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A chromosomal investigation of some European Hydraenidae (Coleoptera: Hydraenidae)

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Abstract

Nineteen European species of Hydraenidae have been investigated chromosomally. All were found to have sex chromosomes which are X0 in the male, XX in the female. *Hydraena testacea* CURTIS, *barrosi* D'ORCHYMONT, *corinna* D'ORCHYMONT and *inapicipalpis* PIC have 12 pairs of autosomes, while *H. riparia* KUGELANN, *brachymera* D'ORCHYMONT, *britteni* JOY, *cantabrica* CHIESA, *palustris* ERICHSON, *Ochthebius dilatatus* STEPHENS, *maculatus* REICHE, *minimus* FABRICIUS, *rugulosus* WOLLASTON, *marinus* PAYKULL, *punctatus* STEPHENS, *Limnebius truncatellus* THUNBERG, *papposus* MULSANT, *furcatus* BAUDI and *nitidus* MARSHAM have 9 pairs of autosomes. There are no obvious differences between the karyotypes of the four *Ochthebius* (*Asiobates*) species (*O. dilatatus*, *maculatus*, *minimus* and *rugulosus*), nor between *Limnebius papposus* and *furcatus*, but the other species all show individual peculiarities. It has not been possible to produce a karyotype of *Limnebius nitidus*, though its chromosome number has been ascertained.

In the course of a general investigation of the chromosomes of British Hydrophilidae, a specimen of the Hydraenid *Limnebius papposus* was studied. The resulting preparations, though poor, indicated that this species had X0 sex chromosomes in the male, as against XY or Xyp in all Hydrophilids studied. A three-month visit to Egham by the junior author gave an opportunity to follow up this preliminary observation, to see whether *L. papposus* did indeed have X0 sex chromosomes, and whether such a system was general in the Hydraenidae.

MATERIAL & METHODS: A list of the species studied, with their localities of origin, is given in the Table below. All preparations were obtained from adult beetles, using either mid-gut crypt cells, or testis. The techniques used are given by ANGUS (1982) and SHAARAWI & ANGUS (1991). For Hydraenidae we found that best results were obtained following colchicine treatment for 20-25 min., followed by 10 min. in hypotonic potassium chloride. Colchicine was administered either by injection through the apex of the abdomen (SHAARAWI & ANGUS 1991) or by partially detatching the abdomen and placing the beetle in colchicine solution. Both methods gave satisfactory results.

SPECIES	LOCALITIES OF ORIGIN
Hydraena:	
H. (*Phothydraena*) *testacea* CURTIS	England: Surrey, Runnymede
H. (*H.* s. str.) *barrosi* D'ORCHYMONT	Spain: Provincia de Lugo, Vilariño, tributary of the rio Landro
H. corinna D'ORCHYMONT	Spain: Provincia de Lugo, Vilariño, tributary of the rio Landro
H. inapicipalpis PIC	Spain: Provincia de Lugo, Vilariño, tributary of the rio Landro

H. riparia KUGELANN	England: Norfolk, Lamb's Common, East Walton; Oxfordshire, Otmoor.
H. brachymera D'ORCHYMONT	Spain: Provincia de Lugo, Vilariño, tributary of the rio Landro
H. britteni JOY	England: Norfolk, Lamb's Common, East Walton
H. cantabrica CHIESA	Spain: Provincia de Lugo, Vilariño, tributary of the rio Landro
H. palustris ERICHSON	England: Norfolk, Gayton Thorpe Common
Ochthebius:	
O. (*Asiobates*) *dilatatus* STEPHENS	England: Sussex, Cuckmere Haven; Oxfordshire, Otmoor
O. (*A.*) *maculatus* REICHE	Israel: Berekhat Atlit
O. (*A.*) *minimus* (F.)	England: Norfolk, East Harling Common; Surrey, Runnymede
O. (*A.*) *rugulosus* WOLLASTON	Israel: Sheinorat Hahula - Ber. Eitan
O. (*O.* s. str.) *marinus* (PAYKULL)	England: Sussex, Cuckmere Haven
O. punctatus STEPHENS	England: Sussex, Cuckmere Haven
Limnebius:	
L. truncatellus (THUNBERG)	England: Norfolk, Cockley Cley; Oxfordshire, Woodeaton
L. papposus MULSANT	England: Norfolk, East Walton Common; Surrey, Runnymede
L. furcatus BAUDI	Spain: Provincia de León, near León
L. nitidus (MARSHAM)	England: Surrey, Runnymede

1. Genus *Hydraena*

All the species for which males were obtained had X0 sex chromosomes. *H.* (*Phothydraena*) *testacea* (Figs. 1, 2), *H.* (*H.* s.str.) *corinna* (Figs. 3, 4), *barrosi* (Fig. 5), and *inapicipalpis* (Fig. 6) have 12 pairs of autosomes, while *H.* (*H.* s.str.) *riparia* (Fig. 7), *brachymera* (Fig. 8), *britteni* (Fig. 9), *cantabrica* (Fig. 10) and *palustris* (Fig. 11) have 9 pairs of autosomes.

The karyotype of *H. testacea* (Figs. 1, 2) is clearly different from those of the three *Hydraena* s.str. species with 12 pairs of autosomes (Fig. 3-6) with a different sequence of relative chromosome lengths and centromere positions along the sequence of chromosomes. However, the three *Hydraena* s.str. species have very similar karyotypes. The karyotype of *H. corinna* (Figs. 3,4) differs most clearly from that of *H. barrosi* (Fig. 5) in having distinct short arms on autosome pair 10 (never observed in *barrosi*), while in *H. inapicipalpis* the short arms of autosome pairs 8 and 10 appear longer than in either *corinna* or *barrosi*. Nevertheless these three karyotypes appear very similar, permitting a tentative identification of the X-chromosome in *H. barrosi* and *inapicipalpis*, even though only females of these species were available for study.

The *Hydraena* species with 9 pairs of autosomes comprise three members of the *H. riparia* group (*H. riparia*, *brachymera* and *britteni*), and two others (*H. cantabrica* and *palustris*). The karyotypes of *H. riparia* (Fig. 7) and *brachymera* (Fig. 8) appear very similar, but differ in that autosome pairs 4 and 5 are more similar in size in *brachymera* (pair 5 more distinctly smaller

than pair 4 in *riparia*), and the centromere position in pair 4 is further from the middle of the chromosome in *riparia* than in *brachymera*. These observations appear true in four different karyotypes (from different beetles) of *riparia* and two of *brachymera*. These two species are very similar morphologically, and D'ORCHYMONT (1936) originally described *brachymera* as male form of *riparia*. The karyotypes support the view of BERTHELEMY (1965) and BALFOUR-BROWNE (1978) that it is a distinct species. *Hydraena britteni* is morphologically very distinct from *riparia*, at least in the male, and its karyotype (Fig. 9) is clearly different from the other two in that the X-chromosome is about the same size as autosomes 8 and 9, while in *H. riparia* and *brachymera* the X-chromosome is clearly smaller than the small autosomes.

The karyotype of *H. cantabrica* (Fig. 10) is not particularly like that of any other species studied, and as only a female is available for study it is not possible to identify the X-chromosome. The karyotype of *H. palustris* (Fig. 11) is also distinctive, and as a male is available, the X-chromosome has been identified. It should be noted that the very long chromosomes in this preparation are a result of their being less condensed than in others and do not indicate that this species has larger chromosomes.

2. Genus *Ochthebius*

The karyotypes of the four *Asiobates* species (*O. dilatatus* (Figs. 12, 13), *O. maculatus* (Fig. 14), *O. minimus* (Figs. 15, 16) and *O. rugulosus* (Fig. 17)) are all very similar, with 9 pairs of autosomes and the X-chromosome slightly larger than autosome pairs 8 and 9. Only female *O. maculatus* was available, and identification of the X-chromosome is therefore tentative. All these species have rather small chromosomes, which are all either metacentric or nearly so. On present evidence it does not seem likely that the chromosomes will be useful in clarifying the status of *Asiobates* species.

The remaining species, *O.* (*O.* s.str.) *marinus* (Fig. 18) and *O.* (*O.* s.str.) *punctatus* (Fig. 19) have very distinctive karyotypes, although the chromosome number is unaltered and the chromosomes are small. The X-chromosome of *marinus* is acrocentric, with a heavily condensed centromere region, and, in the preparation shown, the short arms extended horizontally.

3. Genus *Limnebius*

The karyotype of *L. truncatellus* (Figs. 20, 21) differs from those of *L. papposus* (Fig. 22) and *L. furcatus* (Fig. 23) in having the X-chromosome rather shorter, and metacentric. The karyotypes of *L. papposus* and *furcatus* show no obvious difference, and have the X-chromosome with its short arms at most half the length of the long arms, and the chromosome itself the longest in the nucleus. All these species have 9 pairs of autosomes, as does *L. nitidus*. The preparations of *L. nitidus* (from testis) were adequate for a chromosome count, but not good enough for preparation of a karyotype.

4. Meiosis

First metaphase of meiosis of four species is shown: *H. testacea* (Fig. 24), *O. dilatatus* (Figs. 25, 26), *O. punctatus* (Figs. 27, 28) and *L. truncatellus* (Fig. 29). In all cases the unpaired X-chromosome is recognisable.

Discussion

The most striking feature of the hydraenid karyotypes presented here is that in all cases where males were available the sex chromosomes are X0. This arrangement, though well documented in Coleoptera (SMITH & VIRKKI 1978), is relatively unusual. Thus, of the 1951 cases for which SMITH & VIRKKI record the sex chromosomes, only 271 (14%) have an X0 system. This type of system is, however, prevalent in the Adephaga, where SERRANO & YADAV (1984) record it in 226 (53%) of 426 cases. If the 143 Adephagan records are removed from SMITH & VIRKKI's list, then 214 (12%) out of 1808 cases have X0 sex chromosomes. The majority of the Polyphaga studied

(about 54%) have a small dot-like Y-chromosome, which associates with the X-chromosome during first division of meiosis by means of a nucleolus, giving a parachute-like appearance (Xyp) (JOHN & LEWIS 1960, SMITH & VIRKKI 1978). Within the Polyphaga the X0 sex chromosome arrangement has a pattern of occurrence which appears sporadic, and, from a taxonomic standpoint, random in its distribution, but including the majority of records for Lampyridae, Cantharidae, Elateridae, Passalidae - Passalini and Chrysomelidae - Galerucinae - Diabroticini (SMITH & VIRKKI 1978). The Hydraenidae thus join this group of unrelated taxa. The Hydraenidae have been associated either with the Hydrophiloidea (eg. CROWSON 1955) or the Staphylinoidea (eg. BÖVING & CRAIGHEAD 1931). X0 sex chromosomes are unknown in the Hydrophilidae (sensu lato) (SHAARAWI 1989), but occur sporadically in the Staphylinoidea. Thus SMITH & VIRKKI (1978) list two species of *Nicrophorus* (Silphidae) as having X0 sex chromosomes, and VORONTSOV et al. (1984) record this arrangement in *Philonthus varius* GYLLENHAL and *Quedius fuliginosus* GRAVENHORST (Staphylinidae). Nevertheless, the majority of both the Silphidae and the Staphylinidae listed by VORONTSOV et al. (1984) have Xyp sex chromosomes, and this, in conjunction with the sporadic occurence of X0 chromosomes already noted, means that the X0 chromosomes of Hydraenidae do not provide evidence for a relationship with either the Staphylinoidea or the Hydrophiloidea. They do, however, reveal a sharp and apparently consistent distinction between the Hydraenidae and other groups included in the Hydrophiloidea.

SMITH (1950), after studying about 190 species of Coleoptera, found that the commonest arrangement was 9 pairs of autosomes plus Xyp sex chromosomes, and suggested that this arrangement was ancestral for the order. Even when all the 2160 species listed by SMITH & VIRKKI (1978) are considered, this remains the commonest arrangement, and PETTITPIERRE (1980) and CROWSON (1981) both repeat the suggestion that this is ancestral for Coleoptera. However, the data for Adephaga listed by SERRANO & YADAV (1984) do not support the idea that this karyotype is ancestral for the suborder, and PETTITPIERRE (1987) refers to 9 pairs of autosomes plus Xyp as ancestral arrangement only for the Polyphaga - which, on present evidence, seems likely. If this is the case, then the Hydraenidae, in most of the cases studied, deviate from the ancestral arrangement only in the loss of the small Y-chromosome. This arrangement, with 9 pairs of autosomes plus X0, occurs in all three of the genera studied, and includes those species (*Ochthebius*, subgenus *Asiobates*) where the chromosomes appear simplest and most uniform. It thus seems likely that it represents the ancentral condition for the Hydraenidae.

The main departures from the presumed ancestral arrangement, as well as the greatest diversity of chromosomal form, have been found in species of *Hydraena*. It is at the moment not clear whether the three species of *Hydraena* s.str. with 12 pairs of autosomes are taxonomically close to subgenus *Phothydraena*, but they may be closely related to each other. *Hydraena barrosi* and *corinna* are morphologically similar to one another, but the association *H. inapicipalpis* with this group came as a surprise. It is, however, supported by details of the female genitalia and associated segments, at present being studied by the junior author.

One final point worthy of consideration is the extent to which chromosomal studies may be useful in clarifying distinctions between species. Thus, among the Hydrophiloidea, ANGUS (1982, 1983, 1986, 1988, 1989) found striking differences between the karyotypes of morphologically similar *Helophorus* species, and SHAARAWI & ANGUS (1991) found important differences between the chromosomes of *Anacaena* species. The evidence given here suggests that the chromosomes may be helpful in *Hydraena* and *Limnebius*, but not, unfortunately, in the species-rich *Ochthebius* (*Asiobates*).

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Zusammenfassung

Neuzehn europäische Hydraenidae wurden karyologisch untersucht. Alle Arten besitzen X0 (Männchen) beziehungsweise XX (Weibchen) Geschlechtschromosomen. *Hydraena testacea* CURTIS, barrosi D'ORCHYMONT, *corinna* D'ORCHYMONT und *inapicipalpis* PIC haben 12 Autosomenpaare, während *H. riparia* KUGELANN, *brachymera* D'ORCHYMONT, *britteni* JOY, *cantabrica* CHIESA, *palustris* ERICHSON, *Ochthebius dilatatus* STEPHENS, *maculatus* REICHE, *minimus* FABRICIUS, *rugulosus* WOLLASTON, *marinus* PAYKULL, *punctatus* STEPHENS, *Limnebius truncatellus* THUNBERG, *papposus* MULSANT, *furcatus* BAUDI und *nitidus* MARSHAM 9 Autosomenpaare besitzen. Es konnten keine auffallenden Unterschiede in der Chromosomenstruktur der 4 *Ochthebius* (*Asiobates*) Arten (*O. dilatatus*, *maculatus*, *minimus* und *rugulosus*), sowie zwischen *Limnebius papposus* und *furcatus* gefunden werden. Alle übrigen Arten zeigen jedoch deutliche individuelle Merkmale. Von *Limnebius nitidus* konnte kein geeignetes Chromosomenpräparat hergestellt werden.

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Figs. 1 - 6: Mitotic chromosomes of *Hydraena* species with 12 pairs of autosomes. All preparations from mid-gut cells. Scale bar = 5μm. 1) *H. testacea*, ♂, Runnymede; 2) *H. testacea*, ♀, Runnymede; 3, 4) *H. corinna*, ♂, Vilariño; 5) *H. barrosi*, ♀, Vilariño; 6) *H. inapicipalpis*, ♀, Vilariño, one replicate of autosome 8 missing. The "?" over the X-chromosome means that the interpretation is based on related species and is tentative.

Figs 7 - 11: Mitotic chromosomes of *Hydraena* species with 9 pairs of autosomes. All preparations from mid-gut cells. The scale bar represents 5μm. 7) *H. riparia*, ♂, Lamb's Common; 8) *H. brachymera*, ♂, Vilariño; 9) *H. britteni*, ♂, Lamb's Common; 10) *H. cantabrica*, ♀, Vilariño; 11) *H. palustris*, ♂ Gayton Thorpe Common. The number 10 over the smallest pair of chromosomes of *H. cantabrica* indicates that it has not been possible to identify the X-chromosome.

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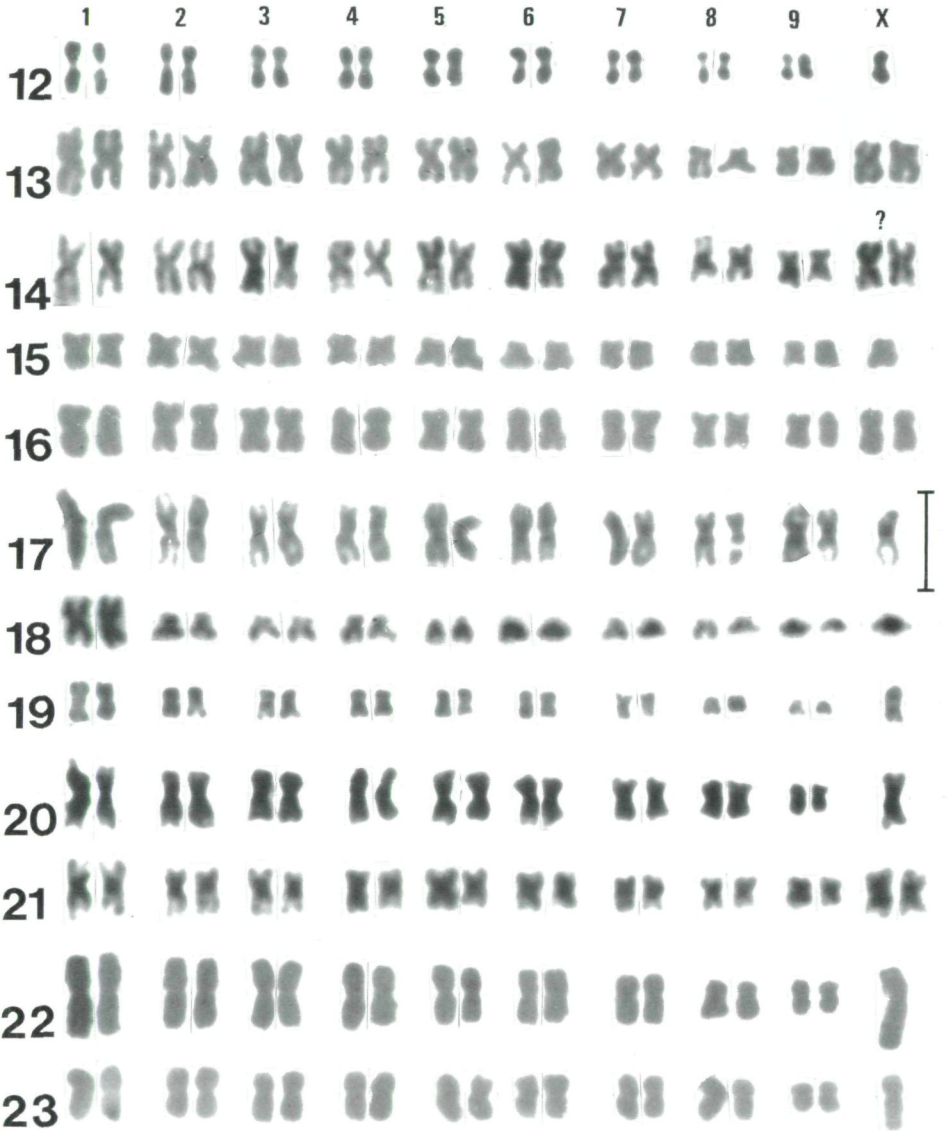
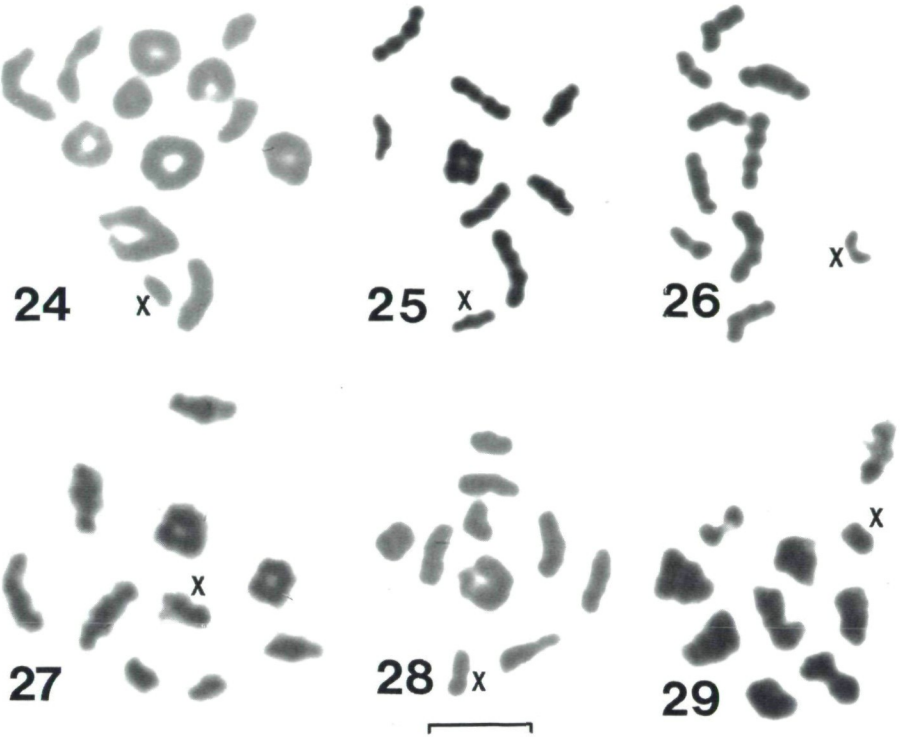


Figs. 12 - 23: Mitotic chromosomes of *Ochthebius* and *Limnebius*. Preparations 12 and 20 from testis (spermatogonium), the rest from mid-gut cells. The scale bar represents 5μm. 12) *O. dilatatus*, ♂, Cuckmere Haven; 13) *O. dilatatus*, ♀, Otmoor; 14) *O. maculatus*, ♀, Berekhot Atlit; 15) *O. minimus*, ♂, Runnymede; 16) *O. minimus*, ♀, East Harling Common; 17) *O. rugulosus*, ♂, Hula Nature Reserve; 18) *O. marinus*, ♂, Cuckmere Haven; 19) O. *punctatus*, ♂, Cuckmere Haven; 20) *L. truncatellus*, ♂, Cockly Cley; 21) *L. truncatellus*, ♀, Woodeaton; 22) *L. papposus*, ♂, East Walton Common; 23) *L. furcatus*, ♂, León. The "?" over X-chromosomes means that the interpretation is based on related species and is tentative.



Figs. 24 - 29: Meiotic chromosomes, first metaphase, from testis of Hydraenidae. The scale bar represents 5 μm. The unpaired X-chromosome is indicated. 24) *H. testacea*, Runnymede; 25, 26) *O. dilatatus*, Cuckmere Haven; 27, 28) *O. punctatus*, Cuckmere Haven; 29) *L. truncatellus*, Cockley Cley.

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