Koleopterologische Rundschau	73	227 – 236	Wien, Juni 2003

A chromosomal analysis of three British species of *Aphodius* ILLIGER subg. *Melinopterus* MULSANT (Coleoptera: Aphodiidae)

C.J. WILSON & R.B. ANGUS

Abstract

The karyotypes of *Aphodius consputus* CREUTZER, *A. prodromus* (BRAHM) and *A. sphacelatus* (PANZER) (Coleoptera: Aphodiidae) are described and illustrated. All three species have nine pairs of autosomes plus sex chromosomes, which are Xy (male), XX (female). The karyotypes of the three species are very distinct from one another in the sizes and shapes of the chromosomes, and in their patterns of C-banding. Chromosome polymorphisms involving the presence or absence of B-chromosomes, variation in C-band size and/or the presence or absence of satellites are shown by all three species.

Key words: Coleoptera, Scarabaeoidea, Aphodiidae, *Aphodius*, chromosomes, karyotypes, polymorphism, C-bands, B-chromosomes.

Introduction

The three species of *Aphodius* ILLIGER subg. *Melinopterus* MULSANT currently found in Britain are *A. consputus* CREUTZER, *A. prodromus* (BRAHM) and *A. sphacelatus* (PANZER), and all were included in a chromosomal analysis undertaken by WILSON (2002) as research for her Ph.D. degree. A fourth species, *A. punctatosulcatus* STURM, shown by KRELL (1991) to be a good species, not a form of *A. sphacelatus*, was added to the British list by MANN (2000) on the basis of material from East Kent housed in old collections. We were unable to find living material of this species, despite extensive searching in the Kentish localities.

There is no published information on the chromosomes of *Melinopterus*. The three species studied all have very distinctive karyotypes, with nine pairs of autosomes and Xy sex chromosomes. Each species has a number of chromosome polymorphisms.

Material and Methods

The material from which chromosomes were obtained is listed in Table 1. It comprises 102 specimens of *A. consputus*, 15 *A. prodromus* and 118 *A. sphacelatus*. British material is listed by Vice-County (DANDY 1969), and the relevant ones are as follows: **4**, North Devon; **11**, South Hants; **14**, East Sussex; **15**, East Kent; **17**, Surrey; **22**, Berks.; **24**, Bucks.; **28**, West Norfolk.

Chromosome preparations were obtained from mid-gut cells, using the methods described by SHAARAWI & ANGUS (1991) and ANGUS (1982). Photographs were printed at a magnification of 3000, and these were used for the preparation of karyotypes. Relative Chromosome Length (RCL, the length of each chromosome expressed as a percentage of half the total autosome length in the nucleus) is used as a rough guide to the comparative sizes of the chromosomes in the karyotypes, without statistical treatment. The exception to this is autosome 1 and the X chro-

mosome of *A. sphacelatus*. Here detailed statistical analysis of large samples is used to investigate the variation in length shown by the X chromosome.

Species	Localities
A. (Melinopterus) consputus	**ENGLAND. 15:** Lydden; Betteshanger; **17:** Box Hill;
A. (Melinopterus) prodromus	**ENGLAND. 4:** Ilfracombe; **11:** New Forest; **14:** Rye; **17:** Bookham Common; Ockham Common; **22:** Old Windsor; **24:** Chalfont St. Giles.
A. (Melinopterus) sphacelatus	**ENGLAND. 4:** Ilfracombe; **11:** New Forest; **14:** Rye; **15:** Lydden; Betteshanger; **17:** Bookham Common; Martyr's Green; **22:** Old Windsor; **28:** East Wretham Heath. **NETHERLANDS. Zuid-Holland:** Leidschendam, Vlietland.

Table 1: Material used for chromosome analysis.

Results

Aphodius consputus: Fig. 1a–i. 2N = 18 + Xy (♂), XX (♀). The karyotype is characterised above all by the extent and variation shown by its C-banding. The autosomes are all either metacentric or submetacentric, and their RCLs range from about 17 to 6. The X chromosome is submetacentric, sometimes with a satellite at the end of its long arm (Fig. 1d,f,i) and its RCL is about 10 or 12. The y chromosome is a small metacentric, RCL about 3. C-banded preparations are shown in Fig. 1c–i. Autosome 1 has a heavy centromeric C-band and a second, slightly smaller, band at the base of its long arm. The C-banding of autosome 2 is similar except that the centromeric C-band is slightly smaller and the band at the base of the long arm is larger. Autosomes 3 and 4 have similar C-bands to 1 and 2, with the centromeric C-band about twice the size of the band on the long arm. Autosome 5 has a heavy centromeric C-band, and the C-band of autosome 6 is polymorphic. In the most commonly encountered form the centromeric C-band is very extensive, occupying about two thirds of the length of the chromosome, but there is also an uncommon form with the C-band very small, only about a quarter of the chromosome length. The total length of the chromosome is about the same (RCL about 10) in both forms. Autosome 7 and the usual form of autosome 8 have heavy centromeric C-bands as well as smaller bands at the base of the long arm, these bands often confluent with the centromeric bands. The RCLs of these chromosomes are about 9. Autosome 9 and the rarer small form of autosome 8 have heavy centromeric C-bands occupying about a third of the length of the chromosome. The RCLs of these chromosomes are about 6. The X chromosome has most of the short arm euchromatic, but with a C-band at its base, and the long arm is entirely heterochromatic. The satellite, also heterochromatic, is normally separated from the rest of the arm by a narrow unstained gap, often with a median longitudinal dark-staining line (Fig. 1d). The y chromosome has a centromeric C-band, accounting for about a third of the length of the chromosome.

Frequencies of the polymorphic forms. The small C-banded form of autosome 6 was encountered only at Lydden, where it occurred in 4 (2 ♂♂, 2 ♀♀) of the 55 beetles analysed, and always as a heterozygote. The small form of autosome 8 occurred at both Lydden (5 ♀♀ out of 55 beetles analysed) and Box Hill (4 ♂♂ + 6 ♀♀ out of 43 beetles analysed), always as a heterozygote. The frequencies in the two populations are not significantly different based on these data. The

probability of the null hypothesis from a 2 x 2 contingency table with χ^2 is 0.099. The satellited and unsatellited forms of the X chromosome were common at both Lydden and Box Hill and both forms occurred as homozygotes as well as heterozygotes in ♀ ♀ (Table 2).

Material	Satellite + +	Satellite + –	Satellite – –	No of beetles	Satellite +	Satellite –	No of X Chromosomes
Lydden ♀	3	14	9	26	20	32	52
Lydden ♂				29	12	17	29
Lydden ♀ + ♂				55	32	49	81
Box Hill ♀	1	10	16	27	12	42	54
Box Hill ♂				16	6	10	16
Box Hill ♀ + ♂				43	18	52	70

Table 2: Occurrences of the various karyotypes with satellited (+) and unsatellited (–) X chromosomes in *Aphodius consputus*.

The observed frequencies of satellited and unsatellited X-chromosomes (males and females combined), and the expected frequencies and expected and observed occurrences of the various karyotypes (in females) are shown in Table 3. The observed frequencies do not differ from those expected from the Hardy-Weinberg equilibrium. The probabilities of the null hypothesis from a two sample χ^2 test are 0.77 for Lydden and 0.78 for Box Hill.

Material	Freq. satellite + (p)	Freq. satellite – (q)	Freq. satellite ++ (p^2)	Freq. satellite +– (2pq)	Freq. satellite — (q^2)	Expected (actual) occurrence satellite ++ (p^2N)	Expected (actual) occurrence satellite +– (2pqN)	Expected (actual) occurrence satellite — (q^2N)
Lydden	0.40	0.60	0.16	0.48	0.36	4.16 (3)	12.48 (14)	9.36 (9)
Box Hill	0.26	0.74	0.068	0.385	0.548	1.83 (1)	10.4 (10)	14.8 (16)

Table 3: Observed and expected frequencies of satellited (+) and unsatellited (–) X chromosomes, and of homozygous and heterozygous karyotypes in ♀ *Aphodius consputus* at Lydden and Box Hill.

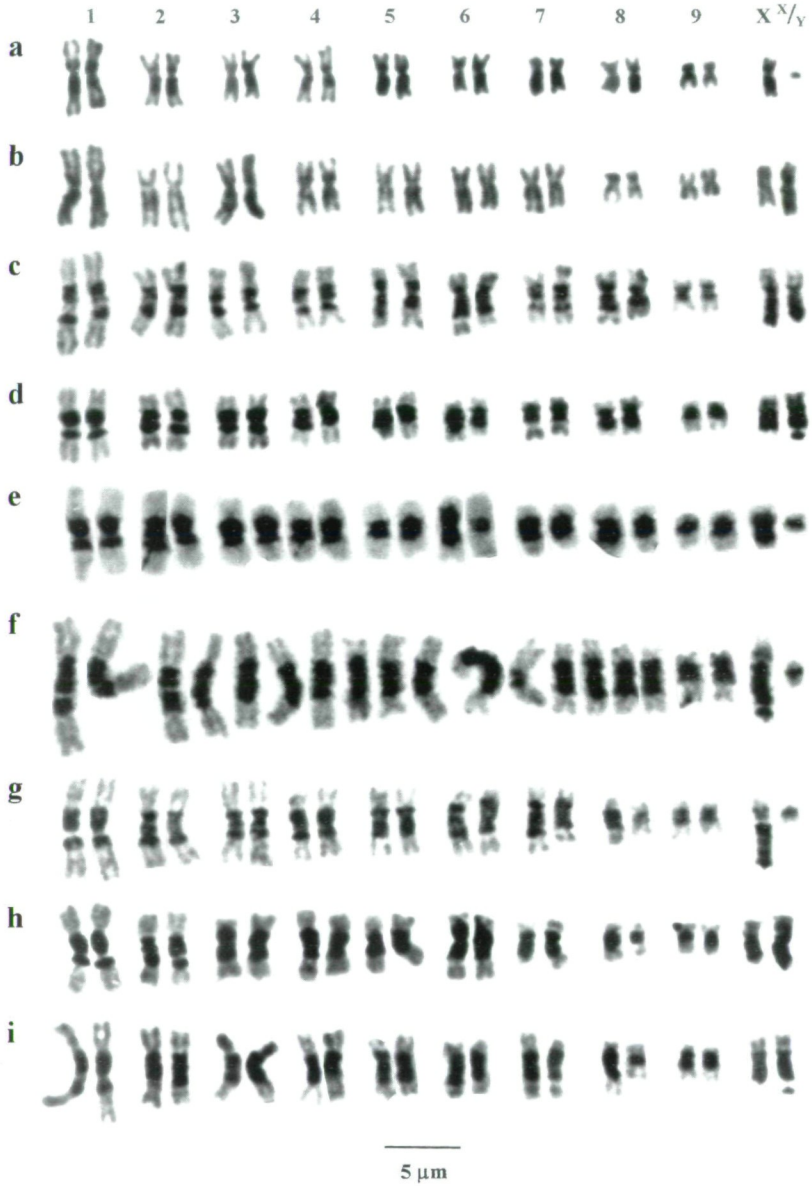

Fig. 1: Mitotic chromosomes of *Aphodius consputus*, mid-gut preparations; **a**: ♂, **b**: ♀, Betteshanger, plain, b X chromosome heterozygous for satellite; **c** – **i**: Lydden, C-banded; **c**: ♀, no autosomal polymorphisms, X-chromosome without satellite; **d**: ♀, no autosomal polymorphisms, X-chromosome heterozygous for satellite; **e**: ♂, autosome 6 heterozygous for small C-band, X-chromosome without satellite; **f**: ♂, autosome 6 heterozygous for small C-band, X-chromosome with satellite; **g**: ♂, autosome 8 heterozygous for small C-band, X-chromosome without satellite; **h**: ♀, autosome 8 heterozygous for small C-band, X-chromosome without satellite; **i**: ♀, autosome 8 heterozygous for small C-band, X-chromosome heterozygous for satellite.

Material	No. of X chromosomes with satellite	No. of X chromosomes without satellite
Lydden ♀	20	32
Lydden ♂	12	17
Box Hill ♀	12	42
Box Hill ♂	6	10
Total ♀	27	79
Total ♂	18	27

Table 4: Numbers of satellited and unsatellited X chromosomes in ♂ and ♀ *Aphodius consputus* at Lydden and Box Hill.

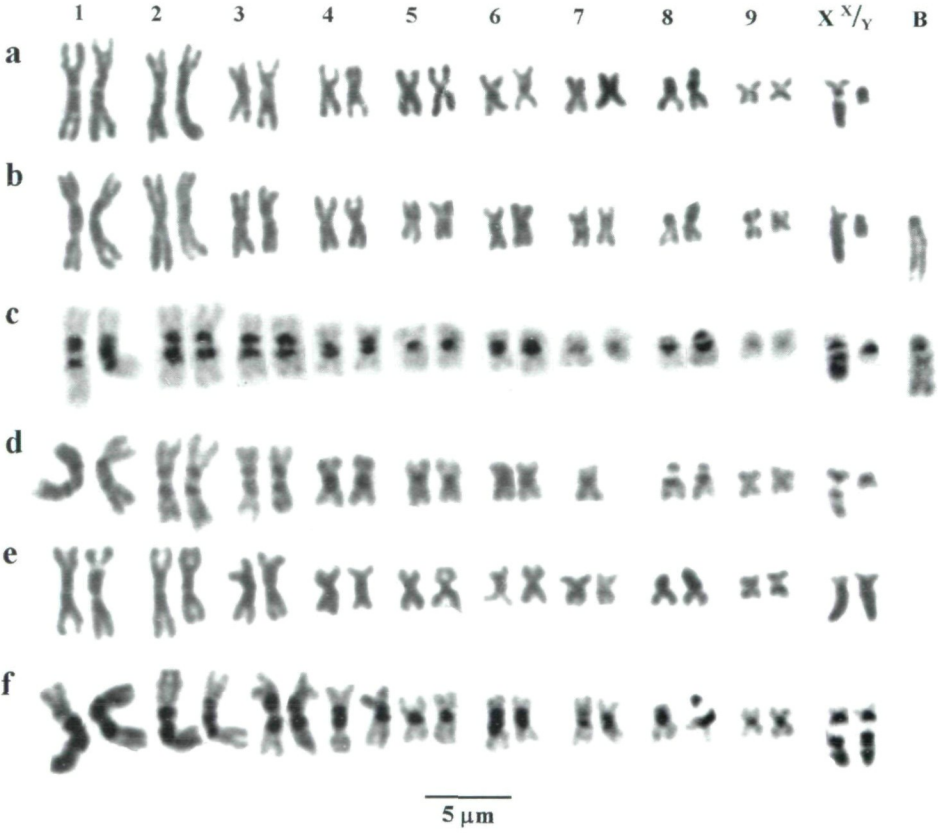

Fig. 2: Mitotic chromosomes of *Aphodius prodromus*; **a**: ♂, mid-gut, New Forest; **b**, **c**: ♂, mid-gut, Bookham, B-chromosome present, b plain, c C-banded, from the same specimen; **d**: ♂, mid-gut, Old Windsor, one replicate of autosome 7 missing, both replicates of autosome 8 with the apical dark section; **e**, **f**: ♀, mid-gut, Bookham, e plain, f C-banded, from the same specimen.

Fig. 3: Mitotic chromosomes of *Aphodius sphacelatus*; **a**, **b**: ♂, mid-gut, Old Windsor, from the same specimen; **c**: ♂, mid-gut, Netherlands, C-banded; **d** – **f**: ♀, mid-gut, Old Windsor, three preparations from the same specimen; **g**: ♂, mid-gut, Old Windsor, with one B-chromosome; **h**: ♀, mid-gut, Old Windsor, with one replicate of autosome 4 missing, and one B-chromosome; **i**: ♀, mid-gut, Old Windsor, with one B-chromosome; **j**, **k**: ♀, mid-gut, Old Windsor, with one B-chromosome, j weakly C-banded, k strongly C-banded, from the same specimen.

The occurrences of satellited and unsatellited X chromosomes in males and females are given in Table 4. Although at a glance the data appear to show a greater proportion of satellited X chromosomes in males, there is no statistically significant difference between the frequencies of the two forms in the two sexes in either of the populations, or when the populations are considered together. The probabilities of the null hypothesis from a 2 x 2 contingency table with χ^2 are 0.98 for Lydden, 0.37 for Box Hill and 0.33 for the two populations together.

Aphodius prodromus Fig. 2a–f. 2N = 18 + Xy (♂), XX (♀), + B-chromosomes. The RCLs of the autosomes range from about 20 to 5. Autosomes 1 – 7 and 9 are metacentric. Autosome 8 may be metacentric with a secondary constriction in the shorter arm, but the distal section of the arm, beyond the gap, may be absent. The X chromosome is subacrocentric, RCL about 10, and the y chromosome is a small acrocentric, RCL about 4. The B-chromosome, when present, is acrocentric, larger than the X chromosome, RCL about 13. C-banding (Fig. 2c,f) shows all the autosomes with distinct centromeric C-bands. These bands are large and heavy in autosomes 1 – 3, rather smaller in autosome 4 and 6. The C-band of autosome 5 is noticeably smaller than those of autosomes 4 and 6, and the C-band of autosome 7 is smaller still, though larger than that of the small autosome 9. Autosome 8 has a heavy centromeric C-band, and the apical part of the short arm, beyond the gap, also appears dark, and is therefore a satellite. In most of the material examined autosome 8 is heterozygous for loss of this apical dark section, but in one specimen (Fig. 2d) the apical section is present in both replicates. The X chromosome is entirely heterochromatic apart from the very short short arm, and the heterochromatic long arm frequently shows an unstained gap adjacent to the centromeric C-band and a further gap about half way down its length. The y chromosome has a distinct centromeric C-band. The B-chromosome appears more or less completely heterochromatic, but the centromere region is more darkly stained than the rest of the chromosome (Fig. 2c). The B-chromosome was found in three specimens, one each from Bookham Common, Old Windsor and the New Forest. In each case one B-chromosome was present, in all the nuclei from which chromosomes were obtained.

Aphodius sphacelatus. Fig. 3a–k. 2N = 18 + Xy (♂), XX (♀), + B-chromosomes. All the autosomes are more or less metacentric and their RCLs range from about 15.9 to 8.3. The X chromosome is subacrocentric and its long arm has the two chromatids closely applied to one another and often shows faintly staining gaps along its length. The RCL value of the X chromosome is about 10, though with some very obvious variation. The y chromosome is unusually long for an *Aphodius*, RCL about 8. C-banding (Fig 3c,j,k) shows autosomes 1 and 2 to have weak centromeric C-bands, which are often obliterated in slightly overtreated preparations. Autosome 3 has stronger centromeric C-bands, and autosome 4 has them stronger still. Autosomes 5, 6 and 7 have the largest bands, while the bands of autosome 8 are similar to those of autosome 5 and those of autosome 9 are similar to those of autosome 4. The long arm of the X chromosome is entirely heterochromatic and in C-banded preparations may appear either solid or with more faintly staining gaps. The y chromosome appears entirely heterochromatic, with some parts denser than others. The B-chromosome is a long acrocentric, RCL about 13. In heavily C-banded preparations it appears to be entirely euchromatic, apart from a very small centromeric C-band (Fig. 3k), but in more lightly treated preparations (Fig. 3j) the middle part of the B-chromosome stains very darkly. B-chromosomes were found in six of the 57 Old Windsor specimens analysed, as well as two of the 41 from Holland. They always occurred singly, and were present in all analysed nuclei of specimens bearing them. There is no significant difference between the frequencies of the B-chromosome in Old Windsor and Dutch material.

One of the striking features of the *A. sphacelatus* karyotype is the wide variation in RCL shown by the X chromosome. In some preparations (Fig. 3c) the X and y chromosomes appear more or less the same size, while in others (Fig. 3g) the X is more than twice the size of the y chromosome, suggesting a well-developed size polymorphism. However, when samples of X

chromosomes are measured, they do not group into more than one size category, even when females with X chromosomes of apparently different sizes are analysed. This eliminates the possibility of a distinct size polymorphism, but still leaves open the possibility of a heteromorphism resulting from variation in the amount of repetitive DNA present in the heterochromatic arm. Fig. 3d–f shows karyotypes prepared from three different nuclei from the same beetle. In Fig. 3d the two X chromosomes are distinctly different in size, but in Fig 3e,f the two X chromosomes are more or less the same size. Autosome 1 in the same nuclei shows a similar size difference between the two replicates (see Tables 5 and 6 for measured lengths and RCL values), suggesting that in this case the observed variation in RCL results from irregularities in chromosome condensation during prophase of mitosis. Fig. 3d–f and Table 6 show that the condensation of the largely heterochromatic X chromosome does not behave differently from that of the autosomes when nuclei at different stages in chromosome condensation are analysed.

Nucleus/ Autosome total	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	***X***
D	4.83	3.67	3.33	3.33	3	3.17	2.83	2.67	2.33	3.67
56.93 µm	4	3.77	3.33	3.33	3	2.67	2.67	2.67	2.33	3.33
E	7	6	5.67	5.33	4.83	4.33	4	4.17	3.67	4.83
89.16 µm	7.67	5.67	5.33	5.17	4.33	4.33	4	3.83	3.83	4.83
F	8.67	6.67	5.67	5.67	5.5	5	5	4.83	3.83	6
101.68 µm	8	6.67	6.67	5.67	5.5	5	5	4.33	4	6

Table 5: Chromosome lengths (µm) in the nuclei shown in Fig. 3d–f.

Nucleus	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	***X***
D	17	12.9	11.7	11.7	10.5	11.7	9.9	9.4	8.2	12
	14.1	13.2	11.7	11.7	10.5	9.4	9.4	9.4	8.2	11.7
E	15.7	13.5	12.7	12	10.8	9.7	9	9.3	8.2	10.8
	17.2	12.7	12	11.6	9.7	9.7	9	8.6	8.6	10.8
F	17	13.1	11.1	11.1	10.8	9.8	9.8	9.5	7.5	11.8
	15.7	13.1	13.1	11.1	10.8	9.8	9.8	8.5	7.9	11.8

Table 6: Relative Chromosome Lengths (RCL) in the nuclei shown in Fig. 3d–f.

Autosome 1 is almost entirely euchromatic and shows no morphological evidence of any polymorphism. It is also clearly the longest chromosome in the karyotype, and is thus reliably identified. It may therefore be taken as a control representing the behaviour of a chromosome without any structural variation, for comparison with the X chromosome. The behaviour of the X chromosome and autosome 1 during condensation at mitosis was examined in detail by means of a series of measurements and statistical comparisons, as follows:

1. 106 karyotypes from 20 ♀♀ from Old Windsor. Analysis of raw data – measurements (mm) from photographs. Autosome 1: average length = 17.142 mm, standard deviation =3.197, 95% confidence interval = +/– 0.608. X chromosome: average length = 11.06 mm, standard deviation = 2.289, 95% confidence interval = +/– 0.436 (z-scores). (Note: at a magnification of 3000 ×, 3 mm = 1 µm.)
2. The same material, analysis of RCL data. Autosome 1: average RCL = 15.951, standard deviation = 1.005, 95% confidence interval = +/– 0.192. X chromosome: average RCL = 10.331, standard deviation = 1.395, 95% confidence interval = +/– 0.267 (z-scores).
3. Average RCL values per beetle from the same 20 ♀♀ from Old Windsor, analysed by t-test. Autosome 1: average RCL = 15.915, standard deviation = 0.730, 95% confidence interval = +/– 0.342. X chromosome: average RCL = 10.102, standard deviation = 1.166, 95% confidence interval = +/– 0.511.

4. Comparison of the differences between the longer and shorter replicates of autosome 1 and the X chromosome in each nucleus from the Old Windsor ♀♀ by paired t-test, since the sample size is the same. This gave a t-statistic of –0.803, 19 degrees of freedom and a 2-tail probability for the null hypothesis of 0.432.

These analyses show no evidence of difference between the variation in RCL and measured length of autosome 1 and the X chromosome. This is further supported by analysis of the X chromosomes from a sample of 18 ♀♀ from Holland. This gave the average RCL as 10.183, with a standard deviation of 1.259 and 95% confidence interval of +/- 0.582, indicating no significant difference from the values obtained from the Old Windsor material.

Discussion

The three species of subgenus *Melinopterus* show a number of chromosomal features of particular interest. They all show clear differences from each other, and all show some polymorphism. The X chromosomes of these species share a common pattern – a short euchromatic arm and a much longer, sometimes variable, heterochromatic arm. The appearance of the X chromosome is however, different in the three species. *A. consputus* is characterised by strong, generally large, C-bands on all the autosomes, with polymorphisms and variation as already described. The y chromosome is small, metacentric and with a distinct C-band. The X-chromosome sometimes has a heterochromatic satellite at the end of the long arm. No chromosome is acrocentric, and no B-chromosomes have been encountered. The karyotype of *A. prodromus* is broadly similar to that of *A. consputus*, but the C-bands are smaller and without polymorphisms, and autosome 8 is polymorphic for the presence of a heterochromatic apical section (satellite) beyond a secondary constriction. The heterochromatic long arm of the X chromosome in C-banded preparations tends to show a segmented appearance. In some specimens a long acrocentric, largely heterochromatic B-chromosome is present. It is, however, rare in the material analysed, so no attempt was made to compare its frequency in different populations. The y chromosome is slightly larger that that of *A. consputus*, acrocentric and with a distinct localised centromeric C-band. The karyotype of *A. sphacelatus* is distinctly different from the other two species in its very small centromeric C-bands on autosomes 1 and 2, and its long, entirely heterochromatic y chromosome, which may in some cases appear as long as the X chromosome. The size variation shown by the X chromosome has been found to result from irregularities in chromosome condensation during prophase of meiosis, with no evidence of heteromorphism. The condensation of the y chromosome also varies, which accounts for the very different appearances of the Xy pair in different preparations. The arrangement of the Xy bivalent at meiosis would be interesting, but no meiotic metaphases were seen in the testes examined, though spermatogonial mitosis was found. The B-chromosome of *A. sphacelatus* is interesting in that it closely resembles that of *A. prodromus* in both size and shape. However, the B-chromosome of *A. prodromus* appears to be clearly heterochromatic, while that of *A. sphacelatus* is more ambivalent. Gentle C-banding treatment shows the B-chromosome with a heavily staining middle section, but a more rigorous treatment shows only a very small C-band at the centromere. The karyotype of the recently recognised *A. punctatosulcatus* would certainly be of interest, but unfortunately no material of this species was found, despite careful examination of material from east Kent.

Acknowledgements

This work is based on Ph.D. research done by the senior author, supervised by R.B. Angus, in the School of Biological Sciences of Royal Holloway, University of London. We thank the School for providing the opportunity to carry out the research. We also thank Dr. Hans Huijbregts and Dr. Jan Huijbregts for collecting and sending the Dutch material of *A. sphacelatus*.

References

ANGUS, R.B. 1982: Separation of two species standing as *Helophorus aquaticus* (L.) (Coleoptera, Hydrophilidae) by banded chromosome analysis. – Systematic Entomology 7: 265-281.

DANDY, J.E. 1969: Watsonian Vice-Counties of Great Britain. - London: Ray Society Publication No. 146, 38 pp., 2 maps.

KRELL, F.-T. 1991: Restituirung und Designation des Hololectotypus von *Aphodius* (*Melinopterus*) *punctatosulcatus* Sturm, 1805 (Insecta, Coleoptera: Scarabaeoidea, Aphodiinae. – Spixiana 14 (3): 293–300.

MANN, D.J. 2000: 1999 Annual Exhibition Imperial College, London, SW7, 27 November 1999. - British Journal of Entomology and Natural History 13: 179.

SHAARAWI, F.A. & ANGUS, R.B. 1991: A chromosomal investigation of five European species of *Anacaena* Thomson (Coleoptera: Hydrophilidae). - Entomologica Scandinavica 21 (1990): 415–426.

WILSON, C.J. 2002: Chromosomal Studies on Dung Beetles (Coleoptera: Scarabaeoidea). - University of London Ph.D. thesis, 230 pp.

Dr. C.J. WILSON & Dr. R.B. ANGUS
School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK.