Studies on the Chromosomes of Five Species of Coccinellidae (Coleoptera)

Uma Agarwal

Received November 17, 1960

Our earlier knowledge about the cytology of the family Coccinellidae is largely due to Stevens (1906, 1909), Yosida (1944, 1946, 1948, 1949, 1951), Bose (1948), Smith (1950, 1953) and Takenouchi (1955, 1957, 1958). Reference to the Smith’s chromosome list of Coleoptera (1953) indicates that the chromosomes have so far been reported in 24 species belonging to this family. The present author has had a chance to study the chromosomes of five species of the Coccinellidae, the results of which are to be presented in this paper.

Material and method

The following is a list of the species used in the present study.

Family Coccinellidae

Sub-family Coccinellinae: Coccinella reponda Thunbg., Coccinella septempunctata Linn., Menochilus semmaculata Fabr.

Sub-family Epilachninae: Epilachna orientalis Zimm., Epilachna vinctiotipectata Fabr.

All the species are of common occurrence in Allahabad. The adult male and female specimens were collected from the fields of solanaceous and umbelliferous plants during periods from February to April in the years 1957, 1958 and 1959. Gonads were fixed in Sanfelice and Corrosive sublimate acetic. Sections (10–12 µ) stained with Gentian violet, Feulgen’s stain and Heidenhains haematoxylin were examined. Diagrams were drawn with the help of a camera lucide at a magnification of 5000 ×.

Observations

Coccinella reponda (Figs. 1–12)

Both spermatogonial and oogonal metaphases contain 20 chromosomes (Figs. 1–2). In males, the complement consists of two pairs of large, fifteen medium and a minute spherical chromosome. The latter has not been observed in the oogonal metaphase and is, therefore, obviously the y-chromosome. The X-chromosome remains morphologically indistinguishable from the autosomes at this stage. The chromosomes according to their

1 Present address—Division of Microbiology, Central Drug Research Institute, Lucknow, India.
comparable shape and size fall into 9 homologous pairs of autosomes and an unequal pair of sex chromosomes. The autosomes consist of one pair of ‘J’-shaped, two pairs of ‘V’-shaped and six pairs of rod-shaped chromosomes.

During prophase the sex chromosomes can easily be distinguished from the autosomes due to their highly condensed nature. They are invariably found associated into a single spherical deeply-stained mass lying excentrically in the nucleus (Fig. 3). The autosomes at leptotene and zygotene appear fine, faintly-stained beaded threads. The pachytene nucleus (Fig. 4) shows ten elements of which nine are autosomal threads and the remainder is a sex chromosome mass. At diplotene (Fig. 5) the autosomes have 1–3 chiasmata each depending on their length. The autosomal bivalents at early diakinesis (Fig. 6) become further condensed but usually carry the same number of chiasmata as at the diplotene. At prometaphase the autosomal bivalents become highly condensed and their chiasmata seem to be nearly terminalized.

The first metaphase shows 10 chromosomes comprising 9 autosomal bivalents and an Xy-complex in the form of a typical parachute (Fig. 7). As seen in the polar view (Fig. 8) the chromosomes appear deeply-stained spherical bodies arranged more or less in a ring with a few elements lying in the interior. At anaphase I the segregation of the X and y elements oc-

Figs. 1-12. Chromosomes of Coccinella reponda. 1, spermatogonial metaphase, 20 chromosomes including spherical y. 2, oogonial metaphase. 3, early prophase showing deeply-stained sex chromosome mass. 4, pachytene. 5, diplotene. 6, diakinesis. 7, first metaphase (side view). 8, first metaphase (polar view). 9-10, anaphase I. 11, second spermatocyte metaphase, x-class. 12, second spermatocyte metaphase, y-class.
curs but the separation of the sex chromosomes is slightly delayed and succeeds that of the autosomal bivalents (Figs. 9-10). As a result of the first division two types of secondary spermatocytes are produced—one with 9 autosomes plus an X and other with the same number of autosomes plus a y (Figs. 11-12).

*Coccinella septempunctata* (Figs. 13-20)

Every spermatogonial division shows 20 chromosomes at metaphase (Figs. 13-14). The complement consists of 14 ‘V’ or ‘J’-shaped meta-centric chromosomes, 5 rod, acrocentric ones and a minute spherical body.

Morphological analysis of the chromosomes revealed that one of the acrocentric elements corresponds to the X-chromosome while the smallest spherical body represents the y. The prophase stage of meiosis in all the species under study is more or less similar to that of *C. reponda*.

At prometaphase (Fig. 15) the autosomal bivalents become highly condensed and their chiasmata seems to be terminalized. Every primary spermatocyte metaphase plate shows the haploid number of 10 (Figs. 16-17). The haploid complement consists of 9 dumb-bell shaped autosomal bivalents and a heteromorphic Xy complex (Fig. 16). In the polar view (Fig. 17) the chromosomes appear deeply-stained spherical bodies. At anaphase I (Fig. 18) the sex chromosomes separate simultaneously with the autosomes resulting into two types of secondary spermatocytes—one with an X-chromosome (Fig. 19) and the other with y (Fig. 20).
Menochilus senmaculata (Figs. 21–26)

The spermatogonial metaphase (Fig. 21) shows 20 chromosomes of various shapes—round, oval, elongated, curved rod, kidney, and V-shaped. In the complement two pairs of large V-shaped metacentric chromosomes and a minute dot-shaped element can be distinctly seen while the remaining fifteen belong to the the medium-sized group. By arranging the diploid chromosomes in pairs according to their comparable shape and size there are established 9 pairs of autosomes and an unequal pair of sex-chromosomes X and y.

Following diakinesis the autosomes assume at first metaphase the form of simple rods with median constriction marking the position of completely terminalized chiasma. The first metaphase (Fig. 22) shows 10 chromosomes of which nine are autosomal bivalents and the remaining one is an Xy bivalent with a heteromorphic structure. In the polar view (Fig. 23) the chromosomes are observed deeply-stained spherical bodies arranged more or less in a ring with few elements lying in the interior. At anaphase of the first division the X and y segregate migrating to the opposite poles along with the autosomes (Fig. 24). There are two types of secondary spermatocytes observable, one with nine autosomes plus an X (Fig. 25) and the other with the same autosome complex plus a y (Fig. 26).

Epilachna orientalis (Figs. 27–34)

The diploid chromosome complement as observed at the spermatogonial and oogonial metaphases consists of 18 elements (Figs. 27–28). The fact that the diploid number of chromosomes is the same in both males and females indicates the presence of an XY:XX sex-chromosome mechanism. In males, all the chromosomes are rod-shaped acrocentric in nature except the two large metacentric ones (Fig. 27). In the complement a V-shaped element largest of all in size and a J-shaped chromosome remain unpaired after homologous pairing. The oogonial complement consists of eight pairs of rod-shaped acrocentric and two V-shaped metacentric chromosomes similar to the larger V-shaped one in males (Fig. 28). It is, therefore, apparent that the largest element in males is the X-chromosome and the J-shaped the Y (approaching the size of the X).

During prophase the sex chromosomes can be readily identified from the autosomes due to their highly condensed nature. The leptotene and zygotene threads are fine and beaded and become gradually condensed. At pachytene (Fig. 29) the autosomal threads appear hairy due to the presence of the fine, Feulgen-positive lampbrush processes. The lampbrush processes disappear completely at diakinesis.

The primary spermatocyte metaphase shows 9 chromosomes consisting of 8 autosomal bivalents and a heteromorphic XY complex. The latter is easily recognised in the side view of the first metaphase (Fig. 30). The XY complex differs in stainability from the autosomal bivalents; one segment is
stained as deeply as the autosomal elements, the other stains much less than the autosomes. In the polar view (Fig. 31) the chromosomes appear spherical deeply-stained bodies. At anaphase I the segregation of the sex-chromosomes follows that of the autosomal bivalents (Fig. 32). As a result, two classes secondary spermatocytes are produced, one possesses 8 autosomal elements and an X, the other carries the same autosomal set and a Y (Figs. 33–34).

Epilachna vigintioctopunctata (Figs. 35–40)

This species has already been studied cytologically by Yosida (1948) and Bose (1948). The diploid number was determined without any doubt to be 18 in every spermatogonium (Figs. 35–36). This proves the correctness of the chromosome counting by Yosida (1948) and Bose (1948). Yosida (1948) observed the presence of minute y chromosome, though the author and Bose (1948) found large Y, approaching the size of the X in this species. The complement consists of eight pairs of oval curved rod-shaped chromosomes and a pair of metacentric ones. The metacentric chromosomes are not identical but vary in their shape and size. After the morphological analysis, it becomes evident that the largest V-shaped element is the X-chromosome and the other metacentric one the Y.

Every first metaphase plate shows 9 chromosomes of which eight are autosomal bivalents while the remaining heteromorphic bivalent represents
the XY complex (Figs. 37–38). The latter differs in stainability from the autosomes, one arm remains negatively heteropycnotic at this stage. As a result of the first division two classes of secondary spermatocytes are produced in respect to the distribution of the X and Y, the one possesses 8 autosomes and an X, and the other carries the same autosomal set and a Y (Figs. 39–40).

Discussion

The frequency distribution of the chromosome number in the sub-family Coccinellinae is represented diagrammatically in Fig. 41 which is based on 21 species. Out of which 18 have been listed by Smith (1953) and three by the author (Agarwal 1960). Figure 41 clearly shows that the chromosome number 20 gives the highest peak, this number being established the modal number of the order Coleoptera as a whole by Smith (1950, 1953). The numbers 12, 16, 17 and 18 are represented by comparatively low frequencies. It may be pointed out that the various species showing deviation from the modal number might have evolved during the course of evolution of the species either due to fusion of autosomes among themselves or with the X-chromosome or simple loss of the minute y-chromosome.

The spermatogonial complement of Coccinella reponda and Coccinella septempunctata, investigated by the author is observed to have 20 chromosomes (18 autosomes+X+y). The diploid complement of Coccinella brukii has been reported to be 18 (16 autosomes+X+y) in the heterogametic sex (Yosida 1944) i.e., two less than what is usual for the genus. Although little can be said positively as to how this change in the typical number is accomplished, yet it appears certain that the decrease in the chromosome number in C. brukii from the modal number is either due to early extrusion of a pair of homologous chromosomes or by fusion of a pair of autosomes. Further investigations of other species may change the shape of this frequency histogram.

In the diploid complement of Epilachna orientalis and Epilachna vingtioctopunctata the author observed 18 chromosomes, and these species have an XY:XX mechanism of sex determination, the Y approaches the X in size (designated as capital Y). The spermatogonial complement of Epilachna vingtioctopunctata has already been reported to be 18 in the heterogametic
sex (Yosida 1948, Bose 1948). Yosida (1948) observed the presence of minute y-chromosome (16 autosomes+X+y), though the author and Bose (1948) found the large Y approaching the X in size (16 autosomes+X+Y) in this species. All the species belonging to the sub-family Epilachninae reported so far have either 18 or 20 chromosomes (Fig. 42) and an XY:XX mode of sex determination (Stevens 1906, 1909, Hoy 1918, Strasburger 1936, Yosida 1944, 1948, Takenouchi 1955).

Yosida (1944, 1948) reported 20 chromosomes (18 autosomes+X+y) for three species of genus Epilachna, E. nipponica, E. vigintioctomaculata and E. pustulosa. If we compare these three species with E. orientalis and E. vigintioctopunctata the difference in the chromosome number and sex chromosome morphology may be well explained either by loss or fusion of chromosomes. It can be suggested that in males of E. orientalis and E. vigintioctopunctata a translocation involving an autosome and X-chromosome and the loss of the minute y-chromosome which is almost near the limit of visibility might have given rise to neo-XY condition during the course of evolution of the species. The possibility nevertheless remains that the minute y-chromosome may have been retained not as a separate entity but by being translocated on to some other member of the complement.

The neo-XY sex-determining mechanism has been reported in nearly one third of the families of order Coleoptera investigated so far i.e. Buprestidae (Asana, Makino and Niiyama 1942, Smith 1949, 1953), Chrysomelidae (Yosida 1944, 1949), Tenebrionidae (Guénin 1950, Smith 1952a, 1952b) Lagriidae, Melandryidae, Curculionidae, Carabidae, Erotylidae, Coccinellidae, Passalidae and Staphylinidae (Smith 1950, 1952b, 1952c, 1953). Buprestidae and Chrysomelidae have, however, been worked out in greater detail than others. In Luperus discrepans and Laperodes praestus (Chrysomelidae) Yosida (1944, 1949a) found even more complex neo-X-Y sex chromosomes as he observed that the neo-X is tripartite and neo-Y chromosome is bipartite (X-A-B: A-B). Such a high degree of complexity is a unique feature with these species among the known neo-XY beetles.

As regards the origin of neo-XY system, Asana, Makino and Niiyama (1942) consider the three Indian buprestids, S. nitidicollis, S. laevigata and J. whithilli having neo-XY sex chromosome mechanism to have originated from more primitive XO forms by what may be termed "conventional centric fusion". But they have not as yet established an XO system as
antecedent to the neo-XY complex of the beetles under consideration. Smith (1949) inferred from his observations that the neo-XY sex-determining mechanism arise from primitive XY:XX system (large X minute y) by loss of the minute y-chromosome and translocating the X onto an autosome resulting into a composite neo-X. The homologue of the autosome involved at the time of translocation becomes neo-Y. The findings of the present study are in agreement with the view expressed by Smith (1949, 1952).

**Summary**

The chromosomes of five species of the family Coccinellidae (Coleoptera) have been studied in germ cells. The chromosome numbers and sex-determining mechanism are summarized in Table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Chromosome number</th>
<th>Sex-chr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2n</td>
<td>n</td>
</tr>
<tr>
<td>1. Coccinella reponda</td>
<td>20 s</td>
<td>10(I, II)</td>
</tr>
<tr>
<td>Coccinella reponda</td>
<td>20 o</td>
<td></td>
</tr>
<tr>
<td>2. Coccinella septempunctata</td>
<td>20 s</td>
<td>10(I, II)</td>
</tr>
<tr>
<td>3. Menochilus senmaculata</td>
<td>20 s</td>
<td>10(I, II)</td>
</tr>
<tr>
<td>4. Epilachna orientalis</td>
<td>18 s</td>
<td>9(I, II)</td>
</tr>
<tr>
<td>Epilachna orientalis</td>
<td>18 o</td>
<td></td>
</tr>
<tr>
<td>5. Epilachna vigintiopunctata</td>
<td>18 s</td>
<td>9(I, II)</td>
</tr>
</tbody>
</table>


**Acknowledgments**

The work was carried out at the Department of Zoology, Allahabad University, Allahabad. The author is deeply grateful to Prof. M. D. L. Srivastava for his kind guidance and encouragement. Thanks are also due to Dr. A. P. Kapur, Zoological Survey of India, for identification of the material.

**References**

   — 1952c. The cytology of Sitophilus (Calandra) oryzae (L), S. granarius (L) and some other Rhynchophora (Coleoptera). Cytologia 17: 50-70.
   — 1946. Chromosome studies in the Coleoptera. II. Seibutu 1: 218-223.
   — 1951. La Kromosomo 9 (Quoted from Smith 1952c).