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Larval salivary gland chromosomes of the blackfly, *Simulium* (*Gomphostilbia*) *yaeyamaense* (Diptera: Simuliidae) from Ryukyu Islands, Japan

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Abstract: Larval salivary gland chromosomes of the blackfly, *Simulium* (*Gomphostilbia*) *yaeyamaense* Takaoka, 1991, from the Yaeyama Island group of the Ryukyu archipelago, were observed and mapped. This species had three pairs of polytene chromosomes. The longest pair, chromosome I, is metacentric and the two shorter pairs, chromosomes II and III, in descending order of length, are submetacentric. The centromeric region of all chromosomes was not expanded and barely recognized by the existence of a heavy band. The nucleolar organizer was situated in section 18 of chromosome I. The Balbiani ring, double bubble and the Parabalbiani ring were situated in sections 47, 46 and 61 of chromosome II, respectively. From three populations examined, one inversion (IIIL-1) was always heterozygous in females, and four other inversions (IIIL-2, IIIL-3, IIIL-4 and IIIL-5), were polymorphic and found as heterozygote in both sexes. This study is the first to map the larval salivary gland chromosomes of the subgenus *Gomphostilbia,* and to record the heterozygous inversion related to the female sex among the Oriental Simuliidae.

INTRODUCTION

Cytotaxonomic studies were extensively carried out on various genera of Simuliidae (Rothfels, 1979). However, the simuliid species of the Oriental Region had so far received little attention. This study was aimed to investigate cytotaxonomically blackflies belonging to the subgenus *Gomphostilbia* Enderlein of the genus *Simulium* s.l. which is mostly distributed in southeast Asia. In this paper we construct a photographic map of the polytene chromosomes of *Simulium* (*Gomphostilbia*) *yaeyamaense* Takaoka, which is distributed on Ishigaki and Iriomote Islands in the Yaeyama Island group of the Ryukyu archipelago (Takaoka, 1991). This species was formerly known as *S.* (*G.*) *batoense* Edwards, 1934, originally described from Java, Indonesia.

MATERIALS AND METHODS

The larvae used were collected from two localities in Ishigaki Island on May 11, 1988 and from one locality in Iriomote Island on May 13, 1988.

The larvae collected were fixed in freshly-prepared acetic alcohol (glacial acetic acid :

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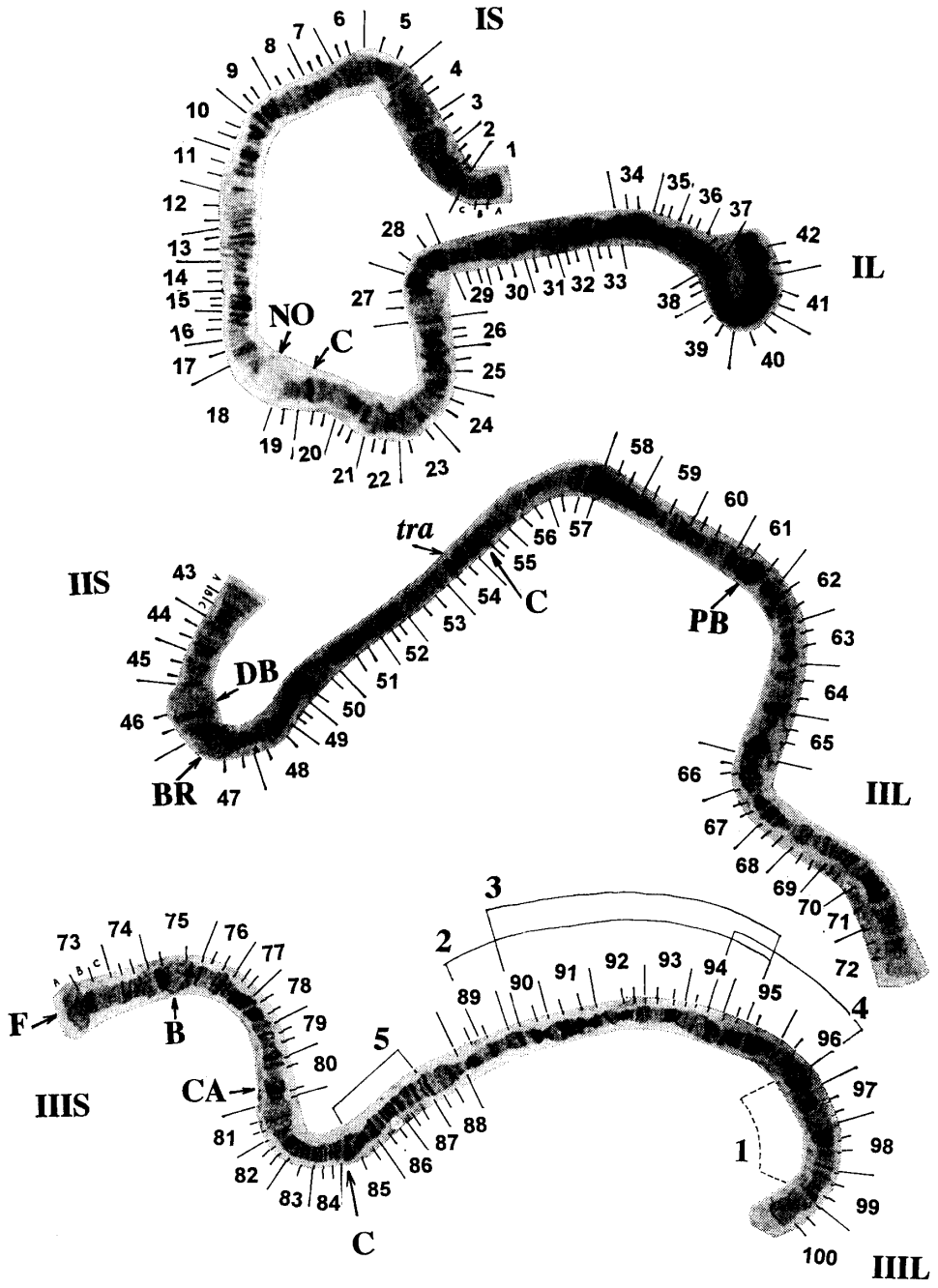

Fig. 1 The standard map of larval salivary gland chromosomes of *Simulium* (*Gomphostilbia*) *yaeyamaense*.

I, II, III, Chromosomes I, II, and III; S, short arm; L, long arm; NO, nucleolar organizer; C, centromere; BR, Ring of Balbiani; DB, double bubble; *tra*, trapezoidal group; PB, Parabalbiani ring; F, flared end; B, blister; CA, capsule. Bracket to the right of chromosome indicates polymorphic inversion; bracket to the left (dotted) indicates sex-linked inversion; 1–5, inversion numbers.

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 condition. Larvae were dissected and left for 2–3 min in 45% acetocarmine (1% carmine, Merck), and the salivary glands were transferred into a medium consisting of 4 parts water, 4 parts acetic

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

Chromosome I		Chromosome II		Chromosome III	
S*	L*	S	L	S	L
18.7±1.4** (19)	23.4±1.2 (23)	12.4±0.6 (12)	17.8±1.2 (18)	11.7±0.5 (12)	15.9±1.4 (16)

* S and L indicate short and long arms, respectively. ** The size of arms is given by the mean value of 10 nuclei ± SD, and banding sections assigned per arm are shown in parentheses.

acid and 1 part lactic acid on a glass slide. After being squashed under a cover slip by pressing, the salivary gland chromosomes 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.

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

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

Results

General chromosome morphology

As expected from the mitotic arrangement, in the larval salivary gland nuclei of *S. (G.) yaeyamaense,* there were three pairs of homologous polytene chromosomes (Fig. 1), each of which was tightly paired. Table 1 summarises relative sizes of the polytene chromosome arms using 10 well spread chromosome complements. The entire chromosome complement was divided into 100 approximately equal sections, beginning with the distal end of the short arm of chromosome I to the distal end of the long arm of chromosome III. For the purpose of mapping, the centromeres were considered to be within the long arm of each chromosome.

Chromosome I

Chromosome I was the longest of the complements. Sections 1–19 and 21–42 were assigned to the short and long arms, respectively (Fig. 1). The centromeric regions did not show any expansion but characteristically had a heavily stained band in section 20. The short arm was characterized by the three heavy bands in section 15 and the nucleolar organizer in section 18. The long arm had a prominent dark broad band in section 28C and a group of three heavy bands in sections 39C–40B. No inversion was found.

Chromosome II

Chromosome II was divided into 30 sections, 43–72 (Fig. 1). The centromere was morphologically similar to that of chromosome I and situated in section 55. Chromosome II was recognized by the Ring of Balbiani (BR), "double bubble" (DB) and the heavy "trapezoidal" group (*tra*) in the short arm, and the Parabalbiani ring (PB) in the long arm. The BR and DB were in sections 47 and 46, respectively, while the *tra* and PB in sections 54 and 61, respectively. No inversion was found.

Chromosome III

Chromosome III, the shortest, was divided into 28 sections, 73–100 (Fig. 1). The centromere was also morphologically similar to those of chromosomes I and II and located in section 85. This chromosome was characterized by the presence of a blister (B) in section 75, and a conspicuous "capsule (CA)" (*i.e.,* saw-toothed puffing entity) in section 80. The short arm was flared to some extent at the distal end. The long arm had three heavy bands in section 98. In the long

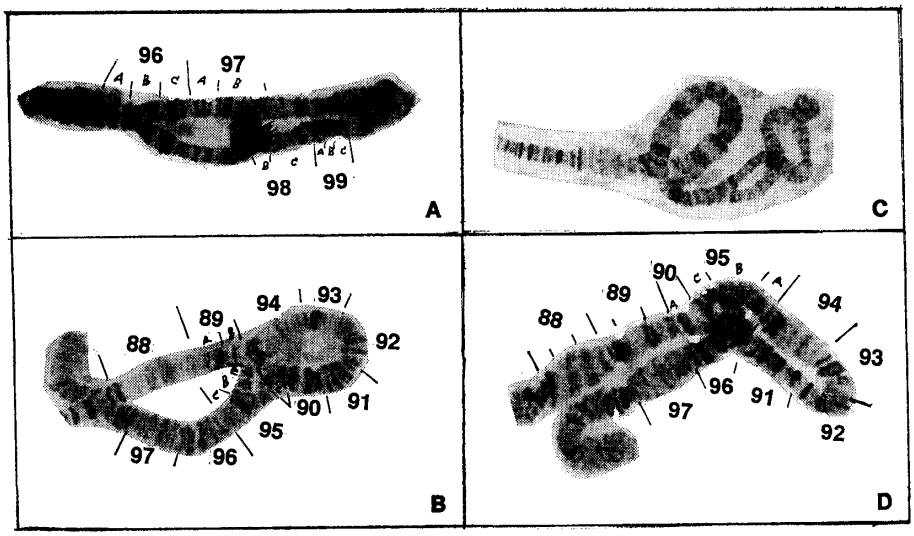

Fig. 2 The pattern of heterozygous inversions of *S.* (*Gomphostilbia*) *yaeyamaense*. A, IIIL-1; B, IIIL-2; C, IIIL-1 and IIIL-2; D, IIIL-3.

Table 2 Frequencies of sex-linked and polymorphic inversion heterozygotes found in *Simulium* (*Gomphostilbia*) *yaeyamaense*.

Locality*	Sex**	No. larvae tested	Sex-linked inversion (IIIL-1)	Polymorphic inversions			
				IIIL-2	IIIL-3	IIIL-4	IIIL-5
1	F	14	1.00	0.36	0.20	0.07	0.00
	M	7	0.00	0.57	0.43	0.00	0.00
2	F	13	1.00	0.23	0.38	0.00	0.00
	M	10	0.00	0.20	0.20	0.00	0.10
3	F	1	1.00	0.00	1.00	0.00	0.00
	M	1	0.00	1.00	0.00	0.00	0.00

* 1 and 2, Hirakubo and Itona (Ishigaki Island); 3, Tsukigahama (Iriomote Island). ** F, female; M, male.

arm, major rearrangements of the banding pattern have occurred (Fig. 2 and Table 2). Distally, a simple inversion IIIL-1 (96B/C–99/100) (Fig. 2A) was recognized to be heterozygous in all females examined from both Ishigaki and Iriomote Islands. On the other hand, all males showed a standard sequence.

Four other different inversions were found as heterozygote in both sexes (Figs. 1 and 2). Table 2 shows the frequencies of sex-linked and polymorphic inversion heterozygotes. IIIL-2 (89A/B–95A/B) (Fig. 2B) and IIIL-3 (90A/B–95/96) (Fig. 2D) were found in all three localities. IIIL-2 and IIIL-3 were independently associated with IIIL-1 in 29% (Fig. 2C) and 32% of female larvae examined, respectively. However, IIIL-4 (94B/C–96B/C) and IIIL-5 (85A/B–87A/B) were each found only in one larva. So far, no inversion homozygote has been detected.

Discussion

S. (*G.*) *yaeyamaense* had three pairs of polytene chromosomes, as reported in most investigated *Simulium* species (Dunbar, 1959; Rothfels, 1979). In Simuliidae, centromeric regions usually form a characteristic expanded region (Rothfels, 1979). The cen-

tromeric region of all chromosomes of this species was not expanded and barely recognized by the existence of a heavy band. This is different from those of the subgenus *Simulium* s. str., *e.g., S.* (*Simulium*) *bidentatum, S.* (*S.*) *arakawae,* and *S.* (*S.*) *aokii,* in which centromeric regions were distinctly expanded and easily recognized (Hadi *et al.,* unpublished data).

Several chromosomal landmarks, *e.g.,* NO, BR, DB, PB, *tra,* B and CA, observed in *S.* (*G.*) *yaeyamaense* are common in taxa throughout the Simuliidae (Rothfels, 1979). The NO appeared in the short arm of chromosome I and was located near the centromere as seen in several species of various subgenera *e.g., S.* (*Nevermannia*) *konoi* and *S.* (*S.*) *quinquestriatum* from Japan, and also *S.* (*G.*) *sundaicum, S.* (*S.*) *eximium,* and *S.* (*S.*) *argyrocinctum* from Java, Indonesia (Hadi *et al.,* unpublished data). In the latter five species the location of *tra,* PB, B and CA was also almost the same as in *S.* (*G.*) *yaeyamaense.* In *S.* (*N.*) *konoi, S.* (*S.*) *eximium* and *S.* (*G.*) *sundaicum* both BR and DB similarly occurred in the short arm of chromosome II. It appears unlikely that any of these landmarks is specific to the subgenus *Gomphostilbia.*

From the three Yaeyama Islands populations examined, no polymorphism was found in chromosomes I and II. However, five inversions were found in the long arm of chromosome III (IIIL-1, IIIL-2, IIIL-3, IIIL-4 and IIIL-5). IIIL-1 existed as a heterozygote in all females tested but not in males. This is the first record of the heterozygous inversion related to the female sex among the Oriental Simuliidae.

Most species of Simuliidae are male heterogametic (Rothfels and Nambiar, 1981). However, the two species, *i.e., S.* (*Inseliellum*) *tahitiense* and *S.* (*I.*) *oviceps* from Tahiti, were reported to be female heterogametic (Rothfels, 1979). In both species three inversions are sex related, which are located in the long arm of chromosome II in *S.* (*I.*) *tahitiense* and in the long arm of chromosome III in *S.* (*I.*) *oviceps.* It would be interesting to clarify whether female heterogamy is restricted to *S.* (*G.*) *yaeyamaense* or found also in other species of the subgenus *Gomphostilbia.*

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摘　要

琉球列島産ヤエヤマナンヨウブユ幼虫の唾液腺染色体の観察

東洋区産ブユ，とくにナンヨウブユ亜属についての染色体の研究はほとんどない．今回，琉球列島の石垣島および西表島に分布するヤエヤマナンヨウブユの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成した．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，不明瞭な動原体部を有していた．おもな特徴のうち，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．第三染色体長腕に5種類の逆位が見いだされ，そのうちの一つは調べた雌幼虫のすべての個体にヘテロの状態で固定しており，雄個体には見られなかった．