The X-O type of sex-chromosome found in *Ovalipes punctatus* (de Haan) (Crustacea: Decapoda)¹⁾

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While working under the guidance of Prof. K. Oguma, on the chromosomes of the decapod Crustacea the author has discovered the sex-chromosomes of X-Y type in the following three species belonging to the family Grapsidae; *Plagusia dentipes* de Haan, *Eriocheir japonicus* de Haan and *Hemigrapsus sanguineus* (de Haan), as reported in the previous papers (Niiyama, '37, '38). In the present paper accounts will be given of the chromosomes of *Ovalipes punctatus*, a species of the family Portunidae (Brachyura: Reptantia), together with clear-cut evidence for the presence of the sex-chromosome belonging to the X-O type.

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Material and Method

Ovalipes punctatus is a yellowish crab with dark spots scattered all over the carapace which is about 56 mm in breadth. According to Sakai ('35) this species distributes widely in Japan from Hokkaido to Ryūkyū. The specimens from which the present material was furnished were obtained by means of the drag-net at Kakisaki, Simoda Bay, Izu, in May 1938. The testes were taken out by vivisection and fixed in weak Flemming's mixture. Iron-haematoxylin method after Heidenhain exclusively was used for staining (for details see Niiyama '34).

Observations

1) Primary spermatocyte The meiotic divisions are found occurring simultaneously in several cysts in the testis, and over one hundred metaphase figures of the primary spermatocyte were observable in a single section. A polar view of the metaphase shows 51 distinct tetrads which

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constitute a beautiful circular equatorial plate. These assume a rectangular or dumb-bell shape such as is common among the Crustacean species. By changing the focus of the microscope from equatorial plate a little upward and downward, the existence is disclosed of a chromosome which lies in a nearly central portion of the equatorial plate situated always in one side of the latter (Figs. 3, 4, 5, 6). Viewed from the side of the equatorial plate, the presence of this peculiar element becomes plainly evident, being a little apart from the equatorial plate in which other elements are contained, as clearly seen in Figs. 7, 8. After closer examination, it becomes certain that this separate chromosome is entirely contained within the spindle of the metaphase. In shape this chromosome is apparently more slender than the ordinary tetrads; it tapers at one of the ends directed toward the equatorial plate. Considered from its structure, this element is certainly of a dyad nature and not a tetrad. At anaphase, this chromosome does not separate at all and migrates towards one of the poles of the spindle. As a result of this division, one of the daughter complexes produced contains this element in its complement. From the facts shown in the morphology and behaviour as above observed, it is proper to consider that this chromosome having a dyad nature is the X-chromosome, just comparable to that generally found in Orthopteran insects. As the peculiar evidence, the X-chromosome of the present species lies always in a central position of the spindle. It is a rare case to find the X in such a position in animals.

Thus the chromosome constitution of the primary spermatocyte of this species is shown by the formula; $n, 52=51+X$.

2) Spermatogonia Figs. 1 and 2 show the metaphase equatorial plate of the spermatogonial division. In these plates, counting of the chromosome number is not so difficult, since the individual elements are well distinguishable from each other. The complement consists of 103 chromosomes of which 4 or 2 pairs are atelomitic V-shaped and the remaining ones are all short rod-shaped.

The occurrence of the odd number of spermatogonial chromosomes confirms further the presence of a single X-chromosome. But it was quite impossible to point out the X-element in the spermatogonial complex, because the X is not provided with any characteristic feature to permit its distinction from the other autosomal elements.

3) Secondary spermatocyte At metaphase of the secondary spermatocyte division the chromosomes having a dyad nature are found scattered in a circular equatorial plate; counting of the number of chromosomes is easily made. Careful examination indicates that there are two kinds of

Figs. 1-2. Metaphase chromosomes of spermatogonia, 103 chromosomes in each. $\times 3700$. **Figs. 3-8.** Metaphase chromosomes of primary spermatocyte. 3-6: polar view, 51 tetrads and a sex-chromosome (x) in each. 7-8: side view. Sex-chromosome (x) is indicated in deep black. $\times 3700$. **Fig. 9.** Photomicrograph of primary spermatocyte metaphase plates; side views and a polar view are shown. $\times 2000$. **Figs. 10-13.** Metaphase chromosomes of secondary spermatocyte. 10-11: no X-class, 51 dyads in each. 12-13: X-class, 52 dyads in each. $\times 3700$.

cells in the secondary spermatocyte with regard to the number of chromosomes. One of them shows 51 chromosomes (Figs. 10, 11) and the other 52 (Figs. 12, 13). Considered from the distribution of the X-chromosome as it occurred in the primary spermatocyte division it is apparent that the cell having 52 elements involves in it an X-chromosome. The X-element is entirely indistinguishable in the complex for the reason noted in the case of the spermatogonial cell. At anaphase, all the elements segregate synchronously, and as the result there are produced two kinds of spermatids, one with the X and the other without it.

Hence the chromosome complex of the secondary spermatocyte is indicated as follows; (a) no X-class having 51 chromosomes and (b) X-class having 52 chromosomes.

Remarks

While in a confused state concerning the sex-chromosomes of decapod Crustacea, the author has reported the definite occurrence of the X- and Y-chromosomes in the three species of the family Grapsidae (Brachyura, Reptantia) (Niiyama, '37, '38). They were clearly detected out at the metaphase of the primary spermatocyte by means of their characteristic behaviour.

In the present species, *Ovalipes punctatus* belonging to the family Portunidae the existence of a peculiar chromosome, which lies always at the central position of the equatorial plate going ahead of the others and migrates undivided to one pole in the primary spermatocyte division, is proved with absolute clearness. The latter peculiar element is quite comparable in its behaviour and other morphological features to the X-chromosome commonly observable in the Orthoptera and some other insects. Moreover, the diploid number of chromosomes attained in the spermatogonial division is proved to be odd, and further there are present two kinds of secondary spermatocytes in respect to the number of chromosomes. These evidences are sufficient to permit the conclusion that *Ovalipes punctatus* possesses an X-chromosome, and that the peculiar chromosome which goes undivided to one pole in the first division is no other than the X-element. As seen in the annexed figures (Figs. 3-8), the presence of the X is beyond question.

With the addition of the data from the present species, *Ovalipes punctatus*, therefore, it may be stated that there are present two types of sex-chromosome, XY and XO, in the decapods so far observed (cf. Niiyama, '37, '38). It is not uncommon in insects to find different types of sex-chromosome among species belonging to the same order. For example, in Diptera, the majority of cases have the XY chromosomes, while *Dasyllis grossa* possesses an X (cf. Metz, '16, '22). In Heteroptera and Coleoptera, there are also known both types of sex-chromosome, in some species the simple XO-type being demonstrated and in some others the XY-type or even the compound XY-type (Wilson, '25; Schrader, '28). In the case of Orthoptera, all the species belonging to the Acrididae (McClung '14 and others) and Locustidae (Asana, Makino & Niiyama, '38) show the XO-type of sex-chromosome, while the majority of species of the Mantidae (Oguma, '21; King, '31) possess the compound XY-type. The Gryllidae offer a more interesting example, that is, a great many species so far studied of this

family have the simple XO-type of sex-chromosome (Ohmachi, '35), while *Oecanthus longicauda* possesses XY chromosomes as the unique exception (Makino, '32).

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