The Sex Chromosomes of a May-fly, *Ameletus costalis* Mats. (Ephemerida)

By

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To the author's knowledge, nothing has ever been published about the chromosome composition from any species of the order Ephemerida; accordingly it is of course unknown which sex is heterogametic. From the viewpoint of insect evolution, the Ephemerida is said to be an order having an archaic nature, being related closely to the Odonata, the Plecoptera and the Neuroptera. The chromosome survey of the Ephemerida is therefore of great interest in connection with the systematic relation with the latter orders in which cytological investigations have already been carried out extensively.

The species selected as the material of this study is *Ameletus costalis* Mats., belonging to the family Siphlonuridae. In the present paper is recorded the chromosome composition in relation to sex determination in this interesting insect.

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Material and Methods

As is well known, the nymphs of the ephemerids are all aquatic. They spend their long nymphal life in the gravellar and stony beds in brooks and rivers, together with the nymphs of other kinds of insects, such as the Plecoptera, the Trichoptera and the Neuroptera. It is noted that one to three years are required for larval development. The greater part of life is thus passed beneath the surface of the water. After it emerges into the air the insect assumes the final adult form after one more ecdysis with winged state and the duration of adult life seems always rather brief.

Ameletus costalis Mats., the present material, is very common in its occurrence, the nymphs leaving the water at the middle of April. The

material for study consists of the testes and the ovaries obtained from old nymphs which were collected during from the last week of March to the beginning of April (1938) in the River Muko near Kobe. The nymphs were dissected out immediately after collecting, with the aid of the dissecting microscope, and the gonads together with the adhering viscera were dropped into vials containing appropriate fixatives. Carothers' solution and Flemming's solution with Benda's formula were chiefly employed for fixation and proved to be good. Following the usual treatment of the paraffin method, they were cut 5–8 micra in thickness and stained according to the iron haematoxylin method after Heidenhain using light-green as the counter-stain.

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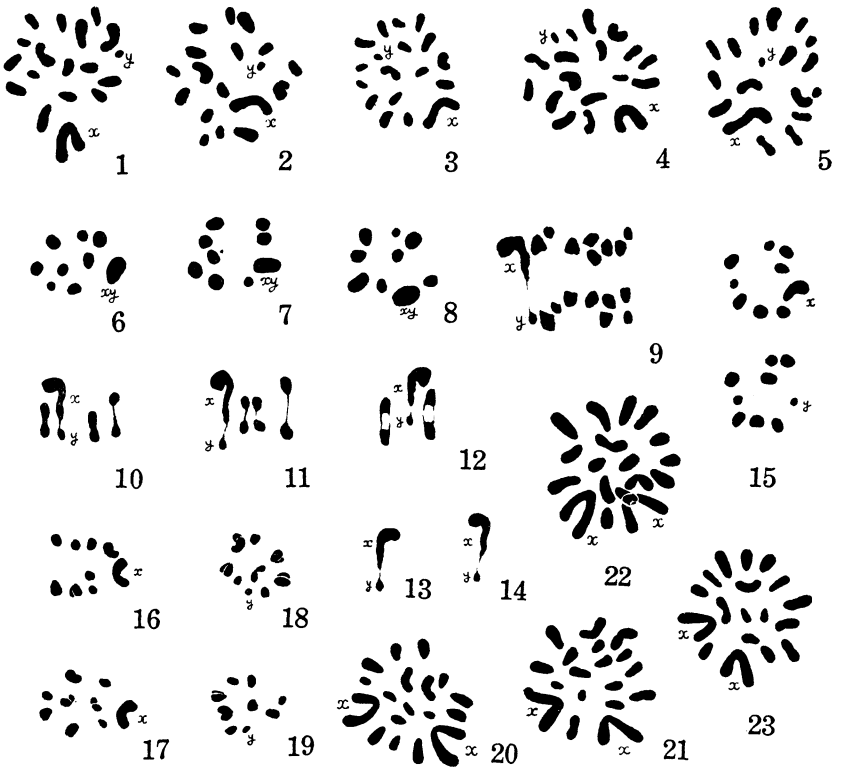
Observations

The spermatogonium: In the metaphase equatorial plate of the spermatogonium are always observed 18 well-defined chromosomes of varying size and shape which take the radial arrangement (Figs. 1–5). The number 18 is exactly constant without any numerical deviation from the basic number. In a complete garniture, as seen in the annexed figures, three elements at least are recognized as being atelomitic in fibre attachment, appearing in a distinct V-shape, while the remaining 15 elements seem to be of telomitic type, assuming rod-shape with tapering end. Of the V-shaped elements one is extremely large, bearing submedian attachment constriction, its corresponding mate cannot be found, but the remaining two of medium size evidently form a homologous pair. Among the telomitic chromosomes, 14 elements constitute seven homologous pairs as determined by comparison of their shape and size, but the last smallest one, again, is without a corresponding one of homologous size. It is these two particular chromosomes, the largest V and the smallest rod, that constitute a heteromorphic pair when homologous chromosomes conjugate and transform into tetrads of ordinary forms. In other words, there are found in a garniture eight homologous pairs composed of two medium sized V's and 14 rod's of varying size, and a particular pair composed of the largest V-shaped one and the smallest rod-shaped. That the latter are nothing other than the X and the Y becomes quite clear after observing their behaviour in the primary spermatocyte and comparing with the chromosome complement of the female cells. They are labelled *x* and *y* respectively in Figs. 1 to 5.



Figs. 1-23. The chromosomes of *Ameletus costalis*. Camera lucida drawings (Zeiss 1.5 mm *obj.* and K20×*oc.*), 4200 ×. 1–5, spermatogonial chromosomes, metaphase polar views. 6–8, primary spermatocyte chromosomes, metaphase polar views. 9, side view of primary spermatocyte chromosomes in anaphase (all elements shown). 10–12, side views of primary spermatocyte chromosomes in meta-anaphase showing *xy*-complex (not all elements drawn). 13–14, side views of *xy*-complex in primary spermatocyte meta-anaphase. 15, sister chromosome groups in anaphase of primary spermatocyte, polar view. 16–17, secondary spermatocyte chromosomes, metaphase polar views; *x*-class. 18–19, the same, *y*-class. 20, oogonial chromosomes, metaphase polar view. 21–23, chromosomes in the cells of follicle epithelia in female. *x*=X-chromosome. *y*=Y-chromosome.

The oogonium and the female somatic cell: The diploid complement of the female was observed in the oogonium as well as somatic cells of the follicle epithelia in the ovaries, for the purpose of comparison with that of the male. In every one of these cells, the metaphase equatorial plate shows constantly 18 chromosomes, consisting of four V's, two extremely large and two medium-sized, and 14 rods with various length (Figs. 20–23). The diploid number of the female is thus the same as that of the male, but between the two sexes there occurs an important difference in respect to the sex chromosomes. When the homologous mates are paired according to their shape and size, it will readily be noticed that all 18 chromosomes constitute nine homologous pairs. Comparing with the male complex, the female differs

from it without the least ambiguity in respect of possessing the largest V elements or the X in a coupled state, and lacking the smallest rod element corresponding to the Y. The XX condition of the female was thus established.

The primary spermatocyte: In the polar view of the primary spermatocyte metaphase the chromosomes appear in distinct clearness well apart from one another and show constant size differences. They are nine in number without exception (Figs. 6–8). Of these elements eight take the ordinary bipartite aspect, appearing as such in lateral view of the metaphase plate, while the remaining one, lying always at the periphery of the spindle and possessing the largest magnitude, shows a quite peculiar form. In polar view it exhibits rather an oblong outline (Figs. 6–8, *xy*). When viewed from the side of the spindle, it is clearly recognized as an asymmetrical tetrad composed of a large V-shaped element associated with a minute one, in striking contrast to the other eight ordinary tetrads (Figs. 9–14). It is legitimate to consider that this asymmetrical tetrad is no other than the X-Y complex resulting from the conjugation of the largest atelomitic X with the minute telomitic Y, which were found as an unequal pair in the spermatogonial cell. The X element maintains its atelomitic nature, and at the free end of the longer arm it comes in contact with an end of the telomitic Y. The mode of the conjugation between X and Y is so quite clearly recognizable in Fig. 9 to Fig. 14, that any further description may be unnecessary.

In the ensuing division the autosomal bivalents separate symmetrically into their daughter halves. The X-Y bivalent also disjoins, X and Y passing to the opposite poles (Fig. 9, side view of the sister chromosome groups; Fig. 15). The separation of chromosomes is synchronous in all elements: the X and Y chromosomes neither lag behind nor precede the autosomes.

The secondary spermatocyte: The asymmetrical distribution of the X and Y chromosomes in the first division results in the production of two kinds of secondary spermatocytes, with nine chromosomes in each case. They differ from each other with regard to the sex chromosome which they contain. The one kind, the X-class, consists of eight autosome dyads plus an X-element which is quite remarkable in its conspicuous V-shape (Figs. 16–17), while the other, the Y-class, includes a minute Y element in addition to a similar complex of autosome dyads (Figs. 18–19).

On the basis of findings obtained from the above observations, it will be easily induced that heterogamety occurs in the male sex and thus the sex-chromosome formula of this species is indicated by XY-XX.

Remarks

Since our knowledge on the chromosomes of the Ephemerida has been *nil* prior to the present observations, one is absolutely unable to compare the present species with any other one of this order so far as the chromosomes are concerned.

The Ephemerida is considered as an order having a close relationship with the order Odonata. The present study was commenced, therefore, with the expectation that some resemblance would exist between these two orders. This expectation, however, was not satisfied during the course of the investigation, as the chromosome complex of *Ameletus costalis* is rather motley or polymorphic due to the coexistance of V's and rods of varying size, in striking contrast in this respect to the dragonflies in which isomorphic karyotype has been widely recorded (Oguma '30, Oguma and Asana '32, Asana and Makino '35). So far as the karyotype is concerned, the May-fly, *Ameletus costalis* much resembles that of the Plecoptera (Nakahara '19, Junker '23, Itoh '33) and the Neuroptera (Naville et de Beaumont, '33, '36, Kichijo '34, Asana and Kichijo '36), rather than the Odonata. In addition to this, the Ephemerida and the Plecoptera practically show a close resemblance in the anatomical features of the testis as may be readily recognized in comparison of *Ameletus* and *Acroneuria* (Plecoptera), studied by Itoh ('33), both having testes similarly composed of spherical follicles surrounding a vas deferens.

In view of the heterogamety the present investigation has revealed that so far as *Ameletus costalis* is concerned, the Ephemerida evidently belongs to the same category with its allied orders Odonata, Plecoptera and Neuroptera as it occurs in the male sex, and absolutely differs from the Trichoptera. In the Neuroptera the XY condition of the male has been known as a common occurrence throughout the studied cases. In the Plecoptera, in which three species have hitherto been cytologically studied, three different types of sex chromosomes, such as X-Y, XX-O and X-O, have been recorded. The Odonata shows a striking uniformity in having X-O type of the sex chromosome, and the X chromosome of this group is characterized by its post-reductional behaviour. In respect of the evidence that the XY constitution of the sex chromosome is discovered in *Ameletus* at least, therefore, the Ephemerida seems to be related most closely to the Neuroptera having X-Y formula. But the X element of the Neuroptera is represented in all cases by one medium sized or smaller one with the rod-shape. Such Neuropteran characteristics of the X chromosome by no means agree with what is found in the X-chromosome of the May-fly, *Ameletus costalis,* in which it is represented by the largest chromosome of V-shape having a submedian fibre

attachment.

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和 文 摘 要

片山久男： マヘグロフタヲカゲロフ（蜉蝣目）の性染色體[1]

マヘグロフタヲカゲロフ（*Ameletus costalis* Mats.）の精原細胞染色體數（♂, 2*n*）は 18 で核型は 1V+2*v*'s+15*r*'s（V, *v*; 夫々大形及び小形 V 形染色體. *r*; 小形棒狀染色體）の構成を持つてゐる。雌の 2*n* 染色體數はやはり 18 であるが, 2V's+2*v*'s+14*r*'s の構成を持つてゐる。雄と比較すると大形の V が雌に 1 個多く, 最小の *r* は雄に 1 個餘計ある。卽ち雄に於いては最大形の 1 個の V が X を, 最小の *r* が Y を代表し, 雌に於いては最大形の 2 個の V が XX を表はすものである事は雄の成熟分裂の研究によつて明白である。卽ち第一精母細胞分裂の側面觀を見ると 9 個の四分染色體の中最大の 1 個だけが X-Y 複合體を形成してゐる。X は V 形でその一方の腕に小形の粒狀染色體（Y）が結合してゐる。第一分裂では X と Y は必ず分離するので, 第二精母細胞には X を含む細胞と Y を含む細胞を生ずる。從つてそれより生ずる精子にも X と Y に關し 2 型が區別されるわけで, これによつて現在まで未知であつた蜉蝣目は雄性接合子が XY 型（ヘテロ）である事が確立された。

本種の核型を近緣關係にある蜻蛉目積翅目及び脈翅目等に比較すると, 前者よりもむしろ後の二者に類似した所の事實が見出される事は興味がある。

1) 兵庫縣川邊郡塚口 岡崎牧場內生物學研究室業績第 3 號.