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## Karyotype of *Potthastia montium* Edwards (Diptera, Chironomidae) from the Russian Far East

Oksana V. IVANCHENKO<sup>1)</sup> and Eugenyi A. MAKARCHENKO<sup>2)</sup>

<sup>1)</sup>Novosibirsk State University, Novosibirsk 630090, Russia

<sup>2)</sup>Institute of Biology and Pedology, Far East Branch of Russian Academy of Sciences,  
Vladivostok 690022, Russia

**Abstract.** For the first time karyotype of *P. montium* Edwards (Dipt., Chironomidae) is described from the Far East.

**Key words.** Chironomidae; Diamesinae; *Potthastia*; karyotype, Far East.

### Introduction

The advantages of karyological analysis for precise determination of the taxonomical status of species and their phylogenetic relationships in the family Chironomidae have become increasingly apparent in recent years (Kiknadze *et al.*, 1989, 1991; Michailova, 1989; Petrova, 1989, 1990). The karyotypes of the species belonging to 20 genera of the subfamily Diamesinae were the most thoroughly studied. Other members were less studied in karyological terms.

At present, there are 5 karyologically studied genera among 13 known genera of Diamesinae in Palaearctic: *Diamesa* Mg. (Kuberskaya, 1984; Michailova, 1989; Petrova, 1989), *Pseudodiamesa* G. (Kuberskaya, 1974, 1983; Zacharias, 1984), *Pagastia* Ol. (Kerkis, 1992; Kerkis *et al.*, 1994), *Sympotthastia* Pag. (Kerkis, 1992), *Lappodiamesa* Ser.-Tos. (Kerkis, 1992).

We present here the results of our studies of salivary gland polytene chromosomes and karyotype features of *Potthastia montium* Edwards from Primorye (Russian Far East). It is the first karyological description of species of the genus *Potthastia*.

### Materials and methods

The chromosomal set of fourth instar larvae were studied. The larvae were fixed in a 3:1 mixture of ethanol and acetic acid and stored in the cold. Preparations of salivary gland polytene chromosomes were made according to the standard acetoorcein method (Keyl & Keyl, 1959). The mitotically dividing cells of imaginal discs were stained with acetoorcein to obtain preparations of metaphase chromosomes. Chromosome numbers were designated by Roman numbers, the chromosome arms by Latin and chromosome regions by Arabic numbers. The above designations, including the centromere, were done according to those for the genus *Sympotthastia* closely related to *Potthastia* (Kerkis, 1992). Because the centromeric regions are indistinct in the two genera, bands, which are thought to be the centromeric regions, are indicated by arrows. The designation system accepted for the subfamily Chironominae was used in analysis of chromosomal polymorphism (Kiknadze *et al.*, 1991). Larvae were collected in the Kedrovaya River (Kedrovaya Pad nature reserve, Khasansk region, Primorye, Russia) in July 1996 by E. Makarchenko. The total number of examined larvae was 125.

## Results

### *Potthastia montium* (Edwards)

*Psilodiamesa montium* Edwards, 1929: 307, ♂; Goetghebuer, 1939: 20, ♂; Sasa, 1988: 51, ♂; *Potthastia montium* Pinder, 1978: 42, fig. 93D, ♂; Makarchenko, 1985: 50, ♂; Langton & Moubayed, 1990: 135-136, pupa; Sasa & Kikuchi, 1995: 207, ♂.

*Potthastia iberica* Serra-Tosio, 1971: 160-165, ♂; Doughman, 1985: 20-25, ♂ ♀, pupa, larva.

*Syndiamesa oiraoctavae* Sasa, 1991: 77, ♂; Sasa & Kikuchi, 1995: 80, ♂.

*Distribution*. Holarctic region: North America, Japan, China, Korea, Russian Far East, Europe.

### Description of karyotype

The salivary glands of *Potthastia* are very asymmetric. One gland lies on top of the other on either side of the intestine. The ducts of the salivary glands are parallel. The glands consist of polyhedral cells.

The left gland lies somewhat higher than the right from segments I-IV. The left gland is  $925 \pm 125 \mu\text{m}$ , its width in the middle part is  $225 \pm 25 \mu\text{m}$ . The gland consists of 75-90 cells. There is a small widening at the anterior end of the left gland. The duct is surrounded by a rosette of conus-like cells with lighter and smoother cytoplasm originating from this widening. The rosette is composed of 9-12 cells. The internal part of the gland is filled with secretion.

The right salivary gland originates from segment II and stretches to segments V-VI. The length of the right salivary gland is  $1238 \pm 263 \mu\text{m}$ . Its width in the middle part is  $100 \pm 3 \mu\text{m}$ . The internal part of the gland is not filled with secretion. Cells contain lighter cytoplasm than cells of the left salivary gland. The gland consists of 65-75 cells.

*P. montium* has a  $2n=8$  (Fig. 1A). The chromosomes of one pair are metacentrics, the



Fig. 1. The chromosomes of *P. montium*. A-mitotic chromosomes; B-a standard photomap of salivary gland polytene chromosomes: I-IV chromosome numbers; IR, IL, IIR, IIL, IIIIR, IIIIL-chromosome arms, N-nucleolar organizer, BR-Balbani Rings. The centromeric regions are indicated by arrows.

chromosomes of two pairs are submetacentrics, and those of one pair are acrocentrics. Three long polytene chromosomes and one short are detected in salivary gland cells. Homologs of polytene chromosomes tightly pair along the entire length of the chromosomes. The chromosomes show clearcut banding patterns, the centromeric regions are indistinct.

Chromosome I is subdivided into 21 regions (Fig. 1B). Markers are thick bands in regions 11, 17, 19-21. A puff is found in region 1.

Chromosome II is subdivided into 19 regions. Markers are the active regions 1, 3, 5, 6 and also a set of bands in region 11. Staining with  $\text{AgNO}_3$  reveals the presence of one nucleolus in chromosome IV; consequently, the giant puffs, Balbiani Rings (BR), are probably the active regions in chromosome II (Fig 1B).

The key features of chromosome III are a set of thick bands in regions 1-3 and a light region defined by two bands in region 15. Chromosome III is subdivided into 15 regions.

Chromosome IV is much shorter than all the chromosomes of the set and it is subdivided into 6 regions (Fig. 1B). Chromosome IV has a nucleolus.

*P. montium* is a highly polymorphic species. Heterozygous sequences were identified in three arms of the long chromosomes. Inversions occurred in arms IR, IIL, IIIL (Fig. 2). Arm IR is most polymorphic (Table 1). Five sequences were found singly or in different combinations.

Table 1. Chromosomal polymorphism of *P. montium*

Arm	IR	IL	IIR	IIL	IIIR	IIIL	IV
sequence	1.1	1.1	1.1	1.1	1.1	1.1	1.1
	1.2			1.2		1.2	
	1.3			1.3		1.3	
	1.4			1.4		1.4	
	1.5			1.5			
	2.3			2.4			
	2.4						
	3.4						
	4.5						

The highest frequencies were observed for the following heterozygous sequences: IR1.2 (Inv 18-14), 0.36 (Fig. 2A); IR1.3 (Inv 19-16), 0.34 (Fig. 2B); IR1.4 (Inv 13-14), 0.25. Sequence IR1.5 (Inv 19-18) was found in single individuals. Homozygous standard sequence IR1.1 occurs with a frequency of 0.2. Rearrangements involved arm L in chromosome II. Five sequences are also identified. Standard sequences IIL1.1 occurs most frequently, 0.58. All the inversion sequences start in region 2 and end as follows: IIL1.2, in region 5 at the boundary with region 6 (Fig. 2 D); IIL1.3, at the boundary with region 6-7; IIL1.4, in region 7-8 (Fig. 2). The frequency of heterozygous inversion IIL1.2 is highest, 0.2; the frequencies of the other sequences are very low. Sequences IIL1.5 (Inv 3-7) is found in one individual (Fig. 2 F). Three heterozygous sequences are found in arm IIIL. Sequence IIIL1.2 forms from an inversion and duplication in regions 1 (Fig. 2G). IIIL1.3 results from an inversion in region 1-3 (Fig. 2H, J). Sequence IIIL1.4 is found in one individual. It presumably results from a pericentric inversion in regions 1-4 (Fig. 2I).

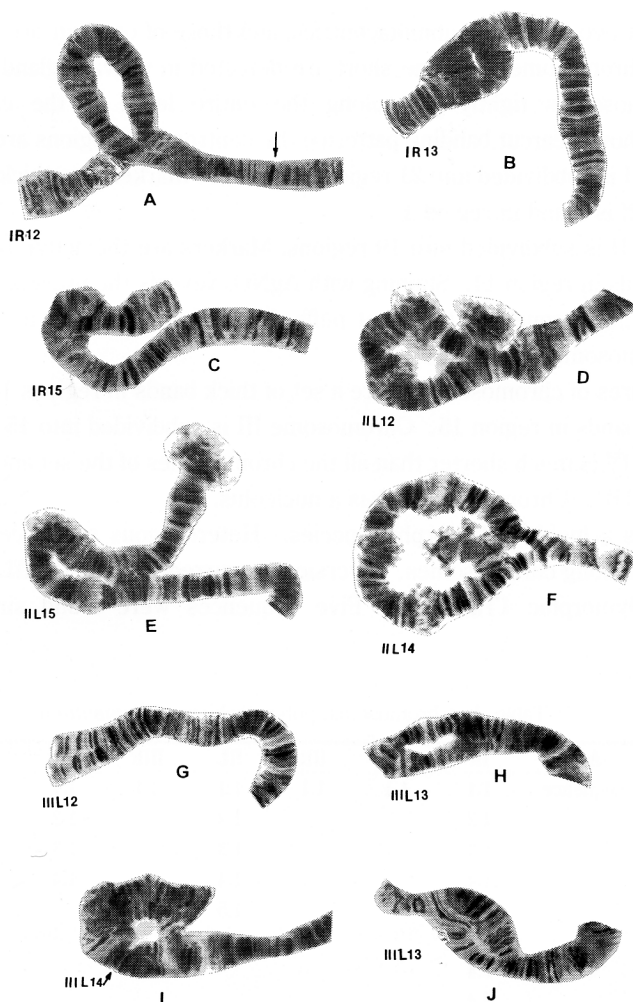


Fig.2. Chromosomal polymorphism in *P. montium*.  
Chromosomal polymorphism in arms: A-C-IR, D-F-  
IIL, G-I-IIIL. The centromeric regions are indicated  
by arrows.

### Discussion

This is the first karyological analysis of the genus *Potthastia*. The karyotype of a representative of *Sympotthastia*, a genus closely related to *Potthastia*, was described by Kerkis (1992). It appeared of interest to determine the structural differences between the karyotypes of these related genera.

Comparisons of the karyotypes of *P. montium* and *Sympotthastia repentina* Makar. allowed us to reveal homologies and differences. The representatives of the two species have a  $2n=8$ , a number probably characteristic of representatives of the subfamily Diamesinae (Petrova, 1989). There is a correspondence between chromosome arms and partly banding patterns. The salivary gland polytene chromosomes of the two species show clear-cut banding patterns, the centromeric regions are indistinct. The homologs of *P. montium* polytene chromosomes always

tightly pair along most of chromosome length. Analysis of banding pattern of the polytene chromosomes demonstrated that the karyotype of *S. repentina* differs from that of *P. montium* by deletions of entire regions and homozygous inversions. Active regions are detected in chromosomes II and IV, but their number is different in the two species. In *S. repentina*, there are two nucleoli, one in chromosome II and one in chromosome IV (Kerkis, 1992). In *P. montium*, one nucleolus is detected in chromosome IV and three BRs in chromosome II. The banding patterns shown by chromosome IV of *P. montium* and *S. repentina* are different in the presence of an inversion in regions 3-5 in *P. montium*. In *P. montium*, the thickness of chromosome IV is comparable to that of the other chromosomes of the set. In *S. repentina*, chromosome IV is twice thinner than the other chromosomes (Kerkis, 1992). A similar structure of chromosome IV has been described for the genus *Sergentia* (Chironominae) (Kiknadze *et. al.*, 1991). *S. repentina* is monomorphic, while *P. montium* is polymorphic. Five sequences are detected in arms IR and IIL, and four in IIIL arms.

Based on this analysis, it may be concluded that the karyotypes of *S. repentina* and *P. montium* are markedly different, although partly homologous with respect to banding patterns. Analysis of a larger number of species of these genera is needed to detect genus-specific features in their karyotype structure.

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### References

- Doughman, J. S., 1985. *Sympotthastia* Pagast (Diptera, Chironomidae), an update based on larvae from North Carolina, *S. diastena* (Sublette) comb.n., and other Nearctic species. *Brimleyana*, **11**: 39-53.
- Edwards, F. W. 1929. British non-biting midges (Diptera, Chironomidae). *Trans. Roy. Entomol. Soc. London* **77**: 279-430.
- Goetghebuer, M. 1939. Tendipedidae (Chironomidae). c) Subfamilie Diamesinae. A. Die Imagines. In Lindner, E. (ed.), *Die Fliegen der Palaearktischen Region*: 1-28.
- Keil, H.-G. & Keil, I. 1959. Die cytologische Diagnostik der Chironomiden. *Arch. Hydrobiol.*, **56**: 43-57.
- Kerkis, I. E. 1992. Karyotypes of four Diamesinae species from Russia. *Netherland. Journ. Aquatic Ecology*, **26** (2-4): 157-162.
- Kerkis, I. E., Ivanchenko, O. V. & Makarchenko, E. A. 1994. A comparative karyological analysis of two sibling-species of the subfamily Diamesinae (Diptera, Chironomidae) from the Russian Far East and Japan. *Citologia*, **36**(5): 479-486 (in Russian).
- Kiknadze, I. I., Blinov, A. G. & Kolesnikov, N.N. 1989. The molecular-cytological organization of the chironomid genome. *Nauka, Novosibirsk*: 4-58 (in Russian).
- Kiknadze, I. I., Shilova, A. I., Kerkis, I. R., Shobanov, N. A., Zelentsov, N. I., Grebenuk, L. P., Istomina, A. G. & Prasolov, V. A. 1991. Karyotypes and morphology of larvae of Chironomini tribe. *Atlas. Nauka, Novosibirsk*: 114 pp. (in Russian).
- Kuberskaya, E. F. 1974. On the characteristics of the karyotype and some special features of the

- nucleus structure in the *Pseudodiamesa nivosa* from the lake Baikal area. *Cytologia* **16**: 1426-1432 (in Russian)
- Kuberskaya, E. F. 1983. Using of cytogenetic analysis methods for definition of the taxonomic status of Diamesinae (Chironomidae). *Diptera (Insecta)*, their systematics, geographic distribution and ecology. Leningrad: 80-81 (in Russian).
- Kuberskaya, E. F. 1984. Morphology of salivary glands and karyotype of *Diamesa tsutsuii* Tok. (Diptera, Chironomidae) from Primorye. *Biology of Far Eastern Fresh Water, Vladivostok*: 102-106 (in Russian).
- Langton P. H. & Moubayed, F. 1990. Redescription of the pupal exuviae of *Potthastia montium* Edwards (= *iberica* Serra-Tosio syn.nov.) (Diptera, Chironomidae). *Br. J. Ent. Nat. Hist.*, **3**: 135-137.
- Makarchenko, E. A. 1985. Chironomids of the Soviet Far East. Subfamilies Podonominae, Diamesinae and Prodiamesinae (Diptera, Chironomidae). Vladivostok, DVNC AN (in Russian). SSSR Press: 1-208.
- Michailova, P. 1989. The polytene chromosomes and their significance to the systematics and phylogeny of the family Chironomidae, Diptera. *Acta Zool. Fennica* **186**: 1-107.
- Petrova, N. A. 1989. Characteristics of the karyotypes of midges (Diptera, Chironomidae) of the world fauna. I. Subfamilies Telmatogetoninae, Podonominae, Tanypodinae, Diamesinae, Prodiamesinae and Orthoclaadiinae. *Entomol. obozr.*, **68**(1): 107-119 (in Russian).
- Petrova, N. A. 1990. Characteristics of chironomid karyotypes (Diptera, Chironomidae) of world fauna. II. Subfamily Chironominae. *Entomol. obozr.* **69**: 194-214 (in Russian).
- Pinder, L. C. V. 1978. A key to the adult males of British Chironomidae. Vol.I, The key; Vol.II, Illustrations of the hypopygia. *Freshwat. Biol. Assoc., Scient. Publ.*, **37**: 169 pp.+189 figs.
- Sasa, M. 1988. Studies on the chironomid midges collected from lakes and streams in the southern region of Hokkaido, Japan. *Res. Rep. Nat. Inst. Envir. Stud.*, **121**: 9-76.
- Sasa, M. 1991. Annex studies on the chironomids of some rivers and lakes in Japan. Part 2. Studies on the chironomids of the Lake Towada area, Aomori. *Rep. Toyama Prefect. Environ. Pollut. Res. Centre*: 68-81.
- Sasa M. & Kikuchi, M. 1995. Chironomidae (Diptera) of Japan. University of Tokyo Press: 1-333.
- Serra-Tosio, B. 1971. Deux Diamesini nouveaux d'Espagne (Diptera, Chironomidae). *Trav. Lab. Hydrobiol. Grenoble*, **62**: 147-167
- Zacharias, H. 1984. Allocyclic behaviour and underreplication of the nucleolus chromosome in *Pseudodiamesa* (Chironomidae). *Chromosoma*, **89**: 263-273.