〔Med. Entomol. Zool. Vol. 47 No. 1 p. 55–61 1996〕

Larval salivary gland chromosomes of the blackfly, *Simulium* (*Gomphostilbia*) *sundaicum* (Diptera: Simuliidae) from Java, Indonesia

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(Received: 11 September 1995; Accepted 1 December 1995)

Key words: blackfly, Simuliidae, *Simulium*, polytene chromosome

Abstract: Polytene chromosome study of the Oriental Simuliidae has been only scantily made. Larval salivary gland chromosomes of the blackfly, *Simulium* (*Gomphostilbia*) *sundaicum* Edwards, 1934, from Java, Indonesia, were observed and their standard maps were constructed. This species had three pairs of polytene chromosomes. The chromosome I and II were metacentric and the chromosome III was submetacentric. Each centromere consisted of a large conspicuous heterochromatic segment in all chromosomes. The large heterochromatin was associated with each other in some cells. The nucleolar organizer was located in section 19 of chromosome I. The Balbiani ring, the double bubble and the Parabalbiani ring were situated in section 49C, 48 and 64C of chromosome II, respectively. Five inversions were found, but none of them was sex-related. C-band staining successfully revealed that band of 84A, centromeric of chromosome III, was heterozygously stained, slightly and heavily in males, while homozygously stained, only heavily in females. Then the slightly stained band could be ascribed to Y-chromosomal, and the heavily stained, to X-chromosomal.

INTRODUCTION

Cytological studies of blackflies in the past few decades have shown that most morphospecies in Simuliidae are comprised of more than two reproductively isolated, biologically distinct species (Rothfels, 1956; Dunbar, 1959). Now, morphotaxonomic and cytotaxonomic data of blackflies are becoming well integrated. However, blackflies in Indonesia, as in other Asian countries, had received little attention so far. This study was aimed to investigate cytotaxonomically blackflies belonging to the subgenus *Gomphostilbia* Enderlein of the genus *Simulium* s. l. which is for the most part distributed in Southeast Asia.

In this study, *Simulium* (*Gomphostilbia*) *sundaicum* Edwards, which is one of the common blackfly species in Java and Sumatra, Indonesia, was examined. This species was once collected while bloodsucking domestic fowls (Edwards, 1934). Takaoka and Davies (1996), making revised descriptions for all stages, suspected that this species is a synonym of *S.* (*G.*) *atratum*.

A photographic map of larval polytene

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chromosomes of this species is presented. Sex-linked bands could also be identified using the C-banding technique.

Materials and Methods

Polytene chromosome preparation. The larvae were collected from Gunung Mas, Sukabumi, West Java, Indonesia on August 27, 1991 by one of us (HT). The larvae collected were fixed in freshly-prepared acetic alcohol (glacial acetic acid:ethanol=1:2) and then stored in a freezer (−20°C) until required for slide preparation.

Final- or penultimate-instar larvae recognized by the presence of pupal gill histoblasts were used to obtain salivary gland chromosomes in optimal conditions. Larvae were dissected and left for 2–3 min in 45% aceto-carmine (1% carmine, Merck), and the salivary glands were transferred in a medium consisting of 4 parts water, 4 parts acetic acid and 1 part lactic acid on a glass slide. The salivary gland chromosomes were squashed under a coverslip and they were observed and photographed under phase contrast microscope. The preparations were subjected to air dry. Each slide preparation was kept in liquid nitrogen for about 20 sec and then the cover slip was removed using a razor. The slides were placed in absolute ethanol for 30 sec, dried for 2–3 days at room temperature, and stained with 2% orcein solution for permanent preparations.

C-banding. Some of the dried preparations were subjected to C-banding. The air dried preparations were placed in a coplin jar with 0.2 N HCl for 30 min. After washed they were treated with 5% barium hydroxide [$Ba(OH)_2 8H_2O$] for 10 min and then treated sequentially with 2×SSC (1×SSC consists of 0.15 M NaCl and 0.015 M sodium citrate) for 15 min. Both treatments were made at 65°C. Finally the slides were stained with 4% Giemsa in Sorensen's buffer (pH 6.8) for 60 min.

Other experimental procedures. Sex was identified by the shape of the gonads found in the stained larval carcass. The male has paired, small spherical testes while the female has elongated ovaries.

Mapping of the polytene chromosomes followed the conventional method of Bedo (1977) and Rothfels *et al.* (1978).

Results

Chromosome characteristics. *S. (G.) sundaicum* had three salivary gland chromosomes (Figs. 1–3), which exhibited tight pairing in all the larvae examined. Table 1 summarizes the results to determine the number of sections mapped on the polytene chromosome arms using 10 well spread chromosome complements. Bands were divided into numbered sections from the IS tip to the IIIL tip and each section was further subdivided into three (A, B, and C) or two subsections (A and B) in the same direction as section numbering. The centromere was formed of a large conspicuous heterochromatic block in all chromosomes. The large heterochromatin was associated with each other in some cells (Figs. 5a and 5b).

Chromosome I was characterized by being the longest among the complements and metacentric and by having a nucleolar organizer (NO) (Table 1 and Fig. 1). The short arm had three heavy bands in sections 17C–18A and the NO in section 19. The long arm had a group of three heavy bands in section 41AB.

Chromosome II was metacentric and remarkably shorter than chromosome I, but had more abundant characteristics. It was easily recognized by the Ring of Balbiani (RB), the double bubble (db) and the basal trapezoidal group (tr) in the short arm and the Parabalbiani ring (PB) in the long arm. The RB and db were in 49C and 48, respectively, while the tr and PB in 54B–55A and 64C, respectively.

Chromosome III (Fig. 3) was the shortest and had the highest arm ratio which served to distinguish it from the other two (Table 1). This chromosome was charac-

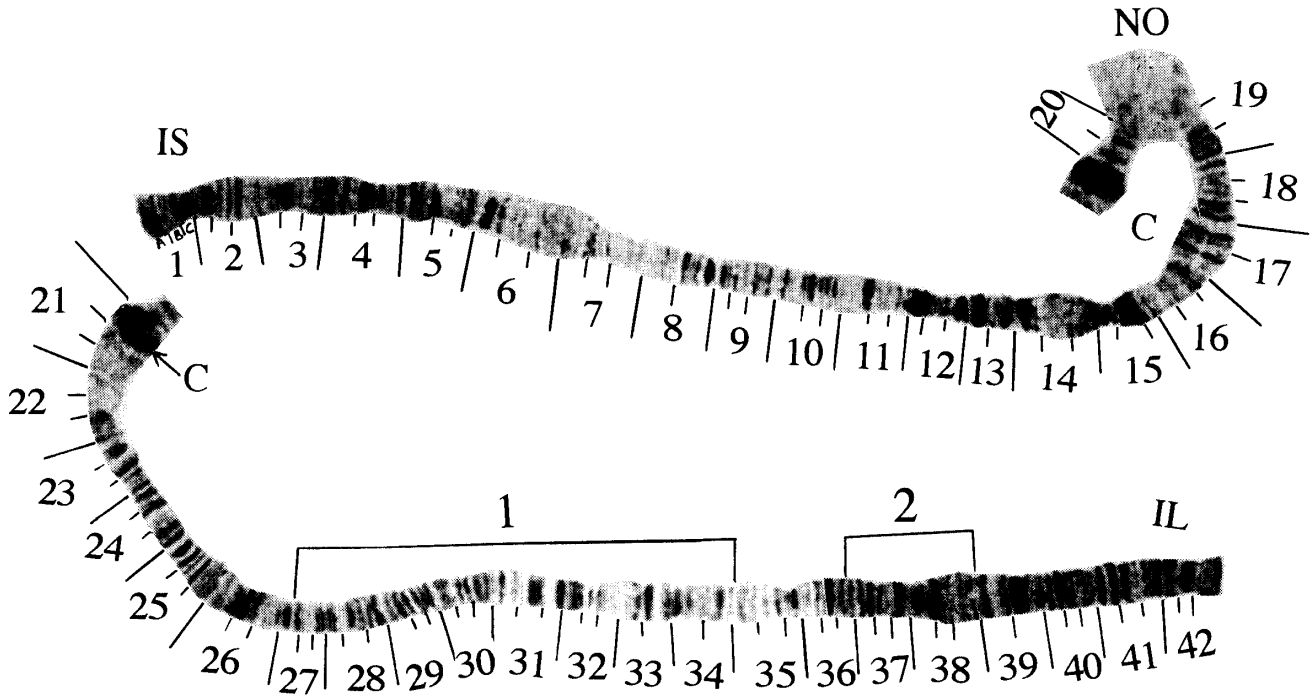

Fig. 1. Map of polytene chromosome I of male *Simulium* (*Gomphostilbia*) *sundaicum*. S, short arm; L, long arm; C, centromere; NO, nucleolar organizer; 1, inversion IL-1; 2, inversion IL-2. Bracket shows location of inversion.

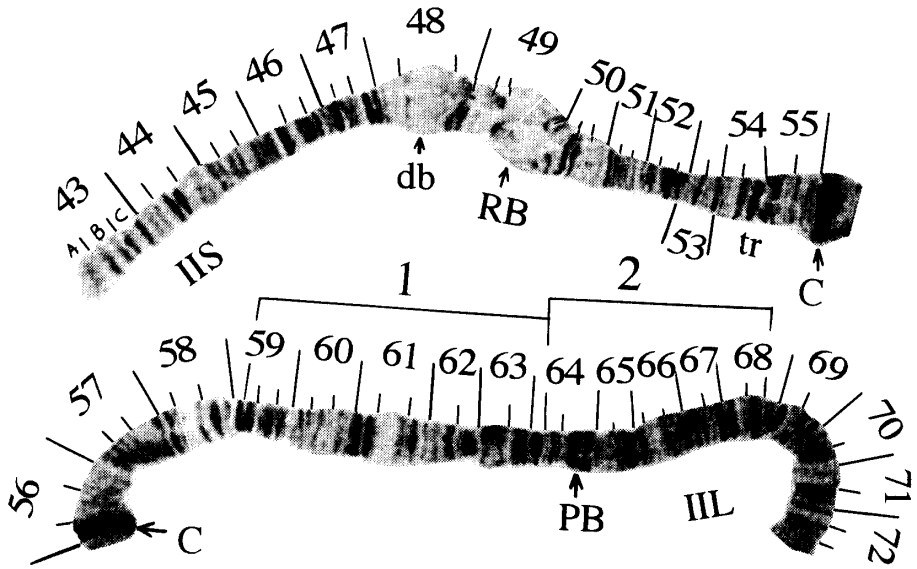

Fig. 2. Map of polytene chromosome II of male *Simulium* (*Gomphostilbia*) *sundaicum*. S, short arm; L, long arm; C, centromere; db, double bubble; RB, Ring of Balbiani; tr, trapezoidal group; PB, Parabalbiani ring; 1, inversion IIL-1; 2, inversion IIL-2. Bracket shows location of inversion.

terized by the presence of the blister (B) in 77B, and the conspicuous 'capsule (Ca)' (*i.e.*, saw-toothed puffing entity) in 80BC. The short arm was flared to some extent at the distal end. The long arm had three heavy bands in 99.

Inversions. In the long arm of chromosome I, two paracentric inversions, IL-1 (27A–34/35) and IL-2 (36C–38/39), were found (Figs. 1 and 4a–c). They were observed as heterozygotes in 13 larvae (59%) and in 10 (45%), respectively. In the long arm of chromosome II, continuous tandem inversions, *i.e.*, IIL-1 (59A–64A/B) and IIL-

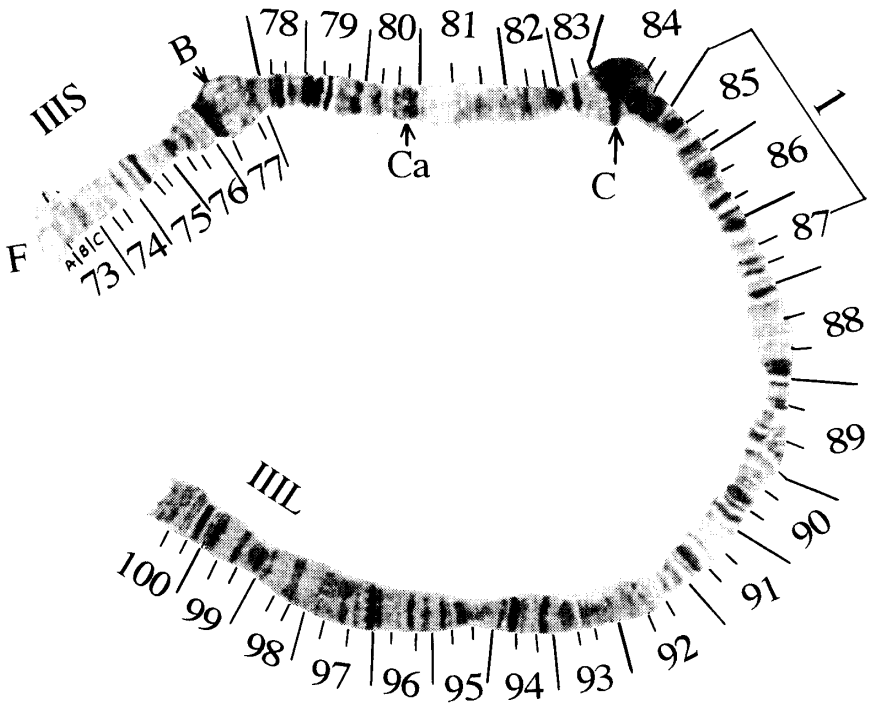

Fig. 3. Map of polytene chromosome III of male *Simulium* (*Gomphostilbia*) *sundaicum*. S, short arm; L, long arm; C, centromere; F, frazzle; B, blister; Ca, capsule; 1, inversion IIIL-1. Bracket shows location of inversion.

Table 1. Relative length of polytene chromosome arms (%) against total complement length in *Simulium* (*Gomphostilbia*) *sundaicum*.

Chromosome I		Chromosome II		Chromosome III	
S*[1]	L*[1]	S	L	S	L
19.8±1.5*[2]	22.0±0.7	12.5±1.3	17.2±1.5	10.0±0.8	18.3±1.1
(20)*[3]	(22)	(13)	(17)	(10)	(18)
1.1 (M)*[4]		1.3 (M)		1.8 (SM)	

*[1] S and L indicate short and long arms, respectively.
*[2] Mean±SD obtained from 10 nuclei.
*[3] Number of banding sections to be assigned per arm.
*[4] Arm length ratio: L/S. M, metacentric; SM, submetacentric.

2 (64A/B–68B/C) (Figs. 2 and 4d), were observed to be heterozygous in 8 (36%) of the 22 larvae examined. These inversions were associated with IL-1 and/or IL-2 in 6 larvae (27% of the total larvae examined). In chromosome III, one inversion, IIIL-1 (84/85–87A/B, Fig. 3) was found to be heterozygous in 1 (5%) of the 22 larvae examined.

C-banding. The C-banding technique utilized showed that band 84A in the centromere of chromosome III was sex-linked. Band 84A was always observed to be heterozygous with wider or densely stained heterochromatin and slightly stained one in all 15 males. In contrast, all seven

Fig. 5. Conventional (a, c, e) and C-banding (b, d, f) stains of salivary gland chromosomes of *Simulium* (*Gomphostilbia*) *sundaicum*. a and b, centromeres of three chromosomes; c–f centromeres of chromosome III (c and d, female; e and f, male). 1, chromosome I; 2, chromosome II; 3, chromosome III. Arrow and arrow head show heavily and slightly stained bands by C-banding, respectively.

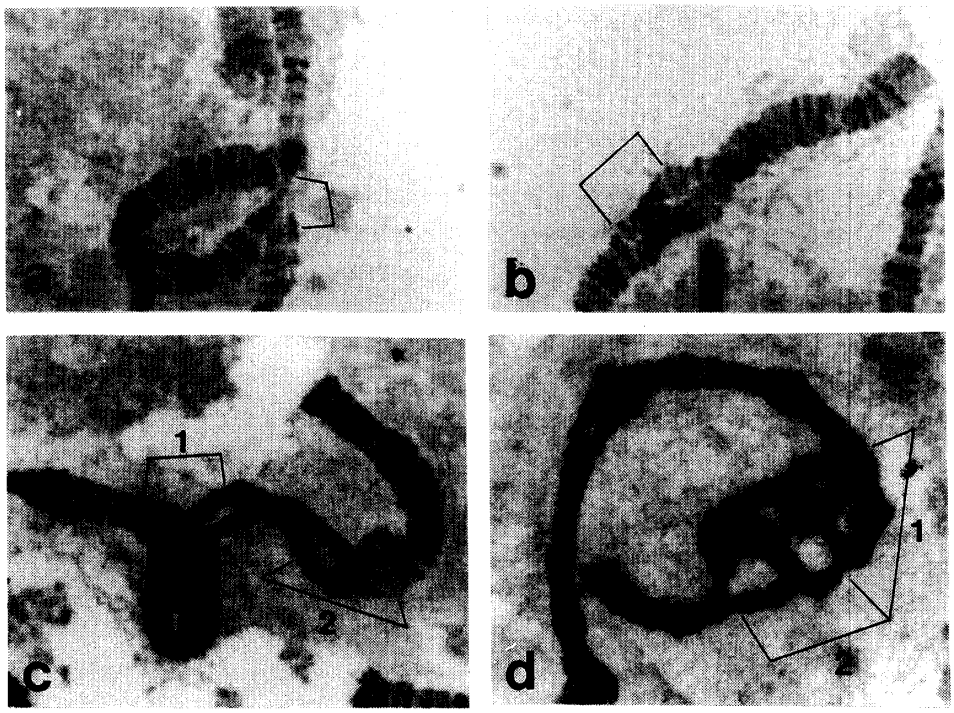

Fig. 4. Four polymorphic inversions found in *Simulium* (*Gomphostilbia*) *sundaicum*. a, inversion IL-1 (27A–34/35); b, IL-2 (36C–38/39); c, inversions IL-1+IL-2; d, inversion IIL-1 (59A–64A/B) and IIL-2 (64A/B–68B/C).

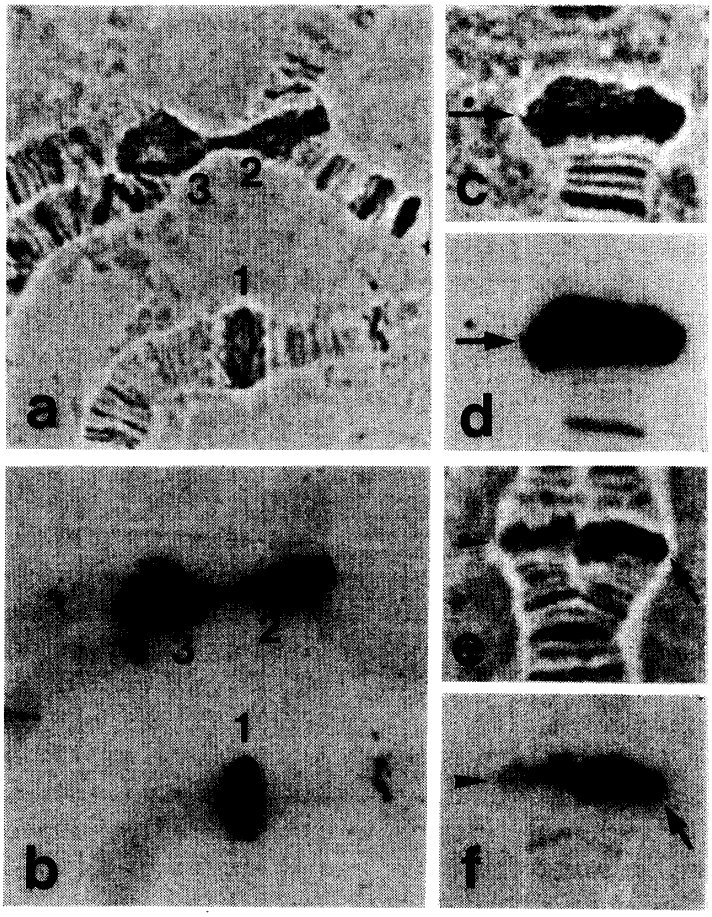

Fig. 5.

females had homozygous dense heterochromatin (Figs. 5c–f).

Discussion

It was revealed in this study that *S.* (*G.*) *sundaicum* had three polytene chromosomes, each with two intimately paired homologues, as reported in most investigated *Simulium* species (Dunbar, 1959; Rothfels, 1979).

The centromeric regions of all chromosomes of this species were expanded and recognized by the heavy heterochromatic band. This was almost similar to those of the subgenus *Simulium* s. str., including species such as *S.* (*S.*) *bidentatum*, *S.* (*S.*) *arakawae*, and *S.* (*S.*) *aokii* (Hadi *et al.*, 1996). However, this was different from *S.* (*G.*) *yaeyamaense*, one of the six Japanese *Gomphostilbia* species, in which centromeric regions were not expanded (Hadi *et al.*, 1995).

A number of chromosomal landmarks, *i.e.*, the NO, RB, db, PB, tr, B, Ca, observed in *S.* (*G.*) *sundaicum* were common in taxa throughout the Simuliidae (Rothfels, 1979). The NO was located in the short arm of chromosome I near the centromere, as seen in various subgenera, examples of species being as follows: *S.* (*G.*) *yaeyamaense* (Hadi *et al.*, 1995), *S.* (*Nevermannia*) *konoi* and *S.* (*S.*) *quinquestriatum* from Japan, *S.* (*S.*) *eximium* and *S.* (*S.*) *argyrocinctum* from Java, Indonesia (Hadi *et al.*, unpublished data). The similar arrangement of the RB and db (the RB being closer to the centromere than db) was found also in *S.* (*G.*) *yaeyamaense*, *S.* (*S.*) *arakawae* and *S.* (*S.*) *eximium* (Hadi *et al.*, 1995, 1996; Hadi *et al.*, unpublished data). These landmarks do not seem to be specific to the subgenus *Gomphostilbia*.

In this study, five inversions: IL-1, IL-2, IIL-1, IIL-2 and IIIL-1 were frequently found except the last inversion. IIL-1 and IIL-2 were continuous tandem inversions. These inversions were observed individually or associated with some other inversions. None of these inversions was sex-related. More individuals are needed to see an exact population structure of the inversions.

The centromere of chromosome III (band 84A) of *S.* (*G.*) *sundaicum* appeared to be sex-linked. Because constitutive heterochromatin (C-band) of this element was heterozygous in males and homozygous in females. Heavily stained C-band of 84A might correspond to X and slightly stained one, to Y. A sex-linked, differential band in the centromere was not observed in *S.* (*G.*) *yaeyamaense* (Hadi *et al.*, 1995), but had been reported in seven species of *Prosimulium* in the *mixtum*-group (Rothfels and Freeman, 1977) and *Eusimulium* (*Hellichiella*) *latipes* (Rothfels and Golini, 1983).

Other instances in which heterobands (not on the centromere) are involved in differentiation of genetic X-chromosomal and Y-chromosomal segments in blackflies have also been documented: Two bands, 17B (representing the NO) and 24C in *Cnephia dacotensis* (Procunier, 1975); NO^+/NO^- in *E.* (*H.*) *saccai* (Rothfels and Golini, 1983) and *S. metallicum* (Conn *et al.*, 1989); 99 in *P. mixtum* (Rothfels and Freeman, 1977); and 37B1 in *S. ochraceum* (Hirai *et al.*, 1994).

Bedo (1975) showed using quinacrine fluorescence that the differentiation of X and Y in the heteroband of *S. pictipes* involved differences in composition as well as in quantity of DNA. The differentiation of X and Y found here was manifested by the difference in quantity of heterochromatin.

In sex-determination, *S.* (*G.*) *sundaicum* showed male heterogamy, as evidenced in the centromeric heteroband. This was contrary to our previous observation on *S.* (*G.*) *yaeyamaense*, of the same subgenus, in which female heterogamy in the inversion, IIIL-1, was found. Our result thus suggests that the sex determination of species in the subgenus *Gomphostilbia* is not simple in terms of the structural difference of the larval polytene chromosomes.

Acknowledgements

We thank Dr. Singgih H. Sigit, Professor of Department Parasitology, Faculty of Veterinary Medicine, Bogor Agricultural University, for his generosity in providing laboratory space and facilities and arranging collecting trips.

Our appreciation also goes to Miss C. Aoki, Division of Medical Zoology, Oita Medical University, for her help in various ways.

This work was supported in part by the Ministry of Education, Science and Culture, Japan, for International Scientific Research Program (No. 03041065; representative Prof. I. Miyagi), and by Indonesian Institute of Science (LIPI) of Indonesian Government.

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摘　要

インドネシア国ジャワ島産ブユ，*Simulium* (*Gomphostilbia*) *sundaicum* 幼虫の唾液腺染色体の観察

東洋区産ブユについての染色体の研究はほとんどない．今回，インドネシア国ジャワ島産ブユ *Simulium* (*Gomphostilbia*) *sundaicum* 幼虫の唾液腺染色体を観察し，基本となる染色体マップを作製した．本種の唾液腺染色体は相同染色体が対合した3本 ($2n=6$) からなり，ヘテロクロマチンに富む明瞭な動原体部を有していた．主な特徴のうち，仁形成部は第一染色体に，また，バルビアニ環および副バルビアニ環は第二染色体に位置していた．逆位は5種観察されたが，いずれも性に連鎖していなかった．性連鎖に関係したバンディングを見るため，C-banding 法を試みたところ，第三染色体の動原体に見られる 84A バンドが，雄では濃淡ヘテロ，雌では濃色ホモで見られたため，濃淡バンドはそれぞれ，X, Y 染色体性をもつと考えられた．