Polytene chromosomes of an Indian Himalayan black fly

*Simulium (Nevermannia) praelargum* (Diptera: Simuliidae)

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Abstract

High quality polytene chromosome maps \((n=3)\) of a Himalayan Simuliid *Simulium praelargum* Datta, 1973 are presented and represent the first cytological description of a taxon found in the feuerborni group, subgenus Nevermannia. Polytene chromosomes one (I) and two (II) are metacentric, chromosome three (III) is submetacentric with the length of each chromosome occupying 37.25%, 31.36% and 31.34% of the total complement length, respectively. Typical simuliid diagnostic intergeneric chromosomal markers are found within the polytene complement of this species. The nucleolar organizer (N.O.) is found at the base of the short arm of chromosome one (IS), the Ring of Balbiani (R.B.), double bubble (D.B.) and triad occur in the short arm of chromosome two (IIS), the Parabalbiani Ring (P.B.) and grey band (gb) occur in the long arm of chromosome two (IIL) and the Blister (BL) and Capsule (Ca) occur in the short arm of chromosome three (IIIS). Terminal bands at the end of IIIIs are heterochromatinized and present atypically with respect to other simuliid fauna. Populations studied so far are unique among the Simuliidae in that they exhibit chromosome structural monomorphism. These high resolution polytene chromosome maps will form the basis for future cytological characterization and phylogenetic comparisons amongst members of the feuerborni group [Current Zoology 56 (4): 437–444, 2010].

Key words

Black fly, Feuerborni group, *Simulium praelargum*, Polytene chromosome maps, Monomorphism

Black flies of the family Simuliidae represent a small group of insects in the order Diptera. Some of these taxa are established vectors of different parasitic diseases (Adler, 2005) including the new and old world tropical vector sibling complexes of human onchocerciasis (Procunier, 1989). Simuliid fauna of the Oriental region and the Indian sub-continent are less studied than fauna from other regions of the world (Adler and Crosskey, 2009) with cytotaxonomic studies on Indian Simuliids being rather scanty. Nevertheless, recent combined molecular and cytological approaches have been encouraging to help resolve the biosystematics of a number of groups within S.E. Asia (Tangkawanit et al., 2009; Kuvangkadirok et al., 2008) including relationships based on molecular data for some members of the feuerborni group (Thanwisi, et al., 2006; Otsuka, et al., 2001). In this regard, the promise of DNA barcoding (Rivera and Currie, 2009), proteomic (Cupp and Cupp 1997; Cupp, et al., 1998) and genomic (Procunier et al., 2005) approaches that target the functionally important salivary gland may also be instructive for understanding evolutionary biology and hierarchal classification of this family. Notwithstanding these integrated taxonomic approaches, cytotaxonomy still remains the gold standard for initially resolving species identity within closely related groups and can contribute significantly to resolving their genetic relationships through the establishment of stepwise paracentric sequential inversion phylogenies (Rothfels 1979, 1989) and in some cases can provide chromosomal biomarkers for supraspecific ranking (Procunier and Muro, 1994).

More than 15 species of black flies belonging to different sub-genera have been reported from the Darjeeling region of North Western Bengal, India (Datta, 1992). *Simulium praelargum*, the taxon of interest for this cytological study occurs in abundance from multiple sites in comparison to other species of *Simulium* including *S. (E).* gracilis, *S. (G).* tenuistylum, *S. (M.)* taktsangense, *S. (M.)* dattai, *S. (M.)* ghoomense, *S. (G.)* williei (unpublished), *S. (G.)* sachini (unpublished), *S. (N.)* sp. 2 (nr. fructicosum). The larval habitat of *S. praelargum* can be characterized by a water body/small
hill stream / trickles that are slow flowing, 2 – 3 inches deep with the breadth of the water body ranging from 10 inches to 3–5 feet. The larvae are found attached mainly to polythene materials, the rough surfaces of the vegetations such as Equisitum as well as small stones. Female flies of this species feed on man and certain other birds and mammals (Datta, 1992).

However, only two species, *Simulium singtamense* (Dey et al., 1993) and *Simulium dentatum* have been mapped chromosomally (Henry, Dey SK, Varma R, 2009). The present investigation is undertaken to present a complete cytological map of salivary gland chromosomes of one of the most abundant species in the region, *Simulium praelargum*, to provide cytological descriptions of populations under study and to use these maps as a starting point for future cytological characterization and phylogenetic comparisons amongst taxa of feuerborni, within which twenty four taxa are recognized (Adler and Crosskey, 2009), and related groups of the subgenus *Nevermannia*.

1 Materials and Methods

Darjeeling, the geographic region of interest for this study, is situated in northern West Bengal at an altitude of 2134 m (7053 ft) and lie at the foot hills of Eastern Sub-Himalayan region, between 26°31’ and 27°13’ North latitude and between 87°89’ and 88°53’ East longitudes, West Bengal, India. The last instar mature larvae of *Simulium praelargum* that were collected in and around Darjeeling town from fresh water stream / trickles constitute the material for this chromosomal study (Table 1 and Fig. 1). Along with the larvae, pupae were also collected from the same habitat and reared in the laboratory to confirm adult stage morphological identification. The larvae were collected, fixed in freshly prepared cold Acetic acid: Ethanol (1:3) mixture and brought to the laboratory. The identification of the larvae was based mainly on the nature of the post-genal cleft, respiratory histoblast and head patterns with other identification characters. The salivary glands were dissected out in 95 % ethanol. The glands were then stained in 2% lacto-propionic orcein. The gel like content of the glands were discarded after the epithelium of the glands were peeled off and restained in 1 % lacto-propionic orcein if required and squashed under a cover glass. The larvae were immediately sexed by the shape of the gonad (testis – oval, ovary – elongate) and were correlated with chromosomal analysis (Table 1).

**Table 1  Collection data for *S. (Nevermannia)* praelargum from Darjeeling**

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Total 620 224 282

Fig. 1 Map of collection site of *Simulium (Nevermannia) praelargum* from Darjeeling
Fig. 2 Mitotic metaphase plate from neural ganglia of *S. (N.) praelargum* showing chromosomes one (I), two (II) and three (III) (arrows point to putative primary constrictions)

The temporary slides were studied using a microscope (Olympus CH 20i). The total complementary length of each polytene chromosome was determined. Well spread plates of the chromosomes or the parts thereof were studied and photographed by a digital camera (Canon A 640 with 10 mega pixel) and transferred to the computers for further image analysis processing with the help of Adobe photoshop CS softwares.

Chromosomes were divided into sections corresponding to their percentage of length (Table 2). Labeling of the cytological maps of *Simulium praelargum* followed the conventions and nomenclature established and used by earlier workers (Rothfels and Dunbar, 1953; Basrur PK, 1959; Rothfels KH, Feraday R and Kaneps A, 1978; Rothfels et al., 1978.). Representative specimens have been deposited in the Museum of the Department of Zoology, Darjeeling Government College, Darjeeling, West Bengal, India.

2 Results

A total of 506 larvae were analysed cytologically from 5 different sites within the environs of the Darjeeling Hill station (Table 1, Fig. 1). *Simulium praelargum* exhibited two metacentric and one submetacentric pairs of mitotic metaphase chromosomes (*n* = 3) with putative primary constrictions (centromere regions) (Fig. 2). The polytene complement presented as three tightly paired homologous chromosomes (Fig. 4–9). The centromere regions of chromosome one and two were prominent and exhibited enhanced centromere bands (Ce) while that of chromosome three was less prominent (C). The first chromosome was 37.25 %, the second 31.36 % and the third chromosome 31.34 % of total complementary length. The sections assigned to different arms of the chromosomes correspond to their % of TCL (Table 2). An idiogram summarizes the salient diagnostic cytological features of the polytene complement (Fig. 3).

2.1 Chromosome I

Chromosome I is sharply distinguished from other two chromosomes by its greater length. It is divided into a short arm and long arm designated as IS bearing sections 1 – 18 with 17.54 % of TCL and IL bearing sections 19 – 38 with 19.71 % of TCL. (Fig. 4, Fig. 5). Chromosome I bears the characteristic nucleolar organizer region (N.O.) at the base of IS in the section 18. The N.O. is readily recognized in good preparations and serves as a distinguished identifying land mark. IL is marked by the presence of a very prominent and thick heterochromatic centromere (Ce) in section 19 A. Section 38 B marks the end of the IL.

2.2 Chromosome II

Chromosome II is characterized by a short arm (IIS) sections 39 - 52 with 13.98 % of TCL (Fig. 6). The short arm of chromosome two is characterized by the presence of double bubble (db or bulge) in the section 42 followed by Balbiani Ring (RB) in section 43. Section 52 bears a trapezoidal (triad) region and marks the proximal end of the IIS. The long arm of chromosome two begins with the section 53 and continues up to section 69 with 17.38 % of TCL (Fig. 7). An enhanced centromere band (Ce) is located in section 53. A very prominent Parabalbiani Ring is located in section 55 A. The distinctive grey band (gb) occurs in section 59 B. A broad well defined region in section 69 marks the end of IIL.

| Table 2 Percentage of total complement length (%TCL) of chromosome arms for 10 Nuclei of *S. (Nevermannia) praelargum* |
|------------------|------------------|------------------|------------------|------------------|------------------|
| % TCL of the Arms | 17.54±0.19 | 19.71±0.18 | 13.98±0.13 | 17.38±0.31 | 10.87±0.18 | 20.47±0.08 |
| % TCL of Chromosomes | 37.25 | 31.36 | 31.34 | 10.87±0.18 | 20.47±0.08 |
| Secs. Assigned per arm | 18 | 20 | 14 | 17 | 11 | 20 |
| Arm ratio | 1.1 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 |

Data are expressed as mean ±SE.
Fig. 3  Idiogram of polytene chromosomes of *S. (N.) praelargum* showing major landmarks

2.3 Chromosome III

The short arm of chromosome three (IIIS) begins with section 70 and terminates in section 80 with 10.87% of TCL. The IIIS end (Section 70A) is heterochromatized. Sections 81 to 100 represents the long arm of chromosome three (IIIIL) with 20.47% of TCL (Fig. 8) (Fig. 9). Two conspicuous landmarks occur in IIIS, the Capsule at section 72 A and the Blister at section 77. Section 81 marks the beginning of the IIIIL and houses a less prominent centromere (C). Section 100 marks the end of IIIIL.

3 Discussion

Although a detailed banding comparison is not within the scope of this study, *S. praelargum* does exhibit conservation in some banding sequence as seen in other taxa within *Simulium s str.* (Rothfels, 1979). Homology is found for chromosome ends of IS and IL (section 1 and section 37/38) as well as for the base of IIIS, the trapezoid region (triad) (section 52); the notable exceptions...

Fig. 4  Photo composite map of the short arm of chromosome one (IS) of *S. (N.) praelargum*
NO: nucleolar organizer. Ce: enhanced centromeres.

Fig. 5  Photo composite map of the long arm of chromosome one (IL) of *S. (N.) praelargum*
Ce: enhanced centromeres.
Fig. 6  Photo composite map of the short arm of chromosome two (IIIS) of *S. (N.) praelargum*

Fig. 7  Photo composite map of the long arm of chromosome two (IIL) of *S. (N.) praelargum*

Fig. 8  Photo composite map of the short arm of chromosome three (IIIS) of *S. (N.) praelargum*
Heterochromatinized end (section 70A)

Fig. 9  Photo composite map of the long arm of chromosome three (IIIIL) of *S. (N.) praelargum.*
C: centromere.
being the closely related taxa *S. samboni* from Brazil and *S. panamense* from Panama (Hirai et al., 1994). In looking at various polytene maps from different subgenera to identify the Parabalbiani Ring and the gb it became apparent that sections 55B – 59A of IIL in *S. praelargum* is homologous to the sections 60–64 including the gb IIL region of *S. ochraceum* A. Of note also, is the finding that the terminal bands of IIIS are heterochromatinized. In many taxa within the Simuliidae this region is flaired (expressed); the functional significance of this observation remains to be clarified.

The present investigation offers high resolution details of the larval salivary gland chromosomes of an Indian black fly, *Simulium* (Nevermannia) *praelargum*, Datta, 1973 collected from Darjeeling hill. The total chromosomal complement consists of three tightly paired homologues (n = 3) as is the case in most other investigated *Simulium* species (Rothfels, 1979, 1989). Diagnostic intergeneric markers were found (Fig. 3) and will aid in species identification of members of the feuerborni group. All populations sampled over multiple decades exhibited structural chromosome monomorphism. The observation of chromosomally polymorphic populations from this study and their relative abundance despite ecological insults (Willie Henry, unpublished observations) is of general interest though chromosomal variation is abundant in the Simuliidae and that this variation has provided insight into population substructuring, adaption, species distributions (Rothfels, 1980) and vector borne disease ecology (Garns and Walsh, 1987; Procunier, 1989; Hirai et al., 1994; Higazi et al., 2000). In the context of zoogeography and how species may change at the molecular genetic/genomic level, further distribution sampling of *S. praelargum* population would be worthwhile to see how widespread the chromosomal phenomenon of structural monomorphism is.

Acknowledgements The authors are grateful to Dr. M. Datta, Diptera Section, Zoological Survey of India, Kolkata for his morphotaxonomic expertise in Simuliiid species identification. Thanks are due to the Principal, Darjeeling Govt. College, Darjeeling for access to various laboratory facilities. The authors express their sincere thanks to Prof. Hiroyuki Takaoka, Division of Medical Zoology, Otta University, Japan and Prof. Peter H Adler, Department of Entomology, Clemson University, U.S.A. for their continued help and guidance. Special thanks are due to Mr. Marvin Henry Gupta for his guidance in the software handling.

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