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A chromosomal investigation of seven Geotrupids and two Aegialiines (Coleoptera, Scarabaeoidea)

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Abstract. — The karyotypes of seven species of Geotrupidae and two Aphodiidae-Aegialiinae are described and illustrated. C-banding has been used to detect heterochromatin. The six species of the tribe Geotrupini (*Anoplotrupes stercorosus* (Scriba), *Geotrupes mutator* Marsham, *G. spiniger* Marsham, *G. stercorarius* (L.), *Thorectes punctatissimus* (Chevrolat) and *Trypocopris pyrenaeus* (Charpentier)) all have 2N = 20 autosomes plus sex chromosomes which are Xy (male) or XX (female), with most of the autosomes as well as the X chromosomes acrocentric, with Relative Chromosome Lengths ranging from about 17 to 5. The y chromosome may be dot-like, or larger, RCL about 5, in which case it is generally heterochromatic. In *A. stercorosus*, *G. spiniger* and *T. pyrenaeus*, from which preparations from meiosis have been obtained, the sex bivalent is Xy_p. *Typhaeus typhoeus* (L.) (Geotrupidae-Chromogeotrupini), *Aegialia arenaria* (F.) and *Psammoporus sabuleti* (Panzer) (Aegialiinae) the karyotypes have 2N = 18 autosomes plus Xy/XX sex chromosomes, and autosome 1 is a conspicuously large metacentric, RCL about 25, about twice as long as autosome 2. All the other chromosomes except autosome 2 of *T. typhoeus* are acrocentric, though the y chromosome may be dot-like. In *T. typhoeus* the sex bivalent is Xy_p. The possible phylogenetic significance of the similarity of the *Typhaeus* and aegialiine karyotypes is discussed, but no firm conclusion is reached.

Résumé. — Les caryotypes de sept espèces de Geotrupidae et de deux Aphodiidae Aegialiinae sont décrits et figurés. Le marquage en bandes C a été employé pour marquer l'hétérochromatine. Les six espèces appartenant à la tribu des Geotrupini (*Anoplotrupes stercorosus* (Scriba), *Geotrupes mutator* Marsham, *G. spiniger* Marsham, *G. stercorarius* (L.), *Thorectes punctatissimus* (Chevrolat) et *Trypocopris pyrenaeus* (Charpentier) ont tous 2N = 20 autosomes et des chromosomes sexuels Xy (mâle) ou XX (femelle), la majorité des autosomes ainsi que les chromosomes X étant acrocentriques, avec des Longueurs Relatives du Chromosome s'étendant d'environ 17 à 5. Le chromosome y peut être punctiforme ou plus large (LRC d'environ 5), auquel cas il est généralement hétérochromatique. Chez *A. stercorosus, G. spiniger* et *T. pyrenaeus*, pour lesquels des préparations de méiose ont été obtenues, le chromosome sexuel est bivalent Xy_p. Chez *Typhaeus typhoeus* (L.) (Geotrupidae Chromogeotrupini), *Aegialia arenaria* (F.) et *Psammoporus sabuleti* (Panzer) (Aegialiinae) les caryotypes ont 2N = 18 autosomes et des chromosomes sexuels Xy/XX, et l'autosome 1 est nettement de type métacentrique long (RCL d'environ 25), près de deux fois aussi long que l'autosome 2. Tous les autres chromosomes à l'exception de l'autosome 2 de *T. typhoeus* sont acrocentriques, mais le chromosome

y peut être punctiforme. Chez *T. typhoeus* le bivalent sexuel est Xy_p. Une signification phylogénique possible de la similitude des caryotypes de *Typhaeus* et aegialien est discutée, mais sans parvenir à une conclusion définitive.

Key words. — Chromosomes, karyotypes, Geotrupidae, Scarabaeoidea, Aphodiidae, Aegialiinae.

Introduction

In the course of chromosomal analyses of various Scarabaeoidea undertaken by the senior author in the course of research for her Ph.D. degree, it was found that the karyotype of *Typhaeus typhoeus* (L.) appeared quite different from of the other Geotrupidae studied, but similar to those of two species of Aegialiinae (Aphodiidae) : *Aegialia arenaria* (F.) and *Psammoporus sabuleti* (Panzer). In view of this, it seems appropriate to present information on the karyotypes of these aegialiines together in one publication.

Material and Methods

The species studied, and their localities of origin, are given below. British localities are referred to their Watsonian Vice-Counties, as set out by Dandy (1969). Spanish localities are listed by Provincia, with the abbreviations used on vehicle number plates. The British Vice-Counties from which material has been obtained are as follows : **England** : **3**, South Devon ; **9**, Dorset ; **11**, South Hants ; **13**, West Sussex ; **15**, East Kent ; **22**, Berks ; **34**, West Gloucester ; **69**, Westmorland. The Spanish Provincias are : **LE**, León ; and **S**, Santander. Classification and nomenclature are based on Baraud (1992) and Dellacasa (1988).

Geotrupidae

Geotrupinae

***Typhaeus* Leach**

T. typhoeus (L.) — **England. 11 :** Denny Wood & Lyndhurst, New Forest ; **15 :** Betteshanger ; **22 :** Windsor Deer Park.

***Anoplotrupes* Jekel**

A. stercorosus (Scriba) — **England. 11 :** Denny Wood, New Forest ; **34 :** Forest of Dean ; **69 :** Helvellyn.

***Geotrupes* Latreille**

G. mutator Marsham — **England. 3 :** Whiddon Deer Park ; **Spain. S :** Reinosa.

G. spiniger Marsham — **England. 15 :** Betteshanger ; **22 :** Old Windsor

G. stercorarius (L.) — **Spain. S :** Puerto de Piedrasluengas ; **LE :** Sanabria.

***Thorectes* Mulsant**

T. punctatissimus (Chevrolat) — **Spain. LE :** Sanabria.

***Trypocopris* Motschulsky**

T. pyrenaeus Charpentier — **England. 9 :** Studland Heath. **Spain. LE :** Sanabria.

Aphodiidae

Aegialiinae

***Aegialia* Latreille**

A. arenaria (F.) ENGLAND. **9** : Studland Heath ; **14** : Camber.

***Psammoporus* Thomson**

P. sabuleti (Panzer) ENGLAND. **13** : Woolbeding.

Methods

Chromosome preparations

The methods used for chromosome preparations are based on those developed by Angus (ANGUS, 1982 ; SHAARAWI & ANGUS, 1991), with mid-gut and testis the sources of dividing cells.

C-banding was carried out routinely wherever possible. Three methods were used, but none was always successful. The species studied and the health (age) of the specimens from which preparations were obtained appear to be the main variables affecting the outcome.

1. Preparations from unstained fresh material. Preparations were left for two days on dry slides. The slides were then immersed in a saturated solution of barium hydroxide at room temperature (about 23° C) for 7 minutes. There were then rinsed in three changes of buffered distilled water (pH 6.8) at room temperature and transferred to salt-sodium citrate (2 X SSC) at 60° C and left for one hour. The slides were then rinsed in three changes buffered distilled water (pH 6.8) at room temperature before being stained for about 10 minutes in 2% Giemsa at pH 6.8. Where preparations were difficult to stain, through apparent overtreatment, they were left in stain for up to 24 hours. This sometimes helped.

2. Preparations from stained fresh material. This technique has been used where preparations were either difficult to obtain or appeared particularly interesting. The preparations were examined and photographed as soon as they had dried after staining. Immersion oil was removed by immersion for 15 minutes in xylene in a Coplin jar. The slide was then rinsed with absolute ethanol and allowed to dry. The slide was then destained by being immersed for 15 min in 2 X SSC at 60° C, then rinsed with unbuffered distilled water. The C-banding schedule was then performed as in **1** (above), except that the barium hydroxide treatment was reduced to 5 min. The C-banding, when produced, was often less clear than with unstained material, but the results can be very good. This technique, by enabling the same preparation to be studied unbanded and C-banded, can be very powerful in enabling accurate karyotypes to be obtained.

3. Preparations from old stained material. In cases where C-banding of unstained material had failed, and where photography of the material had been done months after the preparations were made, C-banding was sometimes attempted where it was felt to be particularly desirable. Immersion oil was removed as in **2** (above), and the preparation was then refixed by immersion in a Coplin jar of 3 :1 fixative at room temperature. The

refixation was performed either by leaving the slides overnight and treating them the next day, or by a 4-hour (morning) fixation followed by afternoon treatment. In both cases the prolonged refixation destains the preparations, so no destaining in 2 X SSC is needed. After refixation the slides were allowed to dry for about 1 hour. The C-banding treatment was carried out as in **2** (above), i.e. the time in barium hydroxide was 5 minutes. The success rate with this treatment was about 50%, but some good preparations were obtained, again in some cases enabling the same preparation to be studied both unbanded and C-banded.

Chromosome preparations were photographed using Agfa high-contrast film, and printed at a magnification of 3000×. Karyotypes were prepared with the autosome pairs arranged in order of decreasing length and the sex chromosomes placed at the right-hand end of the row. Relative Chromosome Length (RCL, the length of each chromosome expressed as a percentage of the total haploid autosome length in the nucleus) has been used only to give an approximate indication of the sizes of the chromosomes – no statistical analyses have been used.

Preservation of the beetles

After removal of the tissues needed for chromosome preparation, the beetles were killed with boiling water and mounted on cards, using gum tragacanth. All material has been kept, mostly in the senior author's collection.

Results

Geotrupidae

Typhaeus typhoeus. Figs. 3 a & b, 2 a. Material analysed : 5 specimens. Published information : none. $2N = 18 + Xy_p$. A very distinctive karyotype, with the autosome 1 metacentric and about twice the length of autosome 2 (RCL about 27, about 12 in autosomes 2 and 3). Autosome 2 is submetacentric, with the shorter arm heterochromatic and bearing a secondary constriction. Autosomes 3 and 4 are almost acrocentric, with very short shorter arms, which are largely heterochromatic. The remaining autosomes, and the X chromosome, are acrocentric. The X chromosome is similar in length to the smaller autosomes (RCL about 6) and the y chromosome is little more than a dot (RCL about 2.5, but measurements are not reliable with such small chromosomes), and is entirely heterochromatic. C-bands (Fig. 3 b) were obtained from four of the specimens (two from the New Forest, one each from Betteshanger and Old Windsor), and are confined to the area round the centromere, with a polymorphism in the size of the C-banding of autosome 1. In all cases observed there is a small centromeric C-band, but there may be an additional, much larger heterochromatic block at the base of the long arm. The four specimens studied were heterozygous for this extra heterochromatin. The C-bands on autosomes 2 – 4 are fairly large, while those on autosomes 6 – 9 and the X chromosome are fairly small, with some differences in the size of the bands on different chromosomes. Autosome 5 has a conspicuously small C-band.

Anoplotrupes stercorosus. Figs. 1 a-c, 2, b & c. Material analysed : 4 specimens. Published information : 2N = 22 (♂), 11 bivalents (Virkki, 1951). 2N = 20 + Xy_p. All the autosomes, and the X chromosome, are acrocentric, with small centromeric C-bands. The RCLs of the autosomes range from about 13 to 7, the X chromosome is similar in size to the smaller autosomes (RCL about 7) and the y chromosome is a large dot-like chromosome (RCL about 5), entirely heterochromatic. First metaphase of meiosis in unbanded preparations shows the Xy_p bivalent (Fig. 2, b (arrowed)) with a large gap between the two chromosomes. A C-banded preparation (Fig. 2, c) shows the heterochromatic y chromosome conspicuously larger than the small centromeric C-band of the X. Fig. 1 a & c shows unbanded preparations from testis and mid gut. The clear separation of the chromatids in the mid-gut preparation (Fig. 1 c) is in sharp contrast to their fused appearance in the testis preparation (Fig. 1 a). The chromosomes are all rather small, with the largest autosomes about 2-3 mm long in these preparations.

Geotrupes mutator. Fig. 1 d - g. Material analysed : 2 specimens. Published information : 2N = 22 (♂) (Virkki, 1951). 2N = 20 + Xy. Autosomes 1 and 2 are submetacentric, with the centromeres set near, but clearly not at, the middle of the chromosome, and autosome 1 has a faintly staining terminal secondary constriction on its short arm (Fig. 1 d & e). The remaining chromosomes are acrocentric. C-banding (Fig. 1 e & g) shows the shorter arms of chromosomes 1 and 2 to be largely heterochromatic, while the X chromosome has a fairly large terminal centromeric C-band. Autosomes 3 – 10 have smaller terminal centromeric C-bands, these being weakest in autosomes 9 and 10. The RCL range of the autosomes is from about 17 to 5, with the X chromosome (RCL about 9) intermediate in length between autosomes 2 and 3.

Geotrupes spiniger. Figs. 1 h, 2 d. Material analysed : 2 specimens. Published information : 11 bivalents (♂) (Salamanna, 1972). 2N = 18 = Xy_p. The only successful preparations were obtained from C-banded material (the slide examined unbanded yielded no chromosomes), and the C-banding pattern is similar to that of *G. mutator* (Fig. 1 g), with heavy centromeric C-bands, clearly not apical, on autosomes 1 and 2. Autosome 3 has the C-band stronger than in *G. mutator*. Of the remaining chromosomes, only autosome 6 shows any trace of C-bands. The RCLs of the autosomes range from about 17 to about 5, with a rather abrupt decrease between autosomes 4 and 5 (RCL's about 11.5 and 8.5 respectively). The X chromosome is similar in size to the smallest autosome, very different from the larger heavily C-banded X chromosome of *G. mutator*. The y chromosome is about half the size of the X. It appears a little darker than the X, and is possibly heterochromatic. This would be in agreement with the other *Geotrupes* species studied. First metaphase of meiosis (Fig 2 d) shows the y chromosome much smaller than the X.

Geotrupes stercorarius. Fig. 1 i-k. Material analysed : 2 specimens. Published information : 2N = 22 (♂), 11 bivalents (Virkki, 1951). 2N = 20 + Xy. All the chromosomes are acrocentric, and the C-bands are small. The RCLs of the autosomes range from about 13 to 6. The X chromosome is distinctly larger than the smallest autosomes (RCL about 8.5), and the y chromosome is only slightly smaller (RCL about 6), but it is entirely heterochromatic and appears as a large dot.

Thorectes punctatissimus. Fig. 1 l-n. Material analysed : 3 specimens. Published information : none. 2N = 20 + Xy. This appears as a rather distinctive karyotype, with autosome 1 metacentric and about 1.5 times the length of autosome 2 (RCLs about 15 for autosome 1, about 11 for autosome 2). The chromosomes all have small centromeric C-bands, and C-banded preparations show that autosomes 3 and 5 have small but distinct short arms, while autosomes 2, 4, 6 – 10, and the X chromosome, are acrocentric. The y chromosome is a small acrocentric (clearest in Fig. 1 m). The RCLs of the autosomes 2 – 10 decrease evenly along the karyotype, from about 11 to 7, the RCL of the X chromosome is about 6 and that of the y about 3.

Trypocopris pyrenaeus. Figs. 1 o-q, 2 e & f. Material analysed : 3 specimens. Published information : none. 2N = 20 + Xy_p. Autosomes 1 – 3 have distinct heterochromatic short arms with secondary constrictions. In the mid-gut preparation (Fig. 1 p) one secondary constriction on autosome 3 is greatly expanded, giving the chromosome an almost metacentric appearance. The centromeric C-bands are all rather heavy and the y chromosome, which is almost as long as the X, is entirely heterochromatic. The RCLs of the autosomes decrease evenly along the karyotype, from about 12 to 6, and the X chromosome is similar in length to the smallest autosomes. The RCL of the y chromosome is about 5. First metaphase of meiosis shows a clear parachute association between the X and y, with the almost equal sized chromosomes separated from one another by a clear space (Fig 2 e). C-banded preparations (Fig. 2 f) show the autosomal bivalents with clearly separated C-bands, and the Xy_p bivalent (arrowed) recognisable by the larger heterochromatic y chromosome compared with the centromeric C-band of the X.

Aphodiidae, Aegialiinae

Aegialia arenaria. Fig. 3 c-e. Material analysed : 7 specimens. Published information : 2N = 20 (♂), 10 bivalents (Virkki, 1951). 2N = 18 + Xy. A very distinctive karyotype with autosome 1 a large metacentric (RCL about 22) and the remaining autosomes, and the X chromosome, acrocentric. Autosome 2 is scarcely more than half the length of

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Fig. 1. Mitotic chromosomes of Geotrupidae.
a, b, *Anoplotrupes stercorosus*, ♂, testis, Helvellyn, **a** plain, **b** C-banded ;
c, *A. stercorosus*, ♀, mid-gut, Forest of Dean ;
d, e, *Geotrupes mutator*, ♂, Whiddon Deer Park, **d**, mid-gut, plain, **e**, testis, C-banded ;
f, g, *G. mutator*, ♀, mid-gut, Reinosa, **f** plain, **g** C-banded ;
h, *G. spiniger*, ♂, mid-gut, Betteshanger, C-banded ;
i, *G. stercorarius*, ♂, mid-gut, Sanabria ;
j, k, *G. stercorarius*, ♂, testis, Piedrasluengas, **j** plain, **k** C-banded ;
l, *Thorectes punctatissimus*, ♂, mid-gut, Sanabria ;
m, n, *T. punctatissimus*, ♂, testis, Sanabria, C-banded ;
o, *Trypocopris pyrenaeus*, ♂, testis, Studland ;
p, *T. pyrenaeus*, ♀, mid-gut, Sanabria ;
q, *T. pyrenaeus*, ♂, testis, Studland, C-banded ;


1
2
3
4
5
6
7
8
9
10
X X/Y
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
5 µm

autosome 1 (RCL about 13.5), and the remaining autosomes decrease in length along the karyotype to RCL about 6.5. The X chromosome is slightly but consistently longer than autosome 2, RCL about 14.4. The y chromosome is small, dot-like, and too small for any attempt to measure its length. All the chromosomes (except the y) have small centromeric C-bands, (Fig. 3 d & e). There are additional C-bands as follows : autosome 1 has a more or less terminal band on its short arm and a band about third of the way from the apex of its long arm ; autosomes 2 – 4 have subterminal C-bands ; and the X chromosome has a band about a quarter of the way from the apex. The y chromosome shows no obvious C-band.

Psammoporus sabuleti. Fig. 3 f-i. Material analysed : 4 specimens. Published information : none. 2N = 18 + Xy. The karyotype closely resembles that of *A. arenaria* in the lengths and centromere positions of the autosomes, but in this case the X chromosome is about the same length as the smallest autosome (RCL about 6.5). All the chromosomes except the y have centromeric C-bands (Fig. 3 h & i), and there are no other C-bands present.

Discussion

The size and extent of the family Geotrupidae is currently under review. In general, the genera *Geotrupes* and *Typhaeus* are regarded as more advanced members of the family (Crowson, 1955, Zunino, 1984), while *Bolboceras* and its relatives have been regarded as primitive. However, While Zunino (1984) places the Bolboceratinae as the subfamily coming nearest to the Geotrupinae, Scholtz & Browne (1996) have made a case for regarding the group as a separate family, Bolboceratidae. Scarabaeoid phylogenies are reviewed by Martin-Piera & Lopez-Colón (2000). The modern consensus appears to place the Bolboceratidae nearer to Trogidae than to Geotrupidae, while the Hybosoridae come in as a sister-group to Geotrupidae. This is important for a consideration of chromosome numbers in Geotrupidae. All species of *Geotrupes* whose karyotypes are known have 2N = 22 chromosomes, while all known Bolboceratidae have 2N = 20 chromosomes, and Hybosoridae include species with both 20 chromosomes (Hybosorinae : Kacker, 1970 ; Yadav et al., 1990) and 22 chromosomes (Dynamopinae : Yadav et al., 1990). 20 chromosomes is the usual number in Scarabaeidae and Aphodiidae, and regarded as the primitive number for the suborder Polyphaga (Smith, 1950). It is in this context that the karyotype of *Typhaeus typhoeus* becomes particularly interesting. *T. typhoeus* has 20 chromosomes, but the longest autosome could have originated by centric fusion of two acrocentric autosomes. This would at a stroke achieve the change from a *Geotrupes*-type karyotype with 11 pairs of acrocentric chromosomes to the *Typhaeus* one with 1 pair of very long metacentrics and nine pairs of shorter acrocentrics. However, Zunino (1984) places the tribe Chromogeotrupini, of which *Typhaeus* is the only genus whose karyotype is known, as the first to branch off from the stem of the Geotrupinae. If the more primitive members or relatives of the Geotrupinae have 2N = 20 chromosomes, the arrangement in *Typhaeus* could just as well be primitive, perhaps with the long metacentric the last stage before the change to a 22-chromosome complement. Chromosome data such as

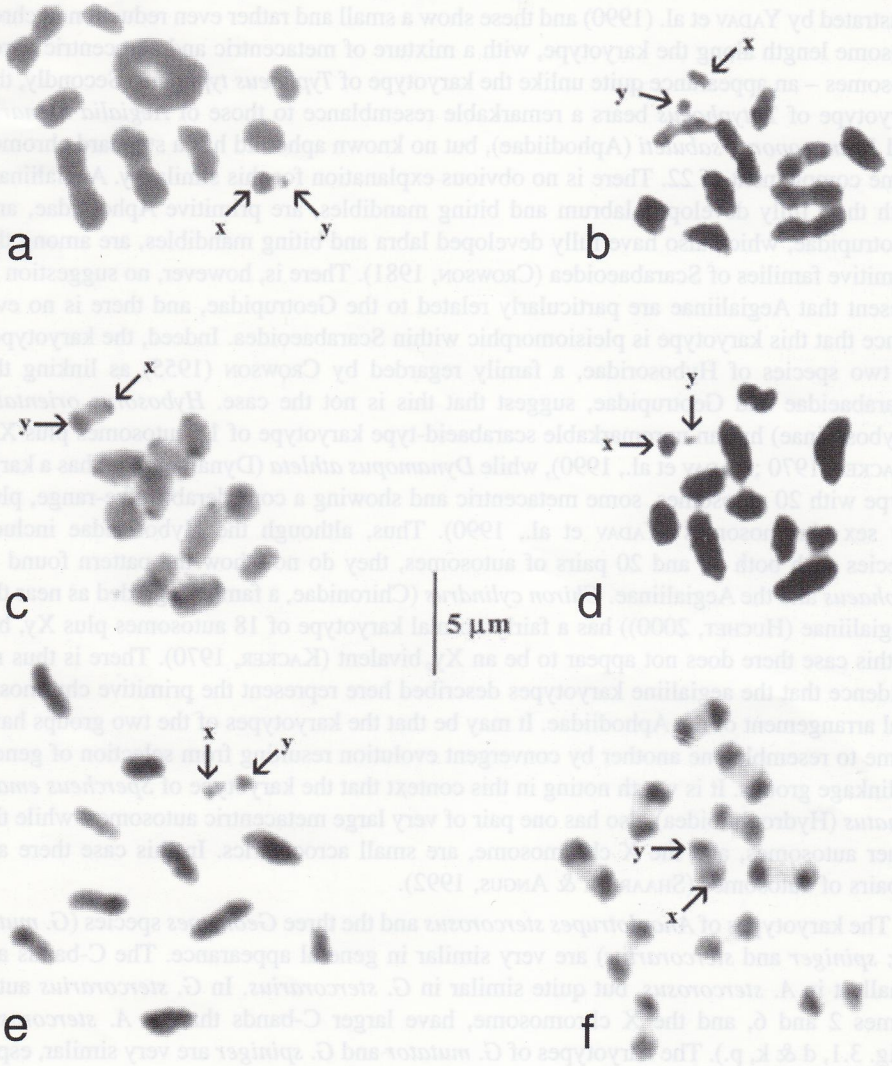

Fig. 2. First metaphase meiotic chromosomes from testis of Geotrupidae.
a, *Typhaeus typhoeus*, Windsor Great Park ;
b, c, *Anoplotrupes stercorosus*, Helvellyn, **b** plain, **c** C-banded ;
d, *Geotrupes spiniger*, Old Windsor ;
e, f, *Trypocopris pyrenaeus*, Studland, **e** plain, **f** C-banded.
The X and y chromosomes are arrowed.

are presented here can never on their own indicate the direction of any change in number, but two other facts are relevant. Firstly, the karyotypes of two species of *Bolboceras* are illustrated by YADAV et al. (1990) and these show a small and rather even reduction in chromosome length along the karyotype, with a mixture of metacentric and acrocentric chromosomes – an appearance quite unlike the karyotype of *Typhaeus typhoeus*. Secondly, the karyotype of *T. typhoeus* bears a remarkable resemblance to those of *Aegialia arenaria* and *Psammoporus sabuleti* (Aphodiidae), but no known aphodiid has a standard chromosome complement of 22. There is no obvious explanation for this similarity. Aegialiinae, with their fully developed labrum and biting mandibles, are primitive Aphodiidae, and Geotrupidae, which also have fully developed labra and biting mandibles, are among the primitive families of Scarabaeoidea (CROWSON, 1981). There is, however, no suggestion at present that Aegialiinae are particularly related to the Geotrupidae, and there is no evidence that this karyotype is pleisiomorphic within Scarabaeoidea. Indeed, the karyotypes of two species of Hybosoridae, a family regarded by CROWSON (1955) as linking the Scarabaeidae and Geotrupidae, suggest that this is not the case. *Hybosorus orientalis* (Hybosorinae) has an unremarkable scarabaeid-type karyotype of 18 autosomes plus Xy_p (KACKER, 1970 ; YADAV et al., 1990), while *Dynamopus athleta* (Dynamopinae) has a karyotype with 20 autosomes, some metacentric and showing a considerable size-range, plus Xy sex chromosomes (YADAV et al., 1990). Thus, although the Hybosoridae include species with both 18 and 20 pairs of autosomes, they do not show the pattern found in *Typhaeus* and the Aegialiinae. *Chiron cylindrus* (Chironidae, a family regarded as near the Aegialiinae (HUCHET, 2000)) has a fairly normal karyotype of 18 autosomes plus Xy, but in this case there does not appear to be an Xy_p bivalent (KACKER, 1970). There is thus no evidence that the aegialiine karyotypes described here represent the primitive chromosomal arrangement of the Aphodiidae. It may be that the karyotypes of the two groups have come to resemble one another by convergent evolution resulting from selection of genetic linkage groups. It is worth noting in this context that the karyotype of *Spercheus emarginatus* (Hydrophiloidea) also has one pair of very large metacentric autosomes, while the other autosomes, and the X chromosome, are small acrocentrics. In this case there are 7 pairs of autosomes (SHAARAWI & ANGUS, 1992).

The karyotypes of *Anoplotrupes stercorosus* and the three *Geotrupes* species (*G. mutator, spiniger* and *stercorarius*) are very similar in general appearance. The C-bands are smallest in *A. stercorosus*, but quite similar in *G. stercorarius*. In *G. stercorarius* autosomes 2 and 6, and the X chromosome, have larger C-bands than in *A. stercorosus* (Fig. 3.1, d & k, p.). The karyotypes of *G. mutator* and *G. spiniger* are very similar, especially in the heavy C-bands on autosomes 1 – 3. The size difference between autosome pairs 4 and 5 appears more marked in *G. spiniger* than in *G. mutator* (Fig. 3.1, f-h). The X chromosome of *G. spiniger* appears strikingly small when compares with those of the other species. The karyotype of *Trypocopris pyrenaeus* (Fig. 3.1, o-q) is similar to those of *Geotrupes* and *Anoplotrupes*, but the C-bands are all rather large and those of autosomes 1 – 3 contain secondary constrictions. The secondary constriction of autosome 3 can appear greatly expanded, giving a metacentric appearance to the chromosome, which is otherwise almost acrocentric.

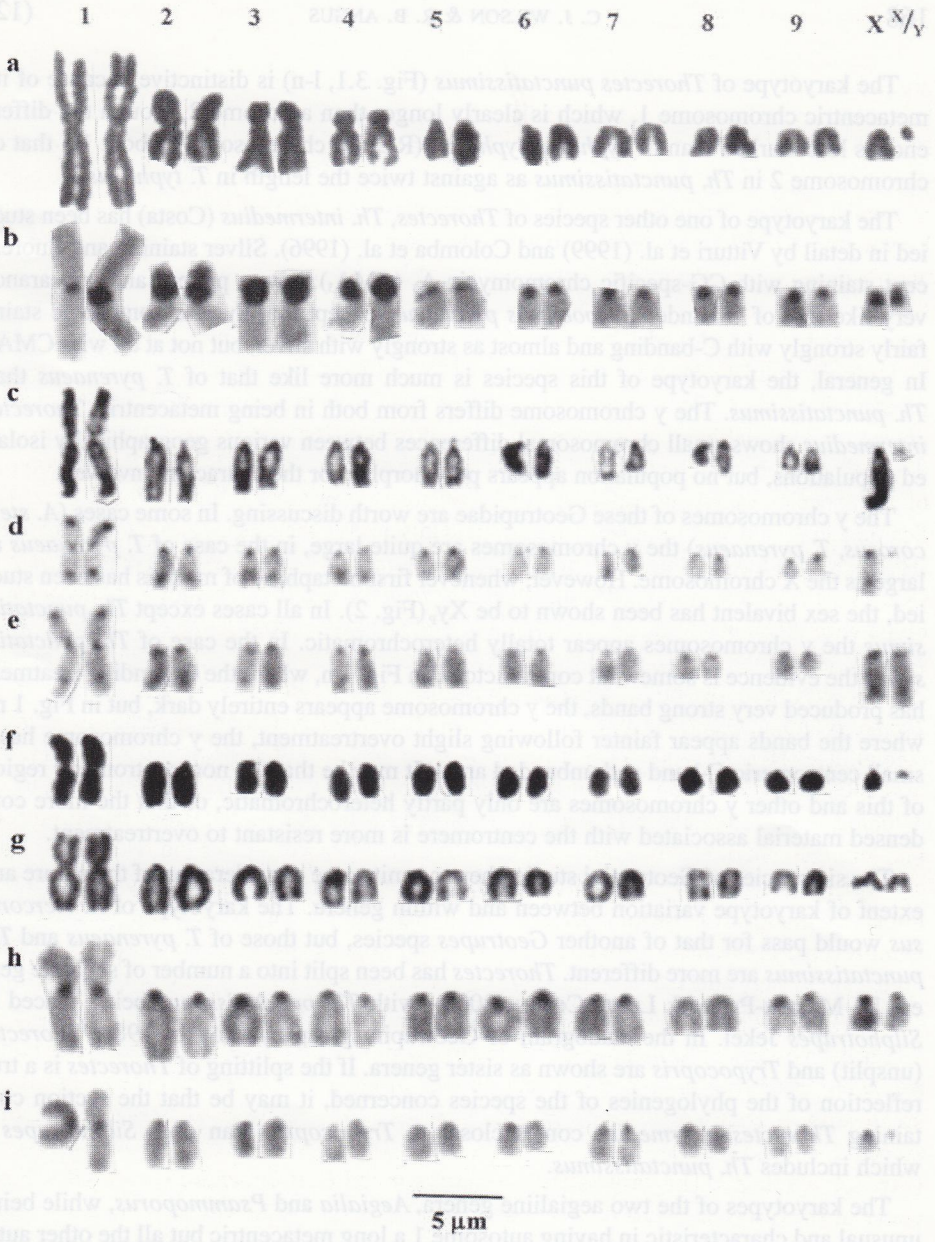

Fig. 3. Mitotic chromosomes of *Typhaeus* and Aegialiinae.
a, b, *Typhaeus typhoeus*, ♂, mid-gut, Windsor Great Park, **a** plain, **b** C-banded ;
c, d, *Aegialia arenaria*, ♂, mid-gut, Camber, **a** plain, **b** C-banded ;
e, A. *arenaria*, ♀, mid-gut, Studland, C-banded ;
f, *Psammoporus sabuleti*, ♂, testis, Woolbeding ;
g, h, *P. sabuleti*, ♀, mid-gut, Woolbeding, **g** plain, **h** C-banded ;
i, *P. sabuleti*, ♂, testis, Woolbeding, C-banded.

The karyotype of *Thorectes punctatissimus* (Fig. 3.1, l-n) is distinctive because of its metacentric chromosome 1, which is clearly longer than autosome 2, though the difference is less marked than in *Typhaeus typhoeus* (RCL of chromosome 1 about 1.5 that of chromosome 2 in *Th. punctatissimus* as against twice the length in *T. typhoeus*).

The karyotype of one other species of *Thorectes*, *Th. intermedius* (Costa) has been studied in detail by Vitturi et al. (1999) and Colomba et al. (1996). Silver staining and fluorescent staining with CG-specific chromomycin A_3 (CMA_3) show a pattern and appearance very like that of C-banded *Trypocopris pyrenaeus*, except that the y chromosome stains fairly strongly with C-banding and almost as strongly with silver, but not at all with CMA_3. In general, the karyotype of this species is much more like that of *T. pyrenaeus* than *Th. punctatissimus*. The y chromosome differs from both in being metacentric. *Thorectes intermedius* shows small chromosomal differences between various geographically isolated populations, but no population appears polymorphic for the characters involved.

The y chromosomes of these Geotrupidae are worth discussing. In some cases (*A. stercorosus, T. pyrenaeus*) the y chromosomes are quite large, in the case of *T. pyrenaeus* as large as the X chromosome. However, whenever first metaphase of meiosis has been studied, the sex bivalent has been shown to be Xy_p (Fig. 2). In all cases except *Th. punctatissimus* the y chromosomes appear totally heterochromatic. In the case of *Th. punctatissimus* the evidence is somewhat contradictory. In Fig. 1 n, where the C-banding treatment has produced very strong bands, the y chromosome appears entirely dark, but in Fig. 1 m, where the bands appear fainter following slight overtreatment, the y chromosome has a small centromeric C-band and unbanded arms. It may be that the non-centromeric region of this and other y chromosomes are only partly heterochromatic, or that the more condensed material associated with the centromere is more resistant to overtreatment.

The six species of Geotrupini studied here permit some consideration of the nature and extent of karyotype variation between and within genera. The karyotype of *A. stercorosus* would pass for that of another *Geotrupes* species, but those of *T. pyrenaeus* and *Th. punctatissimus* are more different. *Thorectes* has been split into a number of separate genera by Martin-Piera & Lopez-Colón (2000), with *Th. punctatissimus* being placed in *Silphotrupes* Jekel. In the cladogram of Geotrupini given by Zunino (1984) *Thorectes* (unsplit) and *Trypocopris* are shown as sister genera. If the splitting of *Thorectes* is a true reflection of the phylogenies of the species concerned, it may be that the section containing *Thorectes intermedius* comes closer to *Trypocopris* than does *Silphotrupes* – which includes *Th. punctatissimus*.

The karyotypes of the two aegialiine genera, *Aegialia* and *Psammoporus*, while being unusual and characteristic in having autosome 1 a long metacentric but all the other autosomes, and the X chromosome, smaller acrocentrics, show a number of clear differences. Firstly, the X chromosome of *Aegialia arenaria* is the largest of the acrocentrics, while that of *Psammoporus sabuleti* is among the smallest. The other obvious differences between the two are shown by the C-banding. In *P. sabuleti* C-bands are confined to the centromere regions of the chromosomes, but in *A. arenaria* there are additional C-bands on autosome 1 and the X chromosomes.

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