| Koleopterologische Rundschau | 69 | 41 - 45 | Wien, Juni 1999 |
|---|---|---|---|

# The karyotype of the Squeak Beetle, *Hygrobia hermanni* (F.) (Coleoptera: Hygrobiidae)

C.L. HUGHES & R.B. ANGUS

**Abstract**

The karyotype of *Hygrobia hermanni* (F.) is shown, on the basis of material from two English localities, to comprise 17 pairs of autosomes plus sex chromosomes which are XY (male) and XX (female). The X-chromosome is a long metacentric, while the Y is small, almost dot-like. At first metaphase of meiosis the Y-chromosome is attached to one end of the X, from which it may be separated by a small gap. There is, however, no real indication of a "parachute" association of the sex chromosomes ($Xy_p$) of the pattern frequently found in Polyphaga.

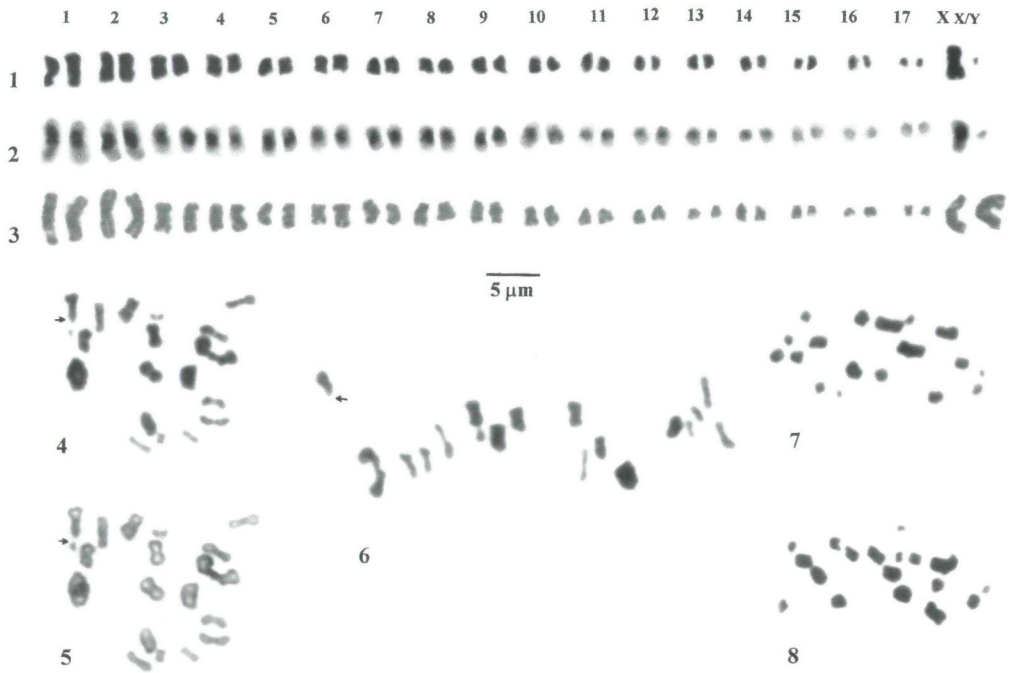
## Introduction

Among the families of adephagan Coleoptera, the Hygrobiidae are noteworthy as being a taxonomically isolated group about which there are as yet no chromosomal data. This makes the sole European species, *Hygrobia hermanni* (F.) an ideal subject for investigation as a third-year undergraduate project of the type currently undertaken by students in the School of Biological Sciences at Royal Holloway. The present study stems from project work done by Clare L. Hughes, supervised by Robert Angus.

## Material and Methods

The material used in present study comprised adult beetles taken at Runnymede (Egham), Surrey and in the New Forest, Hampshire, during July and August 1997. A total of 14 beetles were examined.

Chromosome preparations were obtained from mid-gut and testis cells, following the procedures given by SHAARAWI & ANGUS (1991), with the treatments in colchicine and hypotonic potassium chloride both lasting 10 minutes. C-banding presented considerable difficulty, and the successful preparations were from 2-day old preparations treated for 3 - 5 minutes in saturated $Ba(OH)_2$ at about 25° C, followed by 2 hours in double strength salt sodium citrate (2X SSC) at 60° C. The chromosomes were photographed and printed at a magnification of 3000, and they were then cut out and arranged in idiograms as shown in Figs. 1 - 3, with the chromosomes arranged in order of decreasing size. Many of the chromosome pairs are similar in size and centromere position so that it is in many cases not possible to be sure that the individual chromosomes are allocated to the correct pair. An error of one pair either way would not be detectable, and would not alter the appearance of the idiogram. Because of this, Centromere Indices (CI) and Relative Chromosome Lengths (RCL) (see ANGUS & SHAARAWI 1997) were calculated only for chromosome pairs 1, 2, 3, 10, 11, 12, 13 and X, which appear sufficiently distinctive for the individual chromosomes to be recognised. The differences between the results obtained from these chromosome pairs were analysed statistically, using in the first instance the means and 95% confidence limits calculated by t-test, and then analysis of variance (ANOVA) and Tukey's test. The relative chromosome lengths of these chromosomes are intended to give an indication of the sizes of the chromosomes at their positions in the idiogram arrangement.




Figs. 1 - 8: Chromosomes of *Hygrobia hermanni*. 1 - 3, mitotic chromosomes from mid-gut cells. 1, ♂, New Forest, unbanded; 2, ♂, Runnymede, C-banded; 3, ♀, Runnymede, unbanded. 4 - 6, first metaphase of meiosis from testis, New Forest material. 4 & 5, bright field (4) and phase contrast (5), from the same nucleus; 6, bright field from a different nucleus. Note the XY bivalent (arrowed). 7, 8, second metaphase of meiosis, X-bearing haploid nuclei. The scale line represents 5 µm.

## Results

Idiograms of mitotic chromosomes are shown in Figs. 1 - 3. All the material examined had 17 pairs of autosomes, with pairs 1 and 2 clearly the longest, with RCL values of about 12 and almost metacentric, with one member of pair 1 showing a conspicuous secondary constriction (presumably closed in the other homologue). Pairs 3 - 9 are metacentrics of decreasing size, while pairs 10 - 13 are almost telocentric, with RCL values of about 5 - 3. Pairs 14 - 17 show further reduction in size, with pair 17 being almost dot-like. The X-chromosome is metacentric, about as long as autosome 1, while the Y-chromosome is dot-like, about the same size as autosome 17. C-banding (Fig. 2) shows autosome pairs 1 - 10, and the X-chromosome, to have heavy centromeric C-bands, while autosomes 11 - 17, and the Y-chromosome, have much smaller C-bands. No additional C-bands have been detected, and no chromosome is totally heterochromatic.

Relative Chromosome Lengths and Centromere Indices are shown in Table 1. Chromosomes 1 and 2 cannot be separated on RCL alone, but are clearly separated on CI, and this is also true of chromosome 2 and the X-chromosome when the comparison is made on the basis of means and 95% confidence limits, but in this case the ANOVA and Tukey's test shows a significant difference (Table 3). With chromosome pairs 10 - 13 only RCL is available for comparison, as these chromosomes are more or less telocentric. However, Table 1 shows each of these pairs to

be significantly different from the others at the 95% confidence level, and this is borne out by an ANOVA which gives a significance of 0.000, indicating a very significant degree of inter-chromosome pair differences. Table 4 shows the results of Tukey's test on these data, and this shows no significant difference between pairs 10 and 11, but that all the others are significantly different. ANOVA and Tukey's test is a more rigorous procedure than the mean and 95% confidence limits by t-test given in Table 1.

Figs. 4 - 6 show first meiotic metaphase from testis, with Figs. 4 and 5 being the same preparation photographed using bright field and phase contrast. The XY bivalent (arrowed) shows a gap at one end of the X-chromosome, distal to which is the small Y-chromosome. The sex bivalent does not show the usual "parachute" association ($Xy_p$) characteristic of many polyphagan beetles with dot-like Y-chromosomes (SMITH & VIRKKI 1978), and even under phase contrast (Fig. 5) there is no indication a nucleolus binding the X- and Y- chromosomes together. But for the obvious asymmetry of the bivalent, the appearance is quite similar to some of the smaller autosome bivalents, especially in Fig. 4. These autosomal bivalents appear to be normal chiasmate associations, and this therefore seems likely to be the case for the sex bivalent. This would imply a neo-XY system, with the small Y-chromosome representing an autosome which has become fused to the much larger X. The small centromeric C-band of the Y-chromosome (Fig. 2), and the similarity of the sizes of the Y-chromosome and autosome 17, support this view.

Haploid nuclei in the second metaphase of meiosis are shown in Figs. 7 and 8. Both nuclei appear to show two particularly small chromosomes, apparently from pairs 16 and 17, and both show three chromosomes, presumably 1, 2 and X, which appear larger than the others. Thus these appear to be X-bearing, female-determining, nuclei.

| Chromosome | Mean RCL values and 95% confidence limits (t-test) | Mean Centromere Index and 95% confidence limits (t-test) |
|---|---|---|
| 1 | 13.0125 (12.449-13.576) N=24 | 25.9521 (24.428-27.4774) N=24 |
| 2 | 12.1333 (11.5456-12.721) N=24 | 41.3938 (39.1494-43.638) N=24 |
| 3 | 8.3125 (7.9199-8.7051) N=24 | 32.925 (30.385-35.4651) N=24 |
| X | 11.8474 (11.3466-12.348) N=19 | 31.07368 (29.285-32.863) N=19 |
| 10 | 5.4708 (5.1968-5.7448) N=24 | Telocentric |
| 11 | 5.0333 (4.77627-5.2903) N=24 | Telocentric |
| 12 | 4.37917 (4.1229-4.6355) N=24 | Telocentric |
| 13 | 3.8625 (3.5869-4.13812) N=24 | Telocentric |

Table 1: Relative Chromosome Lengths and centromere indices of the long chromosomes (pairs 1, 2, X) and the telocentric chromosomes (pairs 10 - 13).

## Discussion

One of the aspects of this study which is of particular interest is the arrangement of the sex chromosomes. SMITH (1950), on the basis of a limited data set, suggested that the ancestral arrangement in Coleoptera was 9 pairs of autosomes plus $Xy_p$ sex chromosomes. Subsequent work (SMITH & VIRKKI 1978) has provided ample support for this arrangement being fundamental to the Polyphaga, but there is as yet no good evidence for a parachute association of the X and Y in the Adephaga. Thus, of 426 species of Adephaga whose karyotypes are listed by SERRANO & YADAV (1984), 226 species had XO sex chromosomes, 179 had neo-XY, while the rest had either multiple sex chromosomes or, in about 10 cases, $XY_p$ (or $Xy_p$) is listed. In those species of *Agabus* and *Rhantus* (Dytiscidae) for which a parachute association is claimed, Angus (unpublished data) found the true arrangement to be XO. As already suggested, the sex chromosomes of *Hygrobia hermanni* appear to be a neo-XY, and if this is the case, then the fairly

high number of autosomes plus a neo-XY gives a general resemblance between the karyotypes of Hygrobiidae and such Dytiscidae as *Deronectes* and some *Stictotarsus* (NILSSON & ANGUS 1992). This would be entirely in accord with the phylogenetic studies of BEUTEL (1993), BURMEISTER (1976) and RUHNAU (1986), who place the Hygrobiidae as a sister group to the Dytiscidae. It should, however, be borne in mind that the present results refer to only one of the five known species of Hygrobiidae, and it is quite possible that other species might have different chromosomal arrangements.

| Compare | Difference | Std Error | q statistic | Table q | Null hypothesis |
|---|---|---|---|---|---|
| **X - 1** | 1.1651 | 0.2943 | 3.9593 | 3.360 | Reject |
| **X - 2** | 0.2860 | 0.2943 | 0.9718 | 3.360 | Accept |
| **2 - 1** | 0.879 | 0.2943 | 3.1781 | 3.360 | Accept |

Table 2: Analysis of variance of Relative Chromosome Lengths of chromosomes 1, 2 and X, Tukey's test. F statistic = 4.476. Significance = 0.0152.

| Compare | Difference | Std Error | q statistic | Table q | Null hypothesis |
|---|---|---|---|---|---|
| **1 - 2** | 15.4417 | 0.9351 | 16.5135 | 3.360 | Reject |
| **1 - X** | 5.1216 | 0.9947 | 5.1488 | 3.360 | Reject |
| **X - 2** | 10.3201 | 0.9947 | 10.3749 | 3.360 | Reject |

Table 3: Analysis of variance of Centromere Indices of chromosomes 1, 2 and X, Tukey's test. F statistic = 70.365. Significance = 0.000.

| Compare | Difference | Std Error | q statistic | Table q | Null hypothesis |
|---|---|---|---|---|---|
| **13 - 10** | 1.6083 | 0.1357 | 11.8551 | 3.7200 | Reject |
| **13 - 11** | 1.1708 | 0.1357 | 8.6303 | 3.7200 | Reject |
| **13 - 12** | 0.5167 | 0.1357 | 3.8084 | 3.7200 | Reject |
| **12 - 10** | 1.0917 | 0.1357 | 8.0467 | 3.7200 | Reject |
| **12 - 11** | 0.6542 | 0.1357 | 4.8219 | 3.7200 | Reject |
| **11 - 10** | 0.4375 | 0.1357 | 3.2248 | 3.7200 | Accept |

Table 4: Analysis of variance of chromosomes 10 - 13, Tukey's test. F statistic = 27.327. Significance = 0.000.

## Acknowledgements

We thank the School of Biological Sciences, Royal Holloway, University of London, for providing the facilities to carry out this investigation.

## References

ANGUS, R.B. & SHAARAWI, F.A.I., 1997: Chromosomal analysis of *Chaetarthria seminulum* (Herbst) and six European species of *Laccobius* Erichson. - Koleopterologische Rundschau 67: 181-186.

BEUTEL, R.G. 1993: Phylogenetic analysis of Adephaga (Coleoptera) based on characters of the larval head. - Systematic Entomology 18: 127-147.

BURMEISTER, E.G. 1976: Der Ovipositor der Hydradephaga (Coleoptera) und seine phylogenetische Bedeutung unter besonderer Berücksichtigung der Dytiscidae. - Zoomorphologie 85: 165-257.

NILSSON, A.N. & ANGUS, R.B. 1992: A reclassification of the *Deronectes*-group of genera (Coleoptera: Dytidscidae) based on a phylogenetic study. - Entomologica Scandinavica 23: 275-288.

RUHNAU, S. 1986: Phylogenetic relations within the Hydradephaga (Coleoptera) using larval and pupal characters. - Entomologia Basiliensia 11: 231-271.

SERRANO, J. & YADAV, J.S. 1984: Chromosome numbers and sex determining mechanisms in adephagan Coleoptera. - The Coleopterists Bulletin 38 (4): 335-357.

SHAARAWI, F.A. & ANGUS, R.B. 1991: A chromosomal investigation of five European species of *Anacaena* Thomson (Coleoptera: Hydrophilidae). - Entomologica Scandinavica 21: 415-426.

SMITH, S.G. 1950: The cyto-taxonomy of Coleoptera. - Canadian Entomologist 82: 58-67.

SMITH, S.G. & VIRKKI, N. 1978: Animal cytogenetics, 3. - Gebrüder Borntraeger, Berlin & Stuttgart.

Clare L. HUGHES & Dr. Robert B. ANGUS
*Division of Biology (Bourne), Royal Holloway, University of London, Egham, Surrey TW20 0EX, U.K.*