SCIENTIFIC NOTE

A CHROMOSOME STUDY ON FOUR SPECIES OF THE GENUS *HYLOBIUS* (COLEOPTERA: CURCULIONIDAE)

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Suomalainen (1940a, b) was the first to study the chromosomes of a species belonging to the root-weevil genus *Hylobius*. Since then 16 additional species 1 subspecies have been studied by Smith (1952, 1953, 1956, 1960, 1962) and the present author (Takenouchi 1955, 1958a, b, 1963). Regarding Takenouchi (1955) Smith (1956) commented "Unfortunately Takenouchi's illustrations are in insufficient detail and at too low a magnification to allow comparative analysis, so that the significance of his findings remains at present obscure." Actually *Hylobius* species are difficult to collect in Japan and in the investigation paraffin-sectioned material was used, a method which is not suitable for Coleoptera: the small chromosomes are so condensed that it is very difficult to establish the morphological details of both the autosomes and the sex-determining complex. Also for economic reasons the original figures were reduced too much in reproduction.

This time the author has had a chance to study four species of *Hylobius*, and the results are described in this report.

MATERIALS AND METHOD

The materials used in this study, the collecting localities, and dates are given in Table 1.

Table 1. The species of the genus *Hylobius* studied

Species	Date of Collection	Locality	Collector
Family Curculionidae Subfamily Curculioninae Tribe Hylobiini			
<i>Hylobius galloisi</i> Kôno	July 25, 1971	Maruyama Park Sapporo	Mr. K. Shimaya
<i>H. gigas</i> Kôno	July 31, 1969	Ashiribetsu Fall near Sapporo	Mrs. U. Takenouchi
<i>H. pinastri karafutoensis</i> Kôno	June 1, 1970	Mt. Apoi Samani-gun, Hidaka	Mr. K. Shimaya
<i>H. freyi</i> Zumpt	June 15, 1969	Maruyama-Nishimachi Sapporo	Mr. N. Takenouchi

Testes of adult males were prepared by Smith's squash method (Smith, 1943) and the slides stained in basic fuchsin-methyl green (Smith and Takenouchi, 1969). The camera lucida drawings, made originally at about $\times 3,600$, are reduced to about $\times 3,400$.

OBSERVATIONS

Hylobius galloisi Kôno:

No spermatogonial metaphases were observed, but the slide provided many excellent first metaphase plates. There are 18 bivalent chromosomes without exception (Fig. 1). The complement consists of one large, four medium sized, and twelve smaller bivalents, and a distinctive elongated parachute type of the sex-determining complex. The connection between an X and the y is nearly invisible. The largest bivalent is surely a ring, but the nature of the other autosomes is not clear. Size seriation of the small autosomes is gradual.

Hylobius gigas Kôno:

No spermatogonial metaphases were observed. Many first metaphases showed 18 well defined bivalents (Figs. 2-4). The complement consists of one large ring, seven medium sized, and nine small autosomal bivalents and an typical sex-determining Xy_p . The y is so small as to be nearly invisible. As seen in Fig. 4, the autosomes sometimes appear to form at least four ring bivalents and the y is plainly evident.

Hylobius pinastri karafutoensis Kôno:

No spermatogonial metaphases were observed. The many metaphases showed 11 bivalent chromosomes (Fig. 5). The X is smaller than the smallest autosome and the y again minute. The autosomal bivalents are somewhat rather larger and can be classified into two groups: five large and five smaller. It is supposed that the larger ones are able to form rings.

Hylobius freyi Zumpt:

No spermatogonial metaphases were observed. There were numerous excellent first metaphases which showed 14 bivalents without exception (Fig. 6). Two are relatively large, five medium sized, and six small, and the sex-determining complex forms a typical parachute. No clear ring bivalents were observed.

DISCUSSION

Suomalainen (1940a, b) reported that male *Hylobius abietis* L. had 10 II+X at first metaphase but the result was corrected by Smith (1956) who observed 10 II+ Xy_p . So far eight different diploid chromosome numbers have been found among a total of the 17 *Hylobius* taxons examined (Smith 1952, 1953, 1956, 1960, 1962; Takenouchi 1955, 1958a, b, 1963). One species, *H. montanus* ♀ has 20 diploid chromosomes which suggests that 10 bivalents are formed at first metaphase; six taxons have 22 chromosomes; one (*Hylobius* sp. ♀) 24; one 28; one 32; two 36; three 40; and one 46. Among them, eight species studied by Smith (1956, 1962) in squash preparations without exception clearly

Figs. 1-6. First meiotic metaphases of *Hylobius galloisi* (Fig. 1), of *Hylobius gigas* (Figs. 2-4), of *Hylobius pinastri karafutoensis* (Fig. 5), of *Hylobius freyi* (Fig. 6).

show an Xy_p sex-determining complex, but in males of seven species studied in sectioned preparations only four species showed an Xy_p , and the other three, *H. albosparus*, *H. exsculptus*, and *H. galloisi* did not provide details of the structure of the sex-determining pair (Table 2). This clearly indicates the superiority of the squash method over

Table 2. Summary of chromosome determination in the genus *Hylobius*

Species	2n	MI	Author
<i>Hylobius montanus</i> Kôno	20♀		Takenouchi '55
<i>H. abietis</i> L.		10 II+X	Suomalainen '40a, b
<i>H. abietis</i> (Finland)		10 II+Xy _p	Smith '56, '62
<i>H. abietis haroldi</i> Faust		10 II+Xy _p	Takenouchi '58b
<i>H. congener</i> D. S. & M.	22♂	10 II+Xy _p	Smith '56, '62
<i>H. pales</i> Hbst.	22♂	10 II+Xy _p	Smith '56, '62
<i>H. radialis</i> Buch.	22♂	10 II+Xy _p	Smith '56, '62
<i>H. pinastri</i> Gyll. (Finland)	22♂	10 II+Xy _p	Smith '56, '62
<i>H. pinastri karafutoensis</i> Kôno		10 II+Xy _p	Herein
<i>H. sp.</i>	24♀		Takenouchi '63
<i>H. exsculptus</i> Roel.	28♂	13 II+Xy	Takenouchi '55
<i>H. freyi</i> Zumpt		13 II+Xy _p	Herein
<i>H. warreni</i> Wood	32♂	15 II+Xy _p	Smith '56, '62
<i>H. galloisi</i> Kôno	36♂	17 II+Xy _p	Takenouchi '55, '58a and herein
<i>H. desbrochersi</i> Zumpt	36♂	17 II+Xy _p	Takenouchi '58b
<i>H. perforatus</i> Kôno		17 II+Xy _p	Takenouchi '63
<i>H. gigas</i> Kôno		17 II+Xy _p	Herein
<i>H. pinicola</i> Couper	c40♂	19 II+Xy _p	Smith '56
<i>H. piceus</i> DeG. (Finland)		19 II+Xy _p	Smith '56
<i>H. piceus</i> DeG. (Canada)		19 II+Xy _p	Smith '56
<i>H. albosparsus</i> Boh.		19 II+Xy _p	Takenouchi '55
<i>H. elongatus</i> Roel.		23 II+Xy _p	Takenouchi '58a

the paraffin method for chromosome cytology of these weevils.

In the present paper, the chromosomes of four species of *Hylobius* from Hokkaido, Japan, are described (Table 1). Among them, *H. galloisi*, when previously studied in sectioned preparations, was found to have 2n, 36 and n, 17 II+Xy (Takenouchi, 1955, 1958a). However, to provide details on the method of association of the sex chromosomes, the species was reinvestigated using the squash method. Although no spermatogonial metaphases were observed, the chromosome count obtained in the previous studies was verified. The sex-determining complex proved to be Xy_p but the elongate shape of the parachute suggests that the species has an unusually large nucleolus. An Xy_p of this type has not been observed in any of the other *Hylobius* species so far studied. *H. gigas* showed 17 II+Xy_p in haploid. The formula is similar to that of *H. galloisi* and of two other species, *H. desbrochersi* and *H. perforatus*. *H. pinastri karafutoensis* provided 10 II+Xy_p, which is the commonest formula in the genus, having been reported in six other species or subspecies including the Finnish *H. pinastri* Gyll. (Smith 1956, 1962). *H. freyi* showed 13 II+Xy_p as does *H. exsculptus*. As Smith (1962) has illustrated, identical chromosome numbers in *Hylobius* may conceal profound differences in structure, which become exposed when gonial karyotypes are closely compared. On the other hand, judging by the grasshopper *Eyprepocnemis plorans* Charp., even close karyotypic similarity is no guarantee of structural equivalence (John and Lewis 1965). No new chromosome number was obtained in the species here studied.

SUMMARY

The chromosomes of four Japanese *Hylobius* species were studied in male meiosis using squash preparations. The results are: *H. galloisi* and *H. gigas*, $2n=36$, *H. freyi*, $2n=28$, *H. pinastri karafutoensis*, $2n=22$ and all include an Xy_P sex-determining system.

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