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Joseph E. POWELL & Robert B. ANGUS

A chromosomal investigation of some European species of Haliplidae (Coleoptera)

Abstract - The karyotypes of 15 European species of Haliplidae are described and illustrated. The sex chromosomes are X0 in *Brychius elevatus* and *Peltodytes caesus*, and XY in 13 species of *Haliplus*. The number of autosome pairs is 16 in *Peltodytes caesus*, 19 in *Brychius elevatus*, 17 in *Haliplus* (*Liaphlus*) *fulvus*, 15 in *H.* (*L.*) *variegatus*, 14 in *H.* (*L.*) *flavicollis*, 11 in *H.* (*L.*) *laminatus*, 9 in *H.* (*L.*) *mucronatus*, and 11 in *H.* (*Haliplidius*) *obliquus* and *H.* (*H.*) *confinis*, *H.* (*Neohaliplus*) *lineatocollis* and five species of *H.* (*Haliplus*) It is suggested that the X0 sex chromosome system, the most common in the Adephaga, is plesiotypic for Haliplidae, and that the XY systems are a synapomorphy of the family, and are neo-XY in origin. There is no good evidence of Xy_p systems of the type found in Polyphaga.

The diversity of karyotypes shown by species of the subgenus *Liaphlus* is contrasted with the near uniformity shown by other groups. Interspecific differences between karyotypes are noted.

Riassunto - *Studio dei cromosomi di alcune specie europee di Haliplidae (Coleoptera).*

Sono descritti e illustrati i cariotipi di 15 specie europee di Haliplidae. I cromosomi sessuali sono X0 in due specie (*Brychius elevatus* e *Peltodytes caesus*) e XY in 13 specie di *Haliplus*. Il numero di coppie di autosomi è 16 in *Peltodytes caesus*, 19 in *Brychius elevatus*, 17 in *Haliplus* (*Liaphlus*) *fulvus*, 15 in *H.* (*L.*) *variegatus*, 14 in *H.* (*L.*) *flavicollis*, 11 in *H.* (*L.*) *laminatus*, 9 in *H.* (*L.*) *mucronatus*, e 11 in *H.* (*Haliplidius*) *obliquus*, *H.* (*H.*) *confinis*, *H.* (*Neohaliplus*) *lineatocollis* e in cinque specie di *H.* (*Haliplus*). Si suggerisce che il sistema cromosomico sessuale X0, il più comune negli Adephaga, è plesiotipico per gli Haliplidae, e che i sistemi XY sono una sinapomorfia della famiglia, e sono neo-XY in origine. Non ci sono buone prove di sistemi Xy_p del tipo riscontrato nei Polyphaga. La diversità dei cariotipi osservata nelle specie del sottogenere *Liaphlus* contrasta con la quasi uniformità di altri gruppi. Sono state osservate differenze interspecifiche tra i cariotipi.

Key words: Chromosomes, karyotypes, sex-chromosomes, Haliplidae.

INTRODUCTION

The Coleoptera, with more than 370000 species (McGavin, 2001), are generally regarded as the largest order of insects. Chromosomal investigations of Coleoptera have been undertaken since the work of Stevens (1905) on the sex chromosomes of Chrysomelidae. Beetle cytotaxonomy as a whole was reviewed by Smith & Virkki (1978), where the karyotypes of 2160 species are listed. One of the unexpected features of this work was the suggestion by Smith (1950) that there appeared to be a basic coleopteran karyotype (chromosome formula) comprising 9 pairs of autosomes plus sex chromosomes which were XX in the female and "Xy_p" in the male. The Xy_p arrangement consists of a large X chromosome and a very small Y chromosome, which, at first division of meiosis, are held together by a cytoplasmic vesicle. John & Lewis (1960) regarded this vesicle as a nucleolus, but modern fluorescence in situ hybridisation techniques have demonstrated that in some cases no r-DNA (characteristic of nucleoli and their organisers) is present (Juan et al., 1993).

Smith (1950) based his conclusions on a database of only 191 species, representing 66 families. In the event, his hypothesis has stood up well in the suborder Polyphaga (Smith & Virkki, 1978), but there is as yet no convincing evidence for Xy_p sex chromosomes in the other main coleopteran suborder, the Adephaga. Thus Serrano & Yadav (1984) did not list Xy_p among 426 species of Adephaga (mainly Carabidae), and Serrano & Galián (1998) did not list it among over 900 species of Carabidae.

The chromosomes of the aquatic families of Adephaga (Hydradephaga) are much less well known than those of the Carabidae. Thus Smith & Virkki (1978) list only 27 species, belonging to the families Dytiscidae and Gyrinidae, none with Xy_p. Recent works (Nilsson & Angus, 1992; Nilsson, 2000; Angus, unpublished data) demonstrate X0 systems (lacking Y chromosomes in the male, typical of Carabidae) and neo-XY systems, but never Xy_p. Hughes & Angus (1999) demonstrated an Xy system in *Hygrobia hermanni* (Fabricius, 1775) (Hygrobiidae), but with an apparently chiasmate association at meiosis. In Noteridae both neo-XY systems and systems involving multiple X chromosomes have been reported (Bilton, 1992; Ahmed et al. 1997; Ahmed & Angus, 2000). Of the major adephagan families, only the Haliplidae remain completely unreported chromosomally.

The Haliplidae have a worldwide distribution and probably include about 220 species (van Vondel, 1997). They are highly unusual among Adephaga in being vegetarian (algophagous) as both adults and larvae, though according to van Vondel (1997) the adults also take animal food. The other unique feature of the family is the large coxal plates on the hind legs. These plates were present in the Triassic (fossil) family Triaplidae (Ponomarenko, 1977), and their occurrence suggests that the Haliplidae have had a long period of evolution separate from other adephagan families. Preliminary observations (unpublished) by R. B. Angus indicated that at least *Haliplus ruficollis* (DeGeer, 1774) has a small Y chromosome, raising the possibility that the Haliplidae might have an Xy_p system at meiosis, perhaps retained from an ancestral coleopteran arrangement.

In view of this background, the objectives of this investigation are threefold:

1. To give some account of the karyotypes of Haliplidae.
2. To establish whether there is an Xy_p system in this family, and if there is such a system, whether it is likely to be plesiomorphic.
3. To see to what extent similar and apparently related species have clearly different karyotypes.

MATERIAL

Species from which chromosome preparations have been obtained, along with the localities where they were collected, are listed in Table 1. British localities are referred to their Watsonian Vice-Counties, as reviewed by Dandy (1969). Vice-Counties from which material was collected are as follows: England: 11, South Hants; 17, Surrey; 21, Middlesex; 24, Buckinghamshire; 28, West Norfolk. Scotland: 76, Renfrew; 92, South Aberdeen.

The species are classified according to van Vondel (1997) and Lundmark et al. (2001), but, for chromosomal reasons, species of *Haliplus* (*Liaphlus*) are listed before those of the other subgenera.

Table 1. Species used for chromosome analysis.

Species	Location
Brychius elevatus (Panzer, 1793)	England. V.C 11, River Test at Romsey and Kimbridge
Peltodytes caesus (Duftschmid, 1805)	Netherlands. Haarlem district, dunes lack pools at Bloemendaal. England. V.C. 17, Runnymede, Langham Pond.
Haliplus (Liaphlus) fulvus (Fabricius, 1801)	Scotland. V.C. 92, Loch Kinord.
H. (L.) variegatus Sturm, 1834	England. V.C. 11, New Forest, Crockford Bridge.
H. (L.) flavicollis Sturm, 1834	England. V.C. 28, Thompson Common. Scotland. V.C. 76, Loch Libo
H. (L.) laminatus (Schaller, 1783)	England. V.C. 21, River Colne, Staines Moor
H. (L.) mucronatus Stephens, 1828	Netherlands. Haarlem district, dunes lack pools at Bloemendaal.
H. (Haliplidius) obliquus (Fabricius, 1787)	England. V.C. 17, Runnymede, Langham Pond; V.C. 21, Staines Moor; V.C. 28, Thompson Common.
H. (H.) confinis Stephens, 1828	England. V.C. 21, Staines Moor; V.C. 28, Thompson Common.
H. (Neohaliplus) lineatocollis (Marsham, 1802)	England. V.C. 28, Wolferton
H. (Haliplus) ruficollis (DeGeer, 1774)	England. V.C. 11, New Forest, Hatchet Pond; V.C. 21, Staines Moor; V.C. 28, Thompson Common.
H. (H.) sibiricus Motschulsky, 1860 (= *wehnckei* Gerhardt, 1877)	England. V.C. 28, Wolferton.
H. (H.) fluviatilis Aubé, 1836	England. V.C. 21, R. Colne, Staines Moor; V.C. 28, Wolferton.
H. (H.) lineolatus Mannerheim, 1844	England. V.C. 17, Virginia Water Lake; V.C. 21, R. Colne, Staines Moor; V.C. 24, Wraysbury Gravel Pit
H. (H.) immaculatus Gerhardt, 1877	England. V.C. 21, Staines Moor; V.C. 24, Wraysbury Gravel Pit

Methods

The procedures presented here are based on those developed by Angus (1982), and Shaarawi & Angus (1991). Chromosome preparations were obtained from mid gut, testis and ovary. Treatment with colchicine and hypotonic potassium chloride was for 12 seconds in each case. Relative Chromosomes Lengths (RCL – the length of each chromosome as a percentage of the total haploid autosome length in the nucleus) have not been calculated in this study. This is because the material is in general a small number of preparations from a fairly large number of species. Statistical analysis of RCL values would therefore not be possible, and comparisons using absolute lengths in individual preparations were considered to be sufficiently informative. The specimens from which chromosome preparations were obtained are kept in R. B. Angus' collection.

Results

Brychius elevatus (Panzer, 1793). Material analysed: 3 specimens. 2N = 38 + X0 (♂), XX (♀). Mitotic chromosomes, arranged as karyotypes, are shown in fig. 1 c and d. First metaphase of meiosis, showing the unpaired X chromosome, is shown in fig. 3 a. The chromosomes are all small, with the longest autosomes only about 2.5 mm long, while the X chromosome, and the smaller autosomes are about 1 µm long. The longer autosomes include both metacentrics and acrocentrics. C-banding (fig. 1 d) shows small centromeric C-bands on most of the chromosomes. The individual chromatids are not visible in the preparations obtained from this species.

Peltodytes caesus (Duftschmid, 1805). Material analysed: 4 specimens. 2N = 32 + X0 (♂), XX (♀). Mitotic chromosomes, arranged as karyotypes, are shown in fig. 1 a and b. Autosome pairs 1 – 13, 16, and the X chromosome are either acrocentric or subacrocentric, and C-banding (fig. 1 b) shows that the long arms of autosomes 1 – 13 are almost entirely heterochromatic, and metacentric autosomes 14 and 15 have large heterochromatic C-bands over the centromere. Only autosome 16 and the X chromosome are entirely euchromatic. The chromosomes are fairly large, with autosomes 1 – 10 about 4 µm long, while autosomes 14 and 15, and the X chromosome, are about 2 µm long. Comparison of the karyotypes of *P. caesus* and *B. elevatus* suggests that, despite the different sizes of their chromosomes, the total length of euchromatic chromosome regions is similar in the two species.

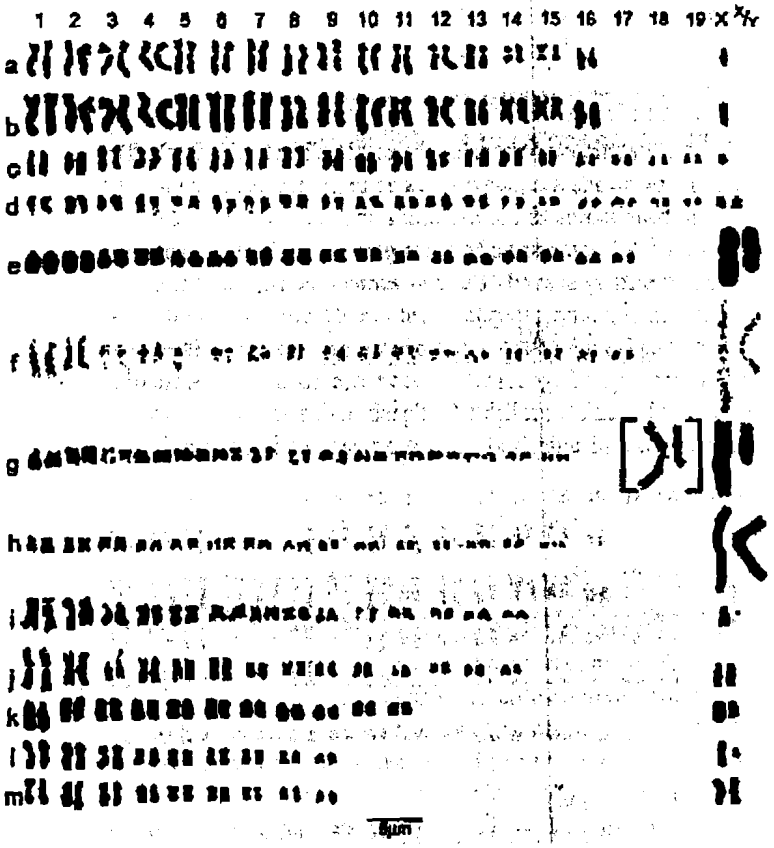

Fig. 1 a-m. Mitotic chromosomes arranged as karyotypes: a - *Peltodytes caesus*, ♂, mid gut, Bloemendaal, plain; b: the same nucleus, C-banded; c, d - *Brychius elevatus*, River Test, c ♂, testis, plain, d, ♀, ovary, C-banded; e, f - *Haliplus fulvus*, ♂, testis, Loch Kinord, e plain, f C-banded; g - *H. variegatus*, ♂, mid gut, Crockford Bridge, sex chromosomes from a second, incomplete nucleus shown in brackets []; h - *H. variegatus*, ♀, ovary, Crockford Bridge; i - *H. flavicollis*, ♂ mid gut, Loch Libo; j - *H. flavicollis*, ♀, mid gut, Thompson Common; k - *H. lamhanus*, ♂, testis, Staines Moor; l - *H. mucronatus*, ♂, mid gut, Bloemendaal; m - *H. mucronatus*, ♀, mid gut, Bloemendaal.

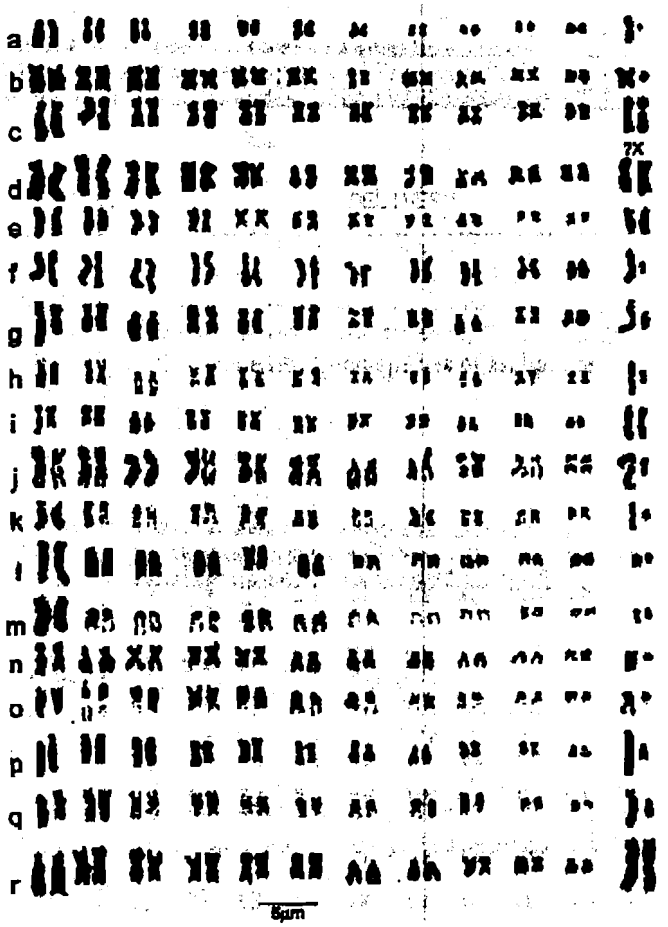

Fig. 2 a-r. Mitotic chromosomes arranged as karyotypes: a - *Haliplus obliquus*, ♂, testis, Thompson Common; b - *H. obliquus*, ♂, mid gut, Staines Moor; c - *H. obliquus*, ♀, mid gut, Thompson Common; d, e - *H. confinis*, ♀, mid gut, Thompson Common, d plain, e C-banded; f - *H. lineatocollis*, ♂, testis, Wolferton; g - *H. ruficollis*, ♂, mid gut, Staines Moor; h - *H. ruficollis*, ♂, testis, Pevensey, C-banded; i - *H. ruficollis*, ♀, mid gut, Thompson Common; j, k - *H. sibiricus*, ♂, testis, Wolferton, j plain, k C-banded; l, m - *H. fluviatilis*, ♂, mid gut, Wolferton, l plain, m C-banded; n, o - *H. lineolatus*, ♂, mid gut, Wraysbury, n plain, o C-banded; p, q - *H. immaculatus*, ♂, mid gut, Wraysbury, p plain, q C-banded; r. *H. immaculatus*, ♀, mid gut, Virginia Water.

Haliplus (*Liaphlus*) *fulvus* (Fabricius, 1801). Material analysed: 2 specimens. 2N = 34 + XY (♂). Mitotic chromosomes, arranged as karyotypes, are shown in fig. 1 e and f. No meiotic preparations were obtained. The autosomes are small, with the longest being approximately 2 µm long. The smallest autosomes are about 1 µm long. In comparison the X and Y chromosomes are very large, about 7 and 5 µm long in fig. 1e. Although only males were obtained the X and Y chromosomes were identified by comparison with those

Fig. 3 a-q. First division of meiosis from testis. The X and Y chromosomes are labelled: a - *Brychius elevatus*, R. Test, metaphase; b - *Haliplus laminatus*, Staines Moor, metaphase; c, d - *H. obliquus*, Thomson Common, c diakinesis, d metaphase; e-h - *H. ruficollis*, e, f, h, Thomson Common, g Hatchet Pond. e, f, early and late zygotene, g diakinesis, h metaphase; i, j - *H. sibiricus*, Wolferton, i diakinesis, j metaphase; k-m - *H. fluviatilis*, Wolferton, k diakinesis, l, m metaphase, the same nucleus, l plain, m after C-banding treatment; n-o - *H. lineolatus*, Wraysbury, metaphase, the same nucleus, n plain, o after C-banding treatment; p, q: *H. immaculatus*, Wraysbury, p late diakinesis, q zygotene.

of *H. variegatus* (fig. 1 g and h). The autosomes include both metacentrics and acrocentrics. The individual chromatids are not visible in the testis preparations obtained from this species. C-banding (fig. 1 f) shows small centromeric C-bands on all the chromosomes, with a larger C-banding area, possibly a secondary constriction, on autosome 1. This is a very striking karyotype because of the extreme size of the sex chromosomes compared with

the autosomes. The X chromosome is equal in length to between a quarter and over a third of the total haploid autosome length in the nucleus, depending on the degree of contraction of the preparation. One by-product of this is that incomplete karyotypes, lacking some autosomes, may look intact. However, the close agreement between the arrangements shown in fig. 1 e and f, differing only in the loss of one replicate of autosome 5 in fig. 1 f, suggests that this is the correct karyotype.

Haliplus (*Liaphlus*) *variegatus* Sturm, 1834. Material analysed: 3 specimens. 2N = 30 + XY (♂), XX (♀). Mitotic chromosomes, arranged as karyotypes, are shown in fig. 1 g and h. Additional sex chromosomes from an incomplete nucleus are shown in brackets [ ]. No preparation of meiosis was obtained as the testes of the material (collected in November) contained only mature sperm. The autosomes are small, the longest being about 2 µm long, and the smallest about 1 µm long. Again, in comparison the sex chromosomes are very large, approximately 5 - 8 µm long. The majority of the autosomes are metacentrics, although some are acrocentrics, the sex chromosomes are metacentric. Individual chromatids are visible on a few chromosomes. Fig. 1 h shows a karyotype of a female *H. variegatus*. In this midgut preparation the individual chromatids are visible in the majority of the autosomes.

Haliplus (*Liaphlus*) *flavicollis* Sturm, 1834. Material analysed: 2 specimens. 2N = 28 + Xy (♂), XX (♀). Mitotic chromosomes, arranged as karyotypes, are shown in fig. 1 i and j. The sex chromosomes are a medium sized acrocentric X chromosome, about 2 µm long, and a dot-like y chromosome. The autosomes range from medium length to small, the longest being about 5 µm long, while the smallest about 0.5 µm long. They include both metacentrics and acrocentrics.

Haliplus (*Liaphlus*) *laminatus* (Schaller, 1783). Material analysed: 1 specimen. 2N = 22 + XY (♂). Mitotic chromosomes, arranged as a karyotype, are shown in fig. 1 k. First metaphase of meiosis, showing paired XY chromosomes, is shown in fig. 3 b. The chromosomes are rather similar in size, with the largest autosomes being about 3.5 µm long, while the smaller autosomes and sex chromosomes being approximately 2 µm long. The X chromosome is in the middle of the size range of the autosomes, while the Y is slightly shorter. The autosomes include both metacentrics and acrocentrics. The individual chromatids are not visible in the testis preparations obtained from this species.

Haliplus (*Liaphlus*) *mucronatus* Stephens, 1828. Material analysed: 3 specimens. 2N = 18 + Xy (♂), XX (♀). Mitotic chromosomes, arranged as karyotypes, are shown in fig. 1 l and m. The autosomes are small, about 2.5 - 0.8 µm long, while the sex chromosomes are a relatively large X chromosome, about 2.5 µm long and a very small y chromosome, about 0.5 µm long. All the chromosomes are metacentric, except autosome 9 and the y chromosome, which are acrocentric. C-banding (not shown) appears very weakly developed, with small indistinct centromeric C-bands on some of the autosomes.

Haliplus (*Haliplidius*) *obliquus* (Fabricius, 1787). Material analysed: 5 specimens. 2N = 22 + Xy (♂), XX (♀). Mitotic chromosomes, arranged as karyotypes, are shown in fig. 2 a-c. First metaphase of meiosis, showing paired Xy chromosomes, is shown in fig. 3 c and d. Diakinesis is shown in fig. 3 c and first metaphase of meiosis is shown in fig. 3 d. The condensed Xy bivalent is labelled, and the arrangement, especially in fig. 3 d, looks a little like Xy_p. The size range of the chromosomes range is such that the longest chromosomes are about three times the length of the shortest, the largest autosome in the preparations shown here being about 3 µm long and the smallest approximately 1 µm long. The X chromosome is about 3 µm long, while the y is dot-like. The autosomes include both metacentrics and acrocentrics. The individual chromatids are visible in preparations shown in fig. 2 b and c, though not in fig. 2 a.

Haliplus (*Haliplidius*) *confinis* Stephens, 1828. Material analysed: 2 specimens. 2N = 22 + XX (♀). Mitotic chromosomes, arranged as karyotypes, are shown in fig. 2 d and e. fig. 2 e shows a C-banded karyotype for this species. There is heavy banding on the suggested X chromosomes. The sex chromosomes cannot be identified from the material studied, as only females were available for analysis. The suggested X chromosome in fig. 2 d and e is based on comparison with *H. obliquus*. The longest autosomes are approximately 4 µm long and the smallest about 2 µm long. C-banding (fig. 2 e) shows secondary constrictions on the long arms of autosome 2 and the suggested X-chromosome. In the X there is some indication of variation in the expansion of this constriction between the two replicates of the chromosome, giving an apparent size and centromere position difference. The chromosomes include both metacentrics and acrocentrics.

Haliplus (*Neohaliplus*) *lineatocollis* (Marsham, 1802). Material analysed: 1 specimen. 2N = 22 + Xy (♂). Mitotic chromosomes, arranged as a karyotype, are shown in fig. 2 f. No meiotic preparation was obtained. The majority of the autosomes are long, being approximately 4.5 µm long for the largest, and 3 µm long for the smallest. The X chromosome is a similar length, although the y is very small, about 1 µm long. The autosomes include both metacentrics and acrocentrics. The individual chromatids are not visible in the preparations for this species.

Haliplus (*Haliplus*) *ruficollis* (DeGeer, 1774). Material analysed: 10 specimens. 2N = 22 + Xy (♂), XX (♀). Mitotic chromosomes from the mid gut and testis of males, arranged as karyotypes, are shown in fig. 2 g and h. respectively. Mitotic chromosomes from female mid gut, arranged as a karyotype, are shown in fig. 2 i. C-banding (fig. 2 h) shows autosome 1 with a secondary constriction in its long arm, and this tends to be expanded to a different extent in the two replicates. This gave great difficulty in arranging karyotypes from unbanded material (fig. 2 g and i) until the cause of the very different sizes of the two replicates of autosome 1 was understood. The largest autosomes are approximately 4.5 µm long, and the smallest about 1 µm long. The X chromosomes are of similar length to the larger autosomes, although the y is considerably smaller being about 1.5 µm long. The autosomes include both metacentrics and acrocentrics. Individual chromatids are visible for some of the chromosomes, particularly in preparations for the mid-gut. fig. 3 e-h

all show meiotic preparations. Fig. 3 e and f shows the chromosomes in early and late zygotene, with the heavily condensed X and y chromosomes lying together. Fig. 3 g shows the chromosomes in late diakinesis, with the X chromosome looped almost in a complete circle, with apparent links to the y from both its ends. Fig. 3 h shows paired X and y chromosomes in metaphase, giving a much more condensed appearance which could be interpreted as Xy_p, but not unequivocally so.

Haliplus (*Haliplus*) *sibiricus* Motschulsky, 1860. Material analysed: 1 specimen 2N = 22 + Xy (♂). Mitotic chromosomes from the testis, arranged as a karyotype, are shown in fig. 2 j and k. C-banding (fig. 2 k) shows heavy banding on the y chromosome and autosomes 1 and 5. The longest autosomes are approximately 5 µm long, while the smallest are about 1.5 µm long. The X chromosome is about the same size as the largest autosomes and the y chromosome is very small, almost dot like. There is a secondary constriction in the short arm of the 8th pair of autosomes, and this can be either extended or condensed. The autosomes include both metacentrics and acrocentrics. The individual chromatids are clearly visible in the unbanded karyotype (fig. 2 j), though not so in the C-banded preparation. Meiotic preparations are shown in fig. 3 i and j. The Xy bivalent at first metaphase could be interpreted as Xy_p (fig. 3 j), but diakinesis (fig. 3 i) shows two apparently terminalised chiasmata linking the sex chromosomes.

Haliplus (*Haliplus*) *fluviatilis* Aubé, 1836. Material analysed: 4 specimens. 2N = 22 + Xy (♂). Mitotic chromosomes from the mid-gut, arranged as a karyotype, are shown in fig. 2 l and m. C-banding (fig. 2 m) shows heavy banding on the long arms of autosomes 1 and at the centromeres of most of the acrocentric autosomes. The first pair of autosomes are clearly the largest, being approximately 4 µm long, the remaining autosomes are considerably smaller, with the smallest about 0.5 µm long. Both of the sex chromosomes are about the same size, although small, approximately 1 µm long. Individual chromatids are visible in the unbanded preparation from the mid-gut. Although the karyotype includes both metacentric and acrocentric autosomes, the vast majority of them are acrocentric, possibly with the exception of the first pair. Meiotic preparations are shown in fig. 3 k-m, with diakinesis shown in fig. 3 k and first metaphase in fig. 3 l and m. These two figures are of the same nucleus, plain in l and C-banded in m. Diakinesis (fig. 3 k) suggests terminalised chiasmata in the Xy bivalent (as in *H. sibiricus*, fig. 3 i), while the condensed sex bivalent at metaphase could be interpreted as Xy_p, though not convincingly so.

Haliplus (*Haliplus*) *lineolatus* Mannerheim, 1844. Material analysed: 4 specimens. 2N = 22 + Xy (♂). Mitotic chromosomes from the mid-gut, arranged as karyotypes, are shown in fig. 2 n and o. C-banding (fig. 2 o) shows heavy regions of banding in most of the autosomes. On metacentric autosomes banding is seen at the centromeres of pairs 4, 5 and 8, and on the short arms of pairs 1-3. Banding is also seen at the centromeres of most of the remaining acrocentric autosomes. The largest autosomes are approximately 3.5 µm long while the smallest are about 1 µm long. The X chromosome is about 2 µm long and the y chromosome is very small and dot-like. Individual chromatids are clearly visible for the unbanded mid-gut preparation (fig. 2 n) The karyotype includes both metacentric and

acrocentric autosomes. Meiotic preparations are shown in fig. 3 n and o. Both figures show metaphase of the same nucleus, showing paired X and y chromosomes, with fig. 3 n being plain and fig. 3 o showing the preparation after C-banding treatment. The sex bivalent appears to show terminalised chiasmata, especially after C-banding treatment, despite the very small size of the y chromosome.

Haliplus (*Haliplus*) *immaculatus* Gerhardt, 1877. Material analysed: 6 specimens. 2N = 22 Xy (♂), XX (♀). Mitotic chromosomes from the mid-gut, arranged as karyotypes, are shown, from a male in fig. 2 p and q and from a female in fig. 2 r. C-banding (fig. 2 q) shows banding on all of the chromosomes. On metacentric autosomes banding is seen at the centromeres of pairs 3-6, 9, 10, and on the short arms of pairs 1 and 2. Banding is also seen at the centromeres of all of the remaining acrocentric autosomes and at the centromere of the metacentric X chromosome. There is a pericentric inversion polymorphism in the longest autosome (autosome 1), which may be either acrocentric (the centromere more or less terminal) or submetacentric (the centromere near, but clearly not in, the middle of the chromosome). Fig. 2 p and q shows a male heterozygous for this polymorphism, while fig. 2 r shows a female with homozygous acrocentric autosome 1. The largest autosomes are approximately 5 µm long in fig. 2 r, and the smallest autosomes are about 1 µm long in this figure. The X chromosomes are the largest in the nucleus, approximately 6 µm long in fig. 2 r. The y chromosomes are small, about a third as long as the X. The individual chromatids are clearly visible in the female preparation from the mid-gut, though less so in the male preparations. The karyotype includes both metacentrics and acrocentrics. Meiotic preparations are shown in fig. 3 p and q. Heavy condensation of the chromosomes in late diakinesis is shown in fig 3 p and the clearly visible, heavily condensed sex chromosomes at zygotene are shown in fig. 3 q. The orientation of the arms of the sex chromosomes in this figure strongly suggests terminalised chiasmata.

DISCUSSION

The results presented here show both a considerable diversity in the karyotypes of Haliplidae, and also a large and apparently diverse assemblage of species with broadly similar karyotypes. The karyotype of *Brychius elevatus*, with 19 pairs of autosomes and an X0 sex chromosome system, is very similar to that recorded by Serrano & Yadav (1984) and Serrano & Galián (1998) (18 pairs of autosomes plus X0) as the commonest karyotype for Carabidae. Beutel & Ruhnau (1990) in their cladistic analysis of Haliplidae, place *Brychius* as the second most basal group to separate from the main stem of the family, which suggests that this karyotype, especially as regards its X0 sex chromosomes, may be plesiomorphic within the Haliplidae. The most basal separation recorded by Beutel & Ruhnau is that of *Peltodytes* Régimbart, 1879. The karyotype of *P. caesus* appears highly aberrant in the very extensive development of heterochromatin, but it does support the idea that X0 sex chromosomes are the original arrangement in the Haliplidae.

Within the genus *Haliplus* Latreille, 1802 the most striking result is the numerically uniform karyotype (11 pairs of autosomes plus Xy sex chromosomes), shown by members of the subgenera *Haliplus* s. str., *Haliplidius* Guignot, 1928 and *Neohaliplus* Netolitzky, 1911, as well

as *H. (Liaphlus) laminatus*. The systematic position of *H. laminatus* has long been questioned, with debate as to whether it belongs in *Liaphlus* Guignot, 1928 or nearer to *Haliplus* s. str. (van Vondel, 1997). The karyotype of 11 pairs of autosomes plus Xy sex chromosomes in *H. laminatus* at first appeared to support its placement near *Haliplus* s. str. However, the subsequent discovery of a diverse selection of karyotypes within *Liaphlus*, both with regard to number and the size of the sex chromosomes, leaves the whole matter once more unclear.

The nature of the XY system of sex chromosomes in Haliplidae may be considered both in terms of whether it is primitive (plesiomorphic) or derived (apomorphic), in terms of whether an Xy_p system is present where the Y chromosome is conspicuously small (Xy). If the X0 systems of *Brychius* and *Peltodytes* are indeed plesiomorphic, the XY system of other Haliplidae, whatever its nature, is apomorphic. The usual way in which XY systems are recorded as emerging from X0 systems is by fusion of the X chromosome with an autosome to give a neo-XY system (Smith & Virkki, 1978). In this system, the original autosome, without the X chromosome, becomes the neo-Y chromosome, while the autosome plus original X becomes neo-X. The association of the neo-XY bivalent at meiosis is by chiasmata between their original autosomal portions. The apparently terminalised chiasmata shown by at least some of the XY bivalents at first division of meiosis (especially *H. sibiricus* at diakinesis, fig. 3 f) suggests that this is indeed a neo-XY system. Although a number of more condensed metaphase preparations (e.g. *H. obliquus*, fig. 3 d, *H. ruficollis*, fig. 3 h and *H. fluviatilis*, fig. 2 l and m) could be interpreted as showing Xy_p, none is unequivocal, and in particular, none shows any sign of a cytoplasmic vesicle. The terminalised chiasmata suggested by preparations of diakinesis seem a more likely explanation. C-banding suggests that the smaller Y chromosome may be almost entirely heterochromatic (*H. ruficollis*, fig. 2 h) or euchromatic with small heterochromatic bands at the centromere (*H. lineolatus*, fig. 2 o). There is also considerable variation in the size of the X chromosome – the largest in the nucleus in *H. immaculatus* (fig. 2, p-r), but among the smallest in *H. fluviatilis* (fig. 2, l and m).

The karyotypes of the species in this "karyotype-group" show interspecific differences in terms of the relative lengths and centromere positions of various chromosomes. Although many small *Haliplus* species may appear similar and difficult to identify (especially as females), none of the species studied here approaches sibling or doubtful status. *Haliplus ruficollis* and *H. sibiricus* (= *wehnckei*) have sometimes been considered as a pair (e.g. Balfour-Browne, 1940), though their aedeagophores are clearly very different. As to their karyotypes, autosome 3 is acrocentric in *H. ruficollis* but submetacentric in *H. sibiricus*, while autosomes 7 and 8 are submetacentric in *H. ruficollis* but acrocentric in *H. sibiricus*. *Haliplus sibiricus* and *H. wehnckei* used to be separated on the form of the right paramere, and Holmen (1987) notes that they have been regarded as possible subspecies but may in fact be conspecific – a view now confirmed by enzyme electrophoresis and morphometric analysis (Lundmark et al., 2001). The British material reported here is of the *H. wehnckei* pattern, and it would be interesting to know the karyotype of specimens with the *H. sibiricus* type of right paramere. It is interesting that there are no obvious differences between the karyotypes of *H. confinis* and *H. obliquus* – easily recognisable species but in the same subgenus (*Haliplidius*). It should be noted that no male karyotype of *H. confinis* has been obtained.

The karyotypes of the *Liaphlus* species studied here are a surprisingly heterogeneous assemblage, especially after the uniformity shown by the other *Haliplus* species. *Haliplus fulvus* and *H. variegatus* have very similar, long sex chromosomes. Their size suggests that they are neo-X and neo-Y. It is notable that *H. fulvus* has 17 pairs of autosomes, as against 15 in *H. variegatus*. The karyotypes of *H. flavicollis*, *H. laminatus* and *H. mucronatus* are all different from one another. *Haliplus flavicollis* has 14 pairs of autosomes, one pair fewer than *H. variegatus*, and three pairs more than *H. laminatus* and the other subgenera, while *H. mucronatus*, with only nine pairs of autosomes, has the lowest chromosome number so far encountered in the Haliplidae. Beutel & Ruhnau (1990) suggest that they have no evidence of monophyly of *Liaphlus*, and the chromosome data presented here support this view. One other conclusion proposed by Beutel & Ruhnau (1990), that *Neohaliplus* may be the first group to branch off within the "*Algophilus-Apteraliplus-Haliplus* complex", is not supported by the chromosome data. Beutel & Ruhnau (1990) suggest that *Algophilus* Zimmermann, 1924 is part of a monophyletic unit including Ethiopian species of *Liaphlus* (not studied here!), but unless these species are karyotypically similar to *H. laminatus* and the other subgenera, it is very unlikely that *Neohaliplus* (*H. lineatocollis*) would have branched off before them. Clearly, there is scope for further chromosomal investigations here.

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