A chromosomal analysis of 15 species of Gymnopleurini, Scarabaeini and Coprini (Coleoptera: Scarabaeidae)

R. B. Angus, C. J. Wilson & D. J. Mann

The karyotypes of one species of Gymnopleurini, two Scarabaeini, five Onitini and seven Coprini are described and illustrated. *Gymnopleurus geoffroyi*, *Scarabaeus cristatus*, *S. laticollis*, *Bubas bison*, *B. bubalus*, *B. bubaloides*, *Onitis belial*, *O. ion*, *Copris lunaris*, *Microcopris doriae*, *M. hidakai* and *Helopcopris gigas* all have karyotypes with 2n=18 + Xy. *Copris hispanus* and *Paracopris* ***ramosiceps*** have karyotypes with 2n=16 + Xy and *Copris sinicus* has a karyotype comprising 2n=12 + Xy. Heterochromatic B-chromosomes have been found in *Bubas bubalus*. Spanish material of *Bubas bison* lacks the distal heterochromatic blocks found in most of the chromosomes of Italian specimens. The karyotype of *Heliocopris gigas* is unusual in that the autosomes and X chromosome are largely heterochromatic.
R. B. Angus* & C. J. Wilson, School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK. r.angus@rhul.ac.uk
D. J. Mann, Hope Entomological Collections, Oxford University Museum of Natural History, Parks Road, Oxford OX1 3PW, UK. darren.mann@oum.ox.ac.uk

Introduction

A previous publication (Wilson & Angus 2005) gave information on the karyotypes of species of Oniticellini and Onthophagini studied by C. J. Wilson in her Ph. D. research (Wilson 2002). The present paper reports on the remaining Scarabaeidae (*Gymnopleurus geoffroyi* Illiger, *Copris lunaris* (Linnaeus) and *Onitis belial* Fabricius) included in that research, with additional material collected by R. B. Angus (European and Cypriot material) and D. J. Mann (Malaysian material).

Material and Methods

A list of the beetle species from which chromosome preparations have been obtained, their taxonomy and their localities of origin are given in table 1. The arrangement of tribes and genera follows Löbl & Smetana (2006). The specimens studied are housed either in C. J. Wilson's or R. B. Angus' collection. Spanish and Italian localities are listed by Provincia, and Cyprus ones by Eparkhia (District). The methods of chromosome preparation and C-banding are given by Wilson (2001). In some cases it has been possible to C-band preparations after they have been photographed plain, giving a very powerful set of data for preparation of karyotypes. The C-banding treatment has to be adjusted to suit ambient room temperature, and in some cases has been progressively adjusted to compensate for unnoticed deterioration of the barium hydroxide solution. With a freshly prepared solution at about 23ºC a treatment for 3 or 4 minutes seems to give the best results. Where preparations are C-banded after being photographed plain, immersion oil is removed from the slide by placing it first in a jar of xylene, then in absolute ethanol. Such preparations are then partially destained by immersion in salt-sodium citrate (standard 2X SSC) at 55ºC for about 10 minutes, rinsed in distilled water, then placed in the barium hydroxide. The SSC treatment is carried out at 55ºC rather than the usual 60ºC, as this seems to give better results. In the case of preparations of *Bubas* chromosomes, it has been found that treatment for 1 hour in SSC, without the

Tijdschrift voor Entomologie 150: 201–211, Figs 1–4, Table 1. [ISSN 0040–7496]. http://www.nev.nl/tve

* *Corresponding author*

Table 1. Material studied and taxonomy.

Scientific name	Localities (number of specimens analysed)
Gymnopleurini	
Gymnopleurus Illiger, 1803	
G. geoffroyi (Fuessly, 1775)	Cyprus. Pafos: Agia Varvara. (1♂)
Scarabaeini	
Scarabaeus Linnaeus, 1758	
S. (*Scarabaeus*) *cristatus* Fabricius, 1775	Egypt. "Egyptian desert." (1♂, purchased from a dealer.)
S. (*Ateuchestus*) *laticollis* Linnaeus, 1767	Spain. Málaga: Frigliani. (1♂)
Onitini	
Bubas Mulsant, 1842	
B. bison (Linnaeus, 1767)	Italy. Sardinia, Nuoro: Laconi. (1♂.) Spain. Málaga: Tarifa; Madrid: El Escorial. (2♂, 1♀)
B. bubalus (Olivier, 1811)	Spain. Málaga: Nerja; Madrid: El Escorial; Ávila: Sierra de Paramera. (4♂, 2♀)
B. bubaloides Janssens, 1938	Cyprus. Pafos: Kritou Marottou; Limassol: Akrotiri, Fassouri (2♂, 1♀)
Onitis Fabricius, 1798	
O. belial Fabricius, 1798	Spain. Cáceres: Abadía. (2♂, 2♀)
O. ion (Olivier, 1789)	Spain. Málaga: Nerja. (2♂)
Coprini	
Copris Geoffroy, 1762	
C. hispanus hispanus (Linnaeus, 1764)	Spain. Málaga: La Línea; La Sauceda; Tarifa. (3♂)
C. lunaris (Linnaeus, 1758)	Spain. Segovia: Valsain; La Salceda. (1♂, 1♀)
C. sinicus Hope, 1842	Malaysia. Sabah (Borneo): Lahad Datu, Ulu Segama Forest Reserve. (1♂)
Paracopris Balthasar, 1939	
P. ramosiceps (Gillet, 1929)	Malaysia. Sabah (Borneo): Lahad Datu, Ulu Segama Forest Reserve. (1♂)
Microcopris Balthasar, 1958	
M. doriae (Harold, 1877)	Malaysia. Sabah (Borneo): Lahad Datu, Ulu Segama Forest Reserve. (1♂)
M. hidakai Ochi & Kon, 1996	Malaysia. Sabah (Borneo): Lahad Datu, Ulu Segama Forest Reserve. (1♂)
Heliocopris Hope, 1837	
H. gigas (Linnaeus, 1758)	Egypt. "Egyptian desert." (1 ♂, purchased from a dealer.)

barium hydroxide treatment, gave clearer banding and separation of the heterochromatic B-chromosomes. SSC treatment alone is a standard method of obtaining G-bands in mammalian chromosomes (Sumner et al. 1971). However, in the case of *Bubas* the banding patterns obtained are the same as those resulting from C-banding treatment, but clearer. It is therefore considered that these bands reflect the presence of constitutive heterochromatin (C-bands) and are not the same as the euchromatic G-bands of mammalian chromosomes. Relative Chromosome Length (RCL) is discussed by Wilson & Angus (2005) and is used here without any statistical analysis (the samples are too small), simply to give an indication of the sequence of chromosome lengths along a karyotype. The standard terms denoting centromere position are as used by Wilson & Angus (2005). The organised arrays of chromosomes are referred to as karyotypes rather than karyograms or ideograms, following the recommended practice for nomenclature of human chromosomes (Paris Conference 1971). The notation Xy_p for the sex chromosomes refers to the association of the small *y* chromosome with the larger X during first division of meiosis, by means of a cytoplasmic vesicle. This notation was introduced by Smith (1950) and the means of association was investigated in detail by John & Lewis (1960). John & Lewis concluded that a nucleolus was involved, but Juan et al. (1993) demonstrated that in some cases no rDNA (ribosome-forming DNA,

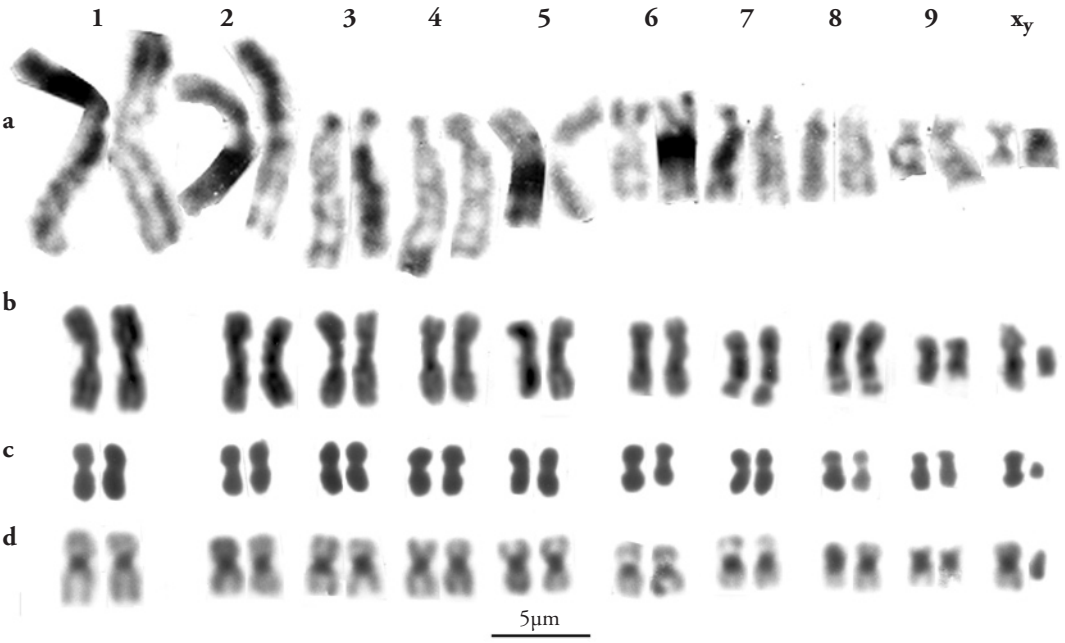

Fig. 1. Mitotic chromosomes from testes of *Gymnopleurus* and *Scarabaeus*, arranged as karyotypes. – a, *Gymnopleurus geoffroyi*, ♂; b, *Scarabaeus cristatus*, ♂; c, d, *S. laticollis*, ♂, c, plain, d, C-banded.

characteristic of nucleoli and their organisers) was present, so the term "cytoplasmic vesicle" is now used. Smith & Virkki (1978) suggested that the X chromosome involved in Xy_p formation was normally a large metacentric. However, the X chromosome need not be metacentric – for instance in *Adalia bipunctata* (Linnaeus, 1758), one of the species studied in detail by John & Lewis (1960), it is clearly acrocentric (R. B. Angus, unpublished data).

Results

Gymnopleurini

Gymnopleurus geoffroyi

Figs 1a, 4a

Published information: none. 2n=18 + Xy_p (♂). The RCLs of the autosomes range from about 20 to 6, and the X and y chromosomes are more or less the same size, and smaller than autosome 9, RCL about 3.5. Autosomes 1, 2 and 5 are metacentric, 6 is submetacentric and 3, 4 and 7 – 9 are acrocentric. The X and y chromosomes are identified on the basis of the Xy_p bivalent at first division of meiosis (Fig. 4a), which suggests the X chromosome is metacentric. No adequate C-banded preparation is available, though there were indications that most, if not all, of the longer chromosomes have small centromeric C-bands. The y chromosome appears rather dark in Fig. 1a and may be heterochromatic.

Scarabaeini

Scarabaeus cristatus

Figs 1b, 4b

Published information: none. 2n=18 + Xy_p (♂). The autosomes are all metacentric, with RCLs ranging from about 16 to 6. The decrease in length is even from pairs 1 to 8 (RCL about 11), but abrupt between pairs 8 and 9 (RCL about 6). There appear to be secondary constrictions (NORs) in the long arms of pairs 7 and 8. The X chromosome (RCL about 11) is also metacentric, and the y chromosome appears to be acrocentric, about half the length of autosome 9. No C-banded preparation is available, but the elongate centromeric constrictions suggest heavy C-bands. First metaphase of meiosis (Fig. 4b) shows the Xy_p bivalent with a distinct gap between the chromosomes.

Fig. 2. Mitotic chromosomes from mid guts of *Bubas* and *Onitis*, arranged as karyotypes. – a, b, *Bubas bison*, ♂, Sardinia, a, plain, b, the same nucleus C-banded; c, d, *B. bison*, ♂, El Escorial, c, treated with SSC, d, C-banded, with one homologue of autosome 9 missing; e, *B. bison*, ♂, Tarifa, treated with SSC; f, *B. bubalus*, ♂, El Escorial, C-banded, with 7 B-chromosomes; g, h, B. *bubalus*, Nerja, with 3 B-chromosomes, g, ♀, C-banded, h, ♂, plain; i, j, *B. bubalus*, Sierra de Paramera, treated with SSC, i, ♂ with 9 B-chromosomes, j, ♀ with 8 B-chromosomes; k, l, *B. bubaloides*, ♂, Fassouri, k, plain, l, treated with SSC; m, *B. bubaloides*, ♀, Kritou Marottou, C-banded, with one homologue of autosome 8 missing; n, o, *Onitis belial*, n, ♂, o, ♀; p, *O. ion*, ♂.

Scarabaeus laticollis

Figs 1c, d, 4c

Published information: 2n=20 (♂), meioformula=9 bivalents + Xy (Virkki 1951). 2n=19 + Xy_p (♂). The autosomes are all either metacentric or submetacentric, and their RCLs range from about 15–9.5, with an even decrease in length along the karyotype. The X chromosome is metacentric, about the same length as autosome 9, and the y chromosome is acrocentric, about a third the length of the X chromosome. C-banding (Fig. 1d) shows heavy centromeric C-bands on all the autosomes and the X chromosome, while the y chromosome appears to be largely heterochromatic. First metaphase of meiosis (Fig. 4c) shows a distinct gap between the X and y chromosomes.

Onitini

Bubas bison

Figs 2 a-e

Published information: 2n=18 + Xy (♂), XX (♀), karyotypes, banding: (Colomba et al. 1996). 2n=18 + Xy. Figs 2a, b show a karyotype from a Sardinian male, standard and C-banded, originated from the same nucleus. All the autosomes, and the X chromosome, are acrocentric. This karyotype resembles Colomba et al.'s Sicilian material in that most of the chromosomes have distal terminal C-bands with the chromatids lying close together in these heterochromatic regions. In this C-banding the Sicilian and Sardinian material is completely different from Spanish material (Figs 2c-e) in which the only C-bands are small centromeric ones and the chromatids are clearly separated from one another distally. The RCLs of the Sardinian material range from about 13.5–9, with an even decrease in length along the karyotype. The X chromosome is among the larger ones, RCL about 11.5. The y chromosome is dot-like. The RCLs of the autosomes of the Spanish material range from about 17–7.5, while the RCL of the X chromosome is about 14.5. If the distal heterochromatic segments of the Sardinian chromosomes are excluded from the RCL calculations, the RCLs of the autosomes range from about 19–8, while that of the X chromosome is about 16. Such estimates can only be approximate, but they do suggest that the differences between the observed RCLs of Sardinian and Spanish material may be simply due to the distal heterochromatic segments of the Sardinian material.

In any evaluation of the chromosomal differences between Sardinian and Spanish *B. bison*, two possibilities have to be considered: either *B. bison* may be a species whose chromosomes show considerable differences in the extent of the C-banding between different populations, or more than one species may be involved. A first question to be answered is whether the chromosomes of the Sardinian and Sicilian material (Colomba et al. 1996) are the same. The Sardinian karyotype presented here has only three chromosomes lacking distal C-bands: autosome pairs 1 and 2, and the X chromosome. Colomba et al. report that in Sicilian material four pairs of autosomes lack distal C-bands, while the X chromosome has such a band, and is also a smaller chromosome than the one suggested for the Sardinian specimen. The different X chromosome would be a problem if only one species is involved, but differences in the number of chromosome pairs with and without distal C-bands would suggest that regional variation within a species is a likely explanation. However, we question the interpretation of the male karyotype by Colomba et al. (1996). The chromosomes placed as pair 1 do not match, and one of them appears to have a distal heterochromatic segment. It is possible to rearrange the chromosomes in this karyotype to match the arrangement given for the Sardinian material in Figs 2 a, b, and while such an arrangement has some mismatches, they appear no worse than in Colomba et al.'s figure. It is to be expected that any karyotype assembled from a single nucleus will have a number of less than perfect pairings, because of irregularities in chromosome condensation through mitotic prophase and early metaphase. Thus, on the evidence so far available it is not clear that the karyotypes of Sardinian and Sicilian material are different, though this may well be the case. If the X chromosome is long in both Sardinian and Sicilian material, then it is possible that Spanish and Italian *B. bison* may be conspecific, with the differences between the karyotypes simply the result of differing quantities of heterochromatin. This is a problem requiring further investigation, possibly involving DNA analysis.

Bubas bubalus

Figs 2f-j

Published information: none. 2n=18 + Xy (♂), XX (♀), + up to 9 B-chromosomes. The autosomes are acrocentric, with their RCLs ranging from about 17–6. C-banding is confined to the centromere regions, and autosomes 1 and 2 sometimes show a very short shorter arm above the centromere. The X chromosome, RCL about 12, is slightly longer than autosome 4, and is submetacentric. The y chromosome is dot-like. B-chromosomes have been found in all the specimens studied. Their number ranges from three (Nerja material, Figs 2g, h) to 9 (material from the Sierra de Paramera, Fig. 2i). The B-chromosomes are all heterochromatic, whether demonstrated by

Fig. 3. Mitotic chromosomes of *Copris*, *Paracopris*, *Microcopris* and *Heliocopris*, arranged as karyotypes. – a, b, *Copris lunaris*, ♂, Valsain, a, plain, b, C-banded; c, *C. lunaris*, ♀, La Salceda, plain; d, *C. hispanus*, ♂, Tarifa, plain; e, f, *C. hispanus*, ♂, La Sauceda, e plain, f, the same nucleus, C-banded; g, h, *C. sinicus*, ♂, g plain, h, the same nucleus C-banded; i, *Paracopris ramosiceps*, ♂, plain; j, *Microcopris doriae*, ♂, plain; k, *M. hidakai*, ♂, plain; l, m, *Heliocopris gigas*, ♂, l, plain, k, the same nucleus C-banded. e, f, j, l and m are from testis, the rest from mid gut.

C-banding (Figs 2 f, g) or SSC treatment (Figs 2 i, j). The RCLs of the B-chromosomes range from about 19–6.

Bubas bubaloides

Figs 2k-m

Published information: none. 2n=18 + Xy (♂), XX (♀). The RCLs of the autosomes range from about 15–5.5, with a marked decrease in length between pairs 6 (RCL about 11) and 7 (RCL about 9). Autosomes 1–4, 8 and 9, are subacrocentric, and 5–7 are submetacentric. The X chromosome, RCL about 13.5, is about as long as autosome 3, and is submetacentric. The y chromosome is a very mall submetacentric, RCL about 5. The centromeric C-bands of autosomes 1–4 are rather heavy, whether shown by C-banding (Fig. 2m) or SSC treatment (Fig. 2l). Autosomes 5–7 appear to show extensive heterochromatin round the centromere. Autosomes 8 and 9 show no detectable C-bands. The X chromosome has a fairly heavy centromeric C-band, comparable with those of autosomes 1–4, and the y chromosome shows no detectable heterochromatin.

Onitis belial

Figs 2n, o

Published information: none. 2n=18 + Xy (♂), XX (♀). The RCLs of the autosomes range from about 15–9 and the X chromosome (RCL about 15) is as long as the longest autosomes. The y chromosome is acrocentric, slightly smaller than autosome 9 (RCL about 8). Autosomes 1–4 and 8, and the X chromosome are more or less metacentric, while the rest are acrocentric. Autosome 3 appears to have a secondary constriction in its short arm. No C-banded preparation is available.

Onitis ion

Fig. 2p

Published information: none. 2n=18 + Xy (♂). The RCLs of the autosomes range from about 14.5–5.5, with pairs 1–4 about the same size and a sharp decrease in size between pairs 4 (RCL about 14.5) and 5 (RCL about 11). The X chromosome (RCL about 13) is intermediate in size between autosomes 4 and 5. The y chromosome is a very small acrocentric, RCL about 2. Autosomes 1–3, 5, 7 and 10 are acrocentric, 6 and 8 are subacrocentric, and 4 and the X chromosome are more or less metacentric. No C-banded preparation is available.

Coprini

Copris lunaris

Figs 3a-c

Published information: 2n=20 (♂) (Virkki 1954). 2n=18 + Xy (♂), XX (♀). The RCLs of the autosomes range from about 17–8, and the sex chromosomes are about the same size as autosome 9. Autosomes 1, 6 and 8 are subacrocentric, 2–4, 7 and 9 are acrocentric and autosome 5 is metacentric. The X chromosome is metacentric and the y is acrocentric. C-banding (Fig. 3b) suggests that the chromosomes have small, localised centromeric C-bands, though the preparation is poor.

Copris hispanus

Figs3d-f

Published information: 2n=20, 9 pairs of autosomes + XY (Salamanna 1966), corrected to 2n=19 and referred to *C. hispanus cavolinii* (V. Petagna, 1792) (Salamanna 1972); 2n=18 + XY (♂), XX (♀), karyotype illustrated – Egyptian material (Ebied et al. 2000). 2n=16 + Xy (♂). Fig. 3d shows a standard karyotype from a mid gut cell of a male from Tarifa, while Figs 3e, f show a karyotype from testis of a specimen from La Sauceda, standard and C-banded. The RCLs of the autosomes range from about 20–5. Autosomes 2 and 3 are the same length (RCL about 16), autosome 4 is a bit smaller (RCL about 14), then there is a marked decrease in size through autosomes 5 and 6 and an abrupt decrease to pairs 7 and 8. Autosome 1 is subacrocentric, autosome 2 is more or less metacentric, autosomes 3 and 5–8 are acrocentric, and autosome 4 is subacrocentric. Autosome 6 shows a pericentric inversion in the La Sauceda specimen, with one homologue acrocentric and the other clearly subacrocentric. The X chromosome is about as long as autosome 5 (RCL about 13), submetacentric with a distinct secondary constriction about a third of the way from the tip of the long arm, and with the distal portion of the arm appearing as a satellite with the chromatids fused together and probably heterochromatic. The y chromosome is a very small subacrocentric, RCL about 2. C-banding (Fig. 3f) shows centromeric C-bands on all the chromosomes. This is a very distinctive karyotype. Salamanna's data for *C. hispanus cavolinii* suggest that this may be a distinct species. Salamanna (1972) figures a spermatogonial metaphase, but the chromosomes do not show sufficient detail for comparison with the material figured here. Ebied et al. (2000) give clear karyotypes showing all the autosomes, and the X chromosome, to be metacentric. Their material cannot be referred to *C. hispanus*.

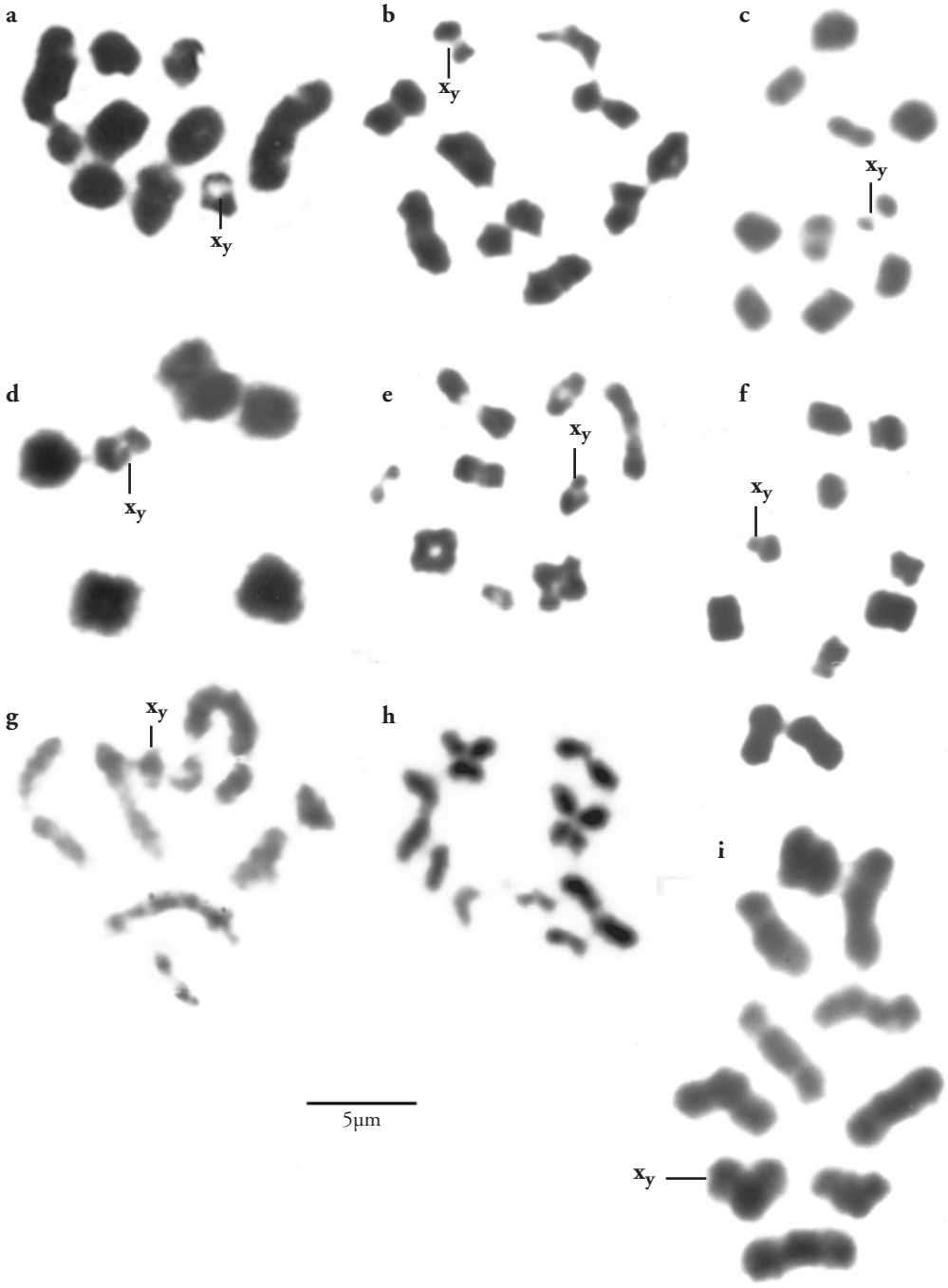

Fig. 4. Preparations of meiosis from testis. a-g, i, first metaphase with the Xy bivalent labelled, h, second metaphase, an X-bearing nucleus. – a, *Gymnopleurus geoffroyi*; b, *Scarabaeus cristatus*; c, *S. laticollis*; d, *Copris sinicus* (two bivalents are lying in contact with each other at the top of the nucleus); e, *Paracopris ramosiceps* (the chromosomes of the bivalent at the top left of the nucleus appear somewhat separated from one another); f, *Microcopris doriae*; g, h, *M. hidakai* (the chromosomes of the bivalent at the left side of the nucleus appear somewhat separated from one another).

Copris sinicus
Figs 3g, h, 4d

Published information: none. 2n=12 + Xy_p (♂). The RCLs of the autosomes range from about 24–9, with abrupt decreases in length between pairs 3 (RCL about 19.5) and 4 (RCL about 14.5) and between pairs 4 and 5 (RCL about 11.5). Autosomes 1–3 are borderline metacentric/submetacentric, autosome 4 is metacentric, and autosomes 5 and 6 are subacrocentric. The X chromosome is submetacentric, about as long as autosome 1. The y chromosome is acrocentric, smaller than autosome 6, RCL about 5. C-banding (Fig 3h) shows distinct centromeric C-bands on all the autosomes and the X chromosome, while the y chromosome appears largely heterochromatic. First metaphase of meiosis shows six large bivalents and the Xy_p, with the X chromosome appearing surprisingly small.

Paracopris ramosiceps
Figs 3i, 4e

Published information: none. 2n=16 + Xy_p (♂). The RCLs of the autosomes range from about 19–6, with abrupt decreases in length between pairs 5 (RCL about 10) and 6 (RCL about 10) and between pairs 6 and 7 (RCL about 7). Autosome 1 is subacrocentric, autosome 2 is metacentric, autosomes 3, 4 and 6–8 are acrocentric, and autosome 5 submetacentric, almost certainly with a secondary constriction in its short arm. The X chromosome is acrocentric, RCL about 14, and the y chromosome is a small metacentric, RCL about 5. No C-banded preparation is available. First metaphase of meiosis (Fig. 4e) shows the 8 autosomal bivalents and the sex chromosomes as a very clear Xy_p.

Microcopris doriae
Figs 3j, 4f

Published information: none. 2n=18 + Xy_p (♂). The RCLs of the autosomes range from about 15–7, with pairs 1–4 submetacentric, and with a gradual decrease in length (the RCL of pair 4 is about 13). Autosome 6 (RCL about 11) is submetacentric, with a secondary constriction in its short arm. Autosomes 7–10 show a gradual decrease in length, from RCL about 9 to RCL about 7. Pairs 7, 8 and 10 are metacentric, while pair 9 is subacrocentric. The X chromosome is metacentric, RCL about 7.5, and the y chromosome appears as a large dot in the available rather condensed preparation, RCL about 4. No C-banded preparation is available. First metaphase of meiosis (Fig. 4f) shows the 9 autosomal bivalents and the Xy_p sex bivalent.

Microcopris hidakai
Figs 3k, 4g, h

Published information: none. 2n=18 + Xy_p (♂). The RCLs of the autosomes range from about 17–6, with an abrupt decrease in length between pair 3 (RCL about 15) and pair 4 (RCL about 10). Autosomes 1 and 3 are subacrocentric, autosome 2 is acrocentric, autosome 4 is submetacentric, and autosomes 5–9 are apparently acrocentric/subacrocentric. The X chromosome is a fairly large submetacentric, RCL about 16, and the y chromosome is dot-like. No C-banded preparation is available. First metaphase of meiosis (Fig. 4g) shows 3 large autosomal bivalents (the chromosomes of the bivalent at the top left of the nucleus are rather widely separated), 3 medium-sized bivalents, 2 small bivalents (1 adjacent to the sex bivalent and 1, with its chromosomes appearing somewhat separated from one another, at the bottom of the nucleus) and the Xy_p sex chromosomes. A second metaphase spread is shown in Fig. 4h. One chromosome is lost, but the large submetacentric autosome 4 and X chromosome are clearly visible. The small autosomes appear more or less acrocentric in this preparation.

Heliocopris gigas
Fig. 3l, m, 4i

Published information: none. 2n=18 + Xy_p (♂). The RCLs of the autosomes range from about 13–10, with an even decrease in chromosome length along the karyotype. The X chromosome, RCL about 12 is also metacentric, with a gap in one arm, while the y chromosome is much smaller, RCL about 4.5, and subacrocentric. C-banding (Fig. 3m) shows that all the autosomes, and the X chromosome, are largely heterochromatic, while the y chromosome appears entirely so. The euchromatic portions of autosomes 1, 2, 4 and 8 account for about 20% of the lengths of the chromosomes, and are located distally on the shorter arms. Autosomes 3 and 6–7 have euchromatic portions accounting for only about 10% of the chromosomes, again located distally on the shorter arms. The euchromatic portion of autosome 9 is located distally on the longer arm, and again accounts for about 10% of the length of the chromosome. In the X chromosome the only apparent euchromatin appears to be in the gap in the longer arm, while there does not appear to be any euchromatin in the y chromosome. First division of meiosis (Fig. 4i) shows the autosomal bivalents as one ring and 8 rods, and the sex bivalent as Xy_p.

Discussion

The species included in this study represent four distinct tribes within the Scarabaeidae. Gymnopleurini, represented by *Paragymnoleurus sinuatus* (Olivier, 1789) and *Gymnopleurus* Illiger, 1803 for which chromosomal data are available for three species, in addition *G. geoffroyi* is reported here. The clearest illustrations are those given by Colomba et al. (2000) for *G. sturmii* MacLeay, 1821. This species agrees with *G. geoffroyi* in the long metacentric autosome 1, but has autosome 2 relatively shorter and submetacentric, with the other autosomes also showing differences from *G. geoffroyi*. In *G. sturmii* the y chromosome is only about half the size of the X. *G. gemmatus* Harold, 1871 is figured by Bisoi & Patnaik (1991) as having some very long metacentric chromosomes in spermatogonial mitosis, and their RCL data suggest that the y chromosome is almost as long as the X. The chromosomes of *G. koenigii* (Fabricius, 1775) are known only from meiosis (Dasgupta 1963) and the figure of diakinesis shows some very long bivalents and the Xy_p with the y chromosome small, dot-like. The remaining species of Gymnopleurini, *G. cyaneus* (Fabricius, 1798) and *Paragymnoleurus sinuatus*, are known only in terms of their chromosome numbers (Kacker 1971; Manna & Lahiri 1972). From the data available it seems that *Gymnopleurus* species may be characterised by a relatively long autosome 1, but that the y chromosome is variable in size.

Scarabaeini are represented by three species for which chromosomal data are available, in addition to the two reported here. Salamanna (1972) figures spermatogonial metaphase of *S. semipunctatus* Fabricius, 1792, with the autosomes metacentric or submetacentric and the y a small acrocentric. His data on *S. sacer* Linnaeus, 1758 refer only to meiosis and he give no information on chromosome morphology. This is also true for Bisoi & Patnaik's (1991) figures of *S. gangeticus* Laporte, 1840. Virkki's (1951) figure of spermatogonial metaphase of S. *laticollis* is in agreement with the data presented here. At the moment it seems that *Scarabaeus* chromosomes are typically metacentric or submetacentric, with C-bands, which may be heavy, confined to the centromere region. In some species at least, the y chromosome is a small acrocentric, not dot-like.

Apart from the data presented here, chromosome information on Onitini is available for *Bubas bison* (Colomba et al. 1996), *Onitis alexis* Klug, 1835 (Ebied et al. 2000), *O. crassus* Sharp, 1875 (Smith & Virkki 1978), *O. philemon* Fabricius, 1801 (Joneja 1960) and *Cheironitis furcifer* (P. Rossi, 1792) (Salamanna 1972). The chromosomes of the *Bubas* species are of particular interest. The presence or absence of terminal C-bands in *B. bison* is described fully in the results section. In all the material studied (Spanish and Italian) all the autosomes, and the X chromosome, are acrocentric. The karyotype of *B. bubalus* is very unusual in possessing up to 9 heterochromatic B-chromosomes. Among the Scarabaeoidea this number of B-chromosomes is matched by *Aphodius lividus* (Olivier, 1789), where there may be 9 heterochromatic B-chromosomes (Angus et al. 2004). However, in *A. lividus* these are all small, comparable in size with the y chromosome, while in *B. bubalus* the B-chromosomes are mainly large, up to twice the length of the longest autosomes. This arrangement is similar to that shown by *Pterostichus nigrita* (Paykull, 1790) and *P. rhaeticus* Heer, 1837 (Angus et al. 2000). The centromeric C-bands of the autosomes and X chromosome of *B. bubalus* are either small or absent. The X chromosome is submetacentric, in contrast to that of *B. bison*. In *B. bubaloides* nearly all of the chromosomes have heavy centromeric C-bands, and the autosomes include acrocentrics, subacrocentrics and submetacentrics. Thus the karyotypes of the three *Bubas* species are all clearly different from one another.

The karyotypes of the two *Onitis* species are clearly different from one another in the number of acrocentric chromosomes. The only other *Onitis* to have its karyotype figured is *O. alexis*, and in this species all the autosomes, and the X chromosome, are acrocentric (Ebied et al. 2000).

For the Coprini, Smith & Virkki (1978) record diploid numbers of 18 and 20 for *Catharsius* Hope, 1837, 20 for *Heliocopris bucephalus* (Fabricius, 1775) and 14 and 20 for *Copris* spp., as well as 19 for *C. hispanicus calvolinii* The diploid number of 18 for *C. hispanus hispanus* is new for the genus and, as mentioned in the Results section, suggests that *C. hispanus calvolinii* is a distinct species. The karyotypes of the two *Microcopris* species (2n=20 (18 + Xy_p)) and *Paracopris ramosiceps* (2n=18 (16 + Xy_p)) fall within the spectrum shown by *Copris*. The chromosomes of *Heliocopris gigas* are remarkable for the extent of the heterochromatin they contain. We know of no Scarabaeoid with such extensive heterochromatinisation, but a similar pattern is shown by the adephagan beetle *Peltodytes caesus* (Duftschmid, 1805) (Haliplidae) (Powell & Angus 2006).

In conclusion, the karyotypes of the species reported here show a wide variation in both number and chromosome form, with clear differences between the various species. In all cases the sex chromosomes are Xy (♂), and where meiosis is known, the pairing is Xy_p.

Acknowledgements

We thank the School of Biological Sciences, Royal Holloway, and the Oxford University Museum of Natural History, Oxford, for the opportunity to carry out this research.

References

Angus, R. B., Wilson, C. J. & D. J. Mann, 2004. Chromosomal and aedeagal distinction between *Aphodius* (*Labarrus*) *lividus* Olivier, 1799 and *A.* (*L.*) *pseudolividus* Balthasar, 1941 (Col., Scarabaeidae, Aphodiinae). – Entomologist's monthly Magazine 140: 255–261.

Angus, R. B., Brown, R. E. & L. J. Bryant, 2000. Chromosomes and identification of the sibling species *Pterostichus nigrita* (Paykull) and *P. rhaeticus* Heer (Coleoptera: Carabidae). – Systematic Entomology 25: 325–337.

Bisoi, M. R. & S. C. Patnaik, 1991. Karyological studies in seven species of Coleoptera (Scarabaeidae) from India. – Entomon 16 (2): 129–137.

Colomba, M. S., Monteresino, E., Vitturi, R. & M. Zunino, 1996. Characterization of mitotic chromosomes of the scarab beetles *Glyphoderus sterquilinus* (Westwood) and *Bubas bison* (L.) (Coleoptera: Scarabaeidae) using conventional and banding techniques. – Biologisches Zentralblätter 115: 58–70.

Colomba, M. S., Vitturi, R. & M. Zunino, 2000. Karyotype analysis, banding, and fluorescent *in situ* hybridisation in the scarab beetle *Gymnopleurus sturmi* McLeay (Coleoptera Scarabaeoidea: Scarabaeidae). – Journal of Heredity 91 (3): 260–264.

Dasgupta, J., 1963. The cytology of *Cybister limbatus*, *Gymnopleurus koenigii*, *Aulacophora intermedia*, and *Alcides* sp. (Insecta: Coleoptera). – Proceedings of the Zoological Society 16: 123–134.

Ebied, A. M., Mostafa, F. M., Yaseen, A. E. & L. S. Kawashti, 2000. Studies on the chromosomal analysis of nine Egyptian species of families Tenebrionidae and Scarabaeoidea (Coleoptera: Insecta.) – Journal of the Egyptian German Society of Zoology 33 (C): 295–315.

John, B. & K. R. Lewis, 1960. Nucleolar controlled segregation of the sex chromosomes in beetles. – Heredity 15: 431–439.

Joneja, M. G., 1960. Chromosome number and sex-determining mechanism in twenty-five species of Indian Coleoptera. – Research Bulletin (N. S.) of the Punjab University 11 (3 – 4): 249–251.

Juan, C., Pons, J. & E. Petitpierre, 1993. Localization of tandemly repeated DNA in beetle chromosomes by fluorescent *in situ* hybridization. – Chromosome Research 1: 167–174.

Kacker, R. K.,1971. Cytology and cytotaxonomy of Indian Coleoptera: Studies on the chromosomes of thirty-one species of Indian Coleoptera. Ph. D. thesis, Banaras Hindu University, Varanasi, India. (Not seen: cited by Smith & Virkki, 1978.)

Löbl, I. & A. Smetana (eds), 2006. Catalogue of Palaearctic Coleoptera. 3: Scarabaeoidea, Scirtoidea, Dascilloidea, Buprestoidea and Byrrhoidea. Apollo Books, Strenstrup. 690pp.

Manna, G. K. & M. Lahiri, 1972. Chromosome complement and meiosis in forty-six species of Coleoptera. – Chromosome Information Service 13: 9–11.

Paris Conference, 1971. Standardization in Human Cytogenetics. Birth Defects: Original Article Series 8: 7, 1972. The National Foundation, New York. (Reprinted in Cytogenetics 11: 313–362 (1972).

Powell, J. E. & R. B. Angus, 2006. A chromosomal investigation of some European species of Haliplidae (Coleoptera). – Memorie della Società entomologica italiana 85: 173–185.

Salamanna, G., 1966. Sui cromosomi di *Pentodon punctatus* Vill., *Copris hispanus* L. e *Geotrupes intermedius* Costa. (Coleoptera, Scarabaeidae). – Atti del VI Congresso Nazionale di Entomologia, Padova 11 – 14 settembre 1965: 83–84.

Salamanna, G., 1972. Aspetti della cariologia degli Scarabaeidae. – Atti del Congres so Nazionale di Entomologia, 1971: 313–324, Figs 1–16.

Smith, S. G., 1950. The cyto-taxonomy of Coleoptera. – Canadian Entomologist 82: 58–68.

Smith, S. G. & N. Virkki, 1978. Animal Cytogenetics 3: Insecta 5: Coleoptera. – Gebrüder Borntraeger, Berlin – Stuttgart. x + 366 pp.

Sumner, A. T., Evans, H. J. & R. A. Buckland, 1971. New technique for distinguishing between human chromosomes. – Nature New Biology 232: 31–32.

Virkki, N., 1951. Zur Zytologie einiger Scarabaeiden (Coleoptera). Studien an der Spermatogenese. – Annales Zoologici Societatis Zoologicae Botanicae Fennicae "Vanamo" 14 (3): vi + 1–105, 2 plates.

Virkki, N., 1954. Weitere Spermatogenesestudien an Skarabäiden (Coleoptera). – Annales Academiae Scientarum Fennicae A. IV. 25: 1–58.

Wilson, C. J. 2001. *Aphodius pedellus* (DeGeer), a species distinct from *A. fimetarius* (Linnaeus) (Coleoptera: Aphodiidae). – Tijdschrift voor Entomologie 144: 137–143.

Wilson, C. J., 2002. Chromosomal studies on dung beetles (Coleoptera: Scarabaeoidea). – University of London Ph.D. thesis. 230 pp.

Wilson, C. J. & R. B. Angus, 2005. A chromosomal analysis of 21 species of Oniticellini and Onthophagini (Coleoptera: Scarabaeidae). – Tijdschrift voor Entomologie 148: 63–76.

Received: 15 January 2007
Accepted: 12 March 2007