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A chromosomal analysis of ten European species of *Aphodius* ILLIGER, subgenera *Acrossus* MULSANT, *Nimbus* MULSANT & REY and *Chilothorax* MOTSCHULSKY (Coleoptera: Aphodiidae)

C.J. WILSON & R.B. ANGUS

Abstract

The karyotypes of *Aphodius* (*Acrossus*) *depressus* (KUGENANN), *A.* (*A.*) *luridus* (F.) and *A.* (*A.*) *rufipes* (L.), *A.* (*Nimbus*) *contaminatus* (HERBST) and *A.* (*N.*) *obliteratus* PANZER, and *A.* (*Chilothorax*) *conspurcatus* (L.), *A.* (*C.*) *distinctus* (O.F. MÜLLER), *A.* (*C.*) *lineolatus* ILLIGER, *A.* (*C.*) *paykulli* BEDEL and *A.* (*C.*) *sticticus* (PANZER) are described and illustrated. In all cases the karyotypes comprise nine pairs of autosomes and sex chromosomes which are Xy (male), XX (female). The karyotypes of all the species are distinctive, but give no clues to the taxonomic groupings of the species. No chromosome polymorphism has been detected.

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

Introduction

WILSON & ANGUS (2003) gave an account of the chromosomes of three British *Aphodius* (*Melinopterus*) species, based on the results of WILSON (2002). This paper, largely from the same research programme, deals with ten species which either have an elytral pattern involving discrete dark spots or streaks on a yellow background, or appear to be closely related to species with such a pattern. The species concerned are placed by BARAUD (1992) in the subgenera *Acrossus* MULSANT, *Nimbus* MULSANT & REY and *Chilothorax* MOTSCHULSKY, three groups which do not form an obvious unit. The taxonomic arrangement used by BARAUD (1992) provides a convenient framework for consideration of European Scarabaeoidea, but is not regarded as definitive. Thus DELLACASA et al. (2000) elevated the subgenera of *Aphodius* to generic rank while DNA-based phylogenetic studies by MATÉ (2002) give various clusters of *Aphodius*, which do not support the generic rank of most of the subgenera. In particular, *Chilothorax* comes out as a number of separate clusters, while *Nimbus* and *Acrossus* tend to be associated, as suggested by KABAKOV & FROLOV (1996). Maté (in litt., 19.xii.2003) remarks that "As for *Chilothorax*, it is now obvious that they only have the spots in common". The "spots" theme is adopted here to give a convenient sized selection of species.

Material and methods

The material from which chromosomes were obtained is listed in Table 1. English material is listed by Vice-County (DANDY 1969), and the relevant ones are as follows: **4**, North Devon; **11**, South Hants; **15**, East Kent; **22**, Berks; **24**, Bucks; **28**, West Norfolk; **69**, Westmorland and North Lancs.

Table 1: Material used for chromosomal analysis.

Species	*Localities and total number of specimens analysed*
A. (Acrossus) depressus	**ENGLAND. 4:** Ilfracombe; **69:** Finsthwaite. (5 specimens)
A. (Acrossus) luridus	**ENGLAND. 24:** Chalfont St Giles. **SPAIN. Provincia de Malaga:** Ronda. (5 specimens)
A. (Acrossus) rufipes	**ENGLAND. 17:** Ockham Common; **22:** Old Windsor. (3 specimens)
A. (Nimbus) contaminatus	**ENGLAND. 22:** East Hendred. (4 specimens)
A. (Nimbus) obliteratus	**ENGLAND. 15:** Betteshanger; **17:** Martyr's Green; **22:** Windsor Deer Park. (3 specimens)
A. (Chilothorax) conspurcatus	**ENGLAND. 11:** New Forest, Hincheslea & Bolderwood Hill. (3 specimens)
A. (Chilothorax) distinctus	**ENGLAND. 22:** Cothill; **28:** Gayton Thorpe. (6 specimens.)
A. (Chilothorax) lineolatus	**SPAIN. Provincia de Cádiz:** Tarifa. (2 specimens)
A. (Chilothorax) paykulli	**ENGLAND. 17:** Box Hill. (4 specimens)
A. (Chilothorax) sticticus	**ENGLAND. 4:** Ilfracombe; **17:** Martyr's Green; **24:** Chalfont St. Giles. (5 specimens)

The methods of preparation and analysis are as described by WILSON & ANGUS (2003), but with no statistical analysis of Relative Chromosome Length (RCL). Initial preparation of karyotypes was by cutting and sticking photographs, but these were then scanned into a computer and handled using Adobe Photoshop.

Results

Subgenus *Acrossus* MULSANT

Aphodius depressus. Fig. 1a-c. Published information: 2N = 20 (♂) (VIRKKI 1951). 2N = 18 + Xy. The autosomes range in RCL from about 16 to 8, with a sharp drop in RCL between autosomes 3 (RCL about 15) and 4 (RCL about 8). The X chromosome is similar in length to the smallest autosomes, and the y chromosome is dot-like. Autosomes 1 - 3 are more or less metacentric, and 1 has a secondary constriction in its shorter arm. Autosomes 4 - 6 are submetacentric, with one arm about half the length of the other, and 7 - 9, and the X chromosome are almost acrocentric. The centromeric C-bands (Fig. 1b, c) are moderately strong and fairly similar in size on all the autosomes and the X chromosome. The y chromosome has a small centromeric C-band. The secondary constriction on autosome 1 shows as an additional C-band.

Aphodius luridus. Fig. 1 d-f. Published information: none. 2N = 18 + Xy. All the autosomes are metacentric, and the decrease in RCL along the karyotype is from about 14 to 8. The X chromosome, also metacentric, appears slightly shorter than the shortest autosomes. Fig. 1e shows autosome 2 with the long arms held together, suggesting a secondary constriction or additional heterochromatin, but C-banding (Fig. 1f) shows strong but fairly small centromeric C-bands on all the autosomes and the X chromosome. No C-banded y chromosome is available for study.

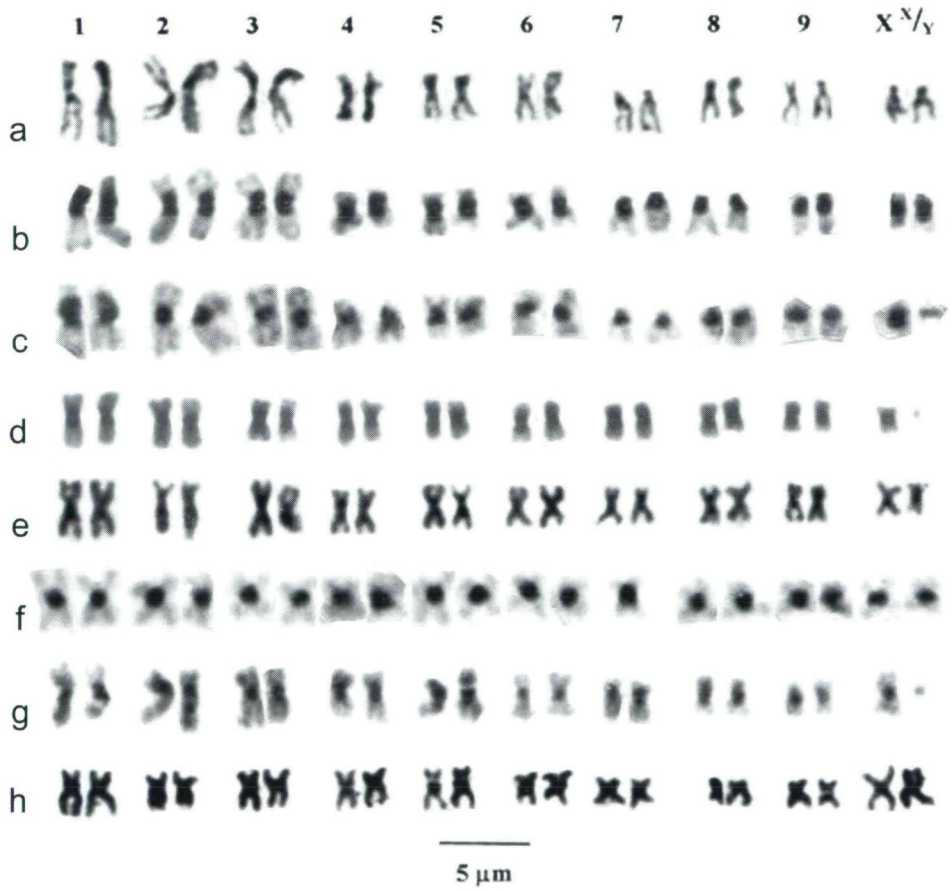

Fig. 1: Mitotic chromosomes from mid-gut cells of *Acrossus* species; **a - c**: *A. depressus*, Ilfracombe, **a** ♀ plain, **b** ♀ C-banded, **c** ♂ C-banded; **d - f**: *A. luridus*, **d** ♂, Chalfont St Giles, plain, **e** ♀, Ronda, plain, **f** ♀, Old Windsor, C-banded; **g, h**: *A. rufipes*, **g** ♂, Old Windsor, C-banded, **h** ♀, Ockham Common, plain.

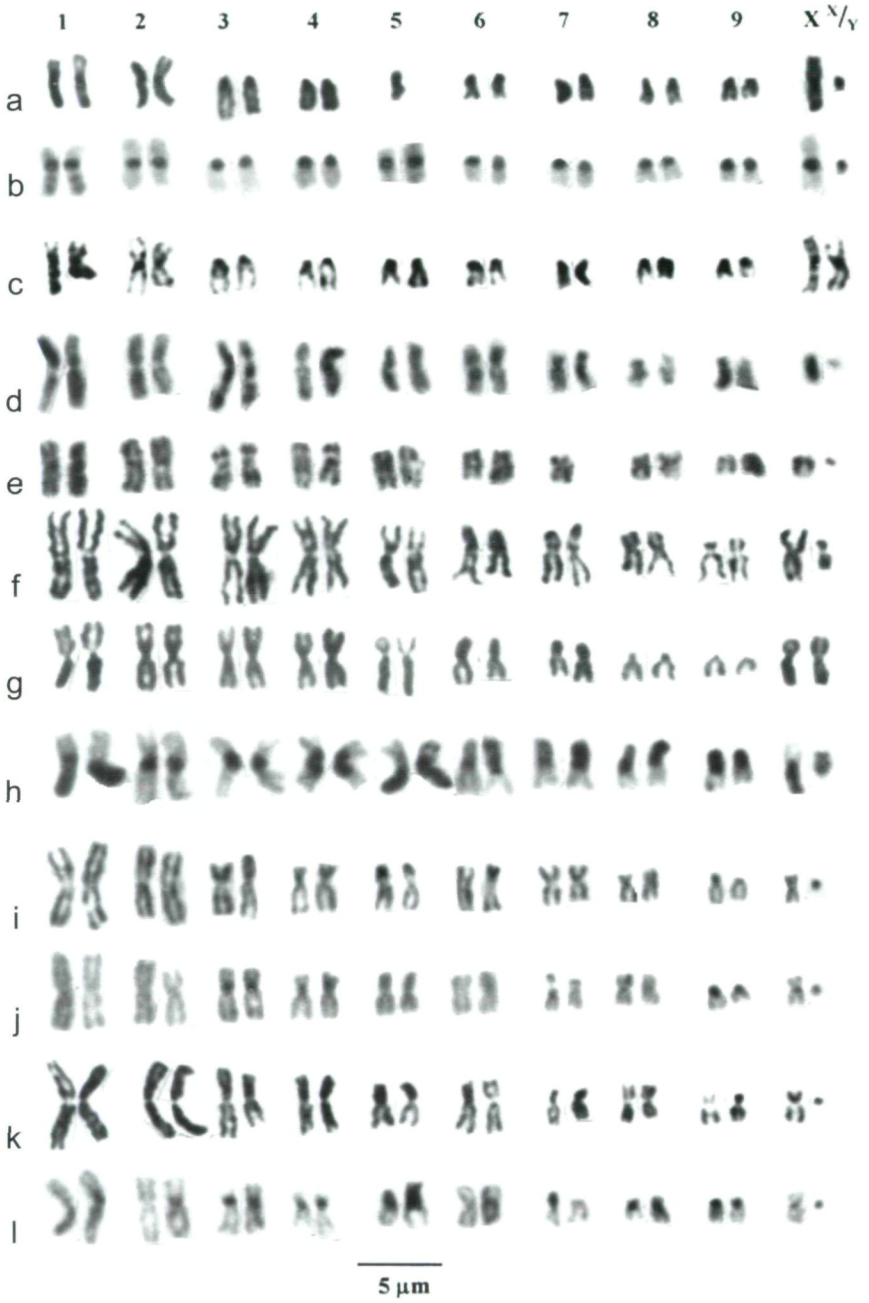

Fig. 2: Mitotic chromosomes from mid-gut cells of *Nimbus* and *Chilothorax* species; **a** - **c**: *A. contaminatus*, East Hendred, **a** ♂, plain, **b** ♂, C-banded, **c** ♀, plain; **d**, **e**: *A. obliteratus*, ♂, **d** Betteshanger, plain, **e** Martyr's Green, partially C-banded, one replicate of autosome 7 missing; **f** - **h**: *A. conspurcatus*, New Forest, **f** ♂, plain, **g** ♀, plain, **h** ♂, C-banded; **i** - **l**: *A. distinctus*, **i**, **j**, ♂, Cothill, **i** plain, **j** C-banded, **k**, **l**, Gayton Thorpe, ♂, the same specimen, **k** plain, **l** C-banded.

Fig. 3: Mitotic chromosomes from mid-gut cells of *Chilothorax* species; **a**, **b**: *A. lineolatus*, Tarifa, ♂, the same specimen, **a** plain, **b** C-banded; **c** - **e**: *A. paykulli*, Box Hill, **c** ♂, plain, **d** ♀, plain, **e** ♀, C-banded; **f** - **h**: *A. sticticus*, **f** ♂, Ilfracombe, plain, **g** ♀, Chalfont St Giles, plain, **h** ♂, Martyr's Green, C-banded.

Aphodius rufipes. Fig. 1g, h. Published information: 2N = 20 (♂), 9 bivalents + Xy (VIRKKI 1951). 2N = 18 + Xy. The autosomes range in RCL from about 14 to 7, and the X chromosome is about as long as autosome 4, RCL about 10.5. Autosomes 1 - 6 and 8 are submetacentric, while autosomes 7 and 9, and the X chromosome, are metacentric. The male karyotype (Fig. 1g) is partly C-banded and shows distinct centromeric C-bands similar in size to those of *A. luridus*. The long arms of autosomes 1 and 2, and the short arm of autosome 5, show distinct secondary constrictions, which in this preparation appear as gaps rather than C-bands.

Subgenus *Nimbus* MULSANT & REY

Aphodius contaminatus. Fig. 2a-c. Published information: none. 2N = 18 + Xy. The RCLs of the autosomes range from about 16 to 8, and the X chromosome (RCL about 19) is the largest in the nucleus. The y chromosome is a very small acrocentric, RCL about 4. Autosomes 1, 2 and 5, and the X chromosome, are metacentric, and the remaining autosomes are acrocentric. C-banding (Fig. 2b) shows all the chromosomes to have strong but localised centromeric C-bands. The long arm of autosome 1 has a secondary constriction. The euchromatic arms of the y chromosome are clearly visible, about as long as the centromeric C-band. This karyotype is unusual in having such a large, almost entirely euchromatic X chromosome.

Aphodius obliteratus. Fig. 2d, e. Published information: none. 2N = 18 + Xy. The RCLs of the autosomes range from about 16 to 6.5, with the X chromosome (RCL about 6.5) similar in size to the smallest autosome. The y chromosome is dot-like. Autosomes 1 - 3 are more or less metacentric, 4 - 6 are submetacentric and 7 - 9 and the X chromosome are subacrocentric. Autosome 3 has a distinct secondary constriction in both arms. C-banding was attempted, but with very limited success (Fig. 2e).

Subgenus *Chilothorax* MOTSCHULSKY

Aphodius conspurcatus. Fig. 2f-h. Published information: none. 2N = 18 + Xy. The RCLs of the autosomes range from about 15 to 5. Autosomes 1 - 4 are metacentric, with little decrease in length (RCL of autosome 4 is about 13) and autosome 5 is submetacentric, RCL about 12. Autosomes 6 and 7 have secondary constrictions in their shorter arms and are variable in appearance because of the degree of expansion of these constrictions. Their RCLs are about 8 - 10. Autosomes 8 and 9 are small, RCL about 6, almost acrocentric. The X chromosome is submetacentric, similar in size to autosomes 5 and 6, RCL about 9. The y chromosome is fairly long (RCL about 5), with a gap about a third of the way along its length. C-banding (Fig. 2h) shows autosomes 1 and 5 and the X chromosome to have heterochromatic long arms, and autosomes 6 - 9 to have heterochromatic short arms. The entire y chromosome is heterochromatic, with the gap somewhat blurred and no evidence that it is at the site of the centromere.

Aphodius distinctus. Fig. 2i-l. Published information: 2N = 20 (♂), Xy sex chromosomes (VIRKKI 1960). 2N = 18 + Xy. The RCLs of the autosomes range from about 18 to 5. The X chromosome is similar in size to autosome 8 (RCL about 7), and the y chromosome is very small, dot-like. Autosomes 1, 2, 6 and 8, and the X chromosome, are metacentric. Autosomes 3 - 5 are submetacentric, autosome 7 has a secondary constriction in the short arm, and autosome 9 is acrocentric. C-banding (Fig. 2j, l) shows autosomes 1 and 2 without any C-bands, and the centromeric C-bands of autosomes 6 - 8 are weak and not reliably obtained by standard treatments. The centromeric C-bands of autosomes 3, 4 and 9 are small but fairly strong and the short arms of autosome 5 may stain heavily after C-banding, suggesting a secondary constriction.

Aphodius lineolatus. Fig. 3a, b. Published information: none. 2N = 18 + Xy. The RCLs of the autosomes range from about 16 to 7, with the X chromosome about as long as autosome 5, RCL about 11. Autosomes 4 and 7 are almost acrocentric, but the remaining autosomes and X chromosome are all more or less metacentric. The y chromosome is small, RCL about 3, and acrocentric. C-banding (Fig. 3b) shows all the autosomes with substantial centromeric C-bands, while the X chromosome has a particularly large C-band, accounting for about 60% of the total length of the chromosome.

Aphodius paykulli. Fig. 3c-e. Published information: none. 2N = 18 + Xy. The RCLs of the autosomes range from about 13 to 8, and the X chromosome is slightly larger than autosomes 8 and 9, RCL about 9. The y chromosome is more or less dot-like, but not very small. All the

autosomes, and the X chromosome, are more or less metacentric. C-banding (Fig. 3e) shows large centromeric C-bands occupying at least half the length of the chromosomes. The C-bands are very heavy in the preparations obtained and their exact extents are not clear.

Aphodius sticticus. Fig. 3f-h. Published information: none. 2N = 18 + Xy. The RCLs of the autosomes range from about 18 to 6, with the X chromosome slightly shorter than autosome 9, RCL about 5, and the y chromosome small, dot-like. There is a sharp decrease in RCL between autosomes 4 and 5 (RCLs about 12 and 9). Autosomes 1 - 4 and 7 - 9 are more or less metacentric, autosome 5 is submetacentric, and autosome 6 and the X chromosome are acrocentric. C-banding (Fig. 3h) shows strong but fairly small centromeric C-bands on all the autosomes, and the X chromosome.

Discussion

The karyotypes of the three *Acrossus* species resemble one another in having the C-bands restricted to the area round the centromere and the y chromosome very small, dot-like. *Aphodius depressus* differs from the other two species in having autosomes 7 - 9, and the X chromosome, acrocentric. *Aphodius luridus* has all the chromosomes (except the y) metacentric or almost so, while *A. rufipes* has autosomes 1 - 5 submetacentric, with their short arms about half the length of the long ones.

The two *Nimbus* species are among the most difficult encountered in this study as sources of chromosomes. Their karyotypes are sharply different from one another – *Aphodius contaminatus* has autosomes 3, 4 and 6 - 9 acrocentric, while these autosome pairs range from metacentric to subacrocentric, always with distinct short arms, in *A. obliteratus*. The X chromosome of *A. contaminatus* is largely euchromatic and at least twice the length of that of *A. obliteratus*. The C-bands of *A. contaminatus* are small but distinct, while those of *A. obliteratus* proved very difficult to demonstrate. Fig. 2e shows weakly developed small centromeric C-bands only on autosomes 1 - 4.

The *Chilothorax* species show considerable karyotype diversity, and, with the exception of *A. lineolatus*, brought back from southern Spain, are among the easiest species as sources of chromosomes. The abundant karyotypes of *A. sticticus* obtained from the Martyr's Green material contrasted very sharply with the almost total failure obtained with *A. obliteratus* from the same place at the same time. The general layout of the *Chilothorax* karyotypes is fairly consistent, with the most striking variations being the very heavy C-bands of *A. paykulli* and the unusually long y chromosome of *A. conspurcatus*. It is worth noting that these species tend to come out together in studies by MATÉ (2002).

None of the species included in this study has shown any chromosome polymorphisms, either in terms of centromere positions (inversions), or of C-band size or number. No B-chromosomes have been encountered. The most important finding is probably that each species has its own characteristic karyotype.

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

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