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**A Study of the Chromosomes of Two Japanese Species of
Spittle-insects belonging to the Family
Cercopidae (Homoptera)¹⁾**

By

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(With 4 Textfigures)

Introduction

The chromosomes of the Cercopidae have been studied by Stevens ('06), Boring ('07, '13) and Boring and Fogler ('15). About eight species of spittle-insects, belonging to this family, were studied by these authors between the years 1906 and 1915, after which no attempt seems to have been made to extent our knowledge of the chromosomes of these interesting insects. Prof. K. Oguma, therefore, suggested to me to undertake a karyological study of the Japanese Cercopidae, as it was likely that these might throw additional light on the number and behaviour of the chromosomes in this group of insects. I am deeply grateful to Prof. Oguma not only for giving me necessary facilities in his laboratory, but also for supervising the work and correcting the manuscript of this paper. I am also indebted to Dr. S. Makino for many an act of friendly assistance during the course of my stay in Sapporo.

Material and Method

The "spittle-insects" or froghoppers belong to the family Cercopidae (Homoptera). These insects pass the winter in the egg

1) Contribution No. 115 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

stage in the stem of the host plants, hatching out in April and May and completing their growth in four to eight weeks. During the nymphal stage, these insects bury themselves under a characteristic white, frothy secretion which, according to Guilbeau (referred to by Comstock) "is obtained from two sources. The greater part of this fluid is voided from the anus; to this fluid is added a mucilaginous substance which renders it viscous and causes the retention of air bubbles which are introduced into it by the insect by means of its caudal appendages". The mucilaginous substance is the excretion of large hypodermal glands, which are in the pleural region of the seventh and the eight abdominal segments. These are known as the glands of Batelli and open through numerous minute pores in the cuticula.

These insects instantly arrest the attention of a passer-by on account of the massy accumulation of the frothy substance upon the twigs of the trees and grasses upon which they live. The specimens for investigation were collected by me during June and July, 1936, in Sapporo from willows and grasses growing by the sides of the roads.

The insects were dissected soon after collecting them from the fields, and the testes together with the adhering viscera were dropped into vials containing appropriate fixatives. Flemming's strong solution was chiefly employed for fixation and proved to be good. Following the usual treatment of the paraffin method, they were cut 7-10 μ in thickness and stained according to the iron haematoxylin method of Heidenhain using light green as the counter stain. The specimens (nymphs) were also reared in the laboratory under suitable conditions to obtain the adults for specific identification. In the museum of the Entomological Institute of this university they were identified to be *Aphrophora intermedia* Uhl. and *Aphrophora coctalis* Mats.

Historical and Critical

The first paper dealing with the chromosomes of Cercopidae is that by Stevens (1906) but this has not been available to me for consultation. However, it appears from Boring's papers ('07, '13) that Stevens had worked on *Aphrophora quadrangularis*. In 1907, Boring furnished us with an account of the chromosomes of four species of American Cercopidae, namely *Aphrophora 4-notata*,

Aphrophora quadrangularis, *Aphrophora* sp., and *Clastoptera obtusa*. She found that the spermatogonial number of chromosomes in *Clastoptera obtusa* is 15, the accessory chromosome "a small, ovoid, smooth-contoured body" taking up an eccentric position in the equatorial plate of the primary spermatocyte and going undivided to one of the two poles.

In *Aphrophora quadrangularis*, she records 21 spermatogonial chromosomes and 10 and 11 chromosomes in the secondary spermatocytes. The accessory chromosome, which does not divide in the maturation division of the primary spermatocyte, has a recognisable identity on account of being *larger* than others in the secondary spermatocyte. An important observation was made by Boring from the study of the chromosomes of the so-called *Aphrophora quadrangularis* in that 12 chromosomes instead of 11 occurred in some cases, and this she reconciled by assuming that the insects which had, till then, been known as *Aphrophora quadrangularis* were in reality referable to two distinct species.

In *Aphrophora quadrinotata*, the same author found the spermatogonium to possess 27 chromosomes, that is, 26 autosomes plus 1 accessory chromosome, and consequently some of the secondary spermatocytes had the 14 chromosomes and others 13 only. In 1913, Boring made further additions to our knowledge by investigating the chromosomes of *Philaenus spumarius* and *Aphrophora spumaria*. In *Philaenus spumarius*, she found 23 chromosomes as the diploid garniture in the spermatogonium, 12 chromosomes in the equatorial plates of the primary spermatocyte, and 11 and 12 chromosomes in the two kinds of secondary spermatocytes. Boring does not mention the spermatogonial number of chromosomes of *Aphrophora spumaria*, but one of her remarks¹⁾ purports to indicate the existence of the same number of chromosomes in *Aphrophora spumaria* as in *Philaenus spumarius*. In the summary furnished by her at the end of the paper, she says that the accessory chromosome is always one of the smallest ones. She also attempted to correlate the somatic variations with the chromosomes, and to this end carefully studied the chromosomes of different individuals with different types of markings, but could

1) "Similar as these two species are in the number of chromosomes and the size relations of the chromosomes" etc., p. 137, Biol. Bull., Vol. XXIV, No. 3, 1913.

not detect any connection between the two. Her remark—"The somatic variation is great, but the chromosomes given no key to it"—is significant for our purpose.

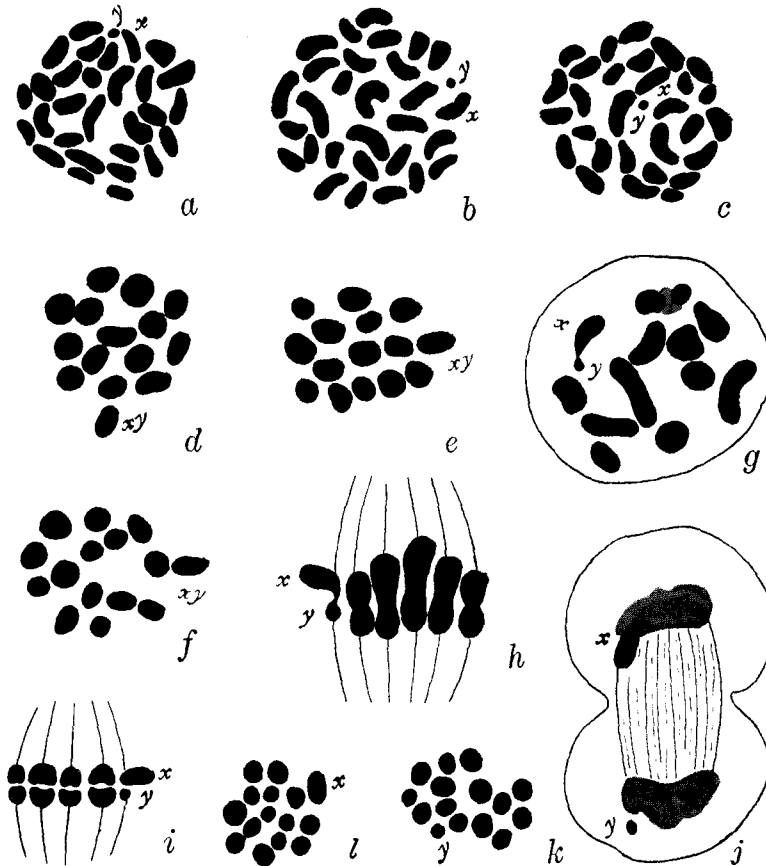
In 1915, Boring and Fogler worked out the chromosomes of three more species—*Philaenus lineatus*, *Aphrophora parallela* and *Clastoptera proteus*. According to these authors, *Philaenus lineatus* has 29 chromosomes in its spermatogonium, including one accessory chromosome. *Aphrophora parallela* also has the same number of chromosomes (29) in its diploid garniture. *Clastoptera proteus*, however, was very incompletely studied by Boring and Fogler ('15) as the material at their disposal was insufficient for the purpose of a fuller study.

Observations

(a) *Aphrophora coctalis* MATS.

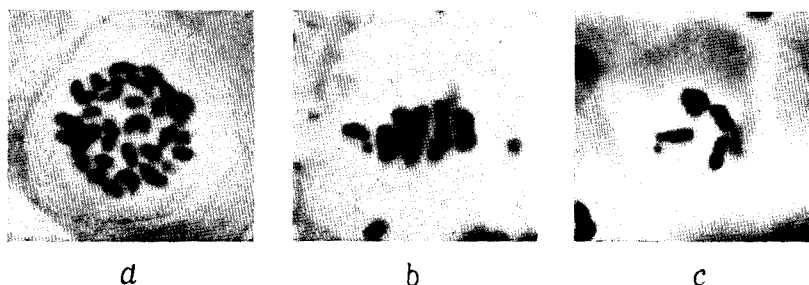
The spermatogonium: The spermatogonium shows 30 chromosomes in the metaphase polar view (Textfig. 1, *a-c* and Textfig. 2, *a*). They take quite a characteristic arrangement similar to the Hemipteran type (Wilson, '25). They are all irregularly rod shaped, some being slightly curved, excepting one chromosome which is distinctly minute and round. In the full garniture, at least two pairs of elongated, large chromosomes can be recognized, especially when the chromosomes are arranged in the serial order as in Textfig. 4, *b*. When homologous chromosomes are paired, twenty eight of them readily make up 14 equal-sized pairs leaving a rod-shaped chromosome of medium size and a minute round chromosome to constitute an unequal pair (Textfig. 4, *b*). The latter constitute the XY complex.

The primary spermatocyte: The metaphase polar view of the first division presents fifteen bivalents which are, more or less, round in outline including the XY bivalent which lies somewhat eccentrically in the equatorial plate (Textfig. 1, *d-f*). The XY complex, which is formed of the pair of unequal chromosomes mentioned above, is clearly recognised in the lateral view of the metaphase plate, in contrast to others which are dumbbell-shaped tetrads (Textfig. 1, *h-i* and Textfig. 2, *b*).



Textfig. 1. The chromosomes of *Aphrophora coctalis* MATS. (1/12 oil imm. and K20x ocular). *a-c*, metaphase groups of spermatogonial chromosomes. *d-f*, metaphase groups of primary spermatocytes. *g*, a nucleus in diakinesis, showing XY complex. *h-i*, side views of the primary spermatocyte metaphase. *j*, late anaphase of first division, showing separation of X and Y elements. *k*, metaphase groups of secondary spermatocyte, Y-class. *l*, the same, X-class.

Here mention must be made of the peculiar mode of conjugation of the X and the Y chromosomes during the first division. Contrary to the general mode of conjugation, the X chromosome is approximately perpendicular to the axis of the spindle, instead of being parallel to it as is generally the case, while the Y element lies parallel



Textfig. 2. Photomicrographs of the chromosomes of *Aphrophora coctalis* MATS, 2000 \times . *a*, spermatogonial metaphase. *b*, side view of first division, showing XY complex. *c*, XY complex in diakinesis.

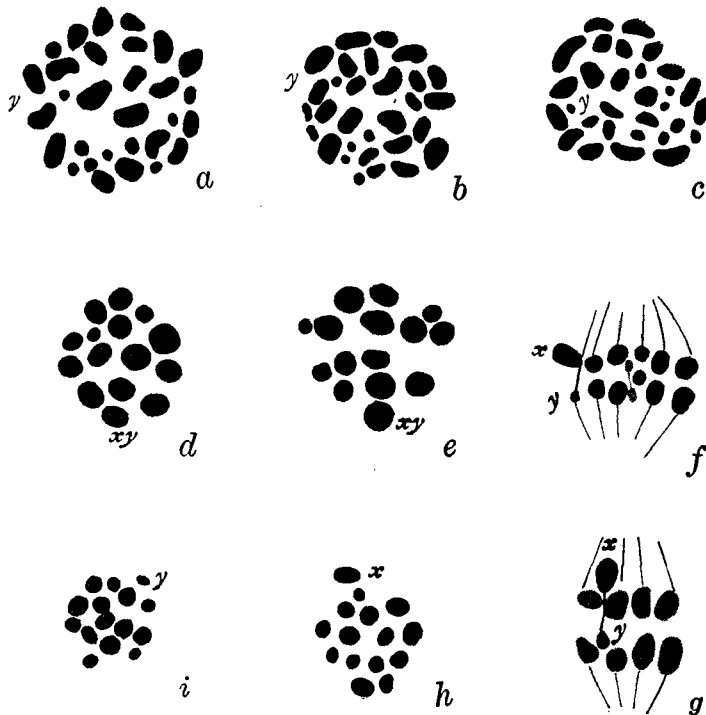
to the axis in the usual way conjugated at its proximal end with one end of the X chromosome, its distal end being attached to the spindle fibre as shown in Textfig. 1, *h-i* and Textfig. 2, *b*. This mode of conjugation is also noticeable in the diakinesis (Textfig. 1, *g* and Textfig. 2, *c*). Similar figure of conjugation between X and Y chromosomes is recorded in the case of a species of stone-fly, *Perla immarginata* (Nakahara, '19) and also in some rats, *Rattus rattus* and *Rattus norvegicus* (Oguma, '34). Evidently this mode of conjugation is of infrequent occurrence in animals. All the Heteroptera, so far studied, have shown the usual type of conjugation in which these two elements lie parallel to the spindle axis (Wilson, '25), and post-heterokinesis, with occasional exception, in the X and the Y chromosomes.

In the ensuing division the autosomal bivalents separate equally into their components. The XY bivalent also does the same, X and Y passing to the opposite poles (Textfig. 1, *j*).

The secondary spermatocyte: The separation of the asymmetrical X and Y chromosomes results in the production of two kinds of secondary spermatocytes, with fifteen chromosomes in each case. But half of them contain the X-element plus fourteen autosome dyads, and the other half possess the Y-element plus an equal number of autosome dyads, as shown in Textfig. 1, *k-l*. In polar view, the dyads are all round in outline and the X and the Y-elements both usually take up a definitely peripheral position in the equatorial plate.

(b) *Aphrophora intermedia* UHL.

The spermatogonium: Thirty well-defined chromosomes can be counted in the metaphase of this species as in the case of *A. coctalis* (Textfig. 3, *a-c*). By pairing homologous chromosomes in the usual manner, fourteen pairs of equal-sized chromosomes and one pair of unequal chromosomes (or XY-pair) are found to constitute the garniture (see Textfig. 4, *a*). All the chromosomes, with the exception of the XY-pair, gradually diminish in size when serially arranged, and have an irregularly ovoidal outline. It is characteristic of the garniture of this species to possess four pairs of somewhat



Textfig. 3. The chromosomes of *Aphrophora intermedia* UHL. (1/12 oil imm. and K20×ocular). *a-c*, metaphase groups of spermatogonial chromosomes. *d-e*, metaphase groups of primary spermatocytes. *f*, side view of primary spermatocyte anaphase. *g*, anaphase side view of first division. *h*, metaphase groups of secondary spermatocyte chromosomes, X-class. *i*, the same, Y class.

spherical and comparatively small-sized chromosomes,¹⁾ and to have elongated rod-shaped chromosomes as were detected in the case of *A. coctalis*.

The primary spermatocyte: From the occurrence of even number of chromosomes in the spermatogonium, 15 bivalents are naturally expected to be present in the primary spermatocyte. That this is so is shown by Textfig. 3, *d-e*. In polar view, the bivalents appear spherical in outline, and in contrast to the preceding species, *A. coctalis*, we find here two strikingly small tetrads. The XY-bivalent always lies at the periphery of the equatorial arrangement. The X- and Y-elements are conjugated in the same way as described for *A. coctalis*; that is, the X-element is perpendicular to the spindle axis, while the Y is placed in the normal way (Textfig. 3, *f*). In the anaphase, all the bivalents separate into their constituents, and the X- and the Y-elements pass to opposite poles (Textfig. 3, *g*).

The secondary spermatocyte: The secondary spermatocytes are of two sorts; (1) those having fourteen autosome dyads plus the X-element, and (2) those possessing an equal number of autosome dyads plus the corresponding Y-element (Textfig. 3, *h-i*).

Remarks

In the literature bearing on these insects no mention of the occurrence of the Y-chromosome has been made by any previous author (see Schrader, '28). Boring ('07, '13) and Boring and Fogler ('15) studied nine species of Cercopidae, but in every case detected only the X-chromosome (Table 1). It is therefore, of particular interest to discover the existence of the Y-chromosome, in addition to the X, in both studied species indigenous to Japan. The peculiar mode of conjugation between these two sex-chromosomes and their prereducational division is also particularly striking.

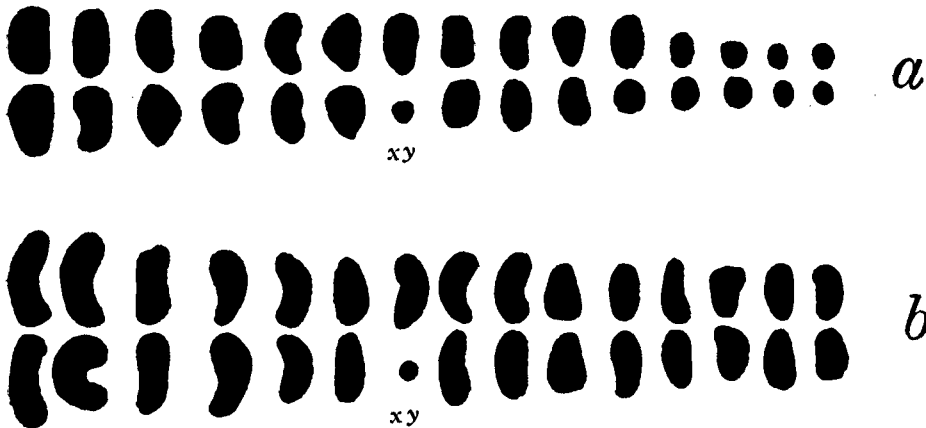
As indicated in the foregoing description, *Aphrophora coctalis* and *Aphrophora intermedia* have the same number of chromosomes,

1) These small chromosomes are regarded as m-chromosomes by Wilson in some species of Heteroptera (Wilson, '25). Prof. Oguma therefore, wished me to bear this in mind whilst examining the slides, but my observation show them not to be of the nature of m-chromosomes. They are just simple chromosome of small size.

Table 1
The chromosome number in Cercopidae

Species	Diploid	Sex-chrom.	Author
<i>Philaenus lineatus</i>	29	XO	Boring & Fogler, '15
<i>P. spumarius</i>	23	XO	Boring, '13
<i>Aphrophora parallela</i>	29	XO	Boring & Fogler, '15
<i>A. spumaria</i>	23	XO	Boring, '13
<i>A. quadrangularis</i>	21	XO	Boring, '07
<i>A. quadrangularis</i> (Harpwell form)	23	XO	Stevens, '06
<i>A. 4-notata</i>	27	XO	Boring, '07
<i>A. coctalis</i>	30	XY	Misra, '37
<i>A. intermedia</i>	30	XY	Misra, '37
<i>Clastoptera proteus</i>	13 (prob.)	XO	Boring & Fogler, '15
<i>C. obtusa</i>	15	XO	Boring, '07

30 in the diploid and 15 in the haploid complex. This number seems to be very characteristic to the Japanese *Aphrophora*, in none of the other species of the same genus hitherto collected and studied in America.



Textfig. 4. Serial arrangement of spermatogonial chromosomes.
a, *A. intermedia*. b, *A. coctalis*.

When compared closely the chromosomes of these two Japanese *Aphrophora*, certain important differences are found irrespective to the number of chromosome. Reader's attention should first be called to Textfig. 4, in which the chromosomes of both species, constituting a spermatogonial garniture, are serially arranged according to their gradation of lengths. In the first place, *Aphrophora intermedia* (Textfig. 4, *a*) possesses eight autosomes in four pairs which are clearly distinguishable from the remaining longer ones by their spherical shape of extremely small size, while the chromosome garniture of *Aphrophora coctalis* (Textfig. 4, *b*) comprises no such spherular chromosomes but all are represented by rod-shaped ones except the Y-chromosome. In the second place, the longest two pairs of chromosomes in *Aphrophora coctalis* are remarkably big as compared with the remainings, while the corresponding ones are not so exaggerated in *Aphrophora intermedia*. By these two points of distinction the two species of *Aphrophora*, now dealt with, are distinguishable with absolute clearness if the chromosomes are compared closely even in cases where we are quite ignorant of taxonomy of the larvae.

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