Larval polytene chromosomes of three Japanese blackfly species (Diptera: Simuliidae)

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Abstract: Standard maps of larval salivary gland polytene chromosomes of three Japanese blackfly species: *i.e.*, *Simulium bidentatum* (Shiraki, 1935), *S. aokii* (Takahasi, 1941) and *S. arakawae* Matsumura, 1915, were presented. All the three species had three paired chromosomes ($2n=6$) and showed other main characteristics in common, such as a Balbiani ring, a double bubble, a parabalbani ring and a nucleolar organizer on the same arm of the same chromosome. However, their precise locations were different among the species. All species had prominent centromeres, the banding patterns of which were different among them. In *S. bidentatum* and *S. aokii* polymorphic inversions were found in low frequencies, and B-chromosomes were also detected. Any sex chromosomes or sex-linked segments could not be detected.

INTRODUCTION

Morphotaxonomic and cytotaxonomic data of blackflies have been accumulated (Rothfels, 1979), but they alone cannot fully describe the true biological nature. Analysis of larval salivary gland polytene chromosomes has often revealed that an isomorphic species is composed of biologically distinct sibling species (*e.g.*, Rothfels, 1956; Dunbar, 1966; and Hirai *et al.*, 1994).

Knowledge of cytotaxonomy of Japanese blackflies is very scarce. Only a few reports have been presented previously (Okada, 1972, 1974; Hirai *et al.*, 1984; Hadi *et al.*, 1995). This paper describes the larval polytene chromosomes of *Simulium bidentatum* (Shiraki, 1935), *S. aokii* (Takahasi, 1941), and *S. arakawae* Matsumura, 1915, all of which are well-known pest to men and domestic animals in Japan

(Ogata, 1955; Takaoka, 1977), and vectors of bovine onchocerciasis in some localities (Takaoka *et al.*, 1992; Takaoka, 1994).

MATERIALS AND METHODS

The larvae of *S. bidentatum*, *S. aokii* and *S. arakawae* were collected from streams and rivers at Hasama, Notsuharu, Taketa, Yabakei, Yufuin (all in Oita Prefecture), and Kikuchi (Kumamoto Prefecture). The number of larvae observed for each species is presented in Table 1. The larvae were removed from the vegetation to which they attached, using forceps and then placed in vials which were filled with freshly-prepared acetic alcohol (glacial acetic acid:ethanol=1:2). Fixed larvae were stored in a freezer (-20°C) until required for slide preparation. Larval identification was carried out according to Takaoka (1977). Chromosome preparation and sexing of larvae were done as described in the previous paper (Hadi *et al.*, 1995).

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Table 1. Samples of larvae of three Japanese blackfly species analyzed cytologically.

Location	Date	No. larvae examined*					
		<i>S. bidentatum</i>		<i>S. aokii</i>		<i>S. arakawae</i>	
		Female	Male	Female	Male	Female	Male
Hasama (Oita)	20 IV 92	23	27	5	15	49	18
Notsuharu (Oita)	27 IV 92	41	21	0	1	0	0
Yabakei (Oita)	12 IV 94	10	5	6	1	0	0
Yufuin (Oita)	12 IV 94	0	0	5	4	0	0
Taketa (Oita)	07 IV 92	15	18	0	0	0	0
Kikuchi (Kumamoto)	08 X 92	13	14	0	0	10	9
Total		102	85	16	21	59	27

* The number of larvae of which chromosome preparation was good enough for analysis.

Mapping of the polytene chromosomes followed the conventional method of Bedo (1977) and Rothfels *et al.* (1978). Measurements of polytene chromosomes were made from photographs of 10 cells spread. The percentage of each chromosome arm to the total complement length (TCL) was used to determine the approximate number of sections assigned to each arm in the standard map. The polytene chromosomes of the three species examined from the Yufugawa river were used for the standard map.

RESULTS

General chromosome morphology. All the three species observed in this study had three pairs of chromosomes ($2n=6$). The longest pair was chromosome I and the shorter pairs were chromosomes II and III in descending order of length. The long arms of the latter two chromosomes were equal in length but the short arms were different, the arm of chromosome II being slightly longer. The relative lengths of three chromosomes and also of their arms were the same in all the three species, then the same number of sections were assigned (Table 2). The centromeres of these three species each formed a characteristic expanded region. Pairing of the homologues was relatively loose in *S. arakawae*, but very tight in the other two species.

Supernumerary chromosomal fragments

(B-chromosome) were detected in four of five populations of *S. bidentatum* and in one of four populations of *S. aokii* examined (Fig. 9a–d). The frequency of the larvae having B-chromosome(s) in the positive populations was 10–22% for *S. bidentatum*, and 15% for *S. aokii*.

S. bidentatum. The standard chromosome maps of *S. bidentatum* are presented in Figs. 1–3. The centromeric regions had an intense dark band in the middle. The short arm of chromosome I (IS) was characterized by a group of two heavy bands in section 4BC, and three heavy bands in 16. The long arm of chromosome I (IL) had a region of fine bands in 20B–21, shield-like pattern in 26B and 34A, and a group of fine light bands in 38B (the 'neck'). The tip of this arm was characterized by some fine bands. The IIS bore the ring of Balbiani (RB) in 43A, the double bubble (db) in 44, and the basal trapezoidal group (tr) in 53. The long arm was distinguished by the Parabalbani ring (PB) in 69C. The IIIS had the puffed alveolar region (blister, B) accompanied distally by two distinct light bands in 75BC, and the conspicuous 'capsular' saw-toothed puffing entity (Ca) in 78C. The IIIS was flared at the tip. In the IIIL were located the nucleolar organizer region (NO) (in 90) and three heavy band groups (in 99).

In all the six populations examined, one heterozygous inversion, IIIL-1 (85B–85/86) (Figs. 3 and 9e), was commonly found in

Table 2. Percentage of chromosome arms to the total complement length (% TCL) from 10 nuclei of larval salivary gland cells of three Japanese blackfly species.

	<i>S. bidentatum</i>	<i>S. aokii</i>	<i>S. arakawae</i>
IS	18.7±1.3 (19)*	18.9±1.6 (19)	18.6±1.6 (19)
IL	22.0±1.0 (22)	22.1±1.2 (22)	21.9±1.1 (22)
R**	1.2 (M)	1.2 (M)	1.2 (M)
IIS	12.8±1.3 (13)	12.9±1.6 (13)	12.9±1.7 (13)
IIL	18.3±1.0 (18)	17.8±1.7 (18)	18.0±1.3 (18)
R	1.4 (M)	1.4 (M)	1.4 (M)
IIIS	10.4±1.1 (10)	10.3±0.6 (10)	9.8±1.2 (10)
IIIL	17.8±1.5 (18)	18.3±1.7 (18)	18.6±1.0 (18)
R	1.7 (SM)	1.8 (SM)	1.9 (SM)

* Parentheses indicate the number of sections assigned to the arms in the standard chromosome map.

** R: arm ratio (long arm/short arm), M, metacentric; SM, submetacentric (identification followed Levan *et al.*, 1964).

Fig. 1. Larval salivary gland chromosome I of female *Simulium bidentatum*. IS, short arm; IL, long arm; C, centromere. Numbers 1–41 indicate sections; A, B and C indicate subsections.

Fig. 2. Larval salivary gland chromosome II of female *Simulium bidentatum*. IIS, short arm; IIL, long arm; C, centromere; RB, ring of Balbiani; db, double bubble; tr, trapezoidal group; PB, parabalbani ring. Serial numbers and alphabets are as Fig. 1.

Fig. 3. Larval salivary gland chromosome III of female *Simulium bidentatum*. IIS, short arm; IIL, long arm; C, centromere; F, flared end; B, blister; Ca, capsule; NO, nucleolar organizer region. Serial numbers and alphabets are as Fig. 1. 1 and 2 show range of inversions.

Fig. 4. Larval salivary gland chromosome I of male *Simulium aokii*. Symbols are as in Fig. 1. 1 shows range of inversion.

both sexes. The frequencies were generally low (7–18%), except for the Hasama population which showed a relatively high frequency (37%). There was another heterozygous inversion, IIL-2 (85/86–86/87) (Figs. 3 and 9e) which, however, occurred in two female larvae.

S. aokii. The standard chromosome maps of this species are shown in Figs. 4–6. The centromeric regions of this species

were expanded and composed of sponge-like, dotted bands. The IS was characterized by three heavy bands in 16. The IL had a region of fine bands in 20B–22A, shield-like pattern in 35A, a group of two heavy bands in 36AB and 39–40, and a group of fine light bands in 38B. The IIS was flared at the tip. The RB and db were located in 44B and 46 of IIS, respectively and the tr and PB in 53 and 64B of IIL,

Fig. 5. Larval salivary gland chromosome II of male *Simulium aokii*. Symbols are as in Figs. 2-3.

Fig. 6. Larval salivary gland chromosome III of male *Simulium aokii*. Symbols are as in Fig. 3.

respectively. The locations of B and Ca were in 75BC and 79A, respectively. The IIIL had the NO in 85 and three heavy band groups in 99.

In *S. aokii*, five different inversions (Fig. 9f-i) were found: four in males from the Yufugawa population, *i.e.*, IS-1 (8/9-9/10), IL-1 (24C-25B/C), IIS-1 (44C-47B/C) and IIL-1 (68/69-70/71), and one in a male from Notsuharu, IIIL-1 (89A-91A/B). Three inversions (IS-1, IL-1 and IIL-1) were also

found in the Yufuin population. All these heterozygous inversions occurred at low rate (15% or less).

S. arakawae. The standard chromosome maps of *S. arakawae* are given in Figs. 7 and 8. The centromeres of all chromosomes were expanded with three distinctive bands. The IS was characterized by a group of two heavy bands in 4, and three heavy bands in 16. The IL had a shield-like pattern each in 26 and 33, a

Fig. 7. Larval salivary gland chromosome I of female *Simulium arakawae*. Symbols are as in Fig. 1.

Fig. 8. Larval salivary gland chromosomes II and III of female *Simulium arakawae*. Symbols are as in Figs. 2-3.

group of fine light bands in 38B, a group of two heavy bands in 39-40, and fine bands in the distal tip (41C). On the IIS which was characterized by the flared basal tip, the db and BR were found in 43 and 44B, respectively and the tr in 53; on the IIL, the PB was in 67C. On the IIIS, the B and Ca were found in 75 and 79A, respectively. The NO was located at the proximal region of the IIII (section 87). Three

heavy band groups were found in 99.

No inversion has been found yet, although pairing of chromosomes was very loose.

DISCUSSION

The three Japanese blackfly species of the subgenus *Simulium* Latreille s. str. here studied were each assigned in the

Fig. 9. B-chromosomes and inversions. a and b, B-chromosomes of female *Simulium bidentatum*; c and d, B-chromosomes of male *Simulium aokii*; e, inversions in *S. bidentatum* (IIIL-1 and IIIL-2); f-i, inversions in *S. aokii*, f, IS-1; g, IIL-1; h, IIS-1; i, IIIL-1.

different species-groups: i.e., *S. bidentatum* to the *malyschevi*-group, *S. aokii* to the *variegatum*-group and *S. arakawae* to the *venustum*-group (Crosskey, 1988).

Our results showed that in all three species, there were three pairs of chromosomes ($2n=6$), as reported in all other investigated species of the subgenus *Simulium* s. str. In all three species, certain chromosomal landmarks, such as the RB, db, PB, and NO were located on the same chromosome arm although their precise locations were different among species. The centromeric regions were prominently expanded but differed in the banding pattern or heterochromatin density by species. The difference of locations of certain landmarks, as well as that of centromeric banding patterns, might be species-group specific. However, more specimens of other related species from various localities are needed to confirm this.

In some populations of *S. bidentatum* and *S. aokii*, B-chromosomes occurred as small metacentrics heavily loaded with heterochromatin and had a nucleolar or-

ganizer (Fig. 9a-d), as reported in *Cnephia dacotensis* and *C. ornithophilia* (Procunier, 1975, 1982). B-chromosomes have been also reported in several other blackfly species such as, *S. (Odagmia) ornatum* (= *S. (O.) iwatense*) (Okada, 1972), *S. venustum* (EFG/C) (Rothfels *et al.*, 1978), *S. (Nevermannia) morisonoi* (Hirai *et al.*, 1984), *S. ochraceum* (Hirai *et al.*, 1994) and *S. (N.) konoii* (Hadi *et al.*, unpublished data). The role and effect of B-chromosomes in Simuliidae remain to be studied.

Rothfels (1956) considered that fixed chromosomal rearrangements, sex chromosome differences and the demonstration of a unique array of chromosomal polymorphisms together, indicate existence of reproductively isolated populations. Although polymorphic inversions were found in *S. bidentatum* and *S. aokii*, there were neither fixed chromosomal rearrangements nor sex chromosome differences among these populations examined, so providing no evidence of sibling speciation. Similarly, examination of *S. arakawae* has revealed no cytotype differences yet. Future studies may disclose

whether these species are composed of more than one sibling species, the presence of which is strongly suggested by their wide distribution in Japan and successful adaptation to various types of habitats.

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摘 要

日本産ブユ 3 種の幼虫唾液腺染色体

Simulium bidentatum (キアシツメトゲブユ) *S. aokii* (アオキツメトゲブユ) および *S. arakawae* (ヒメアシマダブユ) の幼虫の唾液腺染色体を観察し、標準染色体図を作成した。3 種とも染色体の基本数は $n=3$ で, Balbiani ring, double bubble, Parabalbani ring や仁形成部などの主な特徴は 3 種のブユで同一染色体の同じ腕に見られたが、正確な位置は種ごとに違いが認められた。中心体は 3 種とも顕著であったが、ヘテロクロマチンの成す縞模様は種別に異なっていた。逆位はキアシツメトゲブユとアオキツメトゲブユに低率で見いだされた。B 染色体もこの 2 種に見出された。性を決定する染色体の同定は出来なかった。