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A CHROMOSOMAL ANALYSIS OF 21 SPECIES OF ONITICELLINI AND ONTHOPHAGINI (COLEOPTERA: SCARABAEIDAE)

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The karyotypes of 2 species of Oniticellini and 19 species of Onthophagini are described and illustrated. *Euonthophagus pallipes, Caccobius schreberi, Euonthophagus amyntas, E. atramentarius, Onthophagus taurus, O. hirtus, O. furcatus, O. stylocerus, O. vacca, O. similis, O. opacicollis, O. coenobita, O. lucidus, O. ovatus, O. joannae, O. ruficapillus, O. gazella* and *O. albicornis* all have karyotypes with 2N = 18 + Xy. Only female karyotypes have been obtained for *Euoniticellus fulvus, Onthophagus illyricus* and *O. maki*. These species have 2N = 20 chromosomes, including the unidentified sex chromosomes. B-chromosomes have been found in *Euonthophagus amyntas, Onthophagus vacca, O. similis* and *O. gazella*, and chromosome polymorphisms resulting from pericentric inversions have been found in *Onthophagus vacca* and *O. albicornis*. Centromeric C-bands, of varying sizes, are present in nearly all the chromosomes, and the species of *Euoniticellus* and *Euonthophagus* have some chromosomes with a totally heterochromatic arm, a feature not encountered in the *Onthophagus* species. The three species of the *Onthophagus ovatus* group have karyotypes clearly distinct from one another, as do *O. similis* and *O. opacicollis*. However, no difference has been found between the karyotypes of *O. taurus* and *O. illyricus*.

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Key words. – Chromosomes; karyotypes; C-banding; B-chromosomes; *Euoniticellus*; *Caccobius*; *Onthophagus*; Scarabaeidae.

In the course of research for a Ph.D. degree (Wilson 2002) chromosomal data on 18 species of *Euoniticellus*, *Caccobius*, *Euonthophagus* and *Onthophagus* were accumulated, and continuing investigations by R. B. Angus have since added a further three *Onthophagus* species to the list. There is at present no published information on the chromosomes of ten of the species, and where information is available it very seldom includes karyotypes, the usual information being the number of chromosomes and/or the meioformula (the arrangement of the chromosomes at first metaphase of meiosis). The material presented here therefore provides a first opportunity for a comparison of the karyotypes of a suite of small scarabaeid dung beetles.

Material and methods

A list of beetle species from which chromosome preparations have been obtained, and their localities of origin is given in table 1. British localities are referred to their Watsonian Vice-Counties, as set out by Dandy (1969). French localities are listed by Département, written in full. Spanish localities are listed by Provincia, written in full, as are provinces in Cyprus (Eparkhia) and the Dominican Republic (Provincias). The number of specimens from which successful preparations were obtained is given in parentheses after the list of localities for each species. The methods of chromosome preparation and curation of the material are given by Wilson (2001). Relative Chromosome Length (RCL) is the length of each chromosome expressed as a percentage of the total haploid autosome length in the nucleus. It is used, without statistical analysis, to give an account of the approximate sequence of chromosome lengths for the different species. Where only females are available the sex chromosomes cannot be identified, so total haploid autosome length is not available. In these cases a 'standard length' (SL) is used, using the total

Table 1. Material studied and taxonomy.

Scientific name	Localities (number of specimens analysed)
Oniticellini	
Euoniticellus Janssens, 1953	
E. fulvus (Goeze, 1777)	FRANCE. Indre-et-Loire: Souvigné; Indre: La Brenne. (3 specimens.)
E. pallipes (Fabricius, 1881)	CYPRUS. Limassol: Akrotiri, Fassouri. (3 specimens.)
Onthophagini	
Caccobius Thomson, 1859	
C. schreberi (Linnaeus, 1758)	SPAIN. Cáceres: Abadía. (2 specimens.)
Euonthophagus Balthasar, 1959	
E. amyntas (Olivier, 1789)	SPAIN. Cáceres: Abadía. (1 specimen.)
E. atramentarius (Ménétriès, 1832)	CYPRUS. Pafos: Anadhiou; Agia Varvara. (7 specimens.)
Onthophagus Latreille, 1802	
subgenus *Onthophagus* s. str.	
O. taurus (Schreber, 1759)	FRANCE. Indre: La Brenne. SPAIN. Málaga: Torrox. (5 specimens.)
O. illyricus (Scopoli, 1763)	CYPRUS. Limassol: Akrotiri, Fassouri. (3 specimens.)
subgenus *Trichonthophagus* Zunino, 1979	
O. hirtus (Illiger, 1803)	SPAIN. Cádiz: Tarifa. (3 specimens.)
O. maki (Illiger, 1803)	SPAIN. Cádiz: La Linea. (1 specimen.)
subgenus *Furconthophagus* Zunino, 1979	
O. furcatus (Fabricius, 1781)	SPAIN. Cádiz: Tarifa. (1 specimen.)
Subgenus *Palaeonthophagus* Zunino, 1979	
O. stylocerus Graëlls, 1851	SPAIN. León: Sanabria. Cantabria: Corconte. (2 specimens.)
O. vacca (Linnaeus, 1767)	ENGLAND. East Sussex: Rye; Middlesex: Staines Moor. FRANCE. Indre: La Brenne; Maine-et-Loire: Le Lion d'Angers. SPAIN. Cantabria: Corconte. (8 specimens.)
O. similis (Scriba, 1790)	ENGLAND. North Devon: Ilfracombe; East Sussex: Rye; Surrey: Ockham Common; Worcestershire: Wyre Forest. (8 specimens.)
O. opacicollis Reitter, 1893	CYPRUS. Pafos: Pafos; Kholetria; Agia Varvara; Anadhiou; Cape Drepanon. (10 specimens.)
O. coenobita (Herbst, 1783)	ENGLAND. East Kent: Betteshanger; Berkshire: Old Windsor; Windsor Deer Park; Worcestershire: Wyre Forest. (3 specimens.)
O. lucidus (Sturm, 1800)	CYPRUS. Pafos: Anadhiou. (3 specimens).
The *O. ovatus* group	
O. ovatus (Linnaeus, 1767)	FRANCE. Indre: La Brenne; Loir-et-Cher: Pinail. (8 specimens).
O. joannae Goljan, 1953	ENGLAND. East Kent: Lydden; Surrey: Box Hill. (7 specimens.)
O. ruficapillus Brullé, 1832	CYPRUS. Limassol: Akrotiri, Fassouri. (4 specimens.)
subgenus *Digitonthophagus* Balthasar, 1959	
O. gazella (Fabricius, 1787)	DOMINICAN REPUBLIC. Espaillat: Las Flores near Santa Maria; Maria Trinidad Sanchez: Genebra near the Rio Boba; Samana: El Limón. (8 specimens.)
not attributed to a subgenus	
O. albicornis (Beauvois, 1805)	DOMINICAN REPUBLIC. Espaillat: Las Flores near Santa Maria. (5 specimens.)

haploid chromosome (not autosome) length in the nucleus. In practice, the figures obtained would be only slightly different from RCL values. Centromere Index (CI), the length of the short arm as a percentage of the total length of the chromosome is used as a measure of the location of the centromere. In most cases the calculated CIs are not given, but instead standard terms denoting centromere position. Based on Sumner (2003) these may be defined as follows: metacentric – CI 46 – 50; submetacentric – CI 26 – 45; subacrocentric – CI 16 – 25; acrocentric – CI 3 – 15. Unless otherwise stated in the figure captions, all the karyotypes illustrated were obtained from midgut cells.

Fig. 1. Mitotic chromosomes of *Euoniticellus*, *Caccobius* and *Euonthophagus*, arranged as karyotypes.– a, *Euoniticellus fulvus*, ♀, La Brenne; b, *E. fulvus*, ♀, La Brenne, C-banded; c, *E. pallipes*, ♂, Fassouri; d, *Caccobius schreberi*, ♂, testis, Abadía; e, f, *Euonthophagus amyntas*, ♂, Abadía, e plain, with one B-chromosome, f with some spontaneous C-banding and with one replicate of autosomes 4 and 5 missing; g, *E. atramentarius*, ♂, Anadhiou, with one replicate of autosome 5 missing; h, *E. atramentarius*, ♂, Anadhiou, with one replicate of autosomes 6 and 9 missing.

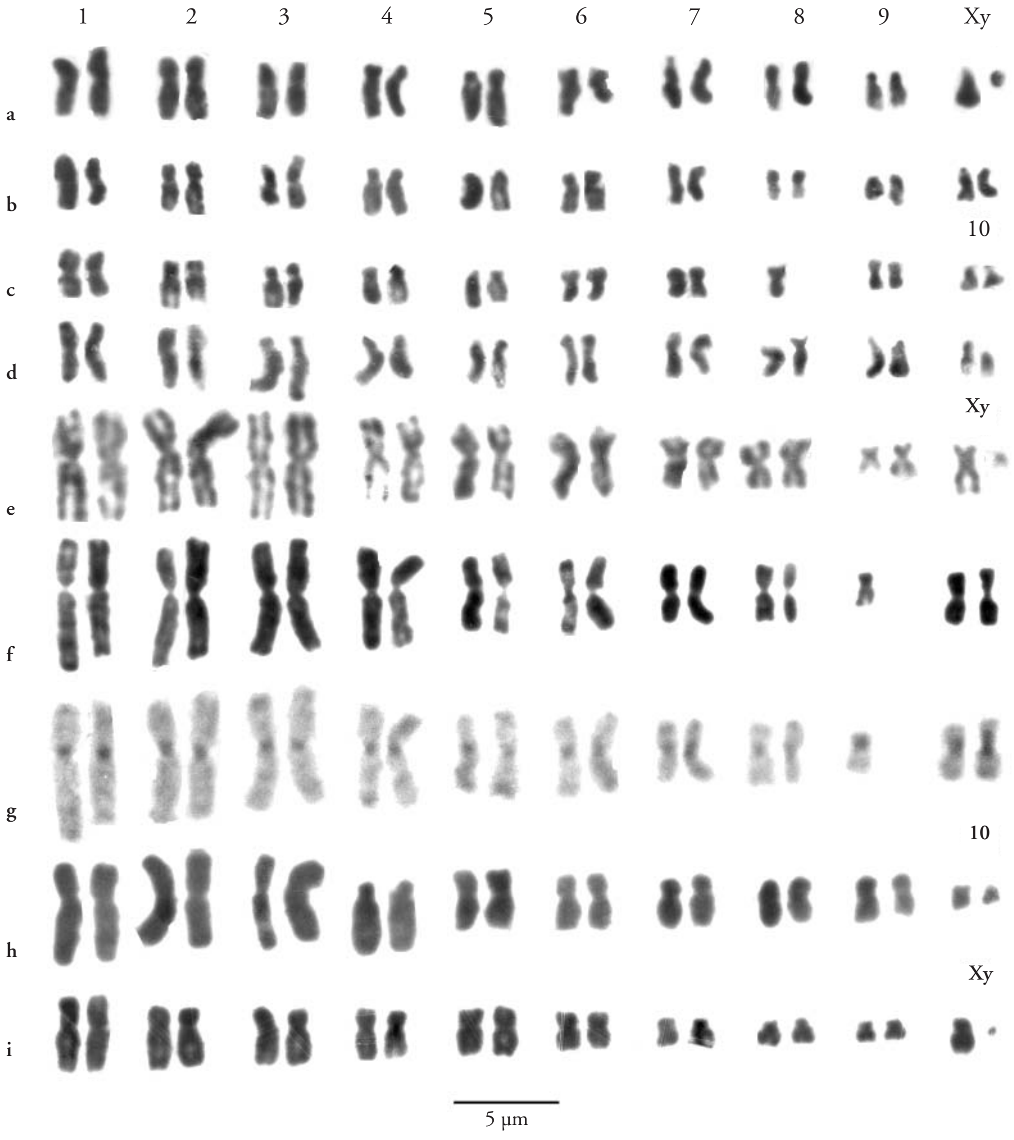


Fig. 2. Mitotic chromosomes of *Onthophagus* s. str., *O.* (*Trichonthophagus*) and *O.* (*Furconthophagus*), arranged as karyotypes. – a, *Onthophagus taurus*, ♂, Torrox; b, *O. taurus*, ♀, La Brenne; c, d, *O. illyricus*, ♀, Fassouri; e, *O. hirtus*, ♂, Tarifa; f, g, *O. hirtus*, ♀, Tarifa, e plain, f the same nucleus C-banded. One replicate of chromosome 9 is missing; h, *O. maki*, ♀, La Linea; i, *O. furcatus*, ♂, Tarifa.

Results

Oniticellini

Euoniticellus fulvus
(figs. 1a, b)

Published information: 10 bivalents (♂) (Virkki 1954). 2N = 20 (♀). The only successful preparations were from females. The standard lengths of the chromosomes range from about 16 to 5. Chromosomes 1, 3 and 5 – 7 are more or less metacentric, 2 and 8 are subacrocentric, and the rest are more or less acrocentric. C-banding (fig. 1b) shows strong centromeric C-bands on all the chromosomes, and chromosome 6 has a heterochromatic long arm.

Euoniticellus pallipes
(fig. 1c)

Published information: 2N = 20, meioformula = 9 bivalents + Xy_p (Yadav et al. 1979). 2N = 18 + Xy. The rcls of the autosomes range from about 21 to 3, with a sharp size decrease between autosomes 3 (rcl about 18) and 4 (rcl about 12) and between autosomes 4 and 5 (rcl about 8). The X chromosome (rcl about 5) is slightly larger than autosome 9 and the y chromosome is dot-like, possibly acrocentric, and about half the size of autosome 9. Autosomes 1-4, and 7 are metacentric, 5, 6 and 8 are subacrocentric, and 9 and the X chromosome are acrocentric. No C-banded preparation is available, but the short arm of autosome 2 consistently shows the chromatids held close together, and has gaps along its length – not always in the same place. It is almost certainly heterochromatic.

Onthophagini

Caccobius schreberi
(fig. 1d)

Published information: 2N = 20 (♂), meioformula = 9 bivalents + Xy_p (Virkki 1954). 2N = 18 + Xy_p. The rcls of the autosomes range from about 16 to 7, with a sharp decrease in size between autosomes 5 (rcl about 12) and 6 (rcl about 8). The X chromosome (rcl about 7.5) is similar in length to autosomes 6 and 7. The y chromosome is dot-like. Autosomes 1 – 5, and 7 and 9 are more or less metacentric, autosome 6 is submetacentric, and autosome 8 and the X chromosome are acrocentric. The preparation shown in fig. 1d is from testis, so the chromatids appear closely applied to one another throughout their lengths. However, partial karyotypes from mid-gut cells showed no arms with the chromatids close together, and no C-banded preparation is available. There do not appear to be any chromosomes with heterochromatic arms in this species. First metaphase of meiosis (not shown) shows the sex bivalent as a normal Xy_p.

Euonthophagus amyntas
(figs. 1e, f)

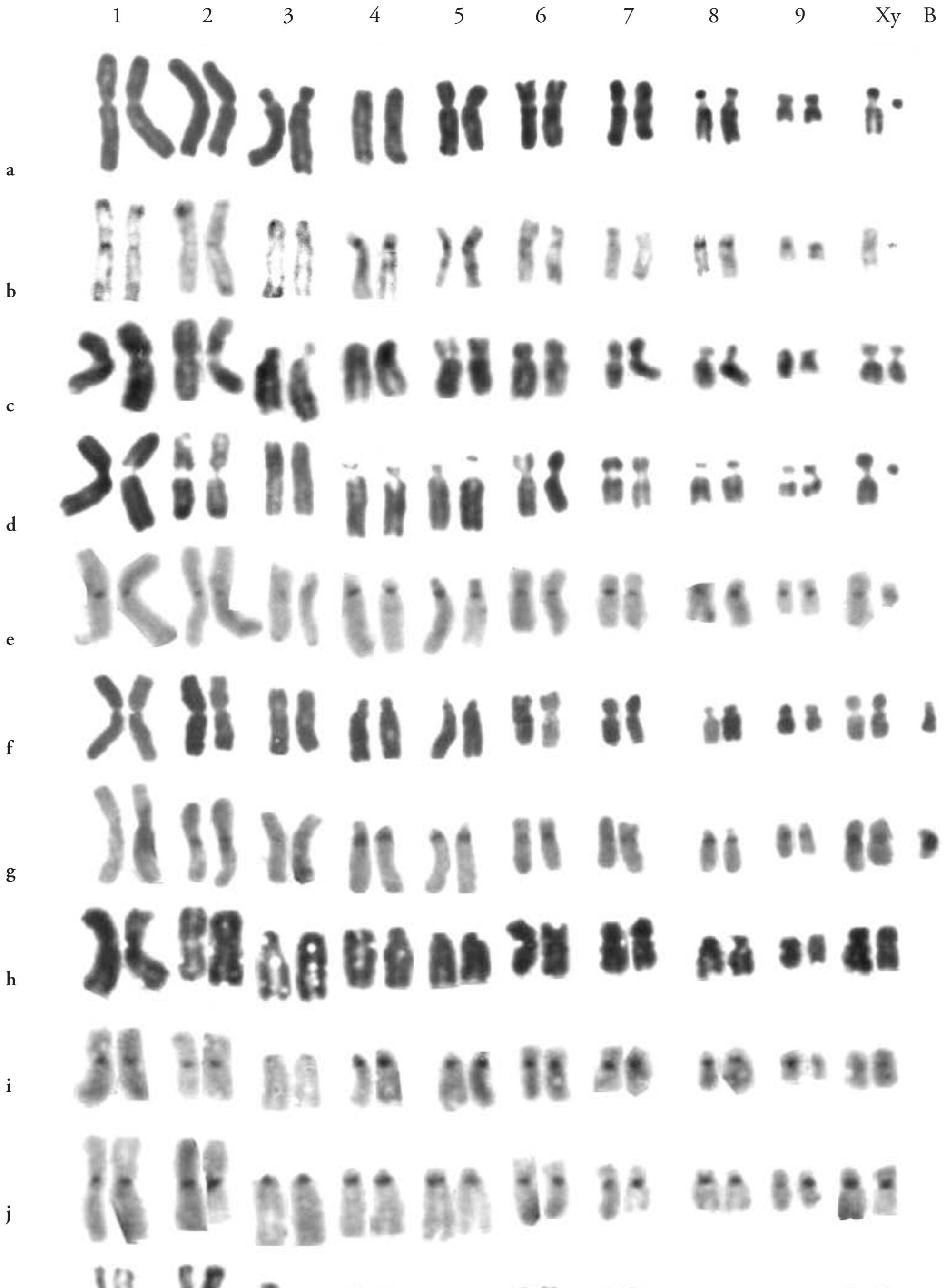
Published information: 2N = 20 (♂) (Virkki 1951). 2N = 18 + Xy + B-chromosomes. The rcls of the autosomes range from about 15 to 6, and the X chromosome (rcl about 9) is about as long as autosome 6. The y chromosome appears acrocentric, and is about a third as long as autosome 9 (rcl about 3). The B-chromosome, not present in all cells from which chromosomes were obtained, looks similar to the y. Autosomes 1 – 5, and 9, and the X chromosome are more or less metacentric, and autosomes 6 – 8 are submetacentric. The karyotype shown in fig. 1f shows some spontaneous C-banding, sufficient to show that autosomes 4, 5 and 8, and the X chromosome, have heterochromatic long arms.

Euonthophagus atramentarius
(figs. 1g, h)

Published information: none. 2N = 18 + Xy. The rcls of the autosomes range from about 15 to 6, with a sharp size decrease between autosome 5 (rcl about 12) and 6 (rcl about 9). The X chromosome (rcl about 9) is about as long as autosome 6. The y chromosome is dot-like. Autosomes 1 – 3, and 9 are more or less metacentric, autosomes 4 – 7 are more or less submetacentric, and autosome 8 is acrocentric. The short arms of autosomes 2 and 3 have secondary constrictions (fig. 1h) and appear heterochromatic in attempted C-banding of partial karyotypes.

Onthophagus (Onthophagus) taurus
(figs. 2a, b)

Published information: 2N = 20 (♂), meioformula = 9 bivalents + Xy (Virkki 1951), 2N = 20, 9 bivalents + Xy_p (Yadav et al. 1979). 2N = 18 + Xy. The rcls of the chromosomes range from about 13 to 6. Chromosomes 1, 3, 7 and the X chromosome are more or less metacentric, 2, 4 and 6 are subacrocentric, and 5 and 9 are acrocentric. The X chromosome has a secondary constriction near the apex of its shorter arm.


1
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8
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Xy
B
a
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e
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g
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i
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5 µm

Onthophagus (Onthophagus) illyricus
(figs. 2c, d)

Published information: Meioformula = 9 bivalents + Xy (♂) (Virkki 1954). 2N = 20 (♀). The karyotype is very similar to that of *O. taurus*, with the sequence of standard lengths very similar to that of the rcl values for *O. taurus*. If chromosome 9 of *O. illyricus* is the X chromosome, then there are no clear differences between the karyotypes of the two species.

Onthophagus (Trichonthophagus) hirtus
(figs. 2e-g)

Published information: none. 2N = 18 +Xy. The rcls of the chromosomes range from about 15 – 4, with abrupt size changes between chromosomes 3 (rcl about 14), 4 (sl about 12) and 5 (sl about 9), and between chromosomes 8 (rcl about 7) and 9 (rcl about 4). The chromosomes are all submetacentric. C-banding (fig. 2f) shows moderate centromeric C-bands on all the chromosomes except the X chromosome, which has a larger and stronger band. One replicate of chromosome 9 is absent from the female preparation.

Onthophagus (Trichonthophagus) maki
(fig. 2h)

Published information: none. 2N = 20 (♀). Only female material is available, so the sex chromosomes cannot be identified and sl values are used for this karyotype. The sequence of sl values is similar to the rcls shown by *O. hirtus*: chromosomes 1-3 have sl values of about 15, chromosomes 4-9 have sl values decreasing fairly regularly from about 10-7, and the sl value of chromosome 10 is about 4. Chromosome 4 is acrocentric, but all the others are submetacentric. No C-banded preparation is available.

Onthophagus (Furconthophagus) furcatus
(fig. 2i)

Published information: Meioformula = 9 bivalents + Xy (Virkki 1954). The rcls of the autosomes range from about 19-5, with an abrupt drop between autosomes 1 (rcl about 19) and 2 (rcl about 16). The X chromosome (rcl about 8.5) is similar in size to autosome 6. Autosomes 1 and 3 – 6 are clearly submetacentric, while autosomes 2, 7 and 8, and the X chromosome are borderline submetacentric/subacrocentric. Autosome 9 is acrocentric and the y chromosome is dot-like.

Onthophagus (Palaeonthophagus) stylocerus
(figs. 3a-c)

Published information: none. 2N = 18 + Xy. The rcls of the chromosomes range from about 16 to 5. Autosomes 1 and 2 are more or less metacentric, 5 and 7 are submetacentric, 3, 6, 8, 9 and the X chromosome are subacrocentric, and autosome 4 is acrocentric. Autosome 3 has a secondary constriction in its short arm. C-banding (fig. 3b) shows small centromeric C-bands, virtually nonexistent on autosome 3 and strongest on autosome 8.

Onthophagus (Palaeonthophagus) vacca
(figs. 3d-k)

Published information: 2N = 20 (♂) (Virkki 1951, 1954). 2N = 18 + Xy + B-chromosomes. The rcls of the autosomes range from about 17 to 5, with a sharp decrease in size between autosome 8 (rcl about 9) and autosome 9 (rcl about 5). The X chromosome (rcl about 8) is about as long as autosome 8 and the y is a small acrocentric, rcl about 4. The B-chromosome is subacrocentric and about the same size as autosome 9 (rcl about 5). Autosomes 1 and 2 are long metacentrics, sharply distinct from all the other chromosomes. Autosome 3 is polymorphic, either submetacentric or more or less acrocentric. Autosomes 5, 6 and 9 are submetacentric, autosome 4 is acrocentric, but with a secondary constriction just above the centromere, autosomes 3 and 8 are more or less acrocentric, and 7 is subacrocentric. C-banding (figs. 3e, g, i, j) shows small centromeric C-bands on all the autosomes and the X chromosome, and a large C-band on the B-chromosome. The C-band on the submetacentric form of autosome 3 is very small and faint (figs. 3e, g), but in the acrocentric form the C-band may be either as weak as in the submetacentric (fig. 3i), or distinctly bolder (fig. 3j). There is no C-banded y chromosome available for study. The material from

Fig. 3. Mitotic chromosomes of *O.* (*Palaeonthophagus*) *stylocerus* and *O.* (*P.*) *vacca*, arranged as karyotypes. a – c, *O. stylocerus*, d – k, *O. vacca*. – a, b, ♂, Corconte, a plain, b C-banded, from the same specimen; c, ♀, Sanabria; d, e, ♂, Corconte, autosome 3 homozygous submetacentric, d plain, e C-banded, from the same specimen; f, g, ♀ with one B-chromosome, Rye Harbour, autosome 3 homozygous submetacentric, f plain, g C-banded, from the same specimen; h, i, ♀, Staines Moor, autosome 3 homozygous acrocentric, h plain, i C-banded, from the same specimen; j, ♀, Lion d'Angers, C-banded, autosome 3 homozygous acrocentric; k, ♀, Lion d'Angers, autosome 3 heterozygous.

Fig. 4. Mitotic chromosomes of subgenus *Palaeonthophagus*, part, arranged as karyotypes. – a, *Onthophagus similis*, ♂, La Brenne, with one B-chromosome; b, *O. similis*, ♂, Wyre Forest, C-banded; c, *O. similis*, ♀, La Brenne, C-banded; d, e, *O. opacicollis*, ♂, Cape Drepanon, d plain, e the same nucleus, C-banded; f, *O. opacicollis*, ♀, Pafos; g, *O. coenobita*, ♂, Betteshanger; h, i, *O. coenobita*, ♀, Wyre Forest, h plain, i C-banded, from the same specimen; j, k, *O. lucidus*, ♂, Anadhiou, j plain, k C-banded, from the same specimen.

Staines Moor is homozygous for the weakly C-banded acrocentric form of autosome 2, while the specimens from Corconte, Rye Harbour and La Brenne are homozygous for the submetacentric. The two specimens from Le Lion d'Angers include one heterozygote and one homozygous for the strongly C-banded acrocentric form. The B-chromosome was found in one female from Rye Harbour, and a single B was present in all cells from which chromosomes were obtained.

Onthophagus (Palaeonthophagus) similis
(figs. 4a-c)

Published information: none. 2N = 18 + Xy + B-chromosomes. The rcls of the autosomes range from about 16 to 5.5, and the X chromosome (rcl about 9.5) is about as long as autosome 7. The y chromosome is small (rcl about 2) and dot-like. The B-chromosome is metacentric, about twice the size of the y (rcl about 4.2). Autosomes 1 – 3, 6 and 9 are metacentric, 4 and the X chromosome are submetacentric, and 5, 7 and 8 are subacrocentric. C-banding (figs. 4b, c) shows distinct centromeric C-bands on all the chromosomes except the y, where there may be a faint band. The X chromosome has a secondary constriction on its short arm. The B-chromosome was found in only one specimen, and was present in all the cells from which chromosomes were obtained.

Onthophagus (Palaeonthophagus) opacicollis
(figs. 4d-f)

Published information: none. 2N = 18 + Xy. The rcls of the autosomes range from about 16 to 6, and the X chromosome (rcl about 10) is intermediate in length between autosomes 5 and 6. The y chromosome is a small submetacentric, rcl about 4. Autosomes 1-4 and 6, and the X chromosome are more or less metacentric, 5, 7 and 8 are submetacentric, and 9 is subacrocentric. C-banding (fig. 4e) shows distinct centromeric C-bands on all the chromosomes including the y. The X chromosome has a secondary constriction in its short arm and the C-band extends over most of this arm as well as the basal part of the long arm, so that at least half the chromosome is heterochromatic.

Onthophagus (Palaeonthophagus) coenobita
(figs. 4g-i)

Published information: none. 2N = 18 + Xy. The rcls of the autosomes range from about 18 to 4, with the X chromosome (rcl about 6) similar in size to autosome 8. The y chromosome (rcl about 3.5) is a small acrocentric. Autosomes 1 and 6 – 9 are acrocentric, 2, 4, and the X chromosome are subacrocentric, and 3 and 5 are submetacentric. Autosome 2 has a secondary constriction in its short arm. C-banding (fig. 4i) shows centromeric C-bands on all the autosomes and the X chromosome. No C-banded y chromosome is available for study. Autosome 4 in unbanded preparations has a gap in the middle of its long arm, and in C-banded preparations this gap appears to have a darker region on each side.

Onthophagus (Palaeonthophagus) lucidus
(figs. 4j, k)

Published information: none. 2N = 18 + Xy. The rcls of the autosomes range from about 16 to 6. The X chromosome (rcl about 8) is about the same size as autosome 8 and the y chromosome is a small metacentric, rcl about 4. Autosomes 1 and 5-8, and the X chromosome are submetacentric, autosomes 2, 3 and 9 are subacrocentric, and autosome 4 is acrocentric. C-banding (fig. 4k) shows strong centromeric C-bands on all the chromosomes, including the y.

Onthophagus (Palaeonthophagus) ovatus
(figs. 5a-c)

Published information: 2N = 20 (♂) (Virkki 1954). 2N = 18 + Xy. The rcls of the autosomes range from about 16 to 6, and the X chromosome (rcl about 8.5) is intermediate in length between autosomes 7 and 8. Autosomes 1-3 are more or less metacentric, 4-7, and the X chromosome, are submetacentric, and 8 and 9 are subacrocentric. The y chromosome is small, dot-like. C-banding (fig. 5b) shows strong localised centromeric C-bands on all the chromosomes, including the y, and less darkly staining secondary constrictions on the short arms of autosomes 4 and 5, and possibly on both arms of autosome 7.

Onthophagus (Palaeonthophagus) joannae
(figs. 5d, e)

Published information: none. 2N = 18 + Xy. The rcls of the autosomes range from about 17 to 7, and the X chromosome (rcl about 8) is intermediate in length between autosomes 8 and 9. The y chromosome is small, dot-like. Autosomes 1-4, 6, 9 and the X chromosome are submetacentric, and 5, 7 and 8 are subacrocentric. Autosome 2 has a secondary constriction in its short arm. C-banding (fig. 5e) shows strong but localised centromeric C-bands on all the chromosomes except the very small y chromosome. Autosome 2 appears more darkly staining than the others.

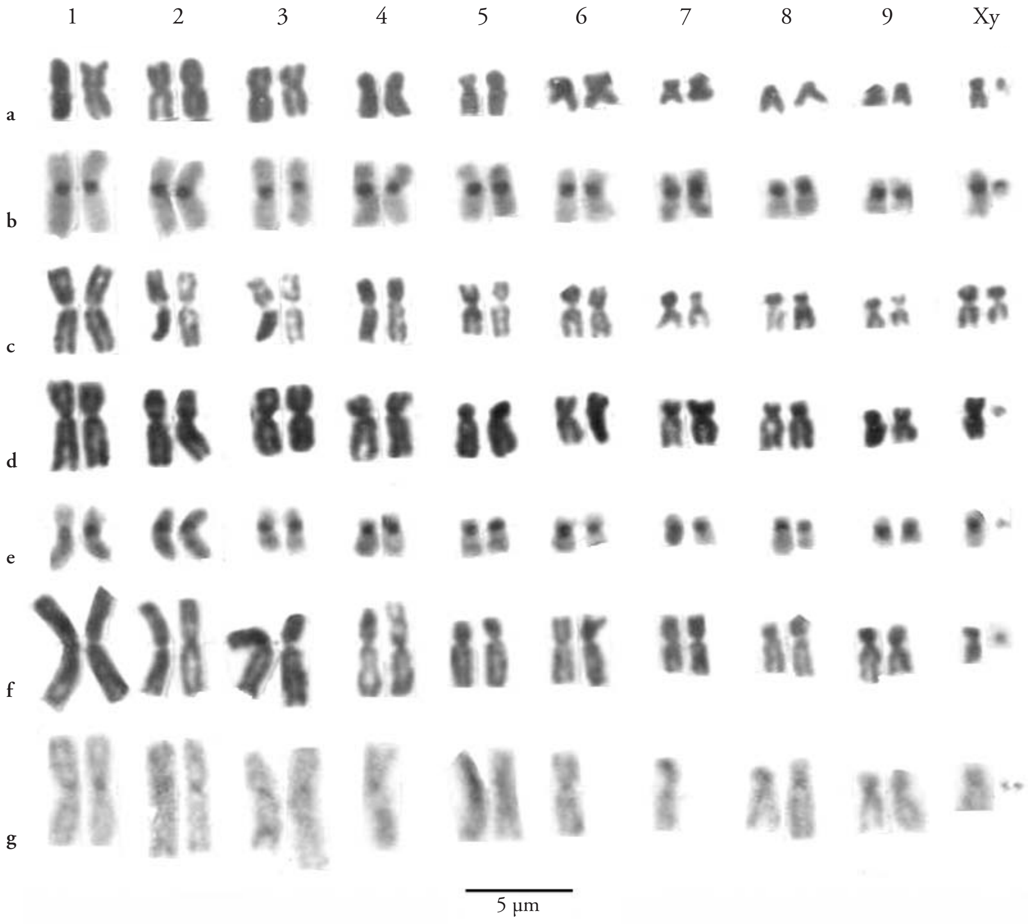

Fig. 5. Mitotic chromosomes of subgenus *Palaeonthophagus*, the *O. ovatus* group, arranged as karyotypes. a, b, *O. ovatus*, ♂, La Brenne, a, plain, b, C-banded, from the same specimen; c, *O. ovatus*, ♀, La Brenne; d, e, *O. joannae*, ♂, Box Hill, d plain, e C-banded; f, g, *O. ruficapillus*, ♂, Fassouri, f plain, g C-banded, from the same specimen, with one replicate of autosomes 4 and 7 missing.

Onthophagus (Palaeonthophagus) ruficapillus
(figs. 5f, g)

Published information: 2N = 20 (♂), meioformula = 9 bivalents + Xy (Virkki 1951). 2N = 18 + Xy. The RCLs of the autosomes range from about 17 to 7.5, and the X chromosome (RCL about 5.5) is smaller than any of the autosomes. The y chromosome is small, dot-like. Autosomes 1 and 2 are metacentric, 3, 4, 6 and 7, and the X chromosome, are submetacentric. Autosomes 5, 8 and 9 are subacrocentric. C-banding has only been partly successful, but indicates very small centromeric C-bands. There appear to be secondary constrictions in the short arms of autosomes 4 and 8, but they are not very clear in the present material.

Onthophagus (Digitonthophagus) gazella
(figs. 6a-c)

Published information: 2N = 20 (♂), meioformula = 9 bivalents + Xy_r (Joneja 1960), 20, 9 bivalents + Xy_p or Xy_r (Kacker 1970), 20 (♂), 9 bivalents + Xy_p (Manna & Lahiri 1972; Bisoi & Patnaik 1991). 2N = 18 + Xy + B-chromosomes. The RCLs of the autosomes range from about 14 to 8.5, and the X chromosome (RCL about 11) is about the same size as autosome 5. The y chromosome is dot-like, RCL about 5,

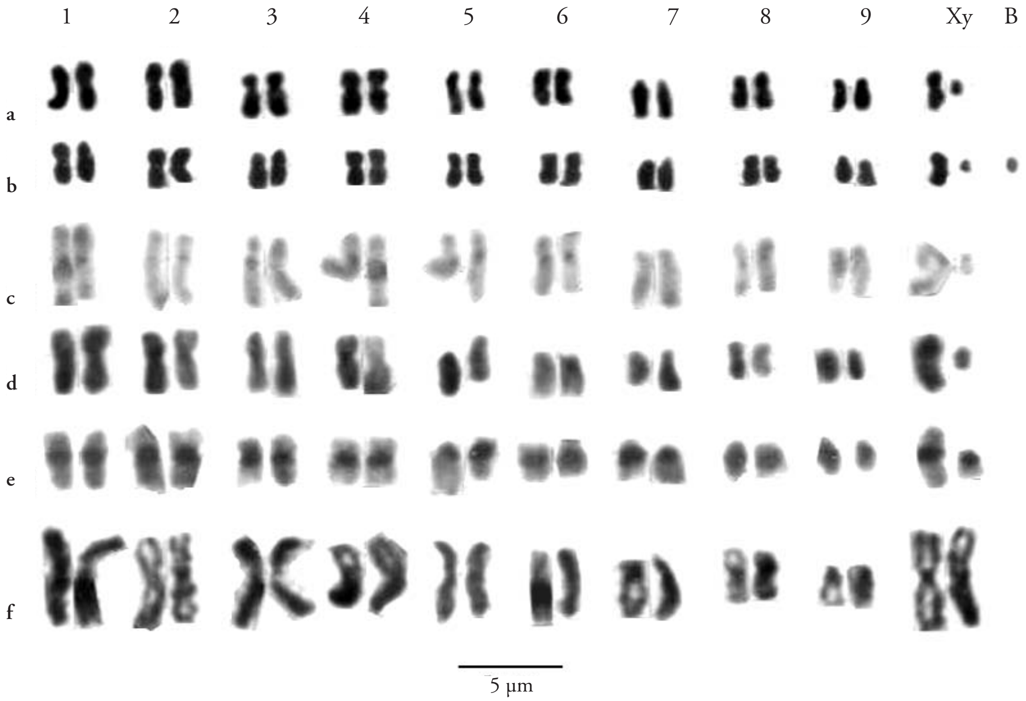

Fig. 6. Mitotic chromosomes of *Onthophagus gazella* and *O. albicornis*, arranged as karyotypes. – a, b, *O. gazella*, ♂, Las Flores, b with one B-chromosome; c, *O. gazella*, ♂, Dominican Republic, C-banded; d, e, *O. albicornis*, ♂, Las Flores, autosome 5 heterozygous metacentric/acrocentric, d plain, e C-banded, from the same specimen; f, *O. albicornis*, ♀, Las Flores, autosome 5 homozygous metacentric.

and the B-chromosome, also dot-like, is slightly larger than the y. Autosomes 1, 2, 4 and 6, and the X chromosome, are more or less metacentric. Autosomes 3, 5 and 8 are submetacentric, and autosomes 7 and 9 are more or less acrocentric. C-banding is not well displayed in the preparation obtained (fig. 6c), but indicates small centromeric C-bands on all the autosomes and the X chromosome, with a hint of a terminal C-band on the y chromosome. The B-chromosome was found in one male from Las Flores, and was present in all the cells where chromosomes were seen.

Onthophagus albicornis
(figs. 6d-f)

Published information: none. 2N = 18 + Xy. The rcls of the autosomes range from about 15 to 7, while the sex chromosomes are a large metacentric X (rcl about 15, as large as autosome 1) and a small more or less metacentric y, rcl about 6. Autosomes 1-4 and 8 are metacentric, autosome 5 is polymorphic, either metacentric or acrocentric, autosome 6 is subacrocentric and autosomes 7 and 9 are more or less acrocentric. C-banding (fig. 6e) shows quite large centromeric C-bands, with some size variation, on all the chromosomes.

Discussion

The karyotypes of the two *Euoniticellus* species are clearly different from one another in their sequences of rcls, with the pronounced difference between the longer and shorter autosome groups of *E. pallipes* and the more even decrease in *E. fulvus*. In both species some of the chromosomes have arms which are entirely heterochromatic.

The material of the tribe Onthophagini comprises one species of *Caccobius*, two *Euonthophagus*, and 16 *Onthophagus*. The karyotype of *Caccobius schreberi* conforms to the normal Scarabaeid pattern with 18 autosomes and Xy_p sex chromosomes, and no chromosome has heterochromatic arms. The two *Euonthophagus* species both have heterochromatic arms, a feature not encountered in any of the *Onthophagus* species studied here. There are clear differences between the karyotypes of *Euonthophagus amyntas* and *E. atramentarius*. Thus autosomes 2 and 3 of *E. atramentarius* have secondary constrictions on their short arms, but in *E. amyntas* there is no trace of these. Autosome 4 appears more or less metacentric in *E. amyntas*, but in *E. atramentarius* it is submetacentric. Autosomes 6-9 are distinctly shorter in *E. atramentarius* than in *E. amyntas*.

The *Onthophagus* species included here show some diversity in their karyotypes, despite the fact that none has heterochromatic chromosome arms. The karyotypes of the two species of *Onthophagus* s. str. are notable for the small sizes of their chromosomes, with autosome 1 less than 3 µm long in both species. This is supported by Virkki's illustrations. Thus Virkki figures spermatogonial mitoses of *O. taurus*, *O. vacca* and *O. nuchicornis*, in which the chromosomes of *O. taurus* are only about a quarter the size of those in *O. vacca* and *O. nuchicornis* (Virkki 1951, figs. 127, 143 and 144). Similarly Virkki's figure of spermatogonial mitosis of *O. illyricus* (Virkki 1954, fig. 19) shows the chromosomes less than a quarter the size of those of *O. nuchicornis* (Virkki 1954, fig. 37). Virkki's material was prepared by serially sectioning testes, in complete contrast to the air-dried preparations from mid-gut figured here (figs. 2a-d). Thus the small size of the chromosomes of *O. taurus* and *O. illyricus* appears genuine. Although the karyotypes of these two species appear morphologically indistinguishable from one another, this does not imply that there is no distinction between these species. Chromosome morphology reflects the spatial arrangement of the genes along the chromosomes, but reveals nothing of the nature of the genes themselves.

The karyotypes of the two *Trichonthophagus* species, *O. hirtus* and *O. maki*, are similar to one another in having three long submetacentric pairs of autosomes and one conspicuously small pair. The karyotypes are easily distinguished from one another by the acrocentric chromosome 4 of *O. maki*. Comparison of the karyotypes of the two species suggests that the X chromosome of *O. maki* is likely to the one figured as either chromosome 7 or 9.

The karyotype of *O. furcatus* appears very distinctive because of the three small pairs of acrocentric/subacrocentric autosomes.

O. stylocerus is an Iberian endemic, black in colour but morphologically similar to *O. vacca*. The sequence of RCLs along the karyotype is very similar to that of *O. vacca*, though autosome 9 appears smaller in *O. stylocerus*, and the centromere indices of autosomes 3 and 5 are clearly different in the two species. The C-bands appear weaker in *O. stylocerus*.

O. vacca has a karyotype notable for the polymorphism shown by autosome 3, involving both a pericentric inversion and variation in C-band strength. This is a species for which Virkki (1960) suggested that one pair of chromosomes were satellited, drawing on his 1951 data. The material figured here (fig. 3d) shows a secondary constriction at the base of the short arm of autosome 5, and this can be open or closed. The distal portion of the arm does not appear to be heterochromatic, so the chromosome does not really appear to be satellited.

O. similis and *O. opacicollis* are a pair of species whose separate identities have been questioned (Baraud 1992). As with the *O. ovatus* group, the karyotypes show clear differences, in this case involving both autosomes and the sex chromosomes. Thus, in *O. similis* autosome 9 is metacentric with a clear median C-band, while in *O. opacicollis* it is subacrocentric with very little euchromatic material in the short arm. The X chromosome of *O. similis* has a clearly localised centromeric C-band and the chromosome is smaller than in *O. opacicollis*, being about as long as autosome 7. In *O. opacicollis* the X chromosome has the C-band extending over the basal half of its long arm, and the chromosome appears longer, intermediate in length between autosomes 5 and 6. The y chromosomes of the species are also different, with that of *O. opacicollis* about twice as long as that of *O. similis*, and with a distinct centromeric C-band.

O. coenobita has a karyotype characterised by the majority of its autosomes being acrocentric or subacrocentric, and a small X chromosome. Autosome 2 has a secondary constriction in the short arm, and when expanded this can give a satellited appearance (fig. 4g).

O. lucidus is a very distinctive species. Its karyotype has five pairs of submetacentric autosomes, and only one acrocentric pair. The X chromosome, also submetacentric, appears similar in size to that of *O. coenobita*.

O. ovatus, *O. joannae* and *O. ruficapillus* are members of the '*O. ovatus* species group', where identification can be difficult, necessitating critical study of the aedeagus (Baraud 1992), and where even now the separate identities of some of the species are questioned. Thus Allen (2001) refers to British *O. ovatus* 'or *O. joannae* Goljan if that is really a good species'. It is therefore very useful that the karyotypes of the three species show clear differences. The most obvious differences between the karyotypes of *O. ovatus* and *O. joannae* are in the centromere positions of autosomes 1 and 2. These chromosomes appear more or less metacentric in *O. ovatus*, but distinctly submetacentric in *O. joannae*. This is confirmed by analysis of the Centromere Index (CI) data for the two species, shown in table 2.

The number of measured chromosomes was 6 in each case. Not only do the 95% confidence limits for the chromosomes of the two species show no overlap,

Table 2. *Onthophagus ovatus* and *O. joannae*, CI of autosomes 1 and 2.

Species & Autosome No.	CI	95% confidence intervals by t-test.
O. ovatus. Autosome 1	45.37	42.81 – 47.92
O. joannae Autosome 1	37.61	33.70 – 41.52
O. ovatus Autosome 2	45.33	41.90 – 48.76
O. joannae Autosome 2	37.37	33.85 – 40.89

but examination of the figures shows that the short arm of autosome 2 has a secondary constriction in *O. joannae*, but no trace of one in *O. ovatus*. This suggests that there are serious differences between the chromosome arrangements of the two species. The karyotype of *O. ruficapillus* appears more like the *O. ovatus* karyotype than that of *O. joannae* in the centromere positions of the long autosomes. Thus autosomes 1 and 2 are very obviously metacentric in *O. ruficapillus*. However, *O. ruficapillus* differs from the other two species in the size of the X chromosome – clearly smaller than autosome 9, while in both *O. ovatus* and *O. joannae* the X chromosome is clearly longer than autosome 9, being longer than autosome 8 in *O. ovatus*, but slightly shorter in *O. joannae*. The centromeric C-bands appear very small and weak in this species.

O. gazella is the only representative of the subgenus *Digitonthophagus* included here. It is an Ethiopian-Oriental species, now widely distributed in the warmer regions of the world, and is one of the species deliberately introduced into Australia as a biological control agent for dung (Waterhouse 1974). A number of authors have given data on its chromosomes, with the most detailed (and recent) account being that of Bisoi & Patnaik (1991). Their account of the karyotype, based on spermatogonial mitosis, lists 6 pairs of metacentrics and 4 of submetacentrics, with the X chromosome one of the smaller metacentrics. The karyotype given here lists 5 metacentrics (including the X chromosome), 3 submetacentrics, and 2 more or less acrocentrics. The absence of any reference to acrocentrics in Bisoi & Patnaik's data appears the most serious discrepancy – but the differences between metacentrics and submetacentrics are often borderline, and 'one of the smaller metacentrics' allows some latitude for the X, here presented as medium sized. Bisoi & Patnaik do not give any figures showing the chromosomes arranged as karyotypes, and examination of their fig. 5 shows at least three chromosomes which might be almost acrocentric. Thus their data are not incompatible with those presented here. It is, however, worth noting that the published data refer to Indian material. According to S. Medrano Cabral of the Natural History Museum in Santo Domingo (unpublished data) the *O. gazella* in the Dominican Republic are derived via Mexico from African material deliberately introduced into Texas. It is therefore possible that there may be some chromosomal differences between the stocks, resulting from pericentric inversions of the sort that give rise to the polymorphisms in autosome 3 of *O. vacca* and autosome 5 of *O. albicornis*.

O. albicornis, endemic to Hispaniola, is a Neotropical species not as yet placed in any particular subgenus. Its karyotype is interesting in having a long metacentric X chromosome, as long as autosome 1. It is the only species in this study which shows this character.

Onthophagus appears to show little departure from the basic number of 2N = 18 + Xy. The published data (Smith & Virkki 1978; Wilson, 2002) cover 31 species (27 of them named), and a further nine species are added here. Of these species, two unnamed ones are described as having 2N= 16 + Xy (Manna & Lahiri 1972), and 1, *O. sellatus* Klug, is described as having 2N = 24 + Xy (Ebied et al., 2000). All the remaining species are listed as having 2N = 18 + Xy. The only variation in chromosome number found in the present study results from the presence of B-chromosomes in some specimens of *Euonthophagus amyntas*, *Onthophagus vacca*, *O. similis* and *O. gazella*.

The main usefulness of karyotype data appears to be in showing differences between often similar species – the *O. ovatus* group and *O. similis* and *O. opacicollis* are good examples. The one instance where the karyotypes fail to show difference between members of a species-pair is that of *O. taurus* and *O. illyricus*. As already discussed, lack of karyotype differences is not in itself evidence of conspecificity.

There is no feature of the karyotypes which appears characteristic of any of the genera studied, though the absence of entirely heterochromatic chromosome arms in any of the *Onthophagus* species studied is worth noting. Such arms are present in both *Euoniticellus* and *Euonthophagus*, and are often associated with interspecific variation in *Aphodius* (e.g. Wilson and Angus, 2004).

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References

Allen, A. A., 2001. *Onthophagus furcatus* (F.) (Col., Scarabaeidae), another exotic beetle found at Kew Gardens. – Entomologist's Monthly Magazine 137: 170.

Baraud, J. 1992. Coléoptères Scarabaeoidea d'Europe. – Faune de France 78: i-ix, 1-856, 11 plates.

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

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

Ebied, A. M., F. M. Mostafa, A. E. Yaseen & 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.

Gillet, J. J. E., & A. Boucoumont, 1911-1927. Scarabaeidae: Coprinae, Termitotroginae. – Coleoptorum Catalogus 90 & 38: 103 – 264. W. Junk, Berlin.

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.

Kacker, R. K., 1970. Studies on chromosomes of Indian Coleoptera IV. In nine species of family Scarabaeidae. – The Nucleus, Calcutta 13: 126-131.

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

Martín-Piera, F. & J. I. López-Colón, 2000. Coleoptera Scarabaeoidea 1. – Fauna Iberica 14: 1-526.

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

Sumner, A. T., 2003. Chromosomes: organization and function. – Blackwell, Oxford. 336 pp.

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.

Virkki, N., 1960. Cytology of some Nearctic Scarabs (Coleoptera, Scarabaeidae). – Annales Academiae Scientarum Fennicae A. IV. 48: 1-48.

Waterhouse, D. F., 1974. The biological control of dung. – Scientific American 230 (4): 100-109.

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, 2004. A chromosomal analysis of the west European species of *Aphodius* Illiger, subgenus *Aphodius* s. str. (Coleoptera: Aphodiidae). – Tijdschrift voor Entomologie 147: 259-264.

Yadav, J. S., R. K. Pillai & L. Karamjeet, 1979. Chromosome numbers of Scarabaeidae (Polyphaga: Coleoptera). – Coleopterists Bulletin 33 (3): 308-318.

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