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Author(s)	TAKENOUCHI, Yasushi
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The Chromosomes of Three Species of Tenebrionid Beetles

By
Yasushi Takenouchi

(Biological Laboratory, Sapporo College, Hokkaido Gakugei University)

(With 24 Text-figures)

The cytological investigation of the Tenebrionidae (Coleoptera) has been carried on extensively in the past several decades by several cytologists with an accumulation of a considerable amount of work (Stevens 1906; Nonidez 1914, 1915, 1920; Wilson 1925; Guénin 1949, 1950, 1951a, b, 1953, 1956; Smith 1950, 1951, 1952a, b, 1953; Dutt 1953). It is interesting that remarkable examples of multiple sex-chromosomes have been reported to occur in this family (Guénin 1949, 1950, 1951a, b, 1953, 1956; Smith 1952a, b, 1953). Reference to the new list of chromosome numbers in animals recently published by Makino (1956) shows that the chromosome numbers of 37 species are known in this family. Quite recently an account on *Caenoblaps nitida* Achüst has been given by Guénin (1956).

Then it is evident that in the tenebrionid beetles the chromosomes have so far been investigated in a total of 38 species. The present author has had a chance to investigate the chromosomes of three species of the Tenebrionidae. The following records the results of investigation with a discussion in relation to species already described cytologically by other authors.

The author feels much pleasure to dedicate this paper to the Jubilee Volume of Professor Tohru Uchida. It is the author's pleasant duty to express his sincere gratitude to Professor Sajiro Makino, Hokkaido University, for his keen interest in the subject and for improvement of this manuscript. The author's thanks are also due to Assist. Prof. Eizi Momma, Dr. H. Kôno of Hokkaido Gakugei University, and Mr. M. Konishi, Entomological Institute, Hokkaido University, for their valuable assistance in the completion of this work.

Material and methods

Alphitobius fagi Panzer, *Tribolium ferrugineum* Fabricius and *Tribolium confusum* Duval are the species coming under study. Several adult males of the first species were kindly supplied by Mr. S. Jin-Oka and Mr. K. Yoshimoto in December 1955. Many adults of the second and third forms were mailed from Kurashiki in January 1955 through the kindness of Dr. Y. Yasue, of the Ohara Institute for Agriculture Research, Okayama University. The testes from adult males supplied the material for each study. In *Alphitobius fagi* squash preparations stained with 10% acetic gentian violet were made, while in the other two species both the squash preparation and the usual paraffin sections after

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fixation with Momma-Bouin's mixture were made and then stained with Heidenhain's iron-haematoxylin and light green.

Observations

(1) *Alphitobius fagi* Panzer (Figs. 1-6)

Several slides showed a few spermatogonia and many spermatocytes in process of division. The diploid number of the spermatogonial chromosomes was found to be 20 (Fig. 1). This number seems to be the primitive cytological constitu-



Figs. 1-6. Chromosomes of *Alphitobius fagi*. 1. Spermatogonial metaphase. 2-3. First metaphase. 4. Side view of the first meta-anaphase. 5. Second metaphase, X-class. 6. The same, Y-class.

Figs. 7-14. Chromosomes of *Tribolium ferrugineum*. 7. Spermatogonial metaphase. 8-9. First metaphase (squashed preparation). 10-11. First metaphase (sectioned preparation). 12. Side view of the first meta-anaphase (sectioned preparation). 13. Second metaphase, X-class. 14. The same, Y-class.

Figs. 15-24. Chromosomes of *Tribolium confusum*. 15-16. Spermatogonial metaphase. 17-18. The first metaphase (squashed preparation). 19-20. The first metaphase (sectioned preparation). 21-22. Side view of the first anaphase. 23. Second metaphase, X-class. 24. The same, Y-class.

tion of the Tenebrionidae, as reported by Smith (1952). The complement consists of eight pairs of metacentric chromosomes, three telocentric elements and a minute spherical one. Morphological analysis of the chromosomes revealed that one of the three telocentric elements corresponds to the X chromosome, while the smallest spherical body represents the Y. In the primary spermatocyte the X and Y constitute a typical "parachute" bivalent as occurred in the Curculionidae and Coccinellidae (Smith 1952a, b; Suomalainen 1955; Takenouchi 1953, 1954, 1955a, b) at metaphase and early anaphase (Figs. 2-4). In the bivalent 10 chromosomes were observable forming a haploid constitution. As the X and Y segregate in the first division, two kinds of secondary spermatocytes are produced: the one contains the X (Fig. 5), and the other the Y (Fig. 6).

(2) *Tribolium ferrugineum* Fabricius (Figs. 7-14)

The present material furnished many dividing spermatogonia and spermatocytes. The size of germ cells is comparatively smaller in this species than that in the above species. Every spermatogonial division shows 20 chromosomes at metaphase (Fig. 7). Most of the chromosomes are of metacentric nature, but at least three smaller elements are rod-shaped. After the morphological analysis, it becomes evident that one of the rod-shaped elements is the X-chromosome and the smallest spherical body is the Y (Fig. 7). The haploid number was determined as 10 (Figs. 8-11). In the haploid complement, nine are autosomal bivalents and the remaining one constitutes the X-Y complex which is represented by a "parachute" bivalent (Figs. 8, 9).

In the sectioned material it is rather difficult to identify the X-Y complex in the metaphase plate (Figs. 10, 11), while it is detected in the anaphase lateral view (Fig. 12). The first division results in the production of two sorts of secondary spermatocytes; the one consists of nine autosomal dyads and an X (Fig. 13), and the other contains the same number of autosomal dyads and a Y (Fig. 14).

(3) *Tribolium confusum* Duval (Figs. 15-24)

This species has already been studied cytologically by Smith (1952a,b), in considerable detail. The findings of this study supplement his results. The testicular tissues from 96 males supplied sufficient material for study of spermatogonial and spermatocyte divisions. The diploid number was determined without any doubt to be 18 in every spermatogonium (Figs. 15, 16). This proves the correctness of the chromosome counting by Smith (1952a,b). Most of the chromosomes are of metacentric structure, a few being rod type in nature. One metacentric element, largest of all in size, and a medium-sized rod-body with slender outline are conspicuous in the spermatogonial metaphase; they remain unpaired after homologous pairing. It is then apparent that the largest element is the X-chromosome, and the medium rod the Y. The X is dissimilar in length of the two arms; the shorter arm is about one-third the length of the longer arm. The latter arm exhibits usually, visible splitting into two chromatides (Figs. 15, 16, 18). According to Smith (1952a, b), the Y also splits, though the present

material shows no such configuration. It is difficult to say with certainty at present whether the Y is telocentric or acrocentric. As seen in Fig. 15, the Y approximately corresponds in size to the longer arm of the X-chromosome.

There are 9 chromosomes at first metaphase in both squashed and sectioned preparations (Figs. 17-20), of which eight are autosomal bivalents while the remaining heteromorphic bivalent represents the X-Y complex. When stretched on the first spindle the X exhibits a V-shape, while the Y is rod-shaped. In superficial magnitude, the longer arm of the X corresponds again to the Y. The Y always associates with the longer arm of the X in an end-to-end connection. The X-Y complex thus formed differs in stainability from the autosomal bivalents through diakinesis to early anaphase: one segment is stained as deeply as the autosomal elements; the other stains much less than the autosomes and exhibits thinner form. In the excellent figure (Fig. 18), the X is seen to be bipartite in structure, consisting of a longer and a shorter arm. In the first division the segregation of the sex-chromosomes follows that of the autosomal bivalents (Figs. 21, 22). As a result two classes of secondary spermatocytes are produced in respect to the distribution of the X and Y: the one possesses 8 autosomal elements and an X (Fig. 23); the other carries the same autosomal set and a Y (Fig. 24). Similar situation as to the sex-determining mechanism was reported to occur in three Indian buprestids by Asana, Makino, and Niiyama (1942). Smith (1952) has reached the conclusion that the sex-determining mechanism of *Tribolium confusum* belongs to a neo-XY type based on his interpretation as follows: the larger one (the neo-X) of the sex-chromosome complex is a multiple consisting of an euchromatic arm (probably an autosome originally) which fused with a shorter non-heteropycnotic arm (a relic of the X), and the smaller one is a rod-shaped element which is supposed to be the neo-Y. The latter is the homologue of the autosomal component of the neo-X. The findings of the present study are in agreement with the view expressed by Smith (1952).

Summary

The chromosomes of three tenebrionid beetles (Tenebrionidae, Coleoptera) were investigated in male germ cells with particular reference to the morphology

Table 1.

Species	Chromosome number		Sex-chr.
	2n	n	
1. <i>Alphitobius fagi</i> Panzer	20 s	10 ♂ (I, II)	X-Y ♂
2. <i>Tribolium ferrugineum</i> Fabricius	20 s	10 ♂ (I, II)	X-Y ♂
3. <i>Tribolium confusum</i> Duval	18 s	9 ♂ (I, II)	X-Y ♂

s, spermatogonium. (I), primary spermatocyte. (II), secondary spermatocyte.

of chromosomes and sex-determining mechanism. The results are summarized in Table 1.

The neo-XY mechanism reported for *Tribolium confusum* by Smith (1952) was substantiated in this study.

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