

## Karyological characters of blackflies (Diptera: Simuliidae)

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**Abstract.** The paper provides a short review of blackfly cytogenetics. The most important characters of blackfly karyotypes, such as centromeres, puffs, Balbiani rings, nucleoli, sex-chromosome determining systems and B-chromosomes are considered. Interchromosomal homozygous translocations and a tandem fusion of polytene chromosomes are described. Evolutionary trends in karyotypic changes are discussed.

**Key words:** Simuliidae, karyotypes, polytene chromosomes, cytogenetics

Blackflies are blood-sucking insects representing a compact distinct cosmopolitan family of Diptera. Many blackfly species are vectors of pathogens of dangerous diseases (Rubzov, 1956). Researchers face great difficulties in the identification of blackflies. Firstly, these are small objects (body length 2-7 mm); secondly, they are weakly differentiated morphologically; thirdly, researchers frequently deal either with females, which are noxious blood-suckers, or with aquatic stages, i.e. larvae, species differences between them being extremely weakly visible. Species differences are more distinctly pronounced in males; male genitalia are the most reliable character for the identification of species; however males are less frequently available for a study. The above-mentioned difficulties stimulated intensive search and usage of new characters, including characteristics of the karyotype (Rotfels, 1956). The karyological method in combination with the classical morphological one is currently successfully used in the systematics of blackflies. Each species is characterized by its own specific pattern of discs in polytene chromosomes. An experienced researcher can identify a genus or a species by this character. The authors have studied the karyotypes of blackflies for 30 years. So far they have

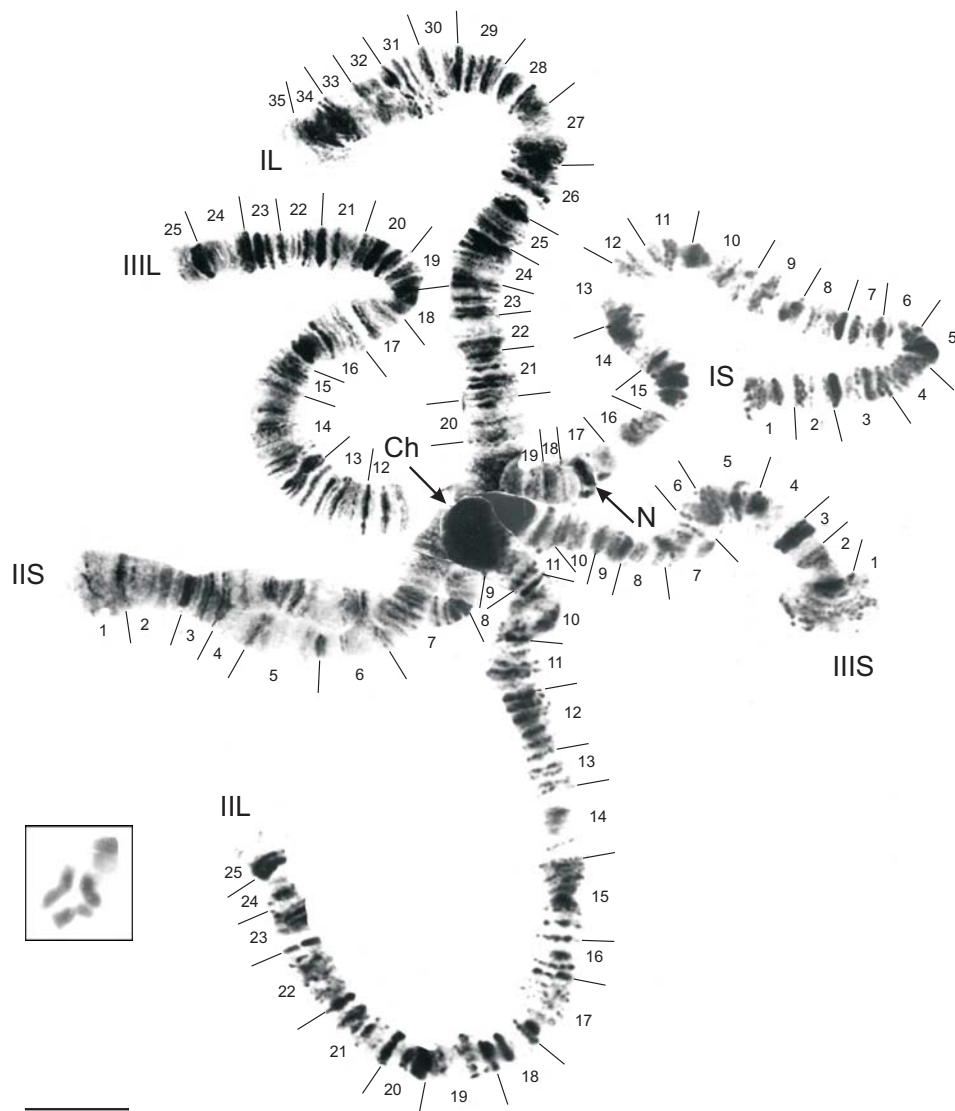
described the karyotypes of 110 species (Chubareva, Petrova, 1979, 2003). The main characters of their karyotypes are generally summarized in the following review.

### Karyotype composition

The modal blackfly karyotype comprises 6 chromosomes, i.e.  $2n = 6$ , however there are species with  $2n = 4$  and  $3n = 9$ . By the length the chromosomes are designated as I, II, III, since homologous chromosomes conjugate in pairs. Chromosome I is meta- or submetacentric, chromosomes II and III are always submetacentric. Most frequently the chromosomes are not equal to each other in size, i.e.  $I > II > III$ , e.g. in genera *Prosimulium* Roubaud, 1906, *Metacnephia* Crosskey, 1969, *Montisimulium* Rubzov, 1974, etc., but it happens that  $II = III$  and then inequality arises resulting in  $I > II = III$ , which occurs in genera *Helodon* Enderlein, 1921, *Sulcicnephia* Rubzov, 1971, *Cnetha* Enderlein, 1921 and others.

### Centromeres

The morphology of the centromere plays a decisive role as a species character. The centromere is protected by heterochromatin comprising highly



**Fig. 1.** Metaphase (in the frame) and polytene chromosomes of *Cnetha garniensis*,  $2n = 6$ . IS, IL, IIS, IIL, IIS, IIIIL - arms of polytene chromosomes with sections designated by Arabic numerals; N - nucleolus; Ch - chromocentre. Bar = 20  $\mu$ m.

repetitive DNA. In karyotypes of the evolutionarily primitive genera, *Twinia* Stone et Jamndack, 1955, *Gymnopais* Stone, 1949 and *Levitinia* Chubareva et Petrova, 1981, centromeres are not pronounced morphologically. This character is regarded as a primitive karyotypical character of the family (Chubareva, Petrova, 2003). In the genera

*Prosimulium*, *Metacnephia*, *Silcicnephia*, *Odagmia* Enderlein, 1921 and many others, the centromeres are distinctly visible and in some species unite into a chromocentre. For instance, in *Cnetha garniensis* Rubzov, 1955 a rounded constant chromocentre is formed, from which 6 arms diverge radially (Fig. 1).

### Nucleolus organizer

The nucleolus is formed on a definite segment of a definite chromosome. This segment is called a nucleolus organizer. It is there that synthesis of the entire ribosomal RNA of a cell occurs. This is one of the most important genetic loci of a chromosome. Blackflies are a "mononucleolar" family. The nucleolus is either connected with the chromosome IS (e.g. in genera *Prosimulium*, *Metacnephia*, *Sulcicnephia*, *Cnetha*, *Montisimulium*, etc., Fig. 1) or with the chromosome III L (in *Helodon*, *Boophthora* Enderlein, 1921, *Odagmia*, *Gnus* Rubzov, 1940, etc., Fig. 2). Increase of the number of nucleoli and change of their localization is an exceptional phenomenon in the blackflies. It is either connected with spontaneous division of the nucleolus into parts and translocation of these parts to other chromosome loci, or with habitation conditions when, without any obvious reasons, single larvae with an additional nucleolus appear.

### Puffs and Balbiani rings

A puff is a unit of local despiralization of a separate locus of a chromosome. Zhimulev (1998) showed a transition of heterochromatin discs into puff and vice versa; the number of discs participating in the formation of puff can vary from 1 to 20. Puffs in the blackflies are arranged in the short arms of chromosomes II and III; in the latter they are separated by a marker heterochromatin disc.

A peculiar group of puffs are giant puffs or Balbiani rings (BR). Discovered in them are genes coding the synthesis of tissue-specific secretory proteins. Balbiani rings are an extremely convenient object for studying gene functioning, because this chromosome locus can be easily observed under a light or electron microscope. Apart from genes, several types of mobile genetic elements have been discovered in BR. Those elements are characterized by unstable position in a genome, during their translocation expression of genes changes, an insertional mutagenesis arises.

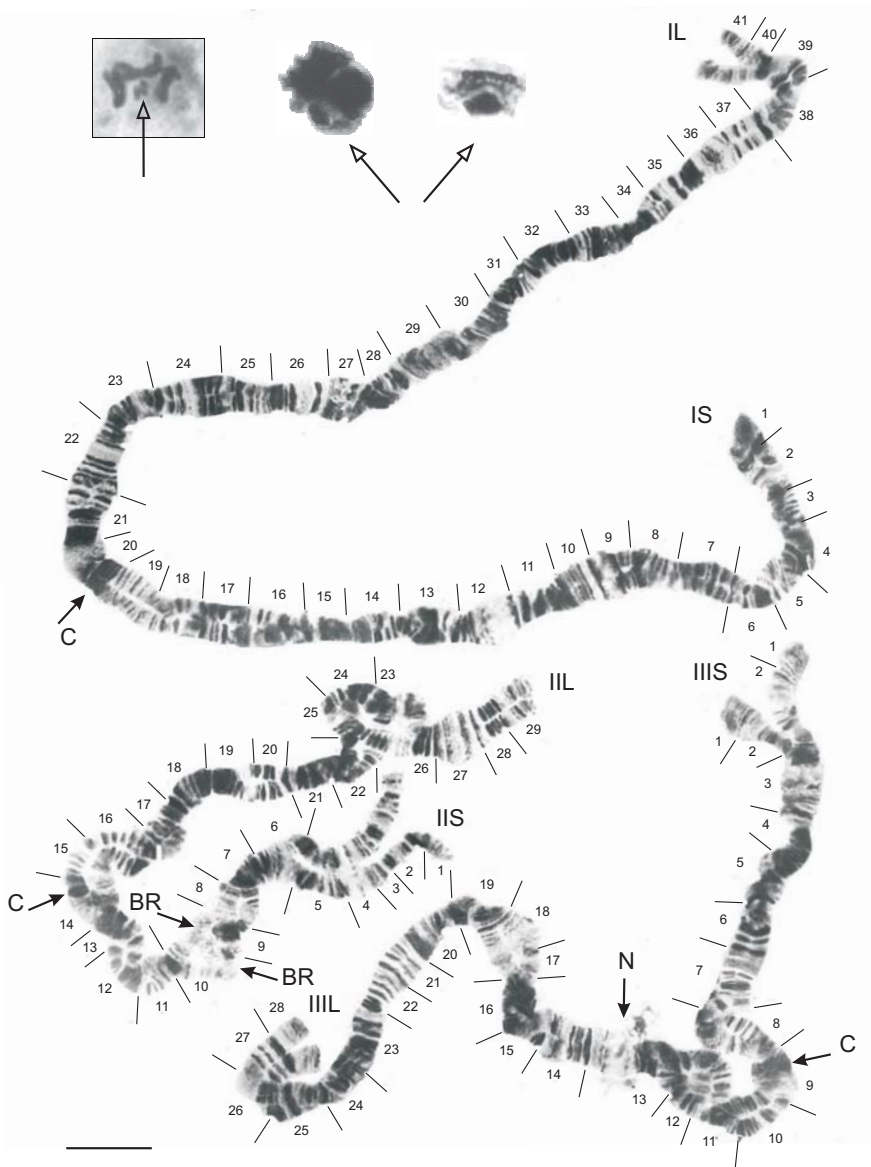
### Sex chromosomes

The sex determining system, when the karyotype of a male is not different from the karyotype of a female, is regarded as primitive (Petrova, 1980). Such system can be observed in the majority of species. But there are some species with a simple or complex system of sex determination. The simple system in the family is determined as XY♂ – XX♀. The male Y chromosome is determined by a heterozygous inversion, or by heterozygous disc, or by heterozygous nucleolus organizer, or by additional heterozygous heterochromatin block near the centromere disc. However there are species with multiple sex chromosomes, e.g. *P. hirtipes* Fries, 1824 (Basrur, 1962), *Byssodon viginquaterni* (Enderlein, 1929) (Rothfels, 1956), *Wilhelmia paraequina* Puri, 1933 (Petrova et al., 2003).

### Polymorphism

The majority of species are monomorphic, inversions in them are single and accidental, e.g. in the genera *Twinia*, *Levitinia*, *Stegopterna* Enderlein, 1930, *Helodon*, and others. There are however some highly polymorphic species, e.g. in the genera *Prosimulium* (Rothfels, 1956, Ottonen, 1966), *Metacnephia*, *Sulcicnephia* (Petrova, 1974), *Boophthora* (Petrova et al., 2007, in press) and others. For instance, 8 natural populations of *B. erythrocephala* De Geer, 1776 originating from Priozersk, Pudozhsky and Luzhsky districts of Leningrad Prov., from Ivanovo Prov. and Samara Prov. (Russia), and from Donetsk Prov. and Chernigov Prov. (Ukraine) were studied. All the populations appeared to be heterozygous: there were 3 to 4 inversions per one individual, i.e. in each population there were from 85 to 100% heterozygous individuals with inversions (Petrova et al., 2007, in press). Noteworthy is *Byssodon maculatus* Meigen, 1804, in which 72 larvae had 529 inversions, i.e., on the average, 7 inversions per one individual. Individuals with 13





**Fig. 2.** Metaphase (in the frame) and polytene chromosomes of *Simulium flavidum*,  $2n = 6+B$ . C - centromere; BR - Balbiani rings. The other designations are as on the Fig. 1. Long arrows indicate B-chromosomes. Bar = 20  $\mu$ m.

through 10 inversions occurred. Such a high frequency of inversions in the karyopool had not previously been observed in blackflies (Chubareva, 1974).

**B-chromosomes**

B-chromosomes have been discovered in several hundreds of species of animals and plants (Camacho et al., 2000). In blackflies, B-chromosomes are shorter than the chromosomes of the

main set (Fig. 2). B-chromosomes are subdivided into 2 types, with disc structure and without it. Type I possesses a species-specific pattern of discs, distinct centromeres and telomeres. This type has been noted in the majority of species, e.g. in *Montisimulium octofiliatum* Rubzov, 1956, *Cnetha zakhariensis* Rubzov, 1955, *Odagmia ornata* Meigen, 1818, *O. maxima* Knos, 1961, *O. variegata* Meigen, 1818, *Tetisimulium alajense* Rubzov, 1939, and others. B-chromosomes of type II are intensively stained small bodies without disc structure. They were discovered in *Montisimulium* sp. (group *M. montium*), *M. inflatum* Rubzov, 1951, *Simulium morsitans* Edwards, 1915. In *S. ovtshinnikovi* Rubzov, 1940 one can see both types simultaneously. Sometimes B-chromosomes are connected with the nucleoli (*C. zakhariensis*), which suggests a high functional activity of those nucleoli. Within an individual the number of B-chromosomes is constant, but it varies between individuals. The number of individuals with B-chromosomes in a population also fluctuates depending on geography and season (Chubareva, Petrova, 2006).

### Chromosome evolution

In the overwhelming majority of species of the blackflies a remarkable constancy of combination of arms of chromosomes occurs: IS+IL, IIS+IIL, IIIS+IIIL. However qualitatively new karyotypes with a different combination of arms, which arose as a result of interchromosomal homozygous translocations, have been discovered in a number of species (Rothfels, 1956; Weber, Grunewald, 1989). In several species of *Twinnia* and *Ahaimophaga* Chubareva, 1978 exchanges of arms of chromosomes II and III (IS+IL; IIS+IIIL; IIL+IIIS) occurred. In other species of *Twinnia* exchanges occurred between other arms of the same chromosomes (IS+IL; IIS+IIIS; IIL+IIIL). In some species of *Prosimulium* exchanges occurred between chromosomes I and II (IS+IIS;

IL+IIL; IIIS+IIIL); and in some species of the genera *Prosimulium*, *Parahelodon* Peterson, 1970 and *Wilhelmia* exchanges occurred in the same chromosomes, but between other arms (IS+IIL; IIS+IL; IIIS+IIIL).

As has been mentioned above, in the majority of species the chromosome number is equal to 6. Only several dozens of species of *Eusimulium* Roubaud, 1906 and a single species of *Astega* Enderlein, 1930 have  $2n=4$ . In *A. lapponica* Enderlein, 1930 decrease of chromosome number occurred as a result of a tandem fusion of chromosomes II and III. In a newly originated dicentric chromosome I both centromeres are marked by heterochromatin blocks, which tend to contact with each other and sometimes fuse into one heterochromatin block. In meiotic and mitotic cells of gonads and ganglia the dicentric chromosome behaves as the monocentric one. In spite of the morphological ambivalence, the newly arisen chromosome is functionally monocentric, i.e. 2 centromere blocks situated close to each other are functioning as a single centromere. It can be suggested that one centromere passes into a latent state, which does not preclude normal functioning of another centromere (Shobanov, Petrova, 1995).

Thus, in the blackflies evolution proceeded on the basis of: 1) fixed homozygous inversions; 2) formation of sex-determining systems; 3) translocation of nucleolus organizer from IS to IIIL; 4) change of morphology of the centromere region; 5) tandem chromosome fusions; 6) homozygous translocations of chromosome arms; 7) appearance of B-chromosomes.

### ACKNOWLEDGEMENTS

The study was supported by the Russian Foundation for Basic Research, Grant No. 05-04-48387 and by the program of the Presidium of the Russian Academy of Sciences "Dynamics of Gene Pools in Animals, Plants and Man".



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Received November 2, 2006

Accepted by V.G. Kuznetsova, January 25, 2007

Published March 14, 2007.