The Chromosomes of Six Species of Ant-lions (Neuroptera)[1]

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(With 6 Textfigures)

Beginning with the work of Oguma and Asana ('32) on *Palpares*, the chromosome studies on insects belonging to the Order Neuroptera have advanced to a great extent during recent years. The chromosomes of two species of the Sialidae belonging to the Suborder Megaloptera have been investigated by Itoh ('33, a, b). Naville et de Beaumont ('33) have examined the chromosomes of fifteen species covering seven families, and their work has thrown much light on the establishment of systematic relationship of the chromosomes between allied orders. Klingstedt ('33) reported on the chromosomes of two species of *Hemerobius* (Hemerobiidae) with special reference to the heteromorphic autosome pair. Brückner ('35), in his paper dealing with the sex organs of *Chrysopa vulgaris*, dwelt upon the morphology and the number of its chromosomes. Recently Kichijo, the junior author ('34, '35), has published two papers dealing with the chromosomes of some Japanese species belonging to the Chrysopidae. More recently, Ikeda and Kichijo ('35) have investigated the chromosomes of two species of Myrmeleonidae found in Japan. The results set forth here deal with the chromosomes in the male germ cells of six neuropterous species belonging to two families, Myrmeleonidae and Ascalaphidae. The material was collected by

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The authors wish to express their best thanks to Professor Kan Oguma for valuable suggestions and guidance in this study. Thanks are also due to Dr. Satoru Kuwayama of the Hokkaido Agricultural Experimental Station, who kindly identified all the specimens used in this investigation. We are also greatly indebted to Assist. Prof. Sajiro Makino for his unstinted assistance throughout this investigation, and for seeing this paper through the press.

Material and Methods

The following investigations are based on material obtained from six species of Neuroptera collected during March to October, 1932. According to Dr. Kuwayama, they are referable to the following two families:

Family Myrmeleonidae

1. *Myrmecaelurus* sp. (*M. acerbus* Walker?)
2. *Macronemurus* sp. ?
3. *Neuroleon* sp.
4. *Myrmeleon* sp. (*M. sagax* Walker?)

Family Ascalaphidae

5. *Ogcogaster segmentator* Westwood.
6. *Glyptobasis dentifera* Westwood.

In order to avoid the difficulty of specific determination the testes of maturing adults were only employed in this study. Immediately after the specimens were captured, the gonads were dissected out by vivisection and put into the fixing solutions. The specimens from which the gonads had been taken out were preserved in alcohol and later on delivered to Dr. Kuwayama for identification. For the fixatives, (1) Flemming's solution diluted with equal volume of distilled water, (2) Modified Bouin's solution (P. F. A. 3), (3) Allen-Bouin's solution, were employed. All these fixatives proved to be satisfactory. Sections were cut eight micra in thickness and stained with iron-haematoxylin according to Heidenhain's method, using light green as the counter stain.

All of the textfigures, were drawn with the aid of a camera lucida using a Zeiss 2 mm objective and a compensating ocular, K 20 ×, giving a magnification of 3700 diameters.

Observations

(a) Family Myrmeleonidae

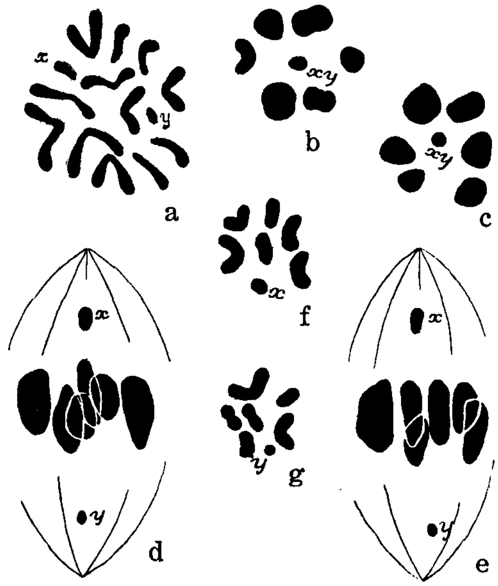
In this family the chromosomes of four genera, *Macronemurus, Palpares, Acanthaclisis* and *Myrmeleon,* have already been reported upon in three papers published by Oguma and Asana ('32; *Palpares* sp.), Naville et de Beaumont ('33; *Macronemurus appendiculatus, Myrmeleon europaeus*), and Ikeda and Kichijo ('35; *Acanthaclisis japonica, Myrmeleon formicarius*). In the present study, additional species from the following four genera, namely *Myrmecaelurus, Macronemurus, Neuroleon* and *Myrmeleon,* have been dealt with, two of which, *Neuroleon* and *Myrmecaelurus,* are quite new material for cytological study. In every species examined in the present study, an unequal pair of X–Y chromosomes was invariably found in accordance with the results already obtained in other species of this family.

1. *Myrmecaelurus* sp. (*M. acerbus* Walker?)

The specific name of this form could not certainly be determined, but according to Dr. Kuwayama, it may be *M. acerbus* Walker, in all probability. A pair of testes from a single specimen caught in August 1932 constitutes the material for study, which was fixed in dilute Flemming's solution with only a trace of acetic acid.

As seen in the equatorial plate of the spermatogonium there are 14 chromosomes of varying size and shape which are arranged radially on the equator (Textfig. 1, a). In this spermatogonial complex V-shaped chromosomes seem to be atelomitic while the remaining six appear to be telomitic. According to shape and size, the atelomitic chromosomes constitute four homologous pairs, while of telomitic chromosomes, larger ones pair into two homologous ones and two smaller ones remain to form a pair made up of two chromosomes of unequal size. It is the latter that is identified as the X–Y pair and they are labelled *x* and *y* respectively in the Textfig. 1, a. The X-element is about two times as large as the Y.

There are found, at metaphase of the primary spermatocyte, seven bivalent chromosomes which are strikingly evident (Textfig. 1, b–c). On the equatorial plate they dispose themselves with their long axes parallel to the spindle axis (Textfig. 1, d–e). Of these seven chromosomes, six are autosome bivalents and the remaining one is an asymmetrical tetrad representing the X–Y complex. In



Textfig. 1. *Myrmecaelurus* sp. (*M. acerbus* ?). a, polar view of spermatogonial metaphase, showing 14 chromosomes. b–c, polar views of primary spermatocyte metaphase, showing 7 chromosomes. d–e, side views of primary spermatocyte meta-anaphase. f–g, polar views of secondary spermatocyte metaphase, showing 7 chromosomes. f, X-class. g, Y-class. 3700×.

the lateral aspect of the spindle the X–Y pair is conspicuously recognized, and the components of this pair are separated precociously in striking contrast to the others. When viewed from the pole, therefore, they can hardly be detected unless the focus is moved up and down to a considerable degree. The size difference between X- and Y-elements is in accordance with what has already been seen in the case of the spermatogonium.

Since the X is completely separated from the Y in the first division, two kinds of daughter cells or the secondary spermatocytes are formed. They differ from each other with regard to their sex chromosome components, although in other respects they are similar. A representative of one class contains an X-element plus 6 autosome dyads (Textfig. 1, f), while the other involves a Y-element with a similar complex of autosome dyads (Textfig. 1, g). In the second division, the X as well as the Y always take a position on the outer circle of the spindle.

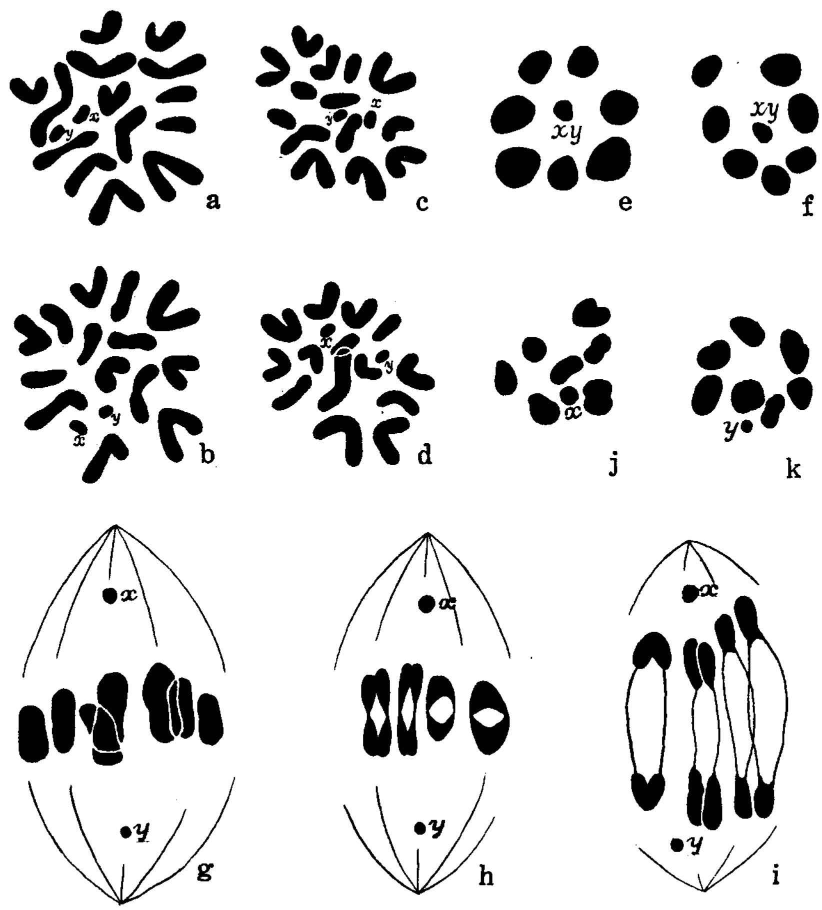
2. *Macronemurus* sp. ?

According to Dr. Kuwayama, the present form probably belongs to the genus *Macronemurus*, but the specific name could not be determined. One member of this genus, *Macronemurus appendiculatus*, has been investigated cytologically by Naville et de Beaumont ('33). Testes from three specimens furnish the material for the present study. They were secured in August, 1932 and fixed in modified Bouin's solution.

A large number of dividing figures are found in the sections. As seen in Textfig. 2, a–d, the spermatogonium contains, without exception, 16 chromosomes of various kinds of V's and rods. After careful examination and by picking out every two homologous chromosomes it was concluded that the two smallest chromosomes, showing a slight size difference between them, are to be regarded as the X- and Y-elements. They are labelled in Textfig. 2, a–d.

Eight well defined chromosomes are observed at the metaphase of the primary spermatocyte (Textfig. 2, e–f). Of these eight chromosomes, seven are autosome bivalents and the one which always occupies a central position on the spindle is the X–Y complex, constituting an asymmetrical tetrad. Since the X–Y complex shows a remarkably precocious segregation into its two components at the first division, as clearly demonstrated in the side view of the spindle (Textfig. 2, g–i), the two separated chromosomes can be detected only by changing the focus in the examination of a polar view. The size difference here too between the X- and Y-elements in the primary spermatocyte is not so remarkable, as is the case with the spermatogonium just observed.

In the anaphase of the first division, each of the autosome tetrads divides into identical sister dyads, and the X–Y complex separates into its components (Textfig. 2, i). As a result there are produced two kinds of secondary spermatocytes, so far as their chromosome garniture is concerned. The one group, the X-class, consists of seven autosome dyads plus an X-element; the other is the Y-class which contains seven autosome dyads and a Y-element (Textfig. 2, j–k).



Textfig. 2. *Macronemurus* sp. ?. a–d, polar views of spermatogonial metaphase with 16 chromosomes in each. e–f, polar views of primary spermatocyte metaphase, showing 8 chromosomes. g–i, side views of primary spermatocyte meta-anaphase. j, polar view of secondary spermatocyte metaphase, showing 8 chromosomes, X-class. k, the same, Y-class. 3700× .

The X and the Y in their respective cells occupy always a peripheral position on the spindle.

From the above mentioned observations it will be clear that the chromosomes of this Indian species of the genus *Macronemurus*, are not materially different in their number, arrangement on the spindle and in their behaviour from those of the taxonomically related species, *M. appendiculatus*, which has been investigated by Naville et de Beaumont ('33). However, the condition of the X- and Y-chromosomes in their relation to autosomes in the Indian form is somewhat different from what obtains in this respect in the chromosomal complex of *M. appendiculatus*. While the X- and Y-chromosome in the figures of the diploid group presented by Naville et de Beaumont ('33) cannot be identified and picked out with any degree of certainty from the autosomes, as has been observed by these authors, these elements in the spermatogonia of the species we have studied can be clearly distinguished from the autosomes, as shown in Textfig. 2, a–d. This may seem to be a noteworthy difference when the chromosomal garnitures of these two species are compared.

3. *Neuroleon* sp.

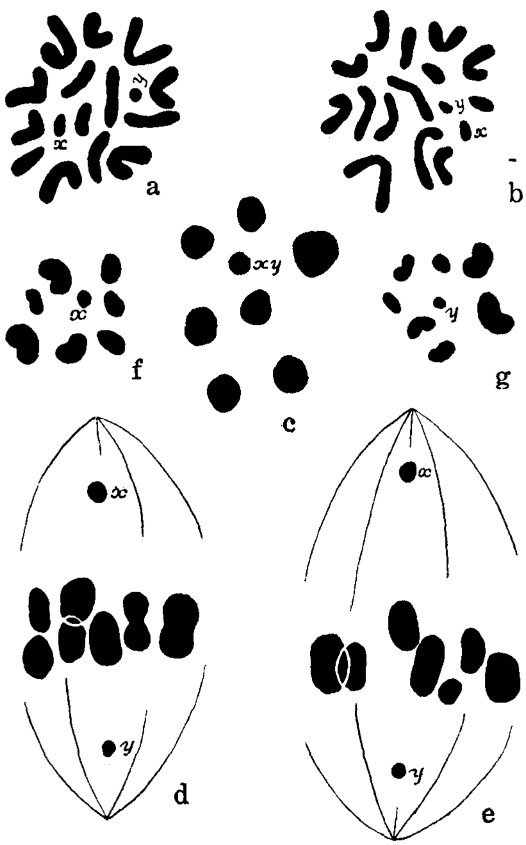
The chromosomes of the member of this genus now to be described have hitherto not been investigated by any authors. The following observations are based on the study of a pair of testes obtained from an individual captured in March, 1932. The fixative was Allen-Bouin's solution.

The chromosomes of the present species resemble in general characteristics, such as the number, shape and behaviour, those of the foregoing form, *Macronemurus* sp. The spermatogonium contains 16 chromosomes consisting of various kinds of V's and rods (Textfig. 3, a–b). When pairs of homologous chromosomes are marked out by adopting the criteria of identity, size and shape between the members of a pair, two small rod-shaped chromosomes are left which are regarded as the X- and Y-elements. There is an appreciable size difference between the X- and Y-chromosomes.

As shown in Textfig. 3, c, eight chromosomes can easily be counted in the metaphase of the primary spermatocyte. In a lateral view of the spindle at this stage the X–Y complex is most conspicuously demonstrated in this case too as was the case with the foregoing species (Textfig. 3, d–e). This complex shows a precocious segregation into its two components in striking contrast to the other autosome tetrads.

In every equatorial plate of the secondary spermatocytes, there are observed eight dyads without exception. In respect to the sex chromosome - components, however, the secondary spermatocytes are discriminated into two groups with different garnitures; the one contains an X-element in addition to seven autosome dyads (Textfig. 3, f) and the other includes a Y-element together with the autosome complex (Textfig. 3, g).

Here it is noticeable that the X as well as the Y take, in the second division, a central position on the spindle, being surrounded by the autosomes. And it needs to be pointed out that this was not the case with the other species previously described.



Textfig. 3. *Neuroleon* sp. a–b, polar views of spermatogonial metaphase with 16 chromosomes. c, polar view of primary spermatocyte metaphase, showing 8 chromosomes. d–e, side views of primary spermatocyte meta-anaphase. f, polar view of secondary spermatocyte metaphase, showing 8 chromosomes, X-class. g, the same, Y-class. 3700× .

4. *Myrmeleon* sp. (*M. sagax* ?)

A pair of testes from an individual of this species was fixed in Allen-Bouin's solution in October, 1932, which served as the material for the present study. Though the specific name of this insect has not been determined with certainty, Dr. Kuwayama is of opinion that this species in question may be *M. sagax* Walker.

The chromosomes observed in two species belonging to this genus, *M. europaeus* and *M. formicarius,* have already been reported upon in two paper, the former by Naville et de Beaumont ('33) and the latter by Ikeda and Kichijo ('35).

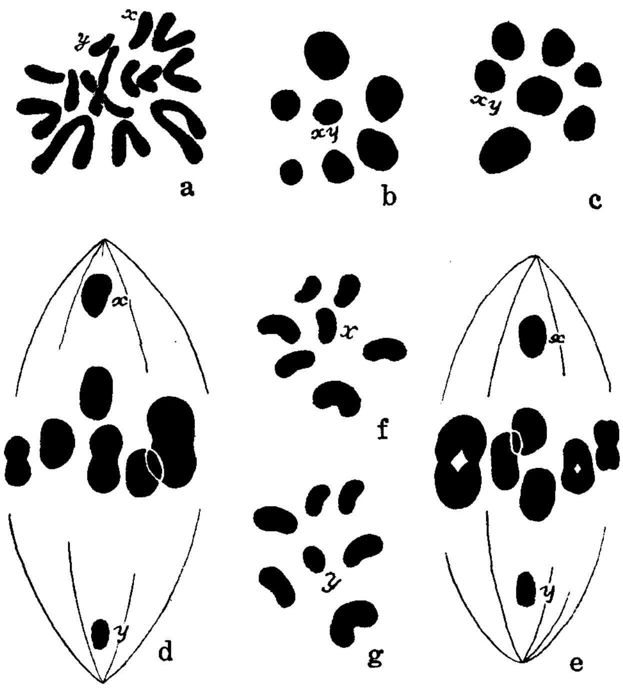
There are 14 chromosomes in the spermatogonium (Textfig. 4, a). When the homologous pairs are picked out according to shape and size, one will soon notice two chromosomes which show a difference in size between themselves. These we regard as a pair of unequal members, the X- and Y-chromosomes (Textfig. 4, a, *xy*).

In *M. europaeus,* Naville et de Beaumont ('33) observed that the chromosomes constituting the diploid complex have atelomitic fibre attachment except the Y-element. In the present species, on the other hand, at least 3 pairs including the XY-elements seem to be telomitic in fibre attachment, as is the case in *Myrmecaelurus* formerly described. On the basis of these more or less scantity observations we are not prepared to make any decided statement as to whether this difference in the chromosome complex between these two species is real and fundamental or it is only a seeming one, a result of an artifact effected by fixing. Judging from the figures alone, as given by Naville et de Beaumont ('33), however, no final answer can yet be given to the question whether all elements (except the Y) are atelomitic in fibre attachment.

Textfig. 4, b and c show the polar views of the primary spermatocyte metaphase where seven tetrads are clearly discovered. The X–Y complex is distinctly demonstrated in a lateral aspect of the spindle; the two components of this complex segregate precociously while the autosome tetrads are still on the equatorial plate (Textfig. 4, d–e).

As a result of the first division there are produced two kinds of secondary spermatocytes, with regard to the X- and Y-elements. Textfig. 4, f and g show the X- and Y-class respectively. The X, as well as the Y, occupies a central position on the spindle surrounded by the autosome dyads.

A comparison of the chromosomes between the present species and the other two species already investigated furnished a striking evidence in regard to the relative magnitude between the X- and Y-chromosomes, though in respect of the number of the chromosomes the three species under discussion are similar. The size difference between the X and the Y is most marked in the European form, *M. europaeus* (Naville et de Beaumont, '33), the Y being smaller than one-third the size of the X. In the Japanese form, *M. formicarius*



Textfig. 4. *Myrmeleon* sp. (*M. sagax* ?). a, polar view of spermatogonial metaphase with 14 chromosomes. b–c, polar views of primary spermatocyte metaphase, showing 7 chromosomes. d–e, side views of primary spermatocyte meta-anaphase. f, polar view of secondary spermatocyte metaphase, showing 7 chromosomes, X-class. g, the same, Y-class. 3700× .

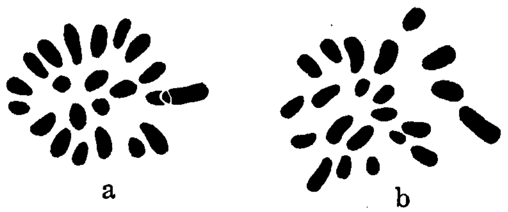
(Ikeda and Kichijo, '35), the relative magnitude between the X and the Y is almost the same as that observed in *M. europaeus*. While in the present Indian form, the Y-element is so much larger as compared with that of the above mentioned two species, as nearly half the size of the X (Textfig. 4, a).

(b) Family Ascalaphidae

Naville et de Beaumont ('33) have studied the chromosomes of *Ascalaphus libelluloides* belonging to this family. An account of the chromosomes of two Indian form, *Ogcogaster segmentator* and *Glyptobasis dentifera*, of the same family is given below. Though in the present study, the spermatogonial chromosomes only have been investigated, owing to the lack of the material, the three species studied, seem to be in agreement, so far as the number and shape of chromosomes are concerned.

5. *Ogcogaster segmentator*

Though the testes from three different specimens obtained in October, 1932 were fixed and examined, the greater part of them seemed to be of no use for the chromosome study, since the germ cells contained in them are much advanced in their course of maturation. Yet fortunately, a cyst was found, in which some sper-



Textfig. 5. *Ogcogaster segmentator*. a–b, polar views of spermatogonial metaphase, showing 22 chromosomes. 3700× .

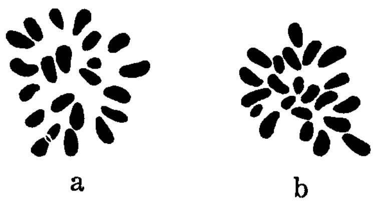
matogonia in the course of division were observed. The fixative was modified Bouin's solution. A careful examination of these spermatogonial division figures reveals 22 chromosomes, all of which are telomitic and rod-shaped, showing gradatory diminution in length (Textfig. 5, a–b). They arrange themselves radially in the equatorial plate, assuming a typical rosette.

As mentioned above, the diploid number of chromosomes is even. This readily suggests the presence of a pair of sex chromosomes, a feature which is of common occurrence in all the species of Neuroptera so far studied. By picking out every two homologous members of a pair on the basis of equality in shape and size, it is supposed that the largest rod without a homologous mate, which is disposed at the outer circle of the spindle, seems to be the X-element, and the Y-element is probably represented by one of the three grain-like chromosomes. This inference, however, can only be confirmed by studying the behaviour of the chromosomes during the maturation division.

6. *Glyptobasis dentifera*

The material for this study consists of a pair of testes from a specimen obtained in September, 1932. It was fixed in diluted Flemming's solution with reduced acetic acid. As in the preceding case, it was not found possible to follow every dividing phase of the chromosomes in the present material; only the spermatogonial chromosomes were observed.

The spermatogonial metaphase shows 22 chromosomes without any possibility of error, as seen in Textfig. 6, a–b. All the chromosomes seem to be telomitic in fibre attachment. So far as the number and shape of the chromosomes are concerned, this species shows no marked difference when it is compared with the two other species of Ascalaphidae so far studied.



Textfig. 6. *Glyptobasis dentifera.* a–b, polar views of spermatogonial metaphase, showing 22 chromosomes. 3700× .

Since there exists an even number of chromosomes in the diploid group, the inference is warranted that the sex chromosome complex of the usual X–Y type is contained in the chromosome garniture of this form also. However, as the chromosomes of this species vary in their length from one another only to a slight degree, it is quite difficult to determine with any degree of certainty the X- and Y-elements in this diploid garniture.

Remarks

So far as the literature shows, the chromosomes of Neuroptera have been investigated in 33 species covering eight families, including the results presented in this paper. The following table records the number of chromosomes in the neuropterous insects hitherto investigated.

Species	Haploid	Diploid	Author
(Subord. Planipennia)			
Myrmeleonidae			
Myrmecaelurus sp. (*M. acerbus* ?)	7	14	ASANA et KICHIJO, '36
Macronemurus appendiculatus	8	16	NAVILLE et BEAUMONT, '33
Macronemurus sp. ?	8	16	ASANA et KICHIJO, '36
Palpares sp.	12	—	OGUMA et ASANA, '32
Neuroleon sp.	8	16	ASANA et KICHIJO, '36
Myrmeleon europaeus	7	14	NAVILLE et BEAUMONT, '33
Myrmeleon formicarius	7	—	IKEDA et KICHIJO, '35
Myrmeleon sp. (*M. sagax* ?)	7	14	ASANA et KICHIJO, '36
Acanthaclisis japonica	7	—	IKEDA et KICHIJO, '35
Ascalaphidae			
Ascalaphus libelluloides	11	22	NAVILLE et BEAUMONT, '33
Ogcogaster segmentator	—	22	ASANA et KICHIJO, '36
Glyptobasis dentifera	—	22	,,
Osmylidae			
Osmylus chrysops	7	14	NAVILLE et BEAUMONT, '33
Chrysopidae			
Chrysopa perla	6	12	NAVILLE et BEAUMONT, '33
Chrysopa vulgaris	6	12	,,
,, ,,	—	16	BRÜCKNER, '35
Chrysopa prasina	6	12	NAVILLE et BEAUMONT, '33
Chrysopa alba	6	—	,,
Chrysopa septempunctata	5	10	,,
Ch. sept. cognata	5	10	KICHIJO, '35
Chrysopa sp.	5	10	NAVILLE et BEAUMONT, '33
Chrysopa japana	6	12	KICHIJO, '34
Chrysopa sapporensis	6	12	,,
Chrysopa intima	6	12	,,

Species	Haploid	Diploid	Author
Chrysopa kurisakiana	6	—	KICHIJO, '34
Chrysotropia japonica	6	12	,,
Hemerobiidae			
Hemerobius pini	7	14	NAVILLE et BEAUMONT, '33
Hemerobius stigma	—	14	,,
,, ,,	7	14	KLINGSTEDT, '33
Hemerobius atrifrons	7	—	NAVILLE et BEAUMONT, '33
Hemerobius humulinus	—	14	KLINGSTEDT, '33
Mantispidae			
Mantispa styriaca	9	18	NAVILLE et BEAUMONT, '33
Coniopterygidae			
Semidalis aleurodiformis	—	18	,,
(Subord. Megaloptera)			
Sialidae			
Chauliodes japonicus	—	20	ITOH, '33
Protohermes grandis	—	23	,,

All the authors are in agreement that in the species they have studied the male is heterogametic. It is also true that in all the cases so far investigated the chromosomal constitution of the male sex cells is of the usual two types, the X- and Y-bearing complexes.

Another noteworthy uniformity among all these species is the behaviour of the two components of X–Y complex at the time of reduction division. They show a remarkably precocious separation in contrast to the behaviour of the autosomal tetrads at this stage of spermatogenesis in Neuroptera. However, with regard to the constitution, particularly the size, of the X- and the Y-elements, when these are compared with each other and as they are seen in various species, the striking uniformity that we witness in other features, as marked above, is not in evidence. For instance, Naville et de Beaumont ('33) have observed that in *M. appendiculatus* the X- and the Y-chromosomes in the spermatogonial complex cannot be identified with certainty, while in the closely allied Indian form of the same genus there is an appreciable difference between the size of the X and that of Y in spermatogonial cells as seen in Text-fig. 2, a–d.

With regard to the chromosomes of a single species of the Ascalaphidae which has come under the observations of Naville et de Beaumont ('33) they say that they have not been able to obtain and examine the spermatogonial chromosomes in their material. This lacuna in our knowledge, they desire, may soon be filled up. The present observations on the spermatogonial chromosomes of the two Indian species of Ascalaphidae here recorded, Textfig. 5, a–b and Textfig. 6, a–b, will, we hope, partially fill this gap in our knowledge of the chromosomes of what promises to be a cytologically interesting group of Neuroptera.

Summary

The numerical relation between the chromosomes of six species of ant-lions from India dealt with in the present paper is given in the following table:

Species	Haploid	Diploid	Sex-chrom.
(a) Myrmeleonidae			
1. *Myrmecaelurus* sp. (*M. acerbus* ?)	7	14	XY
2. *Macronemurus* sp. ?	8	16	XY
3. *Neuroleon* sp.	8	16	XY
4. *Myrmeleon* sp. (*M. sagax* ?)	7	14	XY
(b) Ascalaphidae			
5. *Ogcogaster segmentator*	—	22	XY
6. *Glyptobasis dentifera*	—	22	XY

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