ADDITIONAL DATA TO OUR KNOWLEDGE ON THE DRAGONFLY CHROMOSOME, WITH A NOTE ON OCCURRENCE OF X-Y CHROMOSOME IN THE ANT-LION (NEUROPTERA)[1)]

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In a paper published by OGUMA (1930), one of the present authors, were reported the results obtained by surveying the chromosome in sixteen species of Japanese dragonflies. From what he could observe under the microscope the conclusion has been drawn that the chromosome of the dragonfly differs from species to species in magnitude of the m-chromosome, which in extreme cases disappears entirely. The m-chromosome is clearly observable in the Indian species which are dealt with in the present paper. In one case, moreover, the size diminution of the m-chromosome takes place in several different grades in one and the same species, although the cause and significance can not yet be accounted for sufficiently.

The material employed consisted of testes taken from two species of Indian dragonflies (Odonata), captured and preserved at Ahmedabad, East India, by ASANA, one of the present authors.

In connection with the study of the dragonfly chromosome we are very pleased to record the results obtained from examination of chromosomes of a Neuropteran insect *Palpares* sp., of which the male

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germ-cell contains X-Y pair of sex-chromosomes of conspicuous shape. This insect was also collected at the same locality with the dragonflies above mentioned. Up to this time, nothing has been known about the chromosome of Neuroptera of any species. In this respect, the present results, even though not complete, are of importance and serve to extend our knowledge in this particular order of insect.

1. The Chromosome of *Trithemis aurora* BURM. (Odonata).

This species is classified under the family Libellulidae, having allied characteristics to members of *Sympetrum*, of which two species have already been reported in the previous work (1930).

The testes were fixed by modified BOUIN's fluid, immediately after dissection of an immature adult. In contrast to previous cases, the testes contain not only the spermatocyte but also many spermatogonia in division, in which the diploid chromosomes are to be counted.[1)]

A spermatogonium (Fig. 1, *a*) contains twenty five chromosomes including a single X-chromosome. The autosomes take the form of rod, more or less gently curved, with varied length, and are so arranged on the whole as to have their long axes tangential to the equator, in strong contrast to the case of Orthopteran or Mammalian chromosomes, in which they lie with their long axes radially against the center of the equatorial plate. Among whole twenty five univalents, a pair of quite small chromosomes represents a conspicuous feature. They are the so-called m-chromosomes. It is not easy to point out which is the X-chromosome in this diploid group of chromosomes. But by picking out every two homologous chromosomes by comparison of length, the solitary X-chromosome can be discriminated as shown in Fig. 1, *a*. Its peripheral position in arrangement upon the equatorial plate serves for its identification to some extent.

The primary spermatocyte chromosome is thirteen in number, twelve bivalents and one X-univalent (Fig. 1, *b* and *c*). All the

1) The material used in the study by OGUMA (1930) did not contain spermatogonia in process of division.

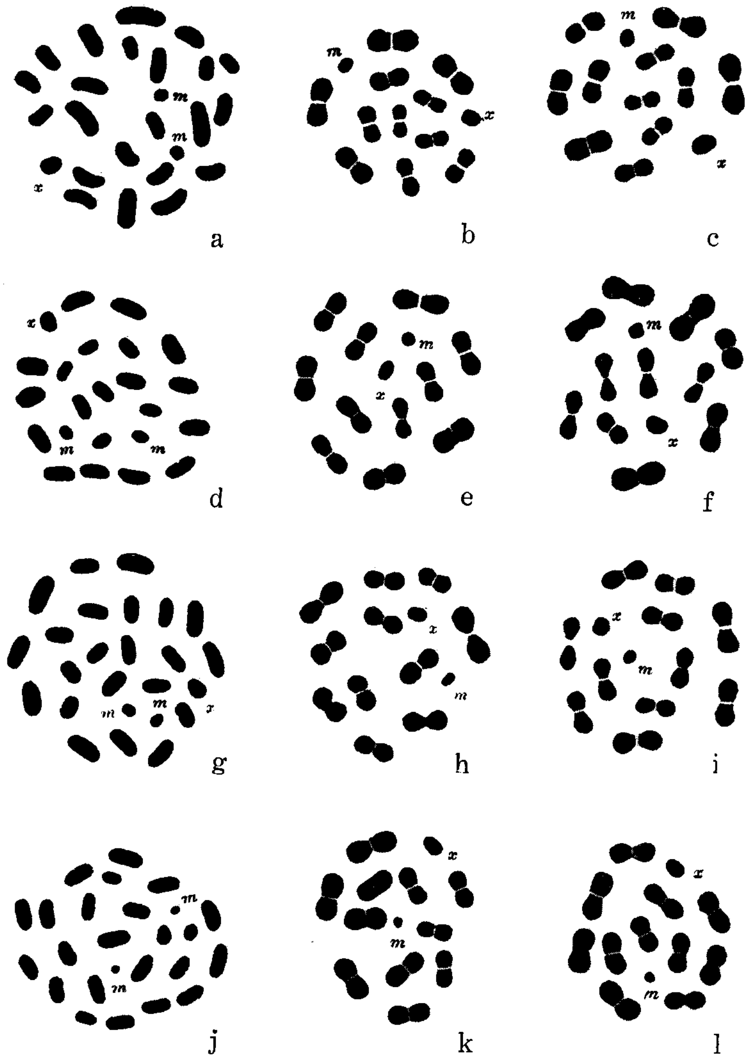

Figure 1. *Trithemis aurora* BURMEISTER (*a–c*), × 5000
a, spermatogonium ; *b*, and *c*, primary spermatocytes.
Tramea chinensis DEGEER (*d–l*), × 5000
d, *g* and *j*, spermatogonia ; *e*, *f*, *h*, *i*, *k* and *l*, primary spermatocytes.
m, m-chromosome ; *x*, x-chromosome.

autosome bivalents, except the m-chromosome, show the dumb-bell shape with a transverse splitting[1] when viewed from the pole of division. The splitting is sometimes so broad that the round heads of the dumb-bell shaped chromosomes may be taken as two separate chromosomes. The X-chromosome is found most frequently at the periphery of the equatorial plate, and is denoted by lacking the splitting of this nature. The m-chromosome bivalent never takes a tetrad form but a dyad, similarly devoid of any splitting when viewed from the pole. The magnitude of the m-chromosome in a polar view, seems to be smaller than half the X. In this respect this dragonfly resembles *Libellula quadrimaculata*.

2. The Size Variation of the m-Chromosome in *Tramea chinensis* DeGeer (Odonata).

This species also belongs to the family Libellulidae and is known to have a wide distribution over southern Asia. The material employed in the study was a pair of testes taken from an immature imago and fixed in Flemming's solution without acetic acid for 24 hours.

Looking over the sections we could discover numerous dividing spermatogonia as well as both primary and secondary spermatocytes, like the preceding species. The chromosome also shows an equal number to the preceding, as twenty five in diploid and thirteen in haploid as shown in Fig. 1, *d* to *l*. But we can not overlook a striking fact concerning the relative size of the m-chromosome which will be described in detail as follows.

1) This transverse splitting denotes the line of contact of chromatids, of which the tetrad is composed, and is also clearly demonstrated in the case of *Anax* studied by Lefevre and McGill (1908). In the previous paper Oguma (1930) described a longitudinal splitting through the longitudinal axis of a dumb-bell like figure of the chromosome, instead of the transverse splitting. Considered from the present result and that obtained in *Anax*, such a longitudinal splitting may not actually exist in tetrad, but is an optical phenomenon caused by superficial reflection on the surface of the tetrad, this being brought about by the rather hard consistency of chromatin due to the use of Hermann's or Carnoy's mixture.

The m-chromosome, either in univalent or in bivalent condition[1], does not exhibit a definite magnitude relative to the remaining autosomes and the X-chromosome. To try to demonstrate this fact it is preferable to observe first the primary spermatocyte, because the X-chromosome, to which the m-chromosome should be compared, is identified without difficulty by absence of splitting. As easily recognized from Fig. 1, *e* and *f*, the m-chromosome bivalent in these two cells shows a fairly large size, about half the X. Such size relation of the m-chromosome seems to be constant in all cells, so far as they belong to one and the same follicle, and these cells will be designated as type number one. Beside cells of this kind we find those of another kind, in which the m-chromosome is much smaller, not larger than one third the X, as shown in Fig. 1, *h* and *i*. These cells are found in a different follicle from that containing cells of the first type, and they will be distinguished as the second type.

The size variation of the m-chromosome extends further, and in extreme cases it becomes to like a spot or a grain as in *Sympetrum pedemontanum*, thus constituting the third type. Fig. 1, *k* and *l* represent actual examples. The other cells found in the same follicle possess quite a smilar nature so far as the m-chromosomes are concerned.

In spermatogonia, a similar variation of size is also observable in parallel to the primary spermatocyte above mentioned. Thus Fig. 1, *d* and *g* show examples of spermatogonia which are comparable with the first and the second type of spermatocytes. There are also examples in which the m-chromosomes take an extremely small size, corresponding to the third type, as can readily be seen from Fig. 1, *j*. These three kinds of spermatogonia belong, without exception, to different follicles, and in no case are cells of different kind found mingled with one another in one and the same follicle. It follows that the cells of every type are those of a definite progeny, or in other words the primary spermatocyte

1) The two m-univalents of the spermatogonium are converted into a single m-bivalent of the primary spermatocyte attaining a double volume as compared with those of the univalent state. When observed from the pole of the first division, however, the m-bivalent also looks like a univalent, as two component halves are overlapped.

with a minute m-chromosome is to be considered a descendant of a spermatogonium having a pair of minute m-chromosomes. When his former paper was published, OGUMA (1930) maintained the hypothesis that in Odonata at least, a pair of certain chromosomes, the m-chromosomes, exhibits a tendency to reduce in size by casting off some amount of chromatin, possibly in parallel to taxonomical differences. At that time, however, it was quite unknown to him that the m-chromosome can vary or show a gradual diminution of size among individuals of one and the same species. Still less it was to be expected that the m-chromosome may present every grade of size reduction among testicular cells of a single individual, as the case of *Tramea chinensis* mentioned above. The fact discovered in the present species brings the view point of diminution of the m-chromosome to a more valid ground, but its significance in taxonomic relation has become still more puzzling.

3. The X-Y Chromosome in Male Germ-Cells of *Palpares* sp.[1] (Neuroptera).

The material consisted of a single pair of testes taken from an imago captured at Ahmedabad on the 4th of September 1930. This insect was parasitized in the abdomen by a certain species of worm, but the testes, as well as the germ-cells contained therein seemed to be healthy. The fixative was FLEMMING's solution without acetic acid as in the case of the preceding species.

The germ-cells showed much advancement in their course of maturation and seemed to be, for the most part, of no use for the study of chromosomes. Yet fortunately, we could find a few follicles, in which a number of dividing figures of the primary spermatocyte could be observed.

1) This species belongs to the family Myrmeleonidae. The specimen, from which the present material was taken, was delivered to Dr. S. Kuwayama, entomologist of the Agricultural Experiment Station of Hokkaido, to whom the authors are greatly indebted for his effort in determining its scientific name. At present he is of opinion that the species is new to science and has promised to propose a new name at a later date.

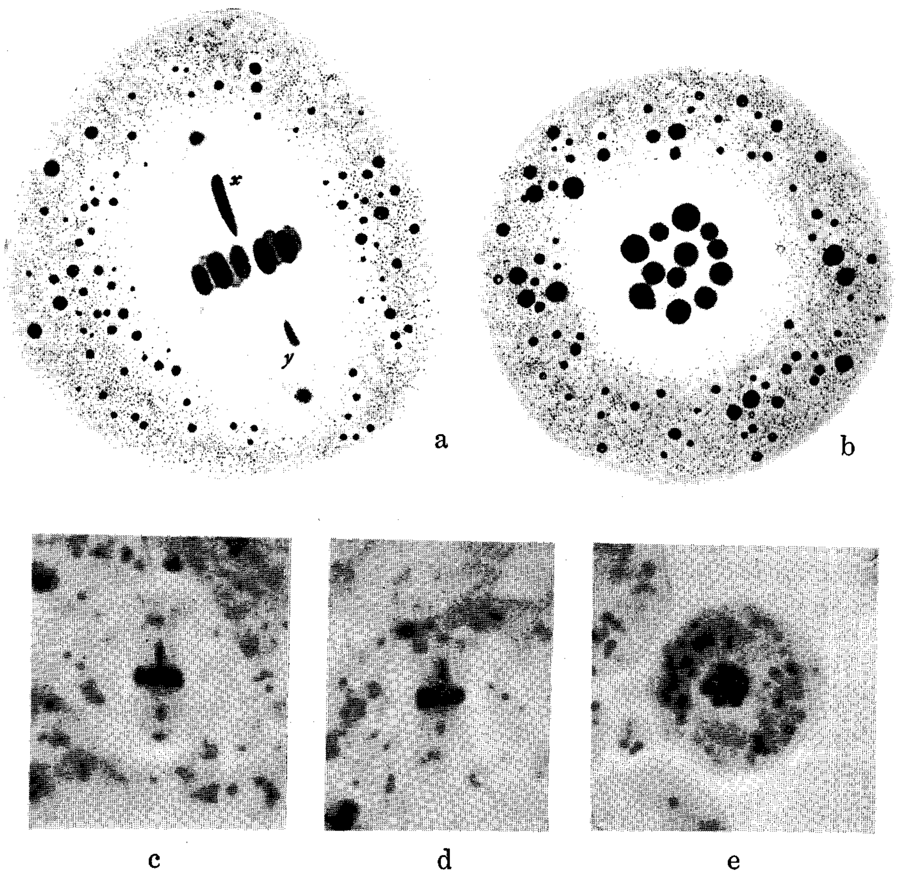

Figure 2. *Palpares* sp. (Primary spermatocytes)
a and *b*, hand drawings, ×5000.
c, *d* and *e*, photomicrographs, ×2000.
The cell *a* is the same with *c*, and *b* with *e*.

The primary spermatocyte is remarkably large in size in comparison with that of the dragonfly, depending on a large volume of cytoplasm, in which spherical plastosomes of various sizes are scattered about. Occupying the central part is observed a clear area, in which a narrow spindle is found. Corresponding to that narrow spindle, the chromosomes constitute an equatorial plate of narrow diameter. Central bodies are very large as clearly shown in the appended figures and photographs (Fig. 2).

When observed from a pole of division, the primary spermatocyte appears to have twelve round chromosomes of various diameters (Fig. 2, *b* and *e*) representing a similar figure to chromosomes of Lepidoptera. In the lateral view, however, it reveals the fact that there exists the X-Y chromosome complex, which is composed of two parts of unequal size (Fig. 2, *a*). This complex appears similarly as a round chromosome if observed from the pole of division. Among twelve chromosomes in Fig. 2, *b* and *e*, therefore, one can hardly distinguish it from the autosome tetrads, unless we change the focus in order to follow it up to down from the equator. Thus it is clear that the primary spermatocyte possesses eleven autosome tetrads and one X-Y complex. The diploid number of chromosomes, therefore, should be twenty four including X and Y chromosome.

The autosome tetrads are oblong in their lateral aspect, and the idiochromosomes (X and Y) are long and distinctly narrow in diameter, representing a striking contrast to the former. Both the X and the Y seem to have similar shape, but remarkably different magnitude. They show somewhat clavate form, directing their blunt ends to the poles, while the opposite ends are more or less pointed. As can readily be recognized from Fig. 2, *a* and *b*, the X-Y complex seems to give up the connection very early as compared with the separation of component halves of the autosomes, and disconnected X and Y chromosome advance to the poles prior to the latter. Owing probably to its smallness the Y approaches the pole earlier than the X.

As the result of the primary spermatocyte division, there should evidently be produced two kinds of secondary spermatocytes, of which one possesses the X and the other the Y in addition to eleven autosomes. Consequently, in the present species the male is heterogametic in relation to chromosomes.

Among insects, Lepidoptera has been the only group in which heterogamety exists in the female, as SEILER (1917, 1920, 1921) shows us in absolute clearness, in contrast to the majority of remaining orders, until KLINGSTEDT (1927, 1931) found a similar occurrence in Trichoptera. Female heterogamety in Trichoptera has been expected by every one who has knowledge of the taxonomic relation between this order and Lepidoptera, and now this expectation has been brought into validity by the efforts in KLINGSTEDT. The order Neuroptera is another group, beside Trichoptera, having a close relation to Lepidoptera. In this respect we had great expectation in what might be discovered in the study of the Neuropteran chromosomes. But investigation shows that in Neuroptera, at least in the ant-lion (Myrmeleonidae), heterogamety is not present in the female, but is found in the male, similarly to Odonata and Plecoptera, other allied groups. The order Neuroptera, therefore, presents more close relation to these groups than Lepidoptera as far as heterogamety is concerned. In contrast to such evidence, the shape of chromosome resembles those of Lepidoptera, as they assume globular form at least in the primary spermatocyte; consequently it suggests to us that some relation still exists between these two orders.

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