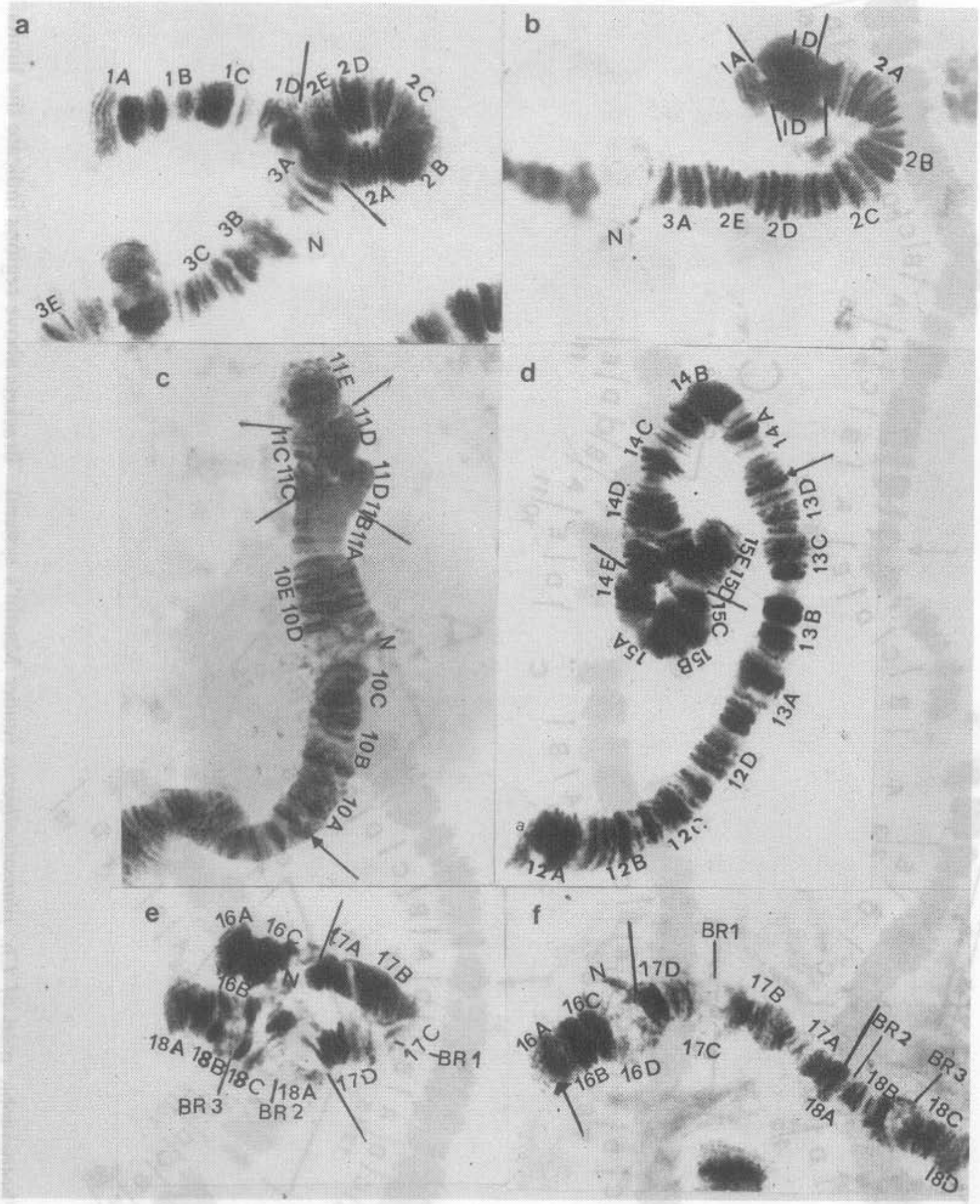

Figure 2. Simple heterozygous inversions of chromosome arms A, B, C and G (a–e) and homozygous for inversion G_2 (f) of *Ch. plumatisetigerus:* (a) B_1/B_2; (b) B_1/B_4; (c) C_1/C_2; (d) A_1/A_2; (e) G_1/G_2; (f) G_2/G_2.

Arm B: Inversion B_1 was the most common sequence in the Bangkok populations. The lowest frequency (57%) of B_1 was found in the Din-Daeng II sample while the others showed high frequency of this inversion ranging from 60–77% (Table 2). All karyotypes in most samples showed agreement with Hardy-Weinberg expected values (Table 2).

Arm G: The G_1 sequence was very common with a high frequency of over 85% in all populations compared with the alternative sequence (Table 3). These karyotypes are in Hardy-Weinberg equilibrium (Table 3).

Arm C: Most populations showed high frequency of the C_2 sequence. In fact the C_1 sequence was found in low frequency (2%) only in the Din-Daeng I sample.

Arm D: The D_2 sequence was the only one inversion observed in all populations examined.

Arm E and F are monomorphic for standard sequence E_1 and F_1 respectively in all populations studied.

Recently the identification of the chromosome arms of *Ch. plumatisetigerus* was based on comparison of the banding pattern to those of European species[10]. This species has the arm combinations AE, BF, CD, G corresponding to the pseudothummi group of Europe[8]. Some conspicuous band groups in each arm are similar to those found in the Australian cytological standard, *Ch. oppositus,* and three members of the *Ch. australis* group; *Ch. australis, Ch. duplex* and *Ch. occidentalis.* The phylogenetic relationship between *Ch. plumatisetigerus* and the Australian species is not known. Detailed information on gene arrangements and the banding patterns among these species await further investigation.

Chironomus plumatisetigerus from the Bangkok populations are polymorphic for at least six inversions, some of which are quite common. It is noted that *Ch. plumatisetigerus* is common in various localities in Bangkok and occurs in variable depths. This observation may reflect the possible correlation between the chromosomal polymorphisms and the wide variety of habitats occupied by larvae of this species.

Although *Ch. plumatisetigerus* is chromosomally quite polymorphic, there is no indication of sex linkage associated with inversion sequences in all populations studied. The localization of sex determining loci in this species should be studied by the technique of analysis of segregation patterns of chromosomal polymorphisms in egg masses[16] or the technique of hybridization of closely related species[17]. This line of approach warrants further extensive studies in Thailand populations.

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Arm A: All populations in this study showed a high frequency of A_2 ranging from 93–100% (Table 1). All karyotypes showed hardy–Weinberg equilibrium in all population samples.

TABLE 1 FREQUENCY OF THE ALTERNATIVE SEQUENCES OF ARM A

Locality	A_1/A_1	A_1/A_2	A_2/A_2	Total	A_1	A_2	χ^2_1	p
Chitralada	–	6	76	82	0.04	0.96	0.02	∿0.87
	(0.13)	(6.30)	(75.57)					
Din–Daeng I	–	1	31	32	0.02	0.98	ct	–
	(0.01)	(1.25)	(30.73)					
Din–Daeng II	–	–	69	69	0.00	1.00	0.00	–
	(0.00)	(0.00)	(69.00)					
Din–Daeng III	–	3	65	68	0.02	0.98	ct	–
	(0.03)	(2.67)	(65.31)					
Yomaraj I	1	5	74	80	0.04	0.96	0.21*	∿0.65
	(0.13)	(6.14)	(73.73)					
Yomaraj II	–	11	69	80	0.07	0.93	0.04*	∿0.85
	(0.39)	(10.41)	(69.19)					

CT – significance test could not be done because of small expected values

* – A_1/A_1 included with A_2/A_2

TABLE 2 FREQUENCY OF THE ALTERNATIVE SEQUENCES OF ARM B

Locality	B_1/B_1	B_1/B_2	B_2/B_2	Total	B_1	B_2	χ^2_1	p
Chitralada	41 (44.90)	39 (31.50)	2 (5.54)	82	0.74	0.26	4.36	∿0.04
Din-Daeng I	19 (18.97)	11 (11.33)	2 (1.69)	32	0.77	0.23	0.01*	∿0.90
Din-Daeng II	23 (22.42)	32 (33.82)	14 (12.76)	69	0.57	0.43	0.23	∿0.65
Din-Daeng III	27 (26.1)	30 (32.04)	11 (9.82)	68	0.62	0.38	0.30	∿0.55
Yomaraj I	33 (31.75)	35 (37.30)	12 (10.95)	80	0.63	0.37	0.23	∿0.65
Yomaraj II	26 (28.80)	45 (38.4)	9 (12.8)	80	0.60	0.40	2.53	∿0.15

* – B_2/B_2 included with B_1/B_1

TABLE 3 FREQUENCY OF THE ALTERNATIVE SEQUENCES OF ARM G

Locality	G_1/G_1	G_1/G_2	G_2/G_2	Total	G_1	G_2	χ^{2*}_1	p
Chitralada	63 (63.50)	19 (17.32)	– (1.18)	82	0.88	0.12	0.21	∿0.65
Din-Daeng I	27 (27.08)	5 (4.71)	– (0.20)	32	0.92	0.08	0.02	∿0.88
Din-Daeng II	54 (54.65)	15 (13.51)	– (0.83)	69	0.89	0.11	0.17	∿0.68
Din-Daeng III	49 (50.3)	19 (16.37)	– (1.33)	68	0.86	0.14	0.55	∿0.45
Yomaraj I	58 (57.80)	20 (20.40)	2 (1.80)	80	0.85	0.15	0.01	∿0.90
Yomaraj II	58 (59.17)	21 (19.26)	1 (1.57)	80	0.86	0.14	0.21	∿0.65

* – χ^2 value of G_2/G_2 included with G_1/G_1

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บทคัดย่อ

ศึกษาโพลีมอร์ฟิซึมของโครโมโซมหนอนแดง *Chironomus plumatisetigerus* หนอนชนิดนี้มีโพลีธีนโครโมโซมของต่อมน้ำลายขนาดใหญ่ที่มีแขนในสภาพ AE, BF, CDและ G ดังนั้นหนอนแดงชนิดนี้จึงจัดอยู่ในพวก *pseudothummi* ซึ่งเป็นกลุ่มของหนอนแดงในยุโรปจัดตามส่วนประกอบของแขนบนแต่ละโครโมโซม

จากการศึกษาโครโมโซมของประชากรหนอนแดงที่อยู่ตามธรรมชาติในเขตต่าง ๆ ของกรุงเทพฯ พบพาราเซนตริกอินเวอร์ชัน (paracentric inversion) 6 แห่งบนแขนต่าง ๆ ของโครโมโซม 5 แขน คือ B_2, B_4; D_2, C_2, A_2 และ G_2 และได้ศึกษาตำแหน่งที่เกิดของอินเวอร์ชันเหล่านี้ ลำดับยีน B_1, F_1, D_2, C_1, E_1, A_2 และ G_1 พบมากในประชากรที่ศึกษา นอกจากนี้ลำดับยีนที่พบส่วนมากอยู่ในสภาวะสมดุลย์ภายใต้กฎของฮาร์ดี - ไวน์เบิร์ก (Hardy-Weinberg equilibrium)