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Title	THE CHROMOSOMES OF THE GRAYFISH, CAMBAROIDES JAPONICUS (DE HAAN) (With 4 Figures in Text)
Author(s)	NIIYAMA, Hidejirō
Citation	北海道帝國大學理學部紀要, 3(2), 41-53
Issue Date	1934-09
Doc URL	https://hdl.handle.net/2115/26964
Type	departmental bulletin paper
File Information	3(2)_P41-53.pdf



THE CHROMOSOMES OF THE CRAYFISH, *CAMBAROIDES JAPONICUS* (DE HAAN)¹⁾

BY

Hidejiro NIYAMA

(With 4 Figures in Text)

As is well known, the chromosome of the decapod Crustacea is very conspicuous for its large number, probably being the largest so far discovered in the animal kingdom (see HARVEY's list, '16, '20). Still further, it exhibits different and unique characteristics when compared with other groups of Crustacea e. g., Copepoda (HÄCKER '08; BRAUN, '09; MATSCHEK, '09; '10, AMMA, '11; HEBERER, '32), Ostracoda (SCHLEIP, '09; SCHMALZ, '11, '12), Cladocera (TAYLOR, '14; CHAMBERS, '13) Isopoda (CARNOY, '85; KOMAI, '20, SUGIYAMA, '32), not only in the number but also in the shape.

Though a number of memoirs have appeared on the cytology of the Decapoda, many important problems as to the chromosome morphology still remain obscure in this group of animals. The present paper embodies the results of a study on the chromosome history in the crayfish, *Cambaroides japonicus*, with the purpose of determining the chromosome number and discovering the sex chromosome, if possible.

The work was carried out under the kind guidance of Professor OGUMA, to whom the author wishes to express his hearty thanks. The author is also greatly indebted to Mr. S. MAKINO, for his kind aid in several ways during the course of the work.

1) Contribution No. 74 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

Material and Method

Cambaroides japonicus (de HAAN) is a common crayfish in the lakes and streams throughout Hokkaido. According to OKADA ('33) it distributes over northern Japan, from Aomori to Sakhalin. The material with which the present study was carried out was mostly obtained in the suburbs of Sapporo, but some are secured from the Lakes of Doya and Kusshara, during June, July and August 1933. The material obtained from the beginning to the end of July proved to be most favourable for the study of chromosomes of various stages. In general, the spermatogonial divisions are encountered in the material obtained at the end of June and the beginning of July, while the spermatocytes are found in the course of division in the testes fixed during the period from the middle part to the end of July. The entire course of spermioteleosis is observed in the material prepared in August. It is noteworthy to find that the testicular contents of this animal are very badly influenced under state of captivity in the laboratory. The germ cells always show a strong tendency to disintegrate even though in material reared for only a few days in the aquarium. For this reason the author used only the material which was killed and fixed immediately after capture.

Although various reagents were employed for fixation of the chromosomes, such as, BOUIN's, CARNOY's, HERMANN's, CHAMPY's, weak CHAMPY's (diluted with an equal volume of dist. water), FLEMMING's and weak FLEMMING's fluids, the most favourable results were obtained merely either weak CHAMPY's or weak FLEMMING's fluid. The former fluid, however, is very good for preservation of the chromosomes, but is much less receptive of iron haematoxylin stain than is the latter fluid. Allowed to rest in the fixing fluids for 20 to 24 hours, the material is washed thoroughly in running water for the same length of time. After being dehydrated with ascending grades of alcohol in the usual ways, the material is cleared in creosote-toluol and toluol, then imbedded in paraffin. Sections were cut 10 micra in thickness. After bleaching with

PAL's method, the sections are chiefly stained with HEIDENHAIN's iron haematoxylin with a counterstain of light-green. For the material fixed with CHAMPY's fluid, CHURA's mixture was used in order to intensify staining power, by leaving for 24 hours in the latter at room temperature before the use of iron-alum. For colour differentiation of the preparations the saturated solution of picric acid was applied; this method was proved to be more satisfactory than the use of iron-alum, especially for the material treated with CHAMPY's fluid.

All the figures were drawn with the aid of an ABBE drawing apparatus at the level of the desk on which the microscope was set.

Observations

I. *The spermatogonium*

The spermatogonium is rather large in size and nearly round in shape. Two kinds of spermatogonia, the primary and secondary may be classified by their size.

The spermatogonium does not undergo simultaneous division in a cyst; the dividing figures are found independently with the neighbouring cells. In the polar view the chromosomes are noticed to be distributed homogeneously throughout the whole area of the equatorial plate, arranging themselves well apart from one another. It is not very difficult, therefore, to count the number of chromosomes in spite of their large number. By careful counting of many equatorial plates it was ascertained at last that the spermatogonium contains invariably 196 chromosomes (Fig. 1. *a, c, d*; secondary spermatogonia. *b*; primary spermatogonium). The chromosomes are mostly short straight rod-shaped, but sometimes slightly curved, and it seems to be impossible to identify the homologous pairs except in the longest ones. These longest chromosomes are always found two in number, and can easily be distinguished from the remaining chromosomes. They are nearly equal in size and sometimes bent at the middle region (Fig. 1, *a* and *c*). In some cases these two take

their position at the periphery of the equatorial plate as seen in Fig. 1, *c*; occasionally they lie at the central region of the plate surrounded by the others (Fig. 1, *b* and *d*). In general appearance the metaphase chromosomes of the spermatogonium much resembles those observed in the Lepidoptera (see BELIAJEFF, '32).

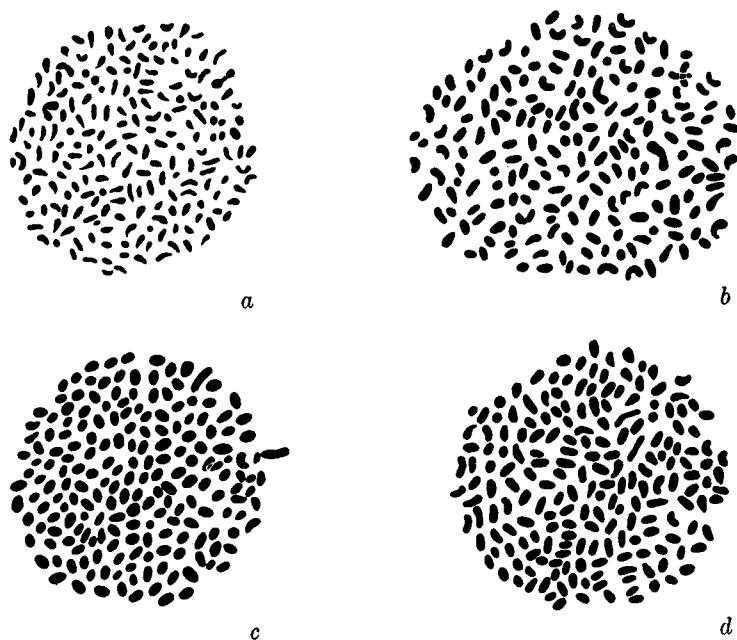


Fig. 1. *a-d*. Metaphase groups of spermatogonia, 196 chromosomes in each. $\times 2600$.

FASTEN ('14) illustrated 200 dot-like chromosomes in the spermatogonium of *Cambarus virilis*, stating that "the chromosomes are spherical in shape and situated close to one another". The present species, therefore, is remarkably different from *Cambarus virilis*, in respect to the shape of chromosomes.

II. *The primary spermatocyte*

In the primary spermatocyte the chromosomes appear in extreme clearness in the metaphase as shown in Fig. 2, *a-i*. Therefore counting of the chromosome number is very easy in the present

stage. In every equatorial plate examined, a garniture of bivalent chromosomes is, without exception, made up of 98 tetrads constituting nearly a round equatorial plate.

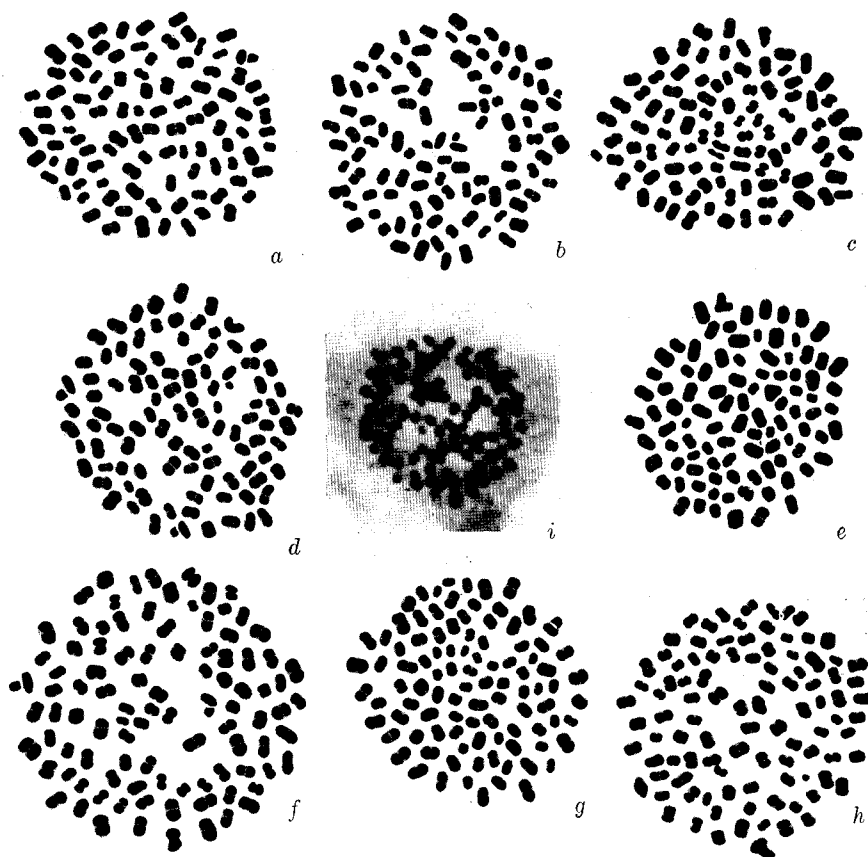


Fig. 2. *a-h*. Metaphase groups of primary spermatocytes, 98 tetrads in each. $\times 2600$. *i*, photomicrograph. $\times 1400$.

The tetrads are generally so disposed in the equatorial plate as to be scattered about throughout the whole space of the latter. Frequently, a few vacant spaces are found in the central region of the equatorial plate as seen in Fig. 2, *b*, *f*, and *h*, being probably due to some mechanical effects in preparing the sections.

When viewed from a pole, the tetrad exhibits a conspicuous transverse suture, across its middle region, by which it is seemingly

separated into two components (Fig. 2, *a-h*). A quite similar condition has already been demonstrated by OGUMA and ASANA ('32) in the primary spermatocyte chromosomes of certain species of the dragonfly, and they consider "This transverse splitting denotes the line of contact of chromatids, of which the tetrad is composed," So far as the observations go, the author inclines to consider that the present tetrads coincide with those found in the dragonfly chromosomes in respect to their nature and structure. Such a structure of the tetrad has never been described by any previous authors who studied the decapod chromosomes (FASTEN, '14, '18, '24, '26 and others). With regard to this fact, FASTEN ('14), in two species of *Cambarus*, wrote thus: "In the metaphase condition the tetrads appear as two large bivalents connected in the form of dumb-bells. No split can be noticed in either of the bivalents, where the equational division of the second maturation stage will occur." Considered from the nature of the tetrad in general, the presence of transverse splitting should be rather natural to find as in the case of the crayfish above mentioned. The tetrad without split as illustrated by previous investigators, seems to be derived from too much hardening of chromatin due to the fixing solutions such as BOUIN's or its modified mixture.

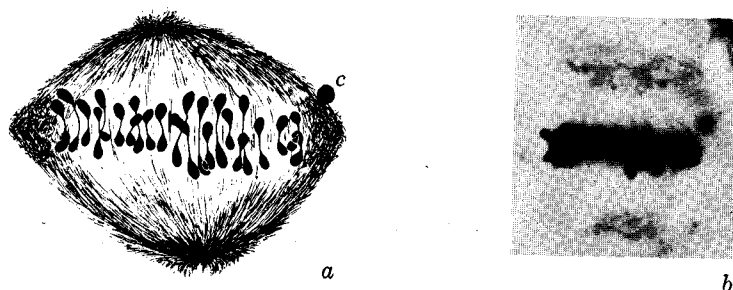


Fig. 3. *a*, side view of primary spermatocyte, early anaphase (not all elements shown). $\times 2600$. *b*, photomicrograph. $\times 1400$. *c*; chromatoid body.

In the anaphase, all the tetrads separate symmetrically into two equal halves, giving rise to the daughter chromosomes composed of

a pair of chromatids in close contact (Fig. 3, *a-b*). The division of the chromosomes is synchronous, neither precession nor succession could be observed in any chromosome. Thus the present division causes only one kind of chromosome garniture in the resulting secondary spermatocytes.

As a constant occurrence the chromatoid body of spherical form was found, one or more in number, in the cytoplasm (*c* in Fig. 3, *a*).

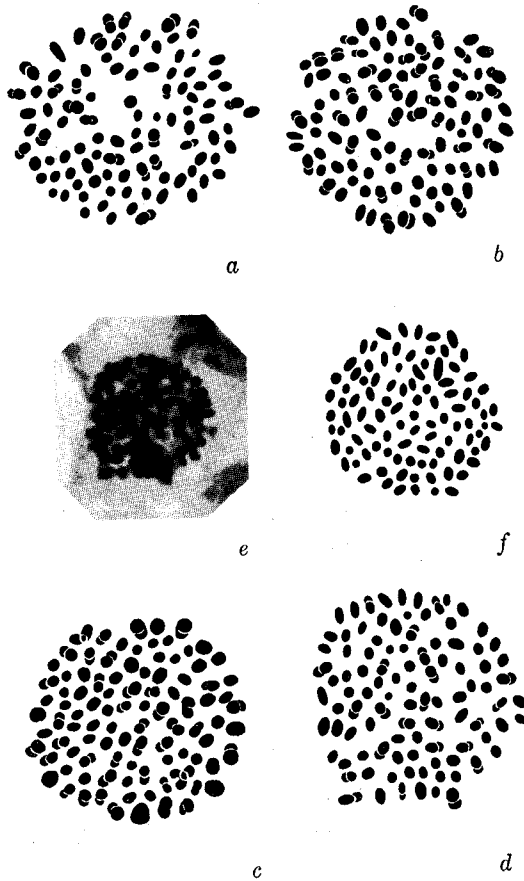


Fig. 4. *a-d*. Metaphase groups of secondary spermatocytes, 98 dyads in each. $\times 2600$. *e*, photomicrograph. $\times 1400$. *f*, a sister complex of chromosomes in the anaphase of secondary spermatocyte, 98 monads. $\times 2600$.

III. *The secondary spermatocyte*

As expected from the mode of distribution of chromosomes, the primary spermatocyte division actually gives rise to only one kind of secondary spermatocyte. The chromosome is generally much smaller in size, compared with that of the primary spermatocyte. Careful counting shows, without any exception, 98 chromosomes in every metaphase equatorial plate (Fig. 4, *a-d*).

They are all rod-shaped but seem so much shorter than the spermatogonial chromosome that one may sometimes mistake them for the primary spermatocyte. But every one does not exhibit tetrad

nature but dyad, of which the composing halves are superimposed each other. There is nothing particular in the mode of the present division; every dyad separates equally into two daughter monads. Fig. 4, *f* shows one of the two daughter chromosome garnitures in the anaphase in which 98 monads are clearly to be counted. In this group of chromosomes we find a long chromosome, which is clearly distinguishable from the remaining ones. This chromosome, as may be readily noticed, is that one which corresponds with one of the longest chromosomes in a pair found in the spermatogonium. The reason why this large chromosome has never been observed in either primary or secondary spermatocyte divisions, is that the difference in size between the latter-mentioned chromosome and the remaining ones is not sufficiently great to distinguish it in a state otherwise than its full extension.

General Considerations

I. *The chromosome number*

Chiefly due to the interesting structure of the sperm, most memoirs hitherto published on the cytology of Decapoda have been limited to the development of the spermatozoa and have not extended so far as to the chromosome (ANDREWS, '04; KOLTZOFF, '03; RETZIUS, '09; NATH, '32, etc.). Thus to enumerate the literature of the chromosome¹⁾, only eleven titles are actually accessible at the present time. The table shows the chromosome number of the Decapoda hitherto studied.

As obvious in the table, the chromosomes of the Decapoda are generally large in number, as compared with the other groups of Crustacea. For example, in the Copepoda, as seen in HARVEY's list ('16, '20), the number of chromosomes varies from sixteen to thirty-two. In the Ostracoda it is generally from twelve to sixteen.

1) Recently a Russian author published a paper concerning the spermatogenesis in the Decapoda, under the title "La spermatogénèse et les rapports phylogénétiques chez les Decapodes". (SELIPOVA, '29). But unfortunately this paper was not accessible to the author.

Species	Diploid	I-cyte	II-cyte	Author
<i>Carcinus menas</i>		30-40		CARNOY, '85
<i>Crangon cataphractus</i>		40-44		"
<i>Eupagurus prideauxii</i>		12 f.	12 f.	WEISMANN and ISHIKAWA, '88
<i>Astacus fluviatilis</i>		ca. 58		PROWAZEK, '02
<i>Homarus</i>		18		LABBÉ, '04
Euphasidae	38 cl.			TAUBE, '09
<i>Hippa talpoides</i>		60		NICHOLS, '09
<i>Menippe mercenaria</i>	51-80 spg.		25-28 f.	BINFORD, '13
<i>Cambarus virilis</i>	200 spg.	100	100	FASTEN, '14
<i>Cambarus immunitis</i> (?)		104	104 (98)	"
<i>Cancer magister</i>		60	60	FASTEN, '18
<i>Cancer productus</i>		58		FASTEN, '24
<i>Cancer oregonensis</i>		56		" "
<i>Cancer gracilis</i>		52		" "
<i>Lophopanopeus bellus</i>		62		FASTEN, '26
<i>Cambaroides japonicus</i>	196 spg.	98	98	NIYAMA, '34

spg.: spermatogonium. I-cyte: primary spermatocyte.

II-cyte: secondary spermatocyte. f.: in female. cl.: in cleavage.

The form of chromosomes in the Decapoda is uniformly short-rod or dot-like. In a study on the spermatogenesis of *Squilla* (the Isopoda), KOMAI ('20) describes a similar form of chromosomes. SUGIYAMA ('33), on the contrary, reports quite interesting evidence in the Isopoda, *Asellus*, in which he found fourteen V-shaped chromosomes.

II. Some remarks on the sex chromosome

It is the most important and interesting problem to examine whether the sexual difference of chromosomes actually exists in the Crustacea, as is the case in Insecta. In some orders of the Crustacea the sex chromosomes have been questioned and discussed by investigators, though a conclusive demonstration has not yet succeeded. In the egg of *Cyclops fuscus* var. *distinctus*, both BRAUN ('09) and MATSCHEK ('10) have described a single, and smaller tetrad which

does not show the "Querkerbe" in addition to 5 ditetrads. This tetrad goes undivided to one pole in the first maturation division. AMMA ('11) believed that in some embryos of this species the diploid number is 11, suggesting the presence of a sex chromosome. Similar conditions were found by these workers in maturing eggs of *Cyclops affinis*, *C. prasinus*, *C. phaleratus* and *C. vernalis*. If these observations be correct, then the female should be heterogametic in respect to chromosomes.

According to KORNHAUSER ('15), in *Hersilia apodiformis* both spermatogonia and oogonia show 22 tetrad-like chromosomes plus 2 chromosomes of the simple type. In the egg pseudoreduction there are found 12 true tetrads, of which 11 still show the "Querkerbe", while in the spermatocytes there are usually 11 tetrads and 2 united chromosomes. The latter occasionally pair, but whatever their pairing behavior may be, they always separate to opposite poles in the first spermatocyte division. Since the second division seems to be equational, all spermatids, therefore, receive 11 ordinary chromosomes plus 1 heterochromosome. It will be apparent that the behavior of the pair of heterochromosomes suggests an XY-pair and consequently the male would be heterogametic.

Recently PALMER ('25) reported the occurrence of an XY-pair of sex chromosomes in the male of *Gammarus chevreuxi*. He emphasizes his claim in his next paper, published in 1926, but he could observe neither the behavior of the heterochromosomes in the pro-phases nor their presence in the female cells.

More recently, HEBERER ('32) has undertaken extensive studies on the chromosomes of the Copepoda, covering five families, Diaptomidae, Temoridae, Calamidae, Centropagidae and Meriidae. Concerning the sex chromosome, however, he could conclude merely as follows: „Das Heterochromosomenproblem bei den Copepoda ist noch fast ganz ungeklärt. Es werden Indizien beigebracht, die eine männliche Heterogametie (XO) bei den Calanoiden sehr wahrscheinlich machen“.

So far as the Decapoda are concerned, FASTEN ('14) seems to be the first and sole author who has dealt with the accessory chromosomes. According to him, in the metaphase of the primary spermatocyte division of *Cambarus immunis* (?) a group of eight chromosomes are found distinctly separated from the other chromosomes, generally along the periphery of the spindle, and seems to be enclosed in the clear vacuole. In the ensuing division this entire group goes to one pole while the other chromosomes divide normally. He stated that: "It is impossible to state whether these chromosomes are accessories, for their behavior could not be traced." In the present material, though closely examined, such a particular group of chromosomes could not be discovered. Still further, there occurred neither precession nor succession in any chromosomes in dividing behavior. As already noticed in the descriptive part, two long chromosomes, clearly distinguishable from the others, were observed in the spermatogonium, but they divide equally in the maturation divisions and there is nothing unusual in the behavior of the last-mentioned chromosomes. At present, therefore, the author hesitates to conclude that these two are accessory chromosomes.

Summary

1. The spermatogonium contains 196 chromosomes, of which two are distinctly large.
2. In the primary spermatocyte metaphase are found 98 tetrads. Every tetrad shows, in the polar view a conspicuous transverse suture in its middle region.
3. The secondary spermatocyte shows 98 dyads. In division each dyad separates equally.
4. Throughout the dividing stages of the spermatogenesis, no peculiar chromosomes in heterotropic behavior, characteristic to the accessory chromosome, could be observed.

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