A COMPARATIVE STUDY OF THE SPERMATOCYTE CHROMOSOME IN ALLIED SPECIES OF THE DRAGONFLY[1)]

By

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With 11 figures in Text

Some fifteen years ago, 1915, I published, in the Japanese zoological magazine, the results of a study on the spermatocyte chromosomes of dragonflies. The material at that time consisted of seven species of Libellulidæ and one of Æschnidæ, and the results are to be summarised as follows: 1, the chromosome number is thirteen (in haploid) in all the species studied, except only one, *Sympetrum pedemontanum*, in which I count twelve chromosomes; 2, the size of the smallest chromosome in a chromosome garniture varies from species to species, in some species it looks like a spot while in other it is hardly distinguished from the next small chromosome by its magnitude; and thus the case of *Sympetrum pedemontanum* may show an example in which it entirely disappears by extreme diminution; 3), the X-chromosome shows apparently constant size in different species.

Since that time some additional material has been gradually accumulated. The studied species now have been increased to sixteen in number, covering three families and eight genera, in which the present study has been carried out.

The chief purpose of the study was to know what relation is present between closely allied species belonging to one genus? Or, whether there is present any kind of the chromosomal difference between those

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species parallel to the taxonomic difference? I collected, therefore, at least two species from a genus, except the genus *Anotogaster*, which has only a single representative in Japan.

In the draganfly, it is very difficult to attempt to indentify any of two allied species of a genus in their nymphal stage, in which the early course of spermatogenesis is to be traced. To avoid error of identification of species, I used the testes of imagos as the material of study. As is well known, the imaginal life of the dragonfly is tolerably long; after emergence from the nymph the adult lives several days with rather soft skin, this stage being usually called the immature form by systematists. The immature adults show no sexual behavior at all. If we dissect them in this stage we can nearly always find the testes in which the later course of spermatogenesis is still going on, and consequently the dividing figures of the spermatocytes, both primary and secondary, are to be observed. The spermatogonial division, however, can hardly be discovered. So the material from the so-called immature adults does not serve for the study of the spermatogonial division. For this reason the present study was naturally restricted in observation to the spermatocyte chromosomes.

The observed species are as follows:

Family LIBELLULIDÆ

Subfamily Libellulinæ

Genus *Orthetrum*
1. 0. *albistylum*
2. 0. *japonicum*

Genus *Sympetrum*
3. *S. pedemontanum*
4. *S. frequense*

Genus *Libellula*
5. *L. quadrimaculata*
6. *L. angelina*

Subfamily Cordulinæ

Genus *Somatochlora*
7. *S. viridiænea*
8. *S. uchidai*

Family ÆSCHNIDÆ

Genus *Gomphus*	9. *G. melampus* 10. *G. suzukii* 11. *G. unifasciatus*
Genus *Anotogaster*	12. *A. sieboldii*

Family CALOPTERYGIDÆ

Genus *Calopteryx*	13. *C. atrata* 14. *C. cornelia*
Genus *Mnais*	15. *M. strigata* 16. *M. costalis*

The material was taken out from the abdomen by vivisection and then fixed with Flemming's strong solution, and Hermann's mixture. Sometimes the mixtures of Carnoy and Gilson were frequently employed, especially in the case when I traveled for collection of material. Osmic vapor was often applied for the smear preparations in addition to the liquid above mentioned. For staining I have used to advantage Heidenhain's hæmatoxylin, thionin and safranin.

In brief, the result of study on the new material obtained after the previous investigation (1915) shows a fair coincidence to that of the latter, and suggests to us how the chromosome number changes from species to species in the dragonfly.

I. DESCRIPTIONS OF CHROMOSOMES

a. Family LIBELLULIDÆ

Eight species, separated into two subfamilies, were observed in this family. The number of chromosome is thirteen in seven out of eight species. The chromosomes of a garniture may be classified into four kinds, viz. a chromosome of enormous size, which is often called

M-chromosome, a remarkably small one, ten of intergrading size and one X-chromosome. These chromosomes are so arranged in the metaphase of primary spermatocyte that they constitute two concentric rings, of which the outer consists of chromosomes of rather large size in comparison with those making up the inner ring. The X-chromosome is found without exception in the outer ring.

The individual autosome assumes a lozenge shape with round corners when viewed *en face*, while X-chromosome looks like a rod. Observed from the pole of division the autosome takes on an appearance of a rod with blunt ends and a slight median constriction (Fig. 1, *b*, *c* & *i*). In a well differenciated preparation we can find a clear line through the axis of the each chromosome (See Fig. 1, *d*; Fig. 2, *b*; Fig. 5, *b*), thus revealing its double nature. The first step of the first division is indicated by the appearance of a clear space at the center of lozenge (See Fig. 2, *b*). In this stage, therefore, it takes on somewhat the similar appearance of a cross tetrad commonly found in Orthoptera. But in reality the lozenge shaped chromosome corresponds rather to the vertical ring tetrad in Amphiba, and not to cross tetrad of other insects.

The chromosomes found in metaphase of the secondary spermatocyte present very different form as compared with the former. All are rod shaped, and arranged on the equatorial plate with their long axes parallel to the spindle axis. Consequently, in their polar view they look like round chromosomes with rather small diameters in contrast to their length (Fig. 1, *e*,*f*; Fig. 2, *c*; Fig. 3, *e*; Fig. 4, *d*; Fig. 5, *d*,*f*). When the division sets in, here arises a striking difference between two subfamilies as to the mode of separation of dividing halves.

In the case of Libellulinæ, the middle portion of a rod-shaped chromosome gradually becomes thinner and constricted off at last in the similar way to pull off any viscous substance (Fig. 1, *f*; Fig. 3. *f*; Fig. 4, *e*). This mode of separation of chromosomes may be called the libellulid type. In the case of Cordulinæ, on the contrary, the rod-shaped

chromosome in the secondary spermatocyte possesses a transverse clear splitting at the point where the separation takes place, and along this splitting the daughter halves separate from each other (Fig. 5, *g*, *h*). Therefore, in early anaphase of this division, every daughter chromosome presents a straight truncated end in striking contrast to the chromosome with tapered end as seen in the libellulid type. Such a mode of separation seems to predominate in the family Æschnidæ, so far as I am aware, so it will be called the æschnid type in this paper.[1)]

The X-chromosome is divided into halves in the first division of spermatocyte, indicating no characteristic behavior as compared with the remaining autosomes. When arranged in the equatorial plate of the secondary spermatocyte it takes without exception an eccentric position (Fig. 1, *e*; Fig. 2, *c*; Fig. 3, *e*; Fig, 4, *d*; Fig. 5, *d*), and migrates to one pole in destitute of division (Fig. 1, *g*, *h*; Fig. 3, *f*; Fig. 4, *e*; Fig. 5, *g*, *h*).

Genus Orthetrum

1. *Orthetrum albistylum* (Fig. 1)

This is a single species in which the spermatogonial chromosomes were actually observed in the present study. The chromosome number of the spermatogonium is twenty five as readily expected from the number in the spermatocyte (Fig. 1, *a*). The X-chromosome could not be identified in this group of chromosomes owing to its similar feature to the autosomes.

The spermatocyte chromosomes found in the first maturation division are shown in Figs. *b* and *c*. Nine chromosomes are so arranged to make up the outer ring, in which the X-chromosome is always found. One chromosome of similar size to the X, labelled with *m*, always takes its position among the peripheral chromosomes, producing a perplexed appearance to the latter. This is the smallest autosome and is charac-

1) A short remark on this point has already been published (1917).

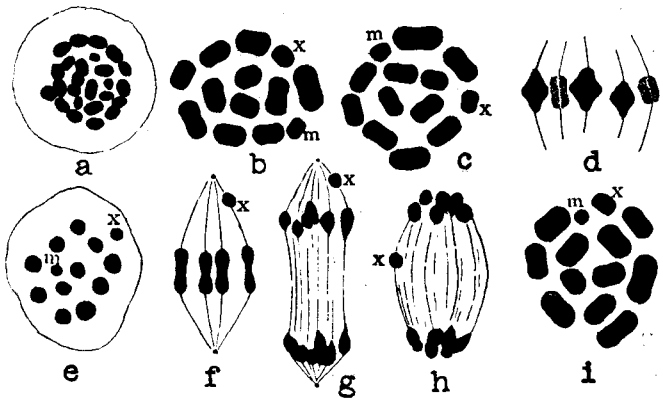

Fig. 1.

Orthetrum albistylum (a-h) and *Orthetrum japonicum* (i). *a*, Polar view of metaphase of spermatogonial division; *b-c*, polar views of metaphase group, primary spermatocyte division; *d*, side view of the same; *e*, polar view of metaphase group, secondary division; *f*, side view of the same; *g-h*, side views of the spindles of secondary division, in the former x-chromosome precedes to autosomes and in the latter it lags from autosomes. Zeiss Apoch. 1.5 mm. × Comp. Oc. 18, t. l. 160 mm.

terized by lozenge shape *en face* and possessing a clear splitting in its polar or lateral aspect.

In the secondary spermatocyte (*e*) the X-chromosome is extruded out of the autosomal group, and very early runs to the pole without division (*f*, *g*). Rarely it lags in its course of migration as shown in *h*. A similar occurrence is also pointed out by LEFEVRE and MACGILL (1908) in *Anax*.

2. *Orthetrum japonicum* (Fig. 1, *i*)

This is one of the closest allies to the preceding species from a taxonomical point of view. The two species, moreover, are found in similar habitats. It is very interesting, however, to observe the fact that these two simulating species are to be distinguished sharply from each other in observing the chromosomes under the microscope.

In the metaphase plate of the first division the chromosomes are found in the same number and arrangement (*i*). But a close observation reveals that there exists an important difference, by which this species is quite obviously distinguished from *Orthetrum albistylum* so

far as the chromosomes are concerned. As we already observed, the smallest autosome in the preceding species, acquires nearly equal size to the X-chromosome, while in the present species, on the countrary, it has a much smaller size—smaller than half of the X-chromosome. In this respect, therefore, it can never be mistaken for the preceding species. The X-chromosome assumes, on the other hand, nearly equal size in both of the two species, so the smallest autosome displays a most conspicuous characteristic in a garniture of chromosomes in the present species.

Genus Sympetrum

3. *Sympetrum pedemontanum* (Fig. 2)

The haploid number of chromosomes is thirteen as in *Orthetrum* (*a* and *c*). The relative magnitude of the X-chromosome to the autosomes shows no marked difference from cases of *Orthetrum*. But, if we turn our attention to the smallest autosome, we can notice a striking peculiarity there, that is the extreme diminution of size. One who looks at Fig. 2, *a*, will find a minute chromosome at the center of the equatorial plate, as I labelled with *m*. When viewed laterally its bipartite structure becomes very obvious (*b*). After the division it appears as a more minute figure in the equatorial plate of the secondary spermatocyte (*c*). It is so small that one can hardly distinguish it from the centrosome.

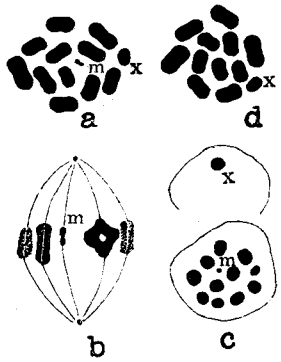

Fig. 2.

Sympetrum pedemontanum (a-c) and *Sympetrum frequense* (d). *a*, Polar view of metaphase group of chromosomes, primary division; *b*, side view of the same; *c*, two successive sections of secondary spermatocyte in metaphase; *d*, polar view of metaphase group of chromosomes, primary spermatocyte division. Zeiss Apoch. 1.5 mm. Comp. Oc. 12, t. l. 160 mm.

4. *Sympetrum frequense* (Fig. 2, *d*)

While the preceding species have a broad brownish band near the apex of the wing, the present species possesses no such opaque portion at all. In spite of such sharp

distinction, however, these two different species often copulate with each other. I have observed three examples of such copulation. It seems to suggest to us a very close phylogenetic relation.

The chromosome number of the spermatocyte is only twelve, one less than in all other libellulid species treated in this study. The smallest chromosome in a chromosome garniture of the primary spermatocyte is the X-chromosome, not the autosome as in the remaining species, as being so concluded from its structure and behavior.

Considering the extreme diminution of size of the smallest chromosome in *Sympetrum pedemontanum*, we can presume a case where the entire fading away of the smallest chromosome takes place through successive diminution of chromatin. The case of *Sympetrum frequense* may prove the case.

Genus Libellula

5. *Libellula quadrimaculata* (Fig. 3)

General feature of the chromosome resembles that of the genus *Orthetrum*, in correspondence with the systematic relation of the two species. Only the size of the largest chromosome seems relatively small, and frequently it can not be distinguished from the next largest

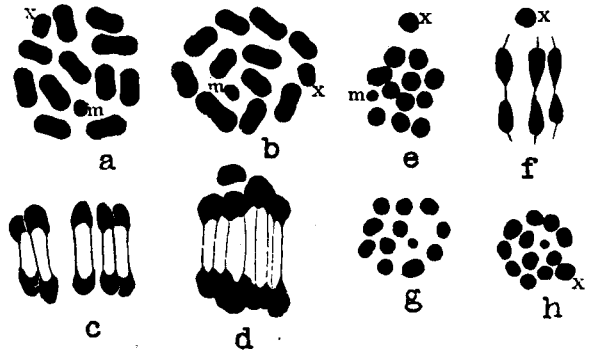

Fig. 3.

Libellula quadrimaculata. *a-b*, Polar views of metaphase group, primary division; *c-d*, side views of anaphase groups, primary division; *e*, polar view of metaphase group, secondary division; *f*, anaphase of the same; *g-h*, polar views of two sister chromosomes, secondary division. Zeiss Apoch. 1.5 mm. Comp. Oc. 12, t. l. 200 mm.

one. The chromosome number is thirteen, including X, in haploid condition (Fig. 3, *a* and *b*). Among these thirteen chromosomes one is represented by a considerably smaller (*m*). It is nearly always, although not conclusively, found inside of the larger chromosome ring in metaphase arrangement, while the X-chromosome, which is seen as the next smallest one, unexceptionally lies in periphery. The smallest autosome is clearly discriminated from the other chromosomes again in the secondary spermatocyte (*e*).

6. *Libellula angelina* (Fig. 4)

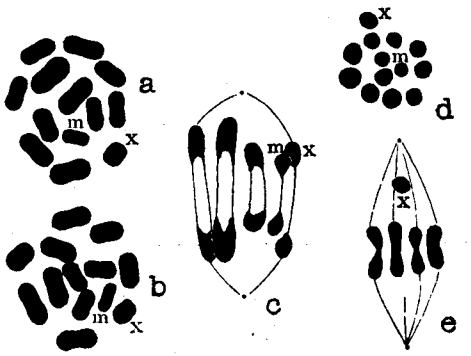

Fig. 4.

Libellula angelina. *a-b*, Polar views of metaphase group of chromosomes, primary division; *c*, side view of the same; *d*, polar view of metaphase group, secondary division; *e*, side view of the same. Zeiss. Apoch. 1.5 mm. Comp. Oc. 12, t. l. 200 mm.

Somewhat parallel to the difference of taxonomical features existing between this species and the preceding, differences in the chromosome are also found between them. The shape and the mode of arrangement on the equatorial plate of the first division allies to that of the preceding. But a sharp distinction can not be overlooked concerning the nature of the smallest autosome; it is not as small in its relative size, as in the preceding species. Measuring in the polar aspect of the chromosome it seems at least three times larger as compared with that of the preceding species; accordingly, the difference between the smallest and the next smallest chromosome or between the former and the X-chromosome is not so conspicuous.

Genus Somatochlora

7. *Somatochlora uchidai* (Fig. 5, *a-d*, *h*)

Out of thirteen chromosomes which are found in the primary spermatocyte (*c*), two are characterized by having roundish contour, in con-

trast to ordinary dumbbell-shaped chromosomes. Of these two, the larger one is the X-chromosome, while the smaller is the smallest autosome. The latter is quite small in magnitude, although still attaining larger volume than that of *Sympetrum pedemontanum*.

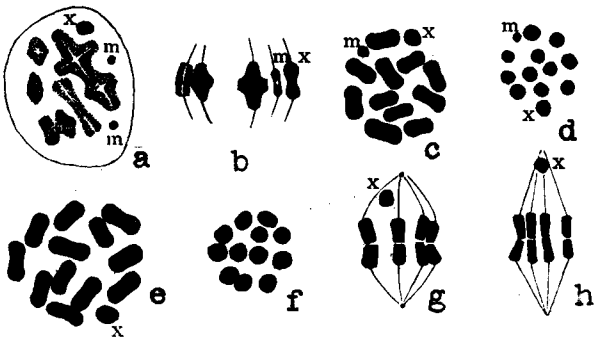

Fig. 5.

Somatochlora uchidai (a-d, h) and *Somatochlora viridiœnea* (e-g). *a*, Prophase of primary division, showing two m-chromosomes still separated; *b*, side view of metaphase, primary division; *c*, polar view of the same; *d*, polar view of metaphase group, secondary division; *h*, side view of the same; *e*, polar view of metaphase group, primary division; *f*, polar view of metaphase group, secondary division; *g*, side view of the same Zeiss Apoch. 1.5 mm. Comp, Oc. 12, t. l. 160 mm.

The gradatory size difference existing in the other autosomes seems to be as much in conspicuousness as in most of the Libellulidæ as already described. With this point too, the present species shows marked difference from the next one involved in the same genus.

8. *Somatochlora viridiœnea* (Fig. 5, *e*, *f*, *g*)

In the following three points at least, this species should be sharply distinguished from the preceding in nature of the chromosome. (1) All the chromosomes are much larger in size as readily recognizable in Fig. 5, *e*; (2) there is no conspicuously small autosome comparable to that found in the preceding species; (3) the size difference between every chromosomes is not so conspicuous as in the preceding.

These three peculiarities of the chromosome are to be seen more clearly in the secondary spermatocyte (*f*). In this chromosome garni-

ture, all twelve autosomes have apparently equal magnitude in striking contrast with Fig. 5, *d*. The X-chromosome is not drawn in this figure; it being arranged in different level with the remaining autosomes.

Family ÆSCHNIDÆ

Four species of this family were investigated, of which three are represented by the genus *Gomphus* and the remaining one the genus *Anotogaster*. In all these species the spermatogonial group of chromosomes could not be studied. The bivalent chromosomes, however, were observed with possible accuracy; they are as in the preceding family, lozenge shaped *en face*, and rod shaped in polar view, but not dumbbell shaped by median costriction as in Libellulidæ.

The chromosome number varies from ten to thirteen in haploid according to species. This is an additional datum that could be made out first in this study, since my previous study shows all investigated species, except one, possessing the constant number thirteen.

The mode of division of chromosomes of the secondary spermatocyte is æschnid type. Fig. 8, *e* and Fig. 9, *d*, will show this fact.

One X-chromosome is also found and it is divided in the first division (Fig. 9, *c*, *d*) but undivided in the second, just like the preceding family. No case where it lags in its migratory course against the remaining autosomes has been observed in this family. In every case it runs precautiously to one pole leaving the autosomes behind.

Genus Gomphus

9. *Gomphus melampus* (Fig. 6)

In the primary spermatocyte we find ten chromosomes including one X (*a* and *b*). This is the least number of chromosome throughout the material treated with in this investigation. The autosomes greatly differ from each other in their size, and the smallest one represents no conspicuous feature, even though being clearly discriminated from the others. The X-chromosome is much larger than the smallest autosome.

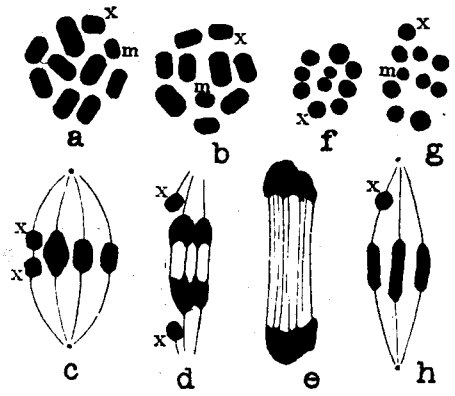

Fig. 6.

Gomphus melampus. *a-b*, Polar views of metaphase group, primary division; *c*, side view of metaphase group, primary division; *d*, anaphase of the same; *e*, telophase of the same; *f-g*, polar views of metaphase groups, secondary division; *h*, side view of the same. Zeiss Apoch. 1.5 mm. Comp. Oc. 12, t. l. 160 mm.

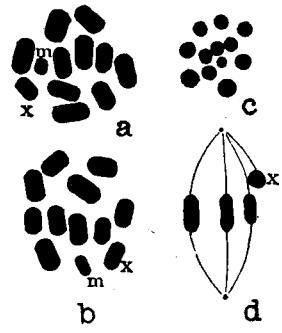

Fig. 7.

Gomphus suzukii. *a-b*, Polar views of metaphase groups, primary division; *c*, polar view of mataphase group, secondary division; *d*, side view of the same. Zeiss Apoch 1.5 mm. Comp. Oc. 12, t. l. 160 mm.

In the secondary spermatocyte (*c* and *d*) are found also ten chromosomes, among which *m*- and X-chromosome can be pointed out.

10. *Gomphus suzukii*[1] (Fig. 7)

In the material, labelled as *Gomphus melampus*, I found a testis which differs markedly from this species in respect to the chromosomes. Every spermatocyte in this testis, both primary and secondary, possesses twelve chromosomes instead of ten as is the case of typical *Gomphus melampus*. Not only in the number of chromosomes but also in the much smaller *m*-chromosome, this kind of spermatocytes is sharply distinguished from the latter, notwithstanding the testis was so labelled by the preparator of the material.

I supposed at first that the present case might be interpreted as certain two bivalent autosomes of ten chromosome species being split

1) This is a new species authorized by Prof. Matsumura (see my paper 1926).

into two original univalents, or in other words, two pairs of univalent chromosomes devoid conjugation at any event, so as to give rise to twelve chromosomes. Careful observation of chromosomes, however, reveals that this is not the case, because of the fact that all the cells in this testis have without exception twelve chromosomes, and that all the autosomes present a bivalent nature. The testis in question, therefore, seems to be, with in all provability, that of a species different from *Gomphus melampus*. The difference of size of *m*-chromosome, moreover, may confirm such conclusion.

The material in question was prepared in Kyoto, where two other species of *Gomphus*, closely related to *Gomphus melampus*, were found in the same season. They were *Gomphus unifasciatus*[1] and *Gomphus suzukii*. The testes of the former species were fixed, after my own identification, during my stay in Kyoto, and the results of study differ, as described below, from the present case and from the preceding species.

For this reason, I wish to conclude presumably that the present species is *Gomphus suzukii*, hoping to ascertain fully this assumption in the future.

11. *Gomphus unifasciatus* (Fig. 8)

In four important points this species greatly differs from the other studied *Gomphus* so far as the chromosomes are concerned. First, the haploid number is eleven including the X-chromosome; secondly, there is no marked difference between autosomes in their relative magnitude; thirdly, the X-chromosome is considerably larger, the largest in all species studied; forth, all chromosomes are quite large in bulk (compare Fig. 8 with Figs. 6 and 7).

Among the chromosomes (Fig. 8, *a* and *b*) we can scarcely recognize gradatory changes in their magnitude, since they attain similar size.

1) This is a new species described by me in 1926.

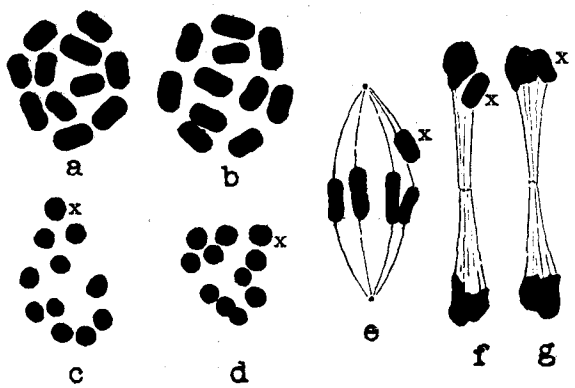

Fig. 8.

Gomphus unifasciatus. *a-b*, Polar views of metaphase groups, primary division; *c-d*, polar views of metaphase groups, secondary division; *e*, side view of the same; *f-g*, side views of telophase groups, secondary division. Zeiss Apoch. 1.5 mm. Comp. Oc. 12, t. l. 160 mm.

Still more, it seems impossible to point out the X-chromosome, owing to its comparatively large size, in a primary spermatocyte. But it is rather easy to distinguish it in the secondary spermatocyte (Fig. 8, *c* and *d*), by eccentric position in metaphase arrangement. At telophase of the division (*f* and *g*), it is still visibly separated from the remaining autosomes which have become aggregated into a mass.

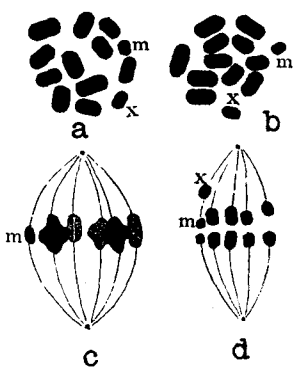

Fig. 9.

Anotogaster sieboldii. *a-b*, Polar views of metaphase groups, primary division; *c*, side view of the same; *d*, early anaphase, secondary division. Zeiss Apoch. 1.5 mm. Comp. Oc. 12, t. l. 160 mm.

Genus Anotogaster

12. *Antogaster sieboldii* (Fig. 9)

While the other genera treated here to fore have at least two species in the same genus for comparison, the present genus affords no other species than *A. sieboldii*, because of its being a sole representative in Japan.

The chromosome number found in a primary spermatocyte is again thirteen including X-chromosome as in most of the Libellulidæ. The smallest autosome attains about half the size of the X-chromosome and is always found with the latter at periphery in metaphase arrangement.

The mode of division of chromosomes in the secondary spermatocyte belongs evidently to the æschnid type as Fig. 6, *d* shows.

Family CALOPTERYGIDÆ

As far as I am aware, no species, belonging to this family, has been studied cytologically by any investigator. The representatives selected for the present study are four species from two different genera.

The haploid number of chromosomes found in both primary and secondary spermatocyte shows do difference, as being thirteen in all four species. A bivalent autosome takes lozenge shape with very blunt corners *en face*, and a rod, without median constriction, when viewed from a pole of the spindle. In this respect we recall the case of Æschnidæ. But the chromosome seems much thinner, and longer in its polar view as compared with that of Æschnidæ. This may be accounted for in the horizontal elongation of the lozenge form.

The X-chromosome is smaller than in the proceding families, and its behavior during two successive divisions is quite similar to that of the other families.

The mode of division of chromosomes in the secondary spermatocyte belongs to the æschnid type with a slight modification; viz. the transverse splitting indistinct (Fig. 10, *d* and *h*).

13. *Calopteryx atrata* (Fig. 10).

Out of thirteen chromosomes in the primary spermatocyte (*a* and *b*) two small ones invite our attention. Of these, one is slightly larger, and represents the X-chromosome, while the other is the smallest autosome. This conclusion is drawn from a study of their structure.

They are both arranged in periphery of metaphase equator. In the secondary spermatocyte (*c*) the X-chromosome is hardly recognized by magnitude, otherwise than its eccentric position (*d*). It may be noticed that the chromosomes are remarkably large in their absolute magnitude, as compared with those of species involved in the other families.

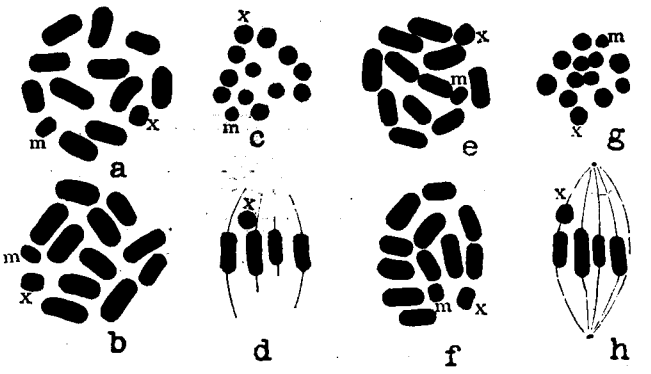

Fig. 10.

Calopteryx atrata (a-d) and *Calopteryx cornelia* (e-h). *a-b*, Polar views of metaphase groups, primary division; *c*, polar view of metaphase group, secondary division; *d*, side view of the same; *e-f*, polar views of metaphase groups, primary division; *g*, polar view of metaphase group, secondary division; *h*, side view of the same. Zeiss. Apoch. 1.5 mm. Comp. Oc. 12, t. l. 160 mm.

14. *Calopteryx cornelia* (Fig. 10, *e-h*).

Fig. 10, *e* and *f* show the metaphase plates of the primary spermatocyte division. At a glance we can not find any remarkable difference in the feature of the chromosomes from that of the preceding species. Even in the smallest autosome, which very often varies in size from species to species, this species shows no difference from the preceding. In spite of such close resemblance in feature of chromosomes, taxonomie characters of these two species exhibit very marked differences, viz. the present species is of the largest in this family, and has the wings of bright brown, while the preceding species is of a median size and has black wings.

Genus Mnais

15. *Mnais strigata* (Fig. 11, *a-d*).

This species is characterized taxonomically by having transparent wings and red pterostigmata in both sexes.

When observing the primary spermatocyte, I was astonished first to find that the chromosome present enormously huge size, not only compared to the next species but also to any species investigated. The smallest autosome acquires a quarter of the size of the X-chromosome,

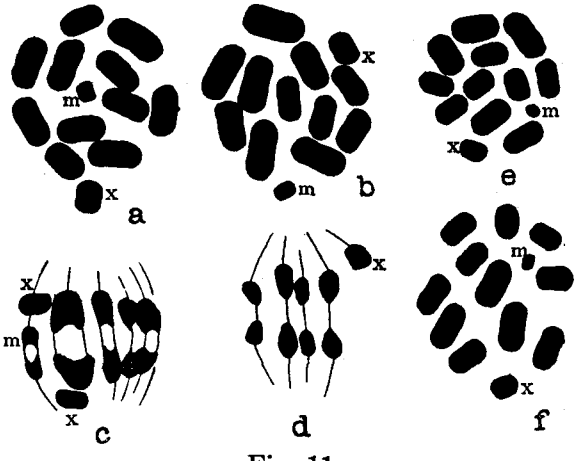

Fig. 11.

Mnais strigata (a-d) and *Mnais costalis* (e-f). *a-b*, Polar views of metaphase groups, primary division; *c*, side view of anaphase group primary division; *d*, side view of anaphase group, secondary division; *e-f*, polar views of metaphase group, primary division. Zeiss Apoch. 1.5 mm. Comp. Oc. 12, t. l. 200 mm.

which is represented by the second smallest one in a series of chromosomes. Either in center or periphery of equatorial plates the smallest autosome takes its position.

16. *Mnais costalis* (Fig. 11, *e-f*).

In colouration of the wing this species is sharply distinguished from the preceding. The wing is clear saffron colour with an opaque

part in male, while in female it is entirely transparent, without saffron tint, but pterostigma being always white.

As is shown in *e* and *f*, (Fig. 11) the chromosomes of a primary spermatocyte have very close resemblance with those of the preceding species. But as a whole, the absolute magnitude is much smaller, as the figures were drawn under the same scale with Figs. *a* and *b*, (Fig. 11) from the material fixed with the same reagent that was used for the preceding species.

The X-chromosome is represented too by the second smallest one in a serial arrangement of chromosomes. The smallest autosome is much smaller than that of the preceding species in relation to the X-chromosome. Seemingly it is smaller than one sixth of the latter.

So far as the chromosomes are concerned, therefore, these two species of *Mnais* can be considered as distinct species, (as previous authors considered), in contrast to my opinion to take them as subspecies of a single species as so described in my former paper (1913). Because, if we recall the case of *Calopteryx*, where there are no marked differences in chromosomes between two species with marked taxonomic characteristics, the chromosome difference shown in the case of *Mnais* will sufficiently serve for specific distinctions in taxonomy.

II. COMPARISON OF CHROMOSOMES IN THE STUDIED SPECIES

(A) Numerical relation

In the male dragonflies, so far as the present study goes, the number of chromosomes in the spermatocyte fluctuates from ten to thirteen as described in the foregoing pages. But the number thirteen is evidently predominant in the majority of species. The fluctuation of the chromosome number can be found not only within species of different families but also within those of a single genus. Thus we discover twelve chromosomes in *Sympetrum frequense*, while thirteen

are found in *Sympetrum pedemontanum*. Comparing three species belonged to *Gomphus*, then, we soon find that three studied species have each a different number of chromosomes.

A few species of the dragonfly have hitherto been investigated by previous authors from cytological point of view. They are three from two families as follows:

Family LIBELLULIDÆ	1. *Sympetrum semicinctum*
	2. *Libellula basalis*
Family ÆSCHNIDÆ	3. *Anax junius*

Of two of Libellulidæ, SMITH (1916) reported thirteen chromosomes in the spermatocyte, and from *Anax* of Æschnidæ was reported fourteen by LEFEVRE and MCGILL (1908).

Keeping this data in mind, therefore, the haploid number of the chromosome shows its minimum in *Gomphus melampus* and maximum in *Anax junius*.

Whatever the number of chromosomes may be, there is constantly present one X-chromosome in all the species studied. The table I will show the numerical relations in detail.

The relation between the chromosome number and taxonomy within species of a genus or a family is by no means simple. In Acrididae, for instance. more than 80 species have hither to been studied already at the time when HARVEY (1916) arranged his list, and in the majority of them we find 23 (♂), 24 (♀) chromosomes in diploid. Those possessing less number than this are to be considered as derived from association of certain definite chromosomes. Thus a fundamental chromosome number, characteristic to Acrididae, can be established.

PAINTER (1925) attempted to settle the fundamental chromosome number of Mammalia (although the evidences are still insufficient), and assumed forty eight as the number in question, from which other numbers are derived by fragmentation of certain chromosomes. There are still many authors believing in a fundamental chromosome number within a species for a family or class.

TABLE I. Chromosome Number

The diploid number with asterik is that actually counted in the spermatoginium, and the others denote expected numbers.

Species	Haploid	Diploid	Author
LIBELLULIDÆ			
Orthetrum albistylum	13	25*	OGUMA ('18)
O. japonicum	13	25	,,
Sympetrum pedemontanum	13	25*	,,
S. frequense	12	23	,,
S. semicinctum	13	25*	SMITH ('16)
Libellula quadrimaculata	13	25	OGUMA ('15)
L. angelina	13	25	,,
L. basalis	13	25*	SMITH ('16)
Somatochlora viridinea	13	25	OGUMA ('15)
S. uchidai	13	25	,,
ÆSCHNIDÆ			
Gomphus melampus	10	19	OGUMA ('30)
G. unifasciatus	11	21	,,
G. suzukii	12	23	,,
Anotogaster sieboldii	13	25	,,
Anax junius	14	27*	LEFEVRE and McGILL ('08)
CALOPTERYGIDÆ			
Calopteryx atrata	13	25	OGUMA ('30)
C. cornelia	13	25	,,
Mnais strigata	13	25	,,
M. costalis	13	25	,,

On the contrary, there exists plenty of facts which do not point in favour of establishing the fundamental number in definite groups. BORING (1907) published a chromosome study of twenty species of Homoptera, distributed in Membracidæ, Cercopidæ, Jassidæ and Fulgoridæ, but this author could not find the fundamental number common to most of the species. Recently OHMACHI (1927) reported the chromosome number of eighteen species of Gryllidae. We find, in his paper, that the chromosome number is so variable from species to species that any definite numerical relation is hardly possible to be pointed out. If we once consider *Gryllotalpa*, which was repeatedly

investigated by VOM RATH (1892), SENNA (1911), VOINOV (1912), PAYNE (1916) and WINIWARTER (1927), we shall soon come to conclusion that it is never easy to assume the fundamental chromosome number in *Gryllotalpa* or Gryllidae in general. In the dragonfly it is also difficult to settle definitely the chromosome number. To discuss this problem I must now consider the m-chromosomes as described below.

B. The m-Chromosome

Out of sixteen species in the present investigation three, *Sympetrum frequense*, *Somatochlora viridicenea* and *Gomphus unifasciatus*, possess no conspicuously small chromosome, which is to be called a m-chromosome. In the remaining thirteen, however, there is always present the so-called m-chromosome, being obviously distinguishable from the other autosomes.

As mentioned above, the smallest autosome or m-chromosome of the dragonfly serves in a great degree for identification of species under the microscope. It varies in its magnitude from species to species, even in most cases between two closely related species in one and the same genus. Thus we can distinguish *Orthetrum albistylum* from *Orthetrum japonicum* by the m-chromosome being of much larger size (c. f. Fig. 1, *b* and *i*). In quite similar way *Libellula quadrimaculata* is sharply distinguished from *Libellula angelina* in possession of the smaller m-chromosome. It is more remarkable that in *Somatochlora uchidai* (Fig. 5, *c*) the m-chromosome is represented by an extraordinarily minute one, while in *Somatochlora viridiaenea* (Fig. 5, *e*) there is no such chromosome comparable to the m-chromosome. In comparison of *Sympetrum pedemontanum* (Fig. 2, *a*) and *Sympetrum freguense* (Fig. 2, *d*) we see in the former species a strikingly small m-chromosome, while in the latter no corresponding one at all.

On the other hand, I have here a single case throughout the present study, where there is no visual difference of m-chromosomes between two species in a genus. That is the case of *Calopteryx*, in

which two representatives, *C. atrata* and *C. cornelia*, have a m-chromosome of almost equal size in spite of their taxonemical divergence.

The following table will show how the m-chromosomes vary within each species of the dragonfly. Twelve species are arranged according to the size of the m-chromosomes. They are measured in their polar view of the equtorial plate, and compared with the X-chromosome, which seems, as dealt with in detail in the next chapter, to be apparently constant in relative magnitude in most species.

TABLE II. The m-chromosome[1)]

Species	m-chromosome (bivalent)
1. *Sympetrum pedemontanum* (Fig. 2)	extremely small
2. *Somatochlora uchidai* (Fig. 5)	extremely small, but larger than the preceding
3. *Mnais costalis* (Fig. 11)	about a sixth of X.
4. *Mnais strigata* (Fig. 11)	about a quarter of X.
5. *Orthetrum japonicum* (Fig. 1)	smaller than a half of X.
6. *Gomphus suzukii* (Fig. 7)	smaller than a half of X.
7. *Libellula quadrimaculata* (Fig. 3)	smaller than a half of X.
8. *Anotogaster sieboldii* (Fig. 9)	about a half of X.
9. *Calopteryx atrata* (Fig. 10)	smaller than X.
10. *Calopteryx cornelia* (Fig. 10)	smaller than X.
11. *Libellula angelina* (Fig. 4)	nearly equal with X.
12. *Orthetrum albistylum* (Fig. 1)	nearly equal with X.

WILSON (1910) enumerates the following possible modes, by which the numerical variation of the chromosomes is caused, viz; (1) the gradual fusion of separate chromosomes into one (linkage) or the reverse process (fragmentation); (2) the sudden mutation of chromosome number; (3) the abnormal process of karyokinesis; (4) the gradual reduction of certain chromosome in size and final disappearance.

1) The m-chromosomes enumerated in this table are of bivalent condition in the primary spermatocyte, X-chromosomes are also those found in the same cell.

Among animals, especially in Insecta, the first mode of WILSON's hypothesis actually takes place most frequently. At present, we have much data to ascertain this possibility. Whenever one considers the results obtained in Orthoptera by WOOLSEY (1914), MCCLUNG (1914, 1917) and ROBERTSON (1916) one may come to the conclusion that this is the common or the general way that gives rise to variation of the chromosome number in the animal kindom. Data proposed by BROWNE (1910) in *Notonecta*, and by DONCASTER (1914) in *Lycia* strongly emphasizes this assumption. Thus in his recent great work on the chromosome, REUTER (1930) seems also to take this view, in reference to summarized data, which has been accumulating during the past years and covering both the animal and vegetable world.

The second and third modes of variation are thought to be rather seldom in actual occurrence in contrast to the former. But the case of WILSON's fourth enumerated point should not be overlooked since the case of the dragonfly proves this point.

Out of my present material, *somatochrora viridiœnea* is the only example in which thirteen chromosomes, in haploid, are represented by 12 large autosomes and one X of relatively small size. I can formulate, accordingly, the whole chromosome number as $12\ a+X$. If in such a group of chromosomes, reduction of size or diminution of volume takes place in any one chromosome, there will appear as a result a different chromosome group, in which one autosome will be replaced by the so-called m-chromosome, and it may correctly be formulated as $11\ a+m+X$. The case of *Orthetrum albistylum*, *Libellula* and others prove this possibility. If the gradual reduction of size still continues in the m-chromosome of above species, then we should find a case, in which it attains extremely minute size as found in *Sympetrum pedemontanum*. By final disappearance, through such gradual reduction of m-chromosome, there should be produced another chromosome group, to be formulated as $11\ a+X$ as in *Sympetrum frequense* for example. Similarly, if reduction again occurs in any one of the $11\ a$ of this group of chromosomes and then becomes smaller and smaller so as to be characterized as m-chromosome again, the resulting chro-

mosome group must be formulated as *10 a+m+X*, as in *Gomphus suzukii*.

In this way, through gradual diminution and final disappearance of an autosome, the chromosome number in dragonflies becomes different from species to species. LEFEVRE and MCGILL (1908) show 14 chromosomes in haploid in *Anax junius*, and one of them being the minute m-chromosome. This is to be formulated as *12 a+m+X*. Therefore, we can expect a predecessor, *13 a+X*, from which, by reduction of one of the autosomes, the case of *Anax* is produced. The following table shows how the number of chromosomes vary in the dragonfly.

TABLE III. The numerical variation of chromosomes

Chromosome formula	Number (haploid)	Examples
13a + X	14	?
12a + m + X	14	*Anax junius*
12a + X	13	*Somatochlora viridiœnea*
11a + m + X	13	*Orthetrum albistylum*
11a + X	12	*Sympetrum frequense*
10a + m + X	12	*Gomphus suzukii*
10a + X	11	*Gomphus unifasciatus*
9a + m + X	11	?
9a + X	10	?
8a + m + X	10	*Gomphus melampus*
8a + X	9	?

Since discovery of a pair of extremely small chromosomes in *Anasa* by PAULMIER (1899), similar occurrences have become gradually discovered among other hemipterous insects such as *Alydus*, *Archymenus* and *Syromastes*. WILSON (1905) was the first to find that the smallest pair of chromosomes does not conjugate throughout the growth period of the primary spermatocyte, but remain separated as a pair of chromatin nucleoli, and are united in the last prophase of division into the smallest dyad. From their peculiar behavior and the

extremely small size, he applied the term microchromosomes or briefly m-chromosomes, to distinguish them from the other autosomes.

LEFEVRE and MCGILL (1908) also found in *Anax* a pair of very small chromosomes and called them by the same name, because they thought that they were homologous to m-chromosomes of Hemiptera. But the behavior during the growth period seems not to be parallel in strict sense to that of the latter. They conjugate, in the growth period of maturating division, in quite a similar way with the remaining autosomes as to give rise to ordinary bivalent chromosome. Such a pair of small chromosomes are also present in most of my material, I could see them clearly at least in the spermatogonial group of chromosomes in *Orthetrum albistylum* (Fig. 1, *a*) and in *Sympetrum pedemontanum*. Still I have often used the name m-chromosomes as LEFEVRE and MCGILL have done for convenience of designation of this kind of chromosomes.

In my case, the entire course of conjugation of univalent m-chromosomes could be followed, stage by stage, in only two species, *Orthetrum albistylum* and *Somatochlora uchidai*. It is very interesting to note that the mode of conjugation differs markedly between these two species. In the former species, on one hand, the univalent m-chromosomes conjugate, like *Anax*, in the usual way to give rise to a bivalent. It is, therefore, evident that the m-chromosomes of the present species are different from the same named ones of WILSON's paper in respect to mode of conjugation. In the latter species, on the other hand, the univalent m-chromosomes conjugate as usual, early in the prophase of the first division, but not unfrequently remain separated until the metaphase sets in. In such an instance we always find in a nucleus three peculiar chromosomes, which are deeply stained with hæmatoxylin in contradiction to chromosomes with vague contour (Fig. 5, *a*). Of these three one is larger than the remaining two. The former corresponds to the X-chromosome, as being readily understood by its magnitude, while the latter represent the m-chromosomes which have failed to conjugate until this time. In this respect, the m-chromosomes of *Somatochlora uchidai* are in perfect accordance

with those of Hemiptera, and deserve correctly to accept the name m-chromosome in the strict sense.

It is of greatest importance to know that a similar fact has already been discovered by BORING (1907) in some Homoptera. This author, as cited above, observed twenty species of Homoptera belonging to four different families, and found that in one of the Membracidae, *Campylenchia curvata*, and in two Fulgoridae, *Pœciloptera septentrionalis* and *P. pruinosa*, a pair of the small chromosomes which do not conjugate during the growth period but remain separated, in spite of the fact that in the remaining species they conjugate to give rise to the smallest bivalent.

Considering these facts, then, it can be supposed that the chromosomes, showing a tendency of diminishing their volume at any time, may acquire a new characteristic so as to behave strictly as m-chromosomes. This shows, at the same time, a phylogenetical significance of the chromosomes in question.

C. The X-Chromosome

So far as known, the dragonfly possesses, without exception, an unpaired X-chromosome in addition to paired autosomes in a nucleus. It is rather small and its relative magnitude seems to be nearly equal in every studied species, excepting *Gomphus unifasciatus*. Even if it be not actually the same in size, it varies only in the slightest degree from species to species at least. In a group of bivalent chromosomes arranged in the metaphase plate of the primary spermatocyte, the X-chromosome always appears approximately as large as the half of the second smallest autosome when viewed from the pole of a spindle. Therefore, in the cases, where the X-chromosome is absent, it is represented by always the smallest chromosome as obvious in *Somatochlora viridiœnea* (Fig. 5, *e*) or *Sympetrum frequense* (Fig. 2, *d*). I have only one example here, in which the X-chromosome in undistinguishable from autosomes so far as the magnitude is concerned. The X-chromosome of *Gomphus unifasciatus* (Fig. 8), for instance, can not be identifi-

ed in the primary spermatocyte unless observing the behavior during division.

At the commencement of the growth period or in the leptotene nucleus, the X-chromosome is always found as a chromatin nucleolus, deeply stained with hæmatoxylin, and distinguishable from the plasmosome. It is ovoid or frequently oblong in shape, and often exhibits a clear split along its long axis. When the leptotene threads begin to contract at a pole of the nucleus, it migrates also to the pole of synizesis, and in majority of cases is closely applied to the nuclear membrane. After the chromosome threads, which grow gradually thicher, give up their polarization at a pole of nucleus and become scattered about through the nuclear cavity, the X-chromosome still remains in contact with the nuclear membrane.

At prophase of the first maturation division it frequently acquires dumbbell shape.

While bivalent autosomes arrange themselves in circles upon the metaphase plate, the X-chromosome always takes a peripheral position as usual. It is sharply distinguished, at this time, from autosomes not only by such peripheral arrangement but also by absence of a clear split when viewed from a pole.

As a rule, the X-chromosome is divided into equal halves at the first naturation division. The division takes place nearly synchronously with the separation of daughter halves of the bivalent autosomes. Its division, however, often precedes in more or less degree from that of the autosomes (see Figs. 6, *c*, *d* and 11, *c*), and migrates to poles in advance of the latter.

Whatever the mode of division of X-chromosome may be, there is produced an equal number of the secondary spermatocytes in which the X-chromosome is equally distributed.

When metaphase of the second maturation division sets in, the X-chromosome always takes an eccentric position against the autosomes as shown in annexed figures, and runs to either pole, without division, in advance of division of autosomes. It plays no part, therefore, in

this division, whereby the resulting two daughter nuclei become different from each other as regards the number of chromosomes. Instead of proceeding, the X-chromosome sometimes lags behind the autosomes in the course of migration as shown in Fig. 1, *h*. A similar example has already been observed in *Anax* by LEFEVRE an4 MCGILL (1908).

Thus the X-chromosome is divided at the first division while undivided at the second in all the studied material. Adverse to this common fact, an interesting but rather particular evidence has been reported by SMITH (1916). She states that in *Libellula basalis* the X-chromosome is not divided at the first maturation division but at the second, as if in Orthoptera, Coleoptera and Diptera. This is evidently an extraordinary mode of division so far as the dragonfly is concerned.

Fortunately I had two representatives of the genue *Libellula*, upon which my attention was turned with special interest in connection with this problem. The results of observation, however, disproved SMITH's evidence, since in both of these two species the X-chromosome behaves no differently from other dragonflies. As can readily be seen in Fig. 4, *Libellula angelina*, there are thirteen chromosomes found in a primary spermatocyte. They are all arranged upon the same plane and divided synchronously at this division. As the X-chromosome is represented by the second smallest one, larger than the m-chromosome, it can clearly be distinguished by its magnitude among the chromosome group. How the X-chromosome is divided in this division is to be understood in Fig. 4, *c*.

A quite similar fact is observable in *Libellula quadrimaculata*, in which the m-chromosome is very small (Fig. 3, *a* and *b*). All chromosomes are similarly divided at the first maturation division (*c*). Although I was unable to identify the X-chromosome in side view of the spindle; still unable at the same time, to find cases in which any one chromosome behaves in a different way from the remaining chromosomes, all were divided at the first division.

The above mentioned facts were verified through repreated observation with close attention to hundreds of cells and there is left in

my mind not the slightest doubt of their truth. I am forced, therefore, to conclude that the evidence described by SMITH (1916) in *Libellula basalis* is particular and unique throughout the dragonfly species.

In addition to this, I find one more strange fact in SMITH's study. That is the shape of the X-chromosome, which assumes a tetrad form as shown in Figs. 99, 100, 101 and 102. In a very few cases, the X-chromosome was reported as assuming a form of bipartite or quadripartite with simulating appearance with autosomes, as we find in Lizards according to PAINTER (1921) and guinea pig according to MOLS (1928). But in the dragonfly it is quite doubtful whether there is a case in which the X-chromosome really assumes such an appearance. I rather believe that she might have mistaken some displaced tetrads for the X-chromosomes, since my recent study of human chromosomes (1930) shows that such a condition is possible.

(D) Modes of Division of Chromosomes in the Secondary Spermatocyte in Relation to Taxonomy

As mentioned in the foregoing pages (see p. 5) there are two distinct types of division of chromosomes in the secondary spermatocyte of dragonflies. All species of Libellulinæ, dealt with in this study, belong to the libellulid type, and those of Æschnidæ to the æschnid type. It is of great importance to find that *Somatochlora* should be classified with Æschnidæ in respect to the mode of division of chromosomes. From a texonomical point of view, this genus evidently belongs to Libellulidæ, not to Æschnidæ at all. But it possesses many distinct characters, by which it is distinguished from other members of Libellulidæ and thereby constitutes with several other genera a subfamily Cordulinæ. Thus the family is separated into two subfamilies, Libellulinæ and Cordulinæ, and the latter has a close relation to Æschnidæ. My present findings, therefore, have proved this fact from cytological aspects.

As regards Calopterygidæ, I have very little cytological data which emphasize taxonomy. I have only one fact and that is the members of this family assume æschnid type in mode of division of chromosomes. This establishes a strong support for a close phylogenetical relation to Æschnidæ. In fact, some Calopterygidæ, *Epiophlebia* for instance, possess so many æschnid characteristics that it is possible to consider this genus as a member of Æschnidæ.

Here it would be better to refer briefly to one of my former studies with reference to relation between cytology and taxonomy.

When spermiotogenesis of three species of dragonfly from three different subfamilies, *Orthetrum* (Libellulinæ), *Anotogaster* (Gomphinæ) and *Gynacantha* (Æschninæ), is compared with each other, I find that *Anotogaster* presents just an intermediate character between *Orthetrum* and *Gynacantha* concerning the morphology of spermatozoa (1915).

Briefly, spermatozoa of *Orthetrum* are remarkably thick and short in length as compared with those of *Gynacantha*, and are found aggregated in quite an irregular way in the follicles. In *Gynacantha*, on the contrary, they are bound together at their "head ends" to form sperm-bundles. *Anotogaster*, then, shows combined characters in these respects: spermatozoa assume similar form to those of *Orthetrum*, but make up sperm-bundles like *Gynacantha*.

These facts will be recognized if we recall taxonomical relationships among these three species. Thus I know that further studies of this kind will play important rôle to account for some perplexing problems in taxonomy.

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