A Study of Chromosomes in Echinoderms[1)]

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(*With 17 Textfigures*)

Reference to the literature appearing in over forty papers evinces the fact that the previous studies done on the chromosomes of echinoderms mainly deal with the behaviour of chromosomes during fertilization and the subsequent cleavage stages, with especial regard to the question of the chromosome individuality. The chromosomes of the cleavage mitosis, however, do not furnish in general any definite feature, but appear in an elongated thread-like form with unusual indefinite outline, representing the frequent occurrence of fragmentation, and on this account they are practically incompetent for the thorough research of the chromosomes. The observations based on such a cleavage material, therefore, cannot yield any conclusive results. The reported evidences in this field by previous investigators, therefore, only presented a mass of utter confusion indicating an astonishing lack of agreement either for the number of chromosomes or as to other morphological details. As Wilson ('25) suggested, it is true that the final solution of the confusing matters there presented might be brought about through a study of the chromosomes in germ-cells. The authors have had an opportunity to investigate the chromosomes of five species of the Echinodermata in spermatogenesis, the data from which form the subject matter of this paper. Though the data are a litte fragmental, to publish this small piece of work would not be entirely of insignificance, in view of the unsatisfactory state of the research work in this field.

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Material and Method: The following four species of the Asteroidea, *Henricia nipponica* Uchida, *Asterina pectinifera* Müller et Troschel, *Asterias amurensis* Lütken, and *Aphelasterias japonica* (Bell), and one species of the Echinoidea, *Strongylocentrotus intermedius* (A. Agassiz), are the animals which came under investigation in the present study. The male gonads treated with weak Flemming's solution recommended for the study of the Crustacean chromosomes by the junior author exclusively provided the material for study. Through the usual paraffin procedure, the testicular material was cut 5 to 8 micra thick and they were stained after the Heidenhain's iron-haematoxylin method with light-green.

Observations

1. *Henricia nipponica*

Henricia nipponica is a rather common sea-star on shore in the northern part of Japan and belongs to the Echinasteridae. The study was based on the material obtained in Osyoro in September of 1941.

Repeated examination was made for final decision of the chromosome number on account of the unusual high number, coupled with the small cell size, and it was decided that the present species possesses 54 chromosomes as diploid in the spermatogonial cell. As seen in Figs. 1–2, the diploid complement of this species is very prominent in containing two conspicuous large V-shaped elements which constitute a homologous pair. The remaining 52 elements are found to be of simple rod-type and show gradual variation in size, ranging from long rods to very short ones.

The haploid number was established as 27 from observations of the primary spermatocyte divisions (Figs. 3–4). This result is sufficient to furnish the corroborative evidence for the statement that the diploid number is 54. The haploid elements are all bivalents with ordinary structure, and there in no evidence for the presence of the heteromorphic bivalent characteristic to the X–Y complex.

By reference to the literature it is noticeable that the chromosome number established for this species is the highest among the Echinodermata so far as the chromosomes have been reported upon

at present. Coupled with this fact the chromosome constitution is likewise unique as compared with that of the other forms so far studied, in showing that the diploid complex comprises two remarkable elements which are very prominent from the rest on account of their enormous size and V-shaped configuration.

2. *Asterina pectinifera*

The present species is one of the most common sea-stars, abundantly found on shore from Hokkaido to Kyusyu and belongs to the Asterinidae. The material on which the study was carried out was derived from the specimens collected near Muroran in February of 1944.

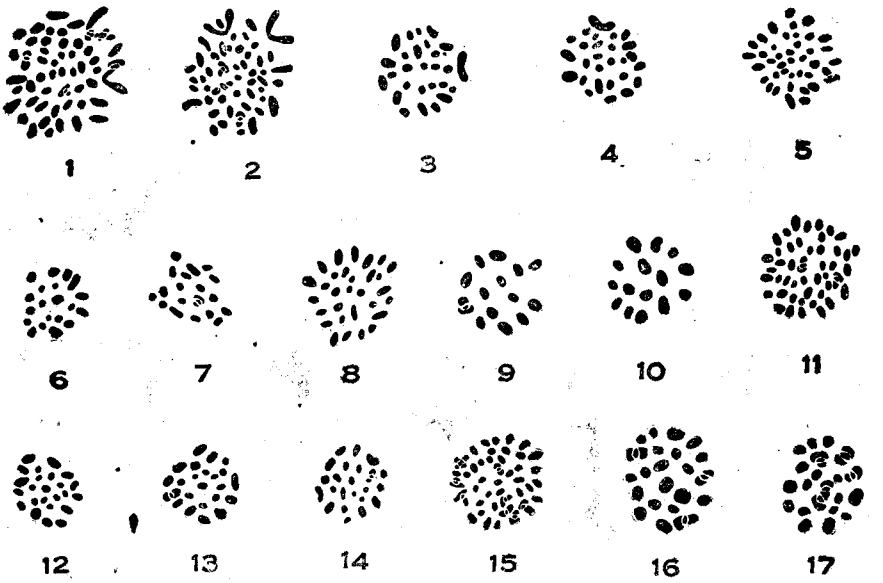
The minute size of the individual chromosomes coupled with the cell size of extreme smallness makes the counting of the chromosome number very difficult. Careful study of several equatorial plates excellently preserved indicated that the diploid number of chromosomes shown in the spermatogonial metaphase is invariably 40 (Fig. 5). The diploid complement seems to be of isomorphic type, because all of the composing elements appear in a form of short rods having no striking fluctuation in length. The chromosome counting in the primary spermatocyte metaphases gave 20 as the reduced number (Figs. 6–7). All the elements of the haploid group are no other than the ordinary bivalents, and no occurrence of any special element could be established in the complement.

3. *Asterias amurensis*

This is also a common species of the sea-star belonging to the Asteriidae. They are widely distributed in the North Pacific regions. The present material was collected and preserved at the Akkeshi Marine Biological station in August of 1944.

The diploid number observed in the spermatogonial metaphase was given to be undoubtedly 30 (Fig. 8). All of the chromosomes assume the form of simple rod showing a slight variation of length, and there occurs no element which is provided with the V-shape. The haploid number, 15, was obtained with extreme clearness by the investigation of the primary spermatocyte division (Figs. 9–10). Thus, the chromosome numbers, 30 for the diploid and 15 for the haploid, were fully established for this species beyond question.

The earlier investigations published by Jordan '07, Tennent '07 and Buchner '11, etc., recorded the diploid number of 36 in the cleavage material of *Asterias forbesii* and *A. glacialis*, while in *A. vulgaris* it is given as 18 by Tennent ('07). The number herein established for *A. amurensis* is not coincident with those statements as cited above.



All are camera-lucida drawings of the metaphase groups, under the magnification of 3700 diameters.

Figs. 1–4. Chromosomes of *Henricia nipponica*. 1–2, spermatogonia. 3–4, primary spermatocytes. Figs. 5–7. Chromosomes of *Asterina pectinifera*. 5, spermatogonium. 6–7, primary spermatocytes. Figs. 8–10. Chromosomes of *Asterias amurensis*. 8, spermatogonium. 9–10, primary spermatocytes. Figs. 11–14. Chromosomes of *Aphelasterias japonicus*. 11, spermatogonium. 12–14, primary spermatocytes. Figs. 15–17. Chromosomes of *Strongylocentrotus intermedius*. 15. spermatogonium. 16–17, primary spermatocytes.

4. *Aphelasterias japonicus*

This species is also a member of the Asteriidae showing a wide distribution from Saghalien to the south. The specimens obtained in Osyoro in September, 1942 furnished the material for study.

By the study of the primary spermatocyte divisions the haploid number obtained for the present species is undoubtedly 24 (Figs.

12–14). On the strength of this finding of the haploid complex, the diploid number was ascertained to be 48 at metaphase of the spermatogonial division (Fig. 11). The chromosomes which show a slight variation in size, appear in a thick rod-shaped structure of minute size and there is no sign of the existence of V-shaped element. The minute size of the chromosomes in this species makes a more precise study difficult.

5. *Strongylocentrotus intermedius*

This is a species of echinoids which is very common in the northern parts of our country. The material on which the study was carried out came from specimens collected and preserved at the Akkeshi Marine Biological Station in August of 1944.

The extreme smallness in the cell-size together with the relatively high number of chromosomes contained make the study rather difficult. Close investigations of the spermatogonial metcphases reveal that the number of chromosomes possessed is evidently 50 (Fig. 15). The corroborative evidence for this finding was furnished from observations of the meiotic chromosomes showing that the primary spermatocyte division lets always see at metaphase 25 chromosomes as the reduced number (Figs. 16–17). It is thus beyond question that the present species possesses the diploid number of 50 and the haploid number of 25.

The diploid group consists of elements assuming a simple rod-shape with very slightly varying length and there is no evidence for the occurrence of the V-shaped element. The chromosome complex of this species is therefore to be of the isomorphic type. Throughout the diploid and haploid stages, any particular element causing the sexual difference of chromosomes could not be pointed out.

Upon looking through the literature references to a considerable number of classical papers regarding the chromosomes of echinoids were found. Working with *Strongylocentrotus lividus* Boveri '02, Baltzer '09, '10, '13, Petrunkewitsch '04 and Schaxell '11 gave the diploid number of 36 in the study of the cleavage material. Hindle '11 also reported the same number in *S. purpuratus*. It is highly probable that such a low count may be attributed to the condition of the cleavage chromosomes unfavourable for accurate study.

Remarks and Summary

On looking up the literature references, a considerable number of classical papers regarding the chromosomes of echinoderms have been accessible. Almost all of these earlier investigations have been concerned with fertilized eggs, with special attention towards the behaviour of the chromosomes during fertilization and the subsequent cleavage stages. Some authors, such as Baltzer '09, '10, '13, Tennent '07, '11, '12, Heffner '10 and Pinney '11, made an attempt to correlate the observed facts in regard to the dimorphism in the chromosome groups in hybrid cultures of echinoids with the dimorphism of sex in this group of animals, in connection with the established fact regarding the occurrence of hetero-chromosomes in insects. These earlier investigations, however, could not furnish any conclusive accounts, either for the number of chromosome or for the sex-mechanism of chromosomes; there has been still left a number of disagreements on these points resulting in an endless controversy among the authors. It is evident that final confirmatory evidences might be obtained through a study of spermatogenesis, but the latter field has long been remained untouched by any authors due probably to the technical obstacles. There is no doubt that the present work which deals with the chromosome research in some echinoderms through the spermatogenetic stage may constitute a valuable contribution to this field of study, though the data are somewhat fragmental.

In Asteroidea, the chromosomes of *Asterias forbesii*, *A. glacialis*, *A. vulgaris* and *Cribrella sanguineolenta* were previously reported. Jordan '07, Tennent '07 and Buchner '11 recorded 36 chromosomes as $2n$ in the cleavages of *A. forbesii* and *A. glacialis*, while in *A. vulgaris* Tennent '07 gave 18 as the diploid number. *Cribrella sanguineolenta* was found to possess 36 somatic chromosomes by the study of Jordan '10.

In the case of Echinoidea the reported observations are very motley and confusing. According to Jordan '12, *Arbacia punctulata* was found to show 40 chromosomes in the cleavage, while Matsui '24 described 38 chromosomes in the same species. In *Chrypeaster rosaceus* Gardiner '27 reported the diploid number of 44, and Matsui '24[a] observed 52 chromosomes in the cleavage of *Echinarachnius*

parma. Doncaster & Gray '13 counted 38 somatic chromosomes for *Echinus acutus* and *E. esculenta*. Stevens '02 and Baltzer '09, '10, '13 reported 38 as the diploid number for *Echinus microtuberculatus bivalens*, while *E. microtuberculatus univalens* was observed to have 18 chromosomes in diploid by Stevens '02 and Boveri '05. According to Pinney '11, *Hipponoë esculenta* and *Moira atropus* was found to show 32 and 46 chromosomes in diploid respectively. The diploid number of *Strongylocentrotus lividus* was reported to be 36 by Baltzer '09, '10, '13, Boveri '02, Petrunkewitsch '04 and Schaxel '11. Hindle '11 found the same number in *S. purpuratus*. The diploid number of *Toxopneustes variegatus* was given as 36 in the studies of Wilson '95, '01, Heffner '10 and Tennent '12.

As already mentioned, it is very difficult and at the same time very dangerous to draw any final conclusion of the chromosome number from the study of the cleavage material, because the chromosomes appearing in the cleavage spindle assume in general an unusually slender outline quite unsuitable for research work, and there is an occasional occurrence of irregular fragmentation of the chromosomes in such a material. On the basis of these facts and also in the light of our recent knowledge, it is evident that the reported evidences by earlier investigators as quoted above, can only furnish an approximate and probable account so far as the chromosome number is concerned. Under this view the results of the present study which were based on observations of both diploid and haploid phases in germ-cells may be of importance. The accounts presented in this study are not in accord with those reported by the previous authors, either in respect of the number of chromosomes or in the other morphological details. The species under study and their chromosome numbers are summarized in a form of table as given below. The chromosome complement is very unique in *Henricia*

Species	2*n*	*n*	Figures
Asteroidea			
Henricia nipponica	54	27	1- 4
Asterina pectinifera	40	20	5- 7
Asterias amurensis	30	15	8-10
Anthelasterias japonicus	48	24	11-14
Echinoidea			
Strongylocentrotus intermedius	50	25	15-17

nipponica showing a pair of prominent V-shaped elements, while the other forms were found to possess karyotypes composed of simple

rod-shaped elements only, in despite of the difference of their chromosome numbers.

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