

**BEHAVIOUR OF THE SPERMATOCYTE CHROMOSOMES OF THE
MAYFLY, *CLOEON DIPTERUM* (LINNAEUS, 1761) s.l.
(EPHEMEROPTERA: BAETIDAE), WITH A NOTE ON
THE CYTOLOGY OF THE ORDER**

B. KIAUTA and A.W.M. MOL

Department of Animal Cytogenetics and Cytotaxonomy,
University of Utrecht, Padualaan 8, Utrecht,
The Netherlands

Received May 16, 1977

The material studied ('s-Hertogenbosch and Utrecht, the Netherlands) morphologically resembles *C. cognatum* Stephens as characterized by SOWA (1975. Ent. scand. 6: 215-223). The male chromosome numbers (from ultimate stage larvae) are: $2n\sigma = 8 + X + Y$, $n(I) = 4 + XY$, $(II) = 4 + X$, or $4 + Y$. The primary and secondary spermatocyte chromosome behaviour is described and figured. A review is given of the hitherto cytologically studied members of the Order, and the chromosome conditions are discussed from the point of view of the phylogenetic affinities of the taxa involved. It is shown that any cytogenetic affinities between the 2 palaeopteran orders, Ephemeroptera and Odonata, are completely lacking. The available evidence on the chromosome cytology of the thysanuran *Zygentoma* is insufficient to enable any comparison with the Ephemeroptera.

INTRODUCTION

The mayfly chromosome cytology has so far hardly received any attention, and the karyotypes of only five species, referable to three families, have become known (cf. Tab. I). This is all the more unfortunate since, along with dragonflies, the ephemeropterans are the most ancient order of the living pterygote insects. They are remotely allied to the dragonflies, but stand entirely apart from all other orders (cf. e.g. MARTYNOV, 1938; HENNIG, 1969). From the cytotoxic and phylogenetic points of view, therefore, a systematic inquiry into

their cytology would be of particular theoretic interest.

Cloeon dipterum is one of the most common European members of the Order, hence it is not surprising that it is one of the five species examined cytologically. Aside from an early note by BERNHARD (1907), its cytology has been described in extenso by WOLF (1960). Nevertheless, Wolf's publication deals, in the first place, with the oogenetic and cleavage stages, and includes but a passing reference to the male (spermatogonial and spermatocyte) complement. For this reason it seems opportune to bring our observations on record in the present note.

The observations are based on lacto-acetic-orcein squash preparations of gonad tissue of ultimate stage larvae (5), collected in the surroundings of the cities of 's-Hertogenbosch (April 18, 1977) and Utrecht (April 21-28, 1977), the Netherlands.

The taxonomic status of the Linnaean *Cloeon dipterum* is uncertain, and it is not unlikely that several closely allied but distinct species and/or infraspecific taxa are united under this classical name. The problem has recently been reviewed by SOWA (1975). For the sake of convenience and in view of the small sample studied in the present note we have refrained from a further taxonomic



Fig. 1. *Cloeon dipterum* (L.) s.l., adult male (Utrecht, the Netherlands), resembling *C. cognatum* Stephens.



Fig. 1. *Cloeon dipterum* (L.) s.l., adult male (Utrecht, the Netherlands), resembling *C. cognatum* Stephens.

analysis of our material, referring it to *C. dipterum* in its classical broader scope. However, our larvae do appear similar to the description and figure 8 given by SOWA (1975) for *C. cognatum* Stephens (cf. also Fig. 1).

OBSERVATIONS

As recorded earlier (WOLF, 1960), the spermatogonial karyotype formula is $2n \delta = 8 + X + Y$, whereas in the spermatocytes $n \delta$ (I) = $4 + XY$, and $n \delta$ (II) = $4 + X$, or $4 + Y$.

Pachytene is the earliest analyzable meiotic stage in our material. The bivalents are often organized in a bouquet. They are more or less similar in size and shape, save for a weakly stained section (secondary constriction) in one of them. The latter is located in the subterminal position, and it is not unlikely, though uncertain, that this element represents the sex bivalent (Figs. 2-3).

At diplotene (Fig. 4) the centromeres of four bivalents appear in terminal position, whereas one bivalent is subacrocentric. This is at variance with the situation described by Wolf, where all elements were telocentric.

At diakinesis (Figs. 5-6) a single chiasma occurs in most bivalents, but in at least one of them two chiasmata are recognizable (Fig. 5).

Chiasma terminalization takes place nearly simultaneously in four metaphase I bivalents, and is somewhat slower in the fifth (Figs. 7-10). The segregation of bivalents is clearly precocious in one bivalent; this possibly is the sex bivalent (Figs. 11-12), the more so, since the two halves are of slightly different size. It is followed by three other bivalents, whereas segregation in the largest bivalent is considerably later than in others, causing many figures to have nine elements at this stage (Fig. 13).

It is probable that at anaphase I the heterochromosomes segregate precociously to the opposite poles in the prereducational way, though, due to their similar size (cf. Figs. 11-12), their identification is uncertain either at this (Fig. 14) or at the subsequent stages of prometaphase II (Fig. 15) or metaphase II (Fig. 17).

At telophase II, the elements of the two daughter cells form a characteristic rosette (Fig. 17).

NOTES ON THE CHROMOSOME CYTOLOGY OF THE ORDER

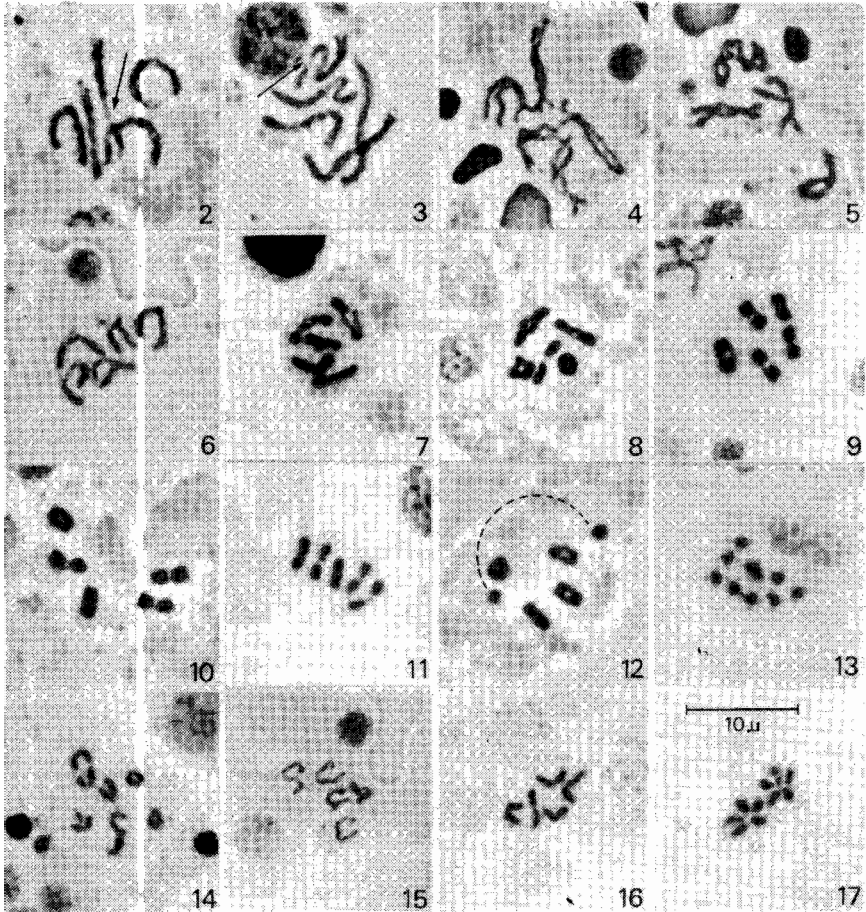
RELATIONSHIPS WITHIN THE ORDER

The cytologically examined mayfly material is far from being sufficiently representative for the Order. Nevertheless, even so, a few general features of the

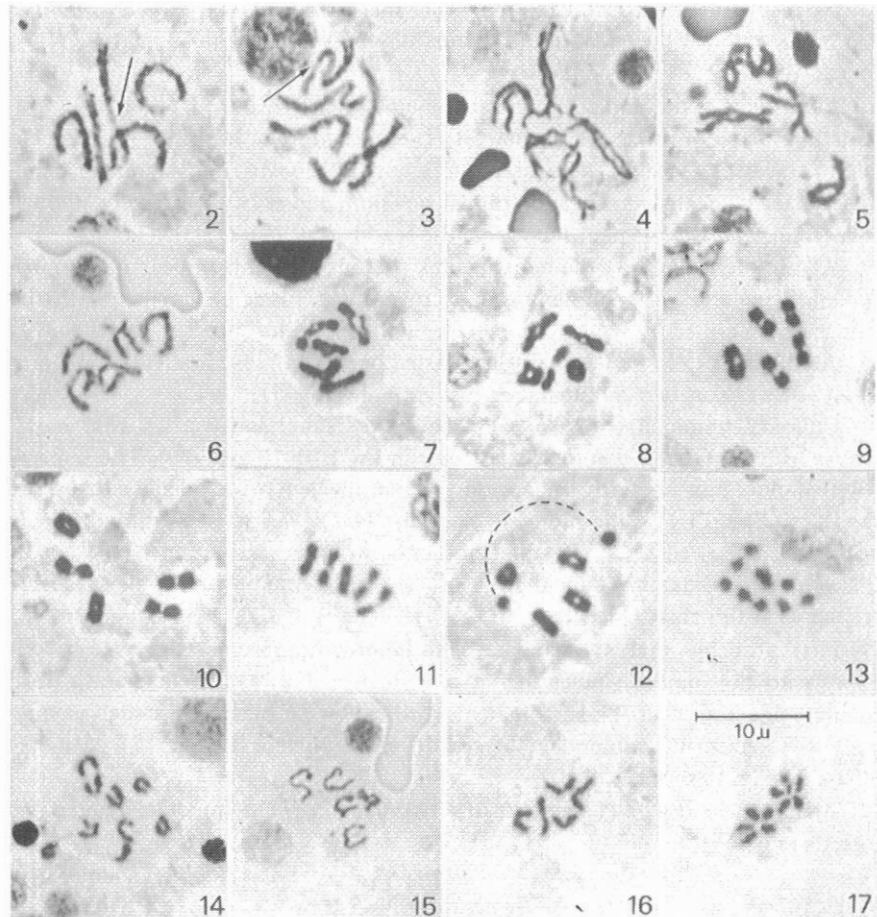
mayfly chromosome cytology became apparent.

In Table I a review is given of the hitherto studied taxa, referable to four genera, three families and two superfamilies. Their phylogenetic relationships are shown schematically in Figure 18.

Various authors have presented different classifications of the higher categories (e.g. EDMUNDS, 1962; LANDA, 1973; RIEK, 1973), but in all systems



Figs. 2-17. Spermatocyte stages of *Cloeon dipterum* (L.) (fig. 13 material from 's-Hertogenbosch, all others from Utrecht; lacto-acetic-orcein squash; 1500 X): (2-3) Pachytene (note secondary constriction in one of the bivalents, indicated by arrow); - (4) Diplotene; - (5-6) Diakinesis; - (7-10) Metaphase I polar view; - (11) Metaphase I, lateral view; - (12) Metaphase I, with one element precociously segregated; - (13) Metaphase I, all bivalents but one segregated; - (14) Anaphase I; - (15) Prophase II, - (16) Metaphase II; - (17) Telophase II.



Figs. 2-17. Spermatocyte stages of *Cloeon dipterum* (L.) (fig. 13 material from 's-Hertogenbosch, all others from Utrecht; lacto-acetic-orcein squash; 1500 X): (2-3) Pachytene (note secondary constriction in one of the bivalents, indicated by arrow); - (4) Diplotene; - (5-6) Diakinesis; - (7-10) Metaphase I polar view; - (11) Metaphase I, lateral view; - (12) Metaphase I, with one element precociously segregated; - (13) Metaphase I, all bivalents but one segregated; - (14) Anaphase I; - (15) Prophase II, - (16) Metaphase II; - (17) Telo-phase II.

Table I
 Review of the hitherto cytologically studied may-fly species (Classification after EDMUNDS, 1962)

Superfamily	Family	Species	n ♂	Sex determination	References
HEPTAGENIOIDEA	Siphonuridae	<i>Ameletus costalis</i> (Matsumura, 1931)	9	XY/XX	KATAYAMA, 1939
	Baetidae	<i>Baetis rhodani</i> (Pictet, 1843-1845)	5	XY/XX*	BOHLE, 1969
		<i>B. vernus</i> Curtis, 1834	5	XY/XX*	BOHLE, 1969
		<i>Cloeon dipterum</i> (Linnaeus, 1761)	5**	---	BERNHARD, 1907
EPHEMEROIDEA	Ephemeridae	<i>Ephemera danica</i> Müller, 1764	5	XY/XX	WOLF, 1960
			5	XY/XX	this paper
			6***	XO/XX	WOLF, 1946

* deduced from spermatocyte and oocyte chromosome numbers; -- ** deduced from oocyte chromosome number; -- *** deduced from spermatogonial and oogonial complements.

the *Siphonuridae* are placed as the most plesiomorphic family, representing a modern survival of the most ancient stem of the order. The Ephemeroidea are a distinct group, not necessarily primitive, but rather specialized in a distinct way. There are, however, difficulties in determining the position of the *Baetidae*. According to BURKS (1973), the family is likely to be of recent origin and is at present in the process of rapid evolution, while RIEK (1973), on the other hand, suggested the possibility that the family is but an artificial group, composed of genera placed together for convenience.

In insect orders characterized by monokinetik chromosomes, higher chromosome numbers are usually found in more "ancient" taxa, though there are many exceptions to this rule, while the situation is just opposite in those possessing holokinetik chromosomes (KIAUTA, 1974). It is interesting, therefore, that the single siphonuride species studied has the highest chromosome number yet known in the Order.

The chromosome numbers and, particularly, the mode of sex determination

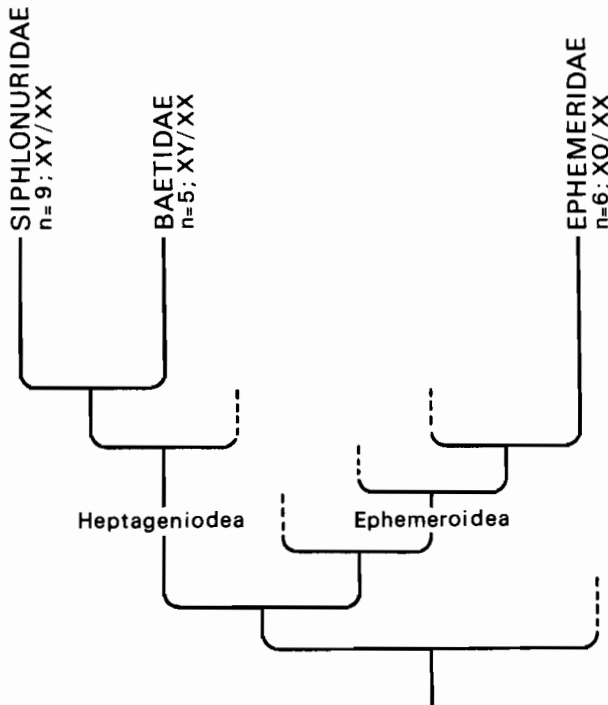


Fig. 18. Phylogenetic affinities in the cytotypologically studied ephemeropteran taxa (schematically after EDMUNDS; 1962).

in *Ephemera* may also indicate the distinct position of the family, though more material should have been examined before any speculation could be made in this field.

CYTOLOGY OF EPHEMEROPTERA IN VIEW OF THE CHROMOSOME
CONDITIONS IN THE PHYLOGENETICALLY "ALLIED"
ODONATA AND "THYSANURA"

Structurally, the mayflies are hardly related to any pterygote order. They show certain remote phylogenetic affinities solely with dragonflies – the only other palaeopteran group. As demonstrated in Table II, however, any cytogenetic similarities between the two orders are completely lacking.

As suggested e.g. by HENNIG (1969) the classical apterygote Thysanura are an artificial group, consisting of the more "original" Archaeognatha on one hand, and the Zygentoma on the other. The latter are considered a sister group of the Pterygota, with which they are classified into Dicondylia. Archaeognatha and Dicondylia form the Ectognatha, the sister group of Entognatha, which covers the apterygote Collembola, Protura and Diplura.

Table II

Comparison of the main cytogenetic features of Ephemeroptera and Odonata

Feature	Ephemeroptera <i>Coverage:</i> 5 species, referable to 3 families (out of 23 living; TSHERNOVA, 1970)	Odonata ¹ <i>Coverage:</i> approximately 500 species, referable to 22 families (out of 28 living)
Centromere condition	Localised	Diffuse
Chiasmata and recombination indices (RI)	Chiasmatic meiosis in both sexes; RI uncertain: probably $2n < RI < 3n$ in δ , $RI \geq 2n$ in ♀ (XY/XX species)	Chiasmatic meiosis in both sexes; $RI = 2n-1$ in ♀ (in primary complements only)
Type number and range	Type number uncertain; range 5-9	Type number $n = 13$; range 9-14 (in primary complements)
Sex determination	XY/XX in most species; XO/XX in one species (family); δ heterogametic	XO/XX in primary complements; δ heterogametic
Mode of reduction of the sex element(s)	Prereduction	Postreduction

¹ KIAUTA, 1972

This being so, the *Zygentoma* are considered more closely related to the ancestors of the Pterygota than are the Archaeognatha, hence they are phylogenetically "related" to the Ephemeroptera. Unfortunately, too little information is available on the cytology of the former, therefore any comparison is impossible.

Taking into account the chromosome picture of the two thysanuran groups, the lower chromosome number of Archaeognatha (cf. ARGILAS, 1941) would indicate that these are less "original" than generally supposed. This conclusion is in perfect agreement with the opinion expressed recently by BIRKET-SMITH (1974), based on the morphology of the somatic abdominal musculature, who suggested that the Ephemeroptera are closer to Archaeognatha than to *Zygentoma*.

ACKNOWLEDGEMENT

For photographic assistance with Figure 1, thanks are due to Mrs. T. ROMANIUK, Department of Population Biology and Biosystematics, University of Utrecht.

REFERENCES

- ARGILAS, A., 1941. Contribution à l'étude de *Dilta littoralis* Wom. (Thysanure Machilidae). Bordeaux, 226 pp.
- BERNHARD, C., 1907. Über die vivipare Ephemeride *Cloeon dipterum*. *Biol. Zbl.* 27: 467-479.
- BIRKET-SMITH, S.J.R., 1974. On the abdominal morphology of Thysanura (Archaeognatha) and Thysanura s. str. *Ent. scand.*, Suppl. (6): 5-67.
- BOHLE, H.W., 1969. Untersuchungen über die Embryonalentwicklung und die embryonale Diapause bei *Baëtis vernus* Curtis und *Baëtis rhodani* (Pictet) (Baëtidae, Ephemeroptera). *Zool. Jb. Anat.* 86: 493-575.
- BURKS, B.D., 1973. Summary of Symposium on phylogeny and higher classification of the Ephemeroptera. *Proc. 1st. int. Conf. Ephemeroptera*, pp. 179-181. Brill, Leiden.
- EDMUNDS, G.F., 1962. The principles applied in determining the hierarchic level of the higher categories of Ephemeroptera. *Syst. Zool.* 11: 22-31.
- HENNIG, W., 1969. Die Stammesgeschichte der Insekten. Kramer, Frankfurt/Main.
- KATAYAMA, H., 1939. The sex chromosomes of a mayfly, *Ameletus costalis* Mats. (Ephemerida). *Jap. J. Genet.* 15 (3): 139-144.
- KIAUTA, B., 1972. Synopsis of the main cytotoxic data in the order Odonata. *Odonatologica* 1 (2): 73-102.
- KIAUTA, B., 1974. Introduction to insect cytotoxicity. Nepal Research Center, Kathmandu. XII + 81 pp.
- LANDA, V., 1973. A contribution to the evolution of the order Ephemeroptera based on comparative anatomy. *Proc. 1st. int. Conf. Ephemeroptera*, pp. 155-159. Brill, Leiden.
- MARTYNOV, A., 1938. Etudes sur l'histoire géologique et de phylogénie des ordres des

Insectes (Pterygota). 1. Palaeoptera et Neoptera – Polyneoptera. Acad. Sci. URSS, Moscou-Leningrad.

- RIEK, E.F., 1973. The classification of the Ephemeroptera. *Proc. 1st. int. Conf. Ephemeroptera*, pp. 160-178. Brill, Leiden.
- SOWA, R., 1975. What is *Cloeon dipterum* (Linnaeus, 1761)? The nomenclatural and morphological analysis of a group of the European species of *Cloeon* Leach (Ephemerida: Baetidae). *Ent. scand.* 6: 215-223.
- TSSHERNOVA, O.A., 1970. On the classification of fossil and recent Ephemeroptera. *Ent. Obozr.* 49 (1): 124-145.
- WOLF, E., 1946. Chromosomenuntersuchungen an Insekten. *Z. Naturf.* 1: 108-109.
- WOLF, E., 1960. Zur Karyologie der Eireifung und Furchung bei *Cloëon dipterum* L. (Bengtsson) (Ephemerida, Baëtidae). *Biol. Zbl.* 79: 153-198.

