A SUPERNUMERARY CHROMOSOME FOUND IN A *PODISMA SAPPOROENSE* SHIRAKI, AND ITS RELATION TO THE SEX-CHROMOSOME.

BY

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(With One Plate)

The occurence of the supernumerary chromosome is of significance from the view point of cytology in relation to the problem of mutation. So it has long attracted the attention of many investigators such as WILSON (1907, 1908), McCLUNG (1914, 1917), CARROL (1920), CAROTHERS (1919) ROBERTSON (1915, 1916, 1917), VOINOV (1914) and WOOLSEY (1914) etc., who have reported emphatically the fact in various cases. The present author during his cytological study of *Podisma sapporoense* SHIRAKI in 1928, one of the common acridids in Hokkaido, found a supernumerary chromosome in the meiotic divisions of germ cells.

The material and the method. *Podisma sapporoense* SHIRAKI was collected abundantly in the suburbs of Sapporo in July 1928, since in this season generally, the meiotic divisions in the testis had been proved to take place very actively. The testis was taken out from the fresh material and was fixed in strong FLEMMING's fixative solution for twenty-four hours. After washing carefully in running water, dehydration was carried out in alcohol. The testis was imbedded in paraffine and stained with HEIDENHAIN's *Fe*-haematoxylin. It was cut in serial sections with a thickness of twenty micra.

The drawings were made by the aid of Abbe's drawing apparatus using a Leiz objective 1/12, and a Leiz ocular 4 with the tube length 190 mm., the drawing plate being placed about 11 cm. beneath the stage of the microscope. By the suggestion of Mr. K. SHIMAKURA, the figures were shaded so as to make any chromosome or any part of a chromosome at the deeper focus to appear darker than the shallower. In this way, the depth of a chromosome is made easily visible which would otherwise be very unclear.

Observations. ***The spermatogonia.*** The supernumerary chromosome is to be identified by its size and form as well as the sex-chromosome already in

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the spermatogonial metaphase; in this stage, twenty-four chromosomes are found of which twenty-two are autosomes, one is the supernumerary chromosome and the remaining one is the sex-chromosome. The supernumerary chromosome marked s in the fiqures is a bipartite V-shaped chromosomes of medium size, as shown in Fig. 2 a, b, c. We see at present that the supernumerary chromosome and the sex-chromosome are in a more diffused state than the others; the condition was more marked in the sex-chromosome. From the fact that every cell in the spermatogonia in different stages, thus far observed, contains one supernumerary chromosome and one sex-chromosome, it is highly probable that the supernumerary chromosome undergoes mitotic division normally like the other chromosomes.

Apparently there is no definite relation in position between the supernumerary chromosome and the sex-chromosome during the spermatogonial metaphase. In the normal* case we find only twenty-three chromosomes and no supernumerary chromosome, as is seen in Fig. 1, which shows the spermatogonial metaphase in the normal *Podisma sapporoense* SHIRAKI. Though V-shaped chromosomes are generally to be seen in the species of Stenobothrus-type (after McCLUNG), it is peculiar that it also occurs in the species of Hippiscus-type (after McCLUNG) such as *Podisma sapporoense* SHIRAKI.

The early leptonema stage. The sex-chromosome appears in this case prior to the supernumerary chromosome in any stage during the prophase of the first spermatocyte, even at the time when the chromosome substances are in a state of condensed karyotin mass. It constantly takes a peripheral position in the nucleus, and is clearly distinguishable from the other autosomes by its heavy heteropycnosis.

The leptonema stage. The sex-chromosome becomes a more condensed karyotin mass, keeping the same position as before, but the supernumerary chromosome, which is now a condensed karyotin thread, approaches nearer to the sex-chromosome so as to be arranged ready for the bouquet stage (Fig. 3).

The bouquet stage. The sex-chromosome condenses intensely in this stage and shows at the same time an irregular U-shaped thread or sometimes an open ring. Here apears then the bouquet stage, in which karyotin threads come into sight between the sex-chromosome and the supernumerary chromosome (Fig. 4). The two chromosomes approach each other still further.

The pachynema stage. The sex-chromosome and the supernumerary chromosome become confused with each other to make a karyotin mass, consisting of unequal halves in size. We see a vesicle attaching to one end which

*The normal nucleus was described by the writer in a recent work (1931) is forked (Fig. 5). Judging from the size and the form of the karyotin mass and the position of the vesicle just mentioned, the larger half may represent the sex-chromosome and the smaller half the supernumerary chromosome. So it is to be understood that we have a modified diakinesis, as regards to these two chromosome. From this stage, the heteropycnosis of the bivalent chromosome, consisting of the supernumerary chromosome and the sex-chromosome, becomes more precocious than in the sex-chromosome of the normal individuals.

The strepsinema, the diplonema and the diakinesis stage. During these stages, the karyotin mass takes a tetrad form, but the sex-chromosome and the supernumerary chromosome are each identified clearly by their size and form (Fig. 6). The terminal vesicle in these stages attaches to the neck part of the pointed end of the sex-chromosome. A peculiar fiber, having the staining quality of karyotin, is distinctly recognized between the tip of the sex-chromosome and the vesicle (Fig. 6, Fig. 14). Now the segregation of the tetrad takes place (Fig. 7).

Even after the segregation, the vesicle always attaches to the sex-chromosome (Fig. 7, Fig. 14 b). There is a question whether the vesicle has some direct functional mechanism in the segregation of the tetrad in this case. However, it is highly probable that the vesicle does have some attraction to the sex-chromosome, while the supernumerary chromosome is separated from the sex-chromosome indirectly (Fig. 5, Fig. 6, Fig. 7, Fig. 14 a, b). It seems true that the behavior of the vesicle in this case differs from what was reported by Dr. CAROTHERS (1916), who is of the opinion that the vesicle actually divides the tetrad into two.

The metaphase of the first spermatocyte. Fig. 8 a, b and Fig. 9 a, b show the stage in which eleven out of the thirteen chromosomes are tetrads, and the remaining two, namely, the sex-chromosome and the supernumerary chromosome are dyads. The supernumerary chromosome is now a compact spherical chromosome with a ring skeletal structure.

While the other tetrads are arranged in the equatorial plate, the supernumerary chromosome appears at the pole region and the sex-chromosome is seen a little apart from the equatorial plate (Fig. 8 b, Fig. 9 b). They never segregate in the metaphase of the first spermatocyte. After this stage the supernumerary chromosome becomes more conspicuous than the sex-chromosome in appearance. The vesicle of the sex-chromosome, however, has disappeared by this time. In some of the cells the supernumerary chromosome and the sex-chromosome, together go toward the same pole (Fig. 8. a, b), while in the other they are separated (Fig. 9 a, b).

With a view to determine the mechanism of division of the sex-chromosome and the supernumerary chromosome, the investigation of 146 nuclei was carried out as follows.

toward one pole	both X & S	X only
frequency	76	70
ratio	1.08	1.00

From the above the conclusion is that they divide only by chance. Consquently, it is obvious that four kinds of daughter nuclei are produced according to the combination of the sex-chromosome, the supernumerary chromosome and the autosomes. In other words, in case the sex-chromosome and the supernumerary chromosome go toward the same pole, one daughter nucleus will contain thirteen chromosomes, namely, eleven autosomes, one supernumerary chromosome and one sex-chromosome, while the other daughter nucleus will contain eleven autosomes. In another case, in which the sex-chromosome and the supernumerary chromosome go toward the opposite poles, both nuclei will contain twelve chromosomes of which eleven are autosomes. The four kinds of nuclei thus produced are shown in the following table.

chromosome number	chromosome formula	type
13 chromosomes	11A+X+S	a-type
12 chromosomes	11A+S	b-type
12 chromosomes	11A+X	c-type
11 chromosomes	11A	d-type

A=autosome, S=supernumerary chromosome, X=sex-chromosome

The second spermatocyte. The sex-chromosome is no longer identified in this stage, but the supernumerary chromosome is clearly recognized by its size and form as well as strong staining capacity. It is a heart shaped chromosome and segregates in this stage as the other chromosome (Fig. 10, Fig. 11).

The four kinds of nuclei which are supposed to come forth in the previous stage are actually traced in this stage as shown in the fiqures representing a-type (Fig. 10), b-type (Fig. 11), c-type (Fig. 12) and d-type (Fig. 13) respectively.

The spermatid. The sex-chromosome and the supernumerary chromosome can no longer be distinguished from other autosomes. However, the four kinds of spermatids, still to be identified in this stage by their chromosomal constitution, develope into spermatozoa respectively without any disturbance. The ripe spermatozoa are seen filling the cysts as normal in this insect.

From the above observations the following facts are summerised: A supernumerary univalent chromosome shows heteropycnosis like the sex-chromosome. A supernumerary univalent chromosome takes a sex-chromosome as a mate for tetrad formation. The abnormal tetrad, consisting of a supernumerary chromosome and a sex-chromosome, undergoes the division precociously. Especially it is worthy of notice that the tetrad is able to divide into two in the early diakinesis stage of the nucleus which has not yet complete achromatic figure. Taking part in the tetrad formation the supernumerary chromosome accelerates the process of heteropycnosis of the sex-chromosome.

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Explanation of Figures

(× 1200)

Fig. 1 Spermatogonial metaphase from a normal individual without super numerary chromosome. (23 in number).

Fig. 2 a, b, c. Spermatogonial metaphase showing V-shaped supernumerary chromosome (S) and rod-shaped sex-chromosome (X). (24 in number).

Fig. 3 Leptonema stage showing supernumerary chromosome (S) and sex-chromosome (X). (optical section).

Fig. 4 Amphinema stage showing supernumerary chromosome and sex-chromosome with vesicle. (optical section).

Fig. 5 Pachynema stage. A tetrad consisting of supernumerary chromosome and sex-chromosome with vesicle. (optical section).

Fig. 6 Early diakinesis stage showing completed tetrad with vesicle. (optical section).

Fig. 7 Early diakinesis stage. Showing segregated supernumerary chromosome and sex-chromosome with vesicle. (optical section).

Fig. 8 a, b. Metaphase of the first spermatocyte; a, polar wiew. b, oblique side view. Supernumerary chromosome and sex-chromosome go toward the same pole. (11 tetrads & 2 dyads).

Fig. 9 a, b. Metaphase of the first spermatocyte; a, polar view. b, side view. Supernumerary chromosome and sex-chromosome go toward opposite poles. (11 tetrads & 2 dyads).

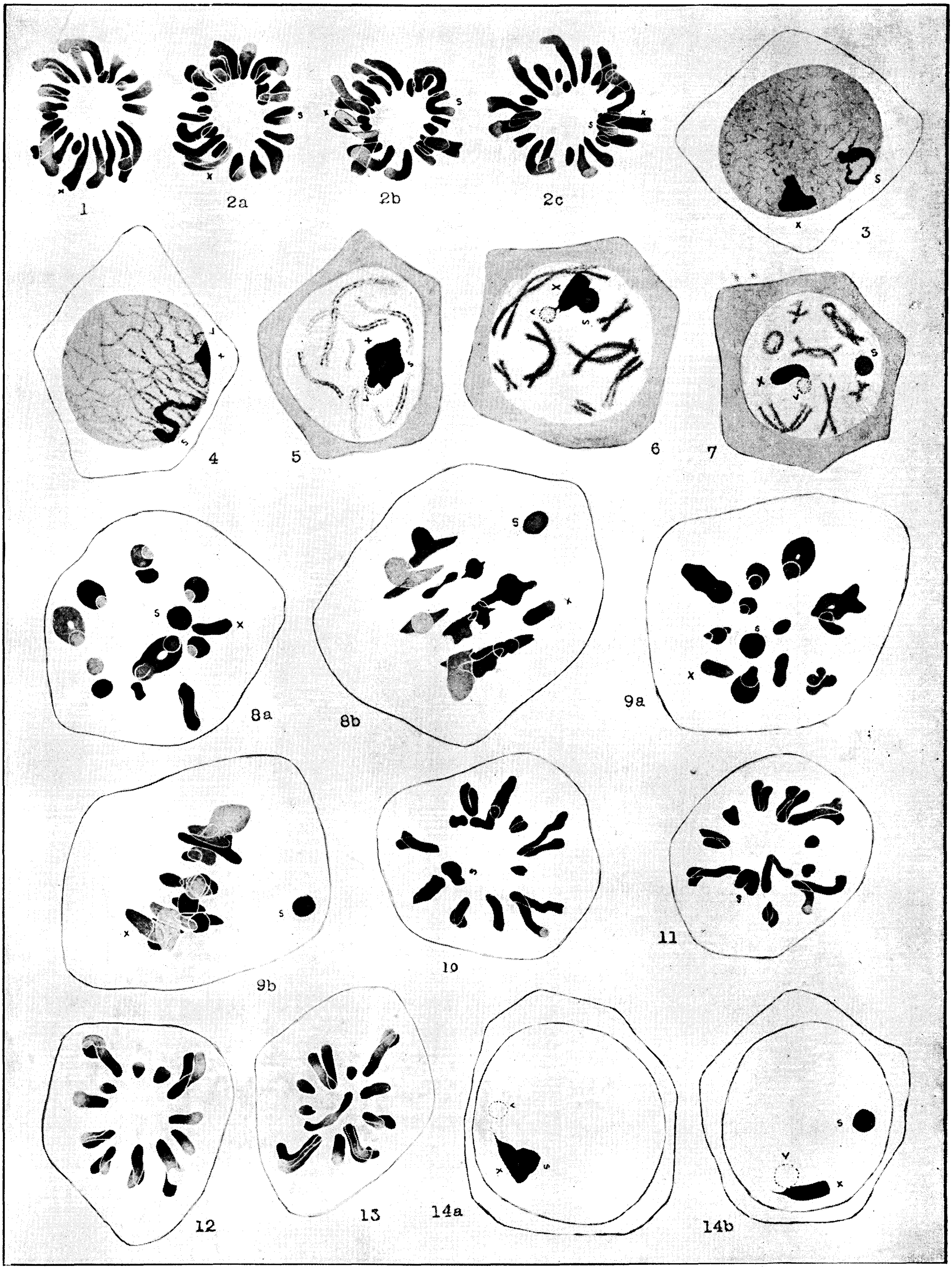
Fig. 10 Metaphase of the second spermatocyte, containing supernumerary chromosome and sex-chromosome. (13 dyads).

Fig. 11 Metaphase of the second spermatocyte, containing supernumerary chromosome. (12 dyads).

Fig. 12 Metaphase of the second spermatocyte, containing the sex-chromosome. (12 dyads).

Fig. 13 Metaphase of the second spermatocyte, containing autosomes only. (11 dyads).

Fig. 14 a, b. Segregation process of a modified tetrad in later prophase of the first spermatocyte.



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摘　　要

アシマダラフキバッタ (*Podisma sapporoense* SHIRAKI) に見出されたる過剰染色體と其の性染色體との關係

名　取　武　光

精蟲發生の過程に於ける過剰染色體の運命は、種の突然變異の問題に關聯して細胞學的に又遺傳學的に興味ある研究問題なり。されば此の問題につき研究を進めたる學者も尠しとせず。就中 WILSON, MCCLUNG, CARROL, CAROTHERS, ROBERTSON, VOINOV 及 WOOLSEY の諸氏は、直翅類の過剰染色體につきて業績を遺す。余も亦 1928年 アシマダラフキバッタに於て過剰染色體を見出し特に其の性染色體との關係に於て特殊なる現象を認めたれば、茲に記して該問題に關する研究補遺となす。

精原細胞に於ける過剰染色體は、V字型の中形染色體にして、性染色體は棒狀の大形染色體なり。兩者間に一定せる位置的關係なきも共に diffused state を示す。本種の如く Hippiscus-type に屬するものに於て Stenobothrus-type 特有の V字型染色體を有する事は奇異と云ふべし。第一精母細胞の分裂前期に於て兩者とも其の早熟性に依りて識別せらる。厚絲期に達るや過剰染色體と性染色體は融合し、不對稱の karyotin mass を形成するに至る。大にして vesicle を附隨せる部分は性染色體にして小なる部分は過剰染色體より成るものなり。此の期より兩者は著しく早熟性を增す。更に核分裂の過程の進むに從ひ此の Karyotin mass は形を整へ tetrad formation を完成するに至る。過剰染色體は外見に於て球形を呈するも環狀の螺旋絲を骨格となす。染色體移動期に達する頃 tetrad は分裂して獨立せる行動をとる。未だ完成せる紡綞絲を備へざる核分裂の過程に於て、過剰染色體と性染色體とより成る tetrad が、完全に分離し得るは興味ある事實なり。Vesicle は分裂後と雖も性染色體の尖端部に附隨す。此の vesicle は tetrad の分裂に關係を有するものゝ如く思惟せらるゝも CAROTHERS (1917) の見たる材料の如く之が能動的に tetrad を二分するや否や速斷し難し。寧ろ余の材料に於ては、此の vesicle が性染色體に對して有する attraction が、間接に過剰染色體の分離を誘導すると解釋するを妥當とす。分裂後に於ける過剰染色體と性染色は、同一極に移行する場合と各異りたる極に移行する場合とあり。余は 146 の分裂像につきての統計により、過剰染色體及性染色體は全く chance に依りて極に移行するものにして、兩者の間に特種の親和性又は反撥性なき事を確めたり。何れも第二分裂の中期に於て正常なる分裂を行ふ。從つて常染色體、過剰染色體及性染色體の組合せによりて四種の相異りたる遺傳質を有する精蟲を生ずるなり。