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RAPID COMMUNICATION

First Description of Polytene Chromosomes in Biting Midges (Diptera: Ceratopogonidae)

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ABSTRACT Polytene chromosomes are described from secretory cells in larvae of *Forcipomyia nigra* (Winnertz). They are present in large glandular-trichogen cells at the bases of secretory setae and in midgut cells that were observed by transmission electron microscopy and light microscopy. Polytene chromosomes, isolated from the glandular-trichogen cells using aceto-orcein squash technique, measure 50–200 μm, have braid-like strands of chromatin and no bands, features that are unique within the Culicomorpha.

KEY WORDS glandular cell, trichogen cell, Culicomorpha, *Forcipomyia*

Polytene chromosomes have been widely reported in various families of Diptera and their banding pattern used to study transcription as well as phylogenetic relationships between taxa. Within the Culicomorphan families Simuliidae, Chironomidae, and Culicidae the banding patterns have been an important tool in discovering cryptic species. They have never been reported in the other five families in this infraorder and here we describe them for the first time in Ceratopogonidae, albeit in a different form. Study of the chromosomes of biting midges has been limited. There are only a few cytogenetic analyses of metaphase chromosomes from different somatic cells and from ovaries (Atchley et al. 1968; Hagan and Hartberg 1986; Isayev 1998, 1999; Nunamaker et al. 1996, 1999a,b). Despite such pressing health and veterinary concerns as Bluetongue and many other diseases (Linley 1985a, Borkent 2004), there have been no reports of attempts to use polytene chromosomes to solve the cryptic species problems present among many of the vectors. The first report of polytene chromosomes, in the trichogen cells secreting viscous fluid at the apices of modified setae of larvae of species of *Forcipomyia* Meigen by Urbanek et al. (2011), prompted us to describe these here in detail. Their different structure explains why they have not been used previously by those studying Ceratopogonidae as a taxonomic tool. We also compare the structure of the nuclei of the secretory trichogen, mature trichogen, and midgut cells.

Materials and Methods

Fourth instar larvae of *Forcipomyia nigra* (Winnertz) (50 specimens) were collected in October 2010 from a pine forest in Gdańsk–Sobieszewo and maintained in the laboratory at T5°± 1°C and relative humidity 80–90%.

For transmission electron microscopy (TEM), material was fixed with 2.5% glutaraldehyde in PBS and postfixed in 1% osmium tetroxide. After dehydrating in a graded alcohol series, sections were embedded in Spurr resin and sectioned using an RMC PowerTome PT-X ultramicrotome. Ultra-thin sections were double stained with acetate and lead citrate and observed under both Philips CM100 and JEM 1200EX II transmission electron microscopes. For light microscopy, semithin sections were stained with toluidine blue or fluorescent stain 4′-6-diamidino-2-phenylindole (DAPI) and observed with Olympus BX51 and Nikon Eclipse E800 microscopes.

To isolate polytene chromosomes, larvae (20 specimens) were dissected in Ringer's solution and placed in a mixture of 96% ethanol and glacial acetic acid (3:1) for 24 h 4°C stained with aceto-orcein technique and Giemsa reagent.

Results and Discussion

Semithin cross-sections stained with toluidine blue and DAPI of the larval abdomen showed that glandular-trichogen cells possess a large nucleus with polytene chromosomes (Figs. 1A, B and 2B). We were able to isolate polytene chromosomes from almost 75% of examined specimens. Individual polytene chromosomes measuring ≈50–200 μm in length and 10–15 μm in width are composed of braided chromatin fibers while the ends of some appear to remain loose and untangled (Fig. 1E, F). Darker spots are probably condensed heterochromatin (Fig. 1D). Chromosomes

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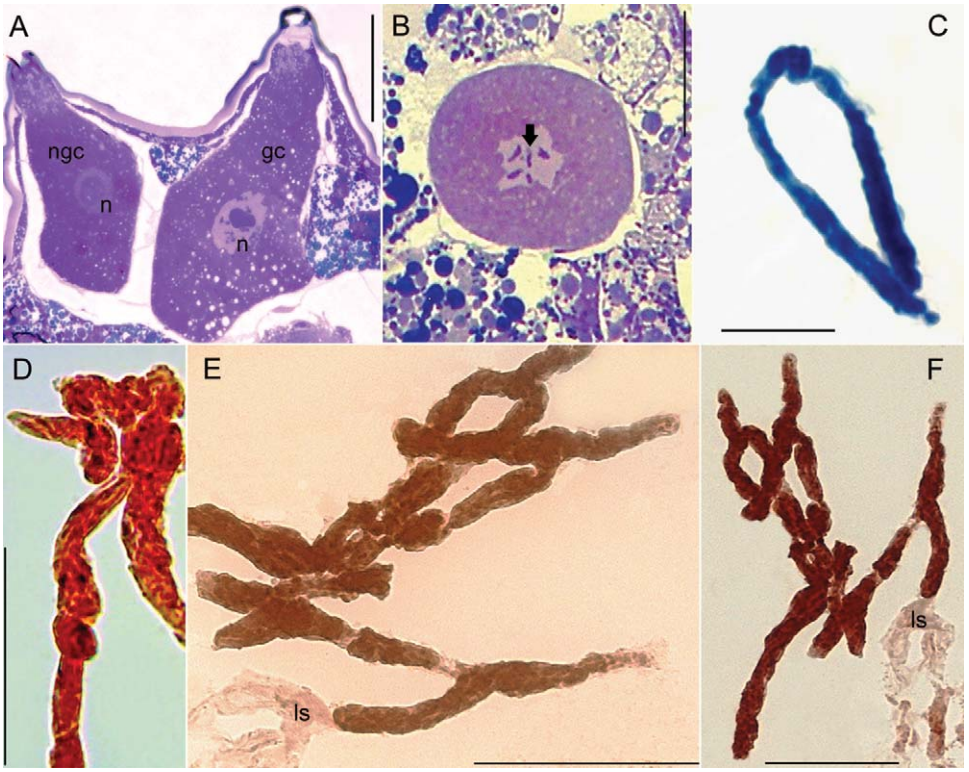

Fig. 1. Light microscope photomicrographs of the glandular cells and polytene chromosomes of a secretory seta of *Forcipomyia nigra* larvae. (A) Longitudinal section of a glandular-trichogen cell and a typical trichogen cell stained with toluidine blue. (B) Cross section of glandular cell with polytene chromosomes (arrow) stained with toluidine blue. (C) Polytene chromosome stained with Gimesa and (D–F) acetoorcein. gc = gland cell; ls = loose chromosomes end; ngc = nonglandular, typical trichogen cell; n = nucleus. Scale bar for (A, B, C) = 25 μm; (D) = 50 μm; (E, F) = 100 μm. (Online figure in color.)

stained with Giemsa are distinguished only by the presence of darker regions of heterochromatin (Fig. 1C). The centromere is also recognizable (Fig. 1C). There are no transverse-banding patterns (Fig. 1D–F) as is frequently observed in the polytene chromosomes from the salivary glands of other dipterans (Kitzmiller et al., 1976, Freitas et al. 1995, Michailova 2003, Adler et al. 2004, Durnova 2009). TEM study also revealed (Fig. 2A, C) concentrations of condensed heterochromatin along the length of each polytene chromosome (Fig. 2A, C).

TEM study of midgut secretory cells also indicated dark stripes inside the nucleus that are likely polytene chromosomes (Fig. 2E, F), similar to those in the midgut of *Drosophila* Fallén or *Melanagromyza* Hendel (Gupta and Sing 1983, Roberts 1988, Zhimulev and Koryakov 2009). We did not observe polytene chromosomes in the cells of the salivary glands of *Forcipomyia* larvae (using the aceto-orcein squash technique). Older trichogen cells of nonsecretory setae lack giant chromosomes, almost certainly because they are no longer producing large amounts of secretion (Figs. 1A and 2D). These seta forming cell probably had polytene chromosomes during growth of the seta, but contrary to glandular-trichogen cell, it decreased in activity what resulted in the loss of polyteny.

Ceratopogonidae are one of eight families in the infraorder Culicomorpha (Borkent 2004). Of these, polytene chromosomes have been previously reported in only Chironomidae, Simuliidae, and Culicidae (Zhimulev 1996, Ashburner 2005) and all species studied have polytene chromosomes with clearly visible bands. However, we cannot unequivocally conclude whether the presence of polytene chromosomes with no banding is a unique feature of Ceratopogonidae within the infraorder because polyteny in the other families (Chaoboridae, Dixidae, Corethrellidae, Thaumaleidae) has not yet been studied.

Our discovery of braided polytene chromosomes in a species of *Forcipomyia* draws into question whether this is true for other members of the family. Banded polytene chromosomes have been previously searched for in *Culicoides sonorensis* Wirth & Jones (W. J. Tabachnick, personal communication) and *Bezzia* sp. (W. L. Grogan, personal communication) without success. As such, it appears that the family may indeed lack banded polytene chromosomes. It is only preliminary assumption because cytogenetic studies on the chromosomes of Ceratopogonidae are very rare

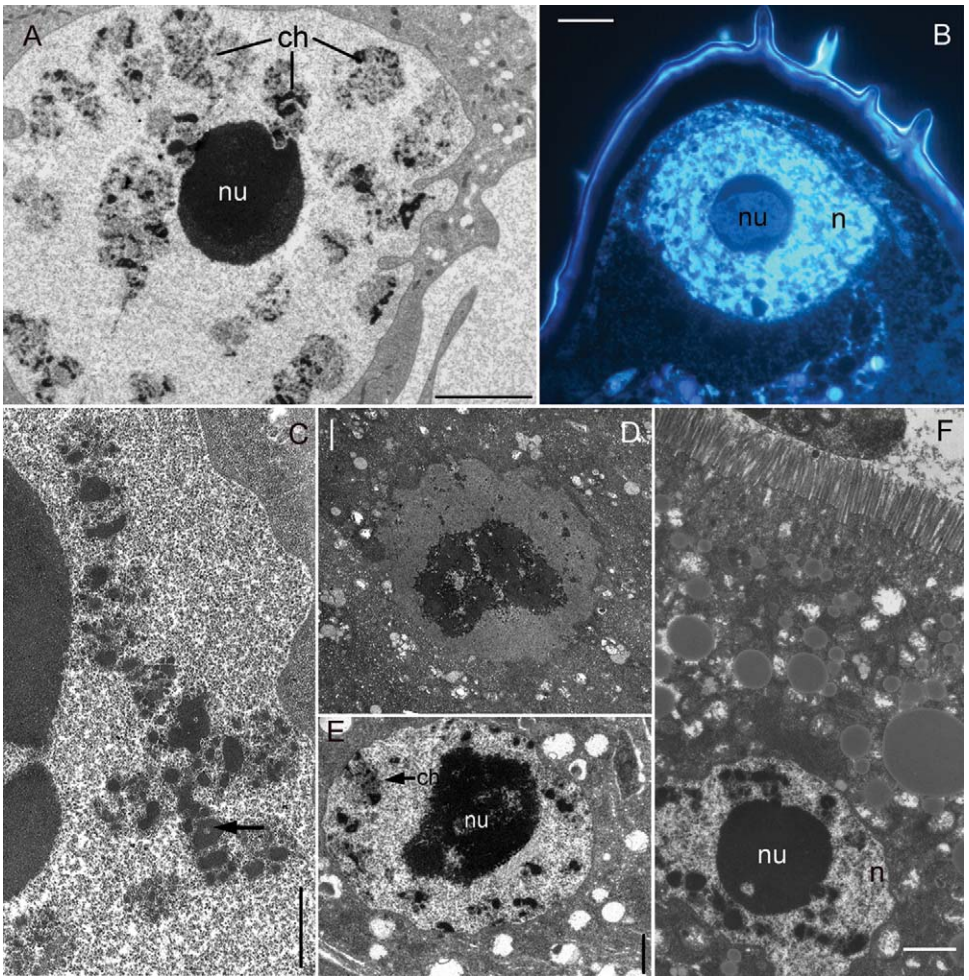

Fig. 2. (A) TEM photomicrograph of the polytene nucleus of the glandular cell of a secretory seta of a *Forcipomyia nigra* larvae. (B) Light microscope photomicrograph of the nucleus of the glandular cell of a secretory seta stained with DAPI. (C–F) TEM photomicrographs of (C) polytene chromosomes (arrow) in the nucleus of a glandular cell (D) nucleus of a typical trichogen cell, (E, F) polytene nucleus of a midgut cell (arrow in E indicates a polytene chromosome). ch = polytene chromosome, n = nucleus, nu = nucleolus. Scale for (A) = 5 μm, (B) = 10 μm, (C-F) = 2 μm. (Online figure in color.)

and therefore further research particularly into potential differences in the process of transcription would be required.

The chromosomes of *Forcipomyia* resemble the polytene chromosomes of some species of plants which also lack bands and where the intimate synapsis between the sister chromatids are probably not present (Barlow 1975, Gostev and Asker 1978, Carvalheira 2000). One reason there is no typical banding pattern in these is low multiplicity, lack of tight pairing, and lower levels of highly endoreplicated chromatids (Therman and Murashige 1984, Guerra 2001). In *Forcipomyia* darker regions of condensed heterochromatin (Fig. 1D–F) are additionally similar to those of plants cells (Guerra 2001). The lack of transverse banding patterns in *Forcipomyia*, and possibly all Ceratopogonidae, limits their application for gene mapping in this family. However, well developed and clearly visible fibers of chromatin could serve for the study of the distribution of different DNA sequences (Guerra 2001). We hope that our preliminary report on the polytene chromosomes will promote further studies on polyteny in biting midges and other families of the infraorder Culicomorpha.

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