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Chromosomal separation of difficult species of *Copris* Geoffroy, 1762 and *Onthophagus* Latreille, 1802 (Coleoptera, Scarabaeidae), with discussion of *O. massai* Baraud as a British Pleistocene fossil

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Abstract

Karyotype analysis shows that *Copris hispanus cavolinii* Petagna should be regarded as a separate species from *C. hispanus* Linnaeus, and that *Onthophagus massai* Baraud is a valid species, not a synonym of *Onthophagus fracticornis* Preyssler. Chromosomal variation between populations of *O. fracticornis* is discussed, and Spanish material is shown to be the most distinct of the populations studied, but it is not considered that it should be placed as a separate species. Pleistocene fossil *O. massai* and Bronze Age *O. fracticornis* from England are discussed and illustrated. The distinctive elytral puncturation of *O. massai* is shown by the Pleistocene material, while Bronze Age *O. fracticornis* resembles modern material of that species.

Keywords

Copris hispanus, *Copris hispanus cavolinii*, *Onthophagus fracticornis*, *Onthophagus massai*, chromosomes, karyotypes, Last (Eemian) Interglacial

Introduction

A number of recent studies on Scarabaeoidea have demonstrated the usefulness of chromosomal analysis in establishing the limits of species which may be difficult to

distinguish from one another morphologically. For example Wilson (2001) showed that the common and widespread European dung beetle *Aphodius fimetarius* (Linnaeus, 1758) (Aphodiidae) in fact comprised two distinct species, *A. fimetarius* itself and *Aphodius pedellus* (DeGeer, 1774) with completely different karyotypes and with absolutely no evidence of hybridisation between them. Further work (Wilson and Angus, 2004) confirmed the initial separation and extended the database. More recently, Angus (2008) was able to show that *Onthophagus similis* (Scriba, 1790) and *Onthophagus opacicollis* Reitter, 1893 (Scarabaeidae) are completely separate species with no evidence of hybridisation between them, contrary to the suggestion of Martín-Piera and Boto (1999), who used allozyme analysis.

The work on *O. similis* and *O. opacicollis* also included *Onthophagus fracticornis* (Preyssler, 1790), whose chromosomes were clearly very distinct from those of the other two species, and this suggests that a detailed comparison of the chromosomes of *O. fracticornis* and *Onthophagus massai* Baraud, 1975, a Sicilian endemic morphologically very similar to *O. fracticornis* would be useful, especially as *O. massai* has been recorded as a fossil from the Last Interglacial (about 120,000 years ago) in England (Coope, 2001).

Work by Angus et al. (2007) showed that the chromosomes of *Copris hispanus hispanus* (Linnaeus, 1764) do not match the published account of those of *Copris hispanus cavolinii* (Petagna, 1792) (Salamanna, 1972), raising the possibility that these are in fact separate species, a view supported by small differences in the form of their male genitalia (e.g. Baraud, 1992). Salamanna's work was done using squash preparations from testis, and his figures do not enable karyotypes to be assembled, so that fresh work is necessary.

Chromosome features used and rationale behind their use

The chromosome features used here, in addition to the total number of chromosomes present, are the size and shape of the chromosomes, expressed as Relative Chromosome Length (RCL: the length of each chromosome as a percentage of the total haploid autosome length in the nucleus) and Centromere Index (CI: the length of the short arm of a chromosome as a percentage of the total length of the chromosome), and the relative amounts and distribution of constitutive heterochromatin (repetitive DNA detected by C-banding). These features allow a high level of distinction between different karyotypes, but care is needed when considering the implication of these differences. Differences in chromosome number may result from the presence of variable numbers of B-chromosomes, e.g. *Pterostichus nigrita* (Paykull, 1790) and *Pterostichus rhaeticus* Heer, 1837 (Angus et al., 2000), or may reflect polyploidy often associated with parthenogenesis, as well as indicating differences between species. Fusion-fission polymorphisms involving different chromosomes are also known in Coleoptera, an example being *Ilybius montanus* Stephens, 1828 (Aradottir & Angus, 2004). The key to understanding these infraspecific differences is the occurrence of heterozygotes. Differ-

ences in CI may result from addition or deletion of heterochromatin, or pericentric inversions, and may occur as polymorphisms within species, e.g. autosome 5 of *Aphodius pedellus* (Wilson, 2001), and differences in RCL may reflect differences in the amount of constitutive heterochromatin present, as in the long and short X chromosome of *Helophorus grandis* Illiger, 1798 (Helophoridae) (Angus, 1989). A key feature of these infraspecific chromosomal variations is that they are likely to occur as heterozygotes, though only if the two arrangements occur sympatrically. Intraspecific variation in chromosome sizes, revealed by differences in the sequence of RCLs along a karyotype, unless reflecting differences in heterochromatic blocks, are likely to result from translocation of material between chromosomes. Such translocational differences may result in mispairings of chromosomes at first division of meiosis, and hence reduced fertility (or even sterility), and are thus prima facie evidence that different species are involved. Some caution is needed here: translocational differences will only be detectable if they result in noticeable alterations in a chromosome's size. There may be reciprocal translocations which would not be detected but which would nevertheless result in reduced fertility or sterility of hybrids. In this sense the results of chromosomal studies are unidirectional – demonstrable differences may indicate that different species are involved, but a lack of such differences does not prove conspecificity.

Material and methods

Table 1 lists the material used in these analyses, with the localities of capture and the number of specimens analysed. This refers to the number of beetles from which distinctive chromosome spreads were used in the RCL and CI analyses. Additional material checked for distinctive chromosomes is given in parentheses. The localities are numbered, and their geographical locations are shown on the map in Fig. 1. Note that when two localities are fairly close together they have been given the same number.

Chromosome preparations are from mid-gut and testis of adult beetles, as described by Angus (1982) and Shaarawi and Angus (1991). Slides were stained in 1– 2 % Giemsa, dried and photographed under oil immersion. Immersion oil was removed using xylene followed by absolute ethanol, and the 2-day old slides were C-banded using saturated barium hydroxide at room temperature (ca 22 °C). Treatment in barium hydroxide was for 3 minutes and was followed by washing in 3 changes of Sörensen at pH 6.8, and incubation in salt-sodium citrate (2 X SSC: 0.3 M sodium chloride and 0.03 M trisodium citrate) for 1 hour at 55 °C. The slides were then washed in a further 3 changes of Sörensen at room temperature, and stained in Giemsa as before. This enables the same nucleus to be studied both plain and C-banded, and has been done throughout this study. Photographs were printed at a magnification of 3000 X, and the chromosomes were cut out and arranged as karyotypes. At this stage they were scanned into a computer and further arrangement and measurement done using Adobe Photoshop. The use of the total

Table 1. Material used, localities, map numbers, numbers of specimens used for chromosome measurements, with additional checked material given in parentheses.

Species	Locality with No. on Map	Number of specimens analysed
Copris hispanus hispanus (L.)	Spain, Provincia de Cádiz, Facinas (No. 1)	3♂
	Spain, Provincia de Cádiz, San Roque (No. 2)	2♂
	Spain, Provincia de Cádiz, La Línea (No. 2)	(1♂)
	Spain, Provincia de Málaga, Parque de los Alcornocales, La Sauceda (No. 2)	2♂
Copris hispanus cavolinii (Pettagna)	Sicily, Provincia di Trapani, Segesta (No. 3)	2♂, (1♀)
	Sicily, Provincia di Trapani, Scopello (No. 3)	1♀
Onthophagus fracticornis (Preyssler)	Spain, Provincia de Madrid, Lozoya (No. 4)	4♂, 2♀
	England, Somerset, Compton Bishop (No. 5)	2♂, (1♀)
	Switzerland, Valais, Chandolin (No. 6)	1♂
	Italy, Abruzzo, Provincia di L'Aquila, Campo Imperatore (No. 7)	3♂
	Czech Republic, southern Moravia, Podyji National Park (No. 8)	2♀
	Macedonia, Šar Planina, (No. 9)	3♂, 1♀
	Macedonia, Mavrovo National Park (No. 9)	1♂
Onthophagus massai Baraud	Sicily, Provincia di Palermo, Parco delle Madonie, Piano Zucchi (No. 10)	2♂, 2♀
	Sicily, Provincia di Messina, Parco dei Nebrodi, Muto (No. 10)	3♂, 1♀

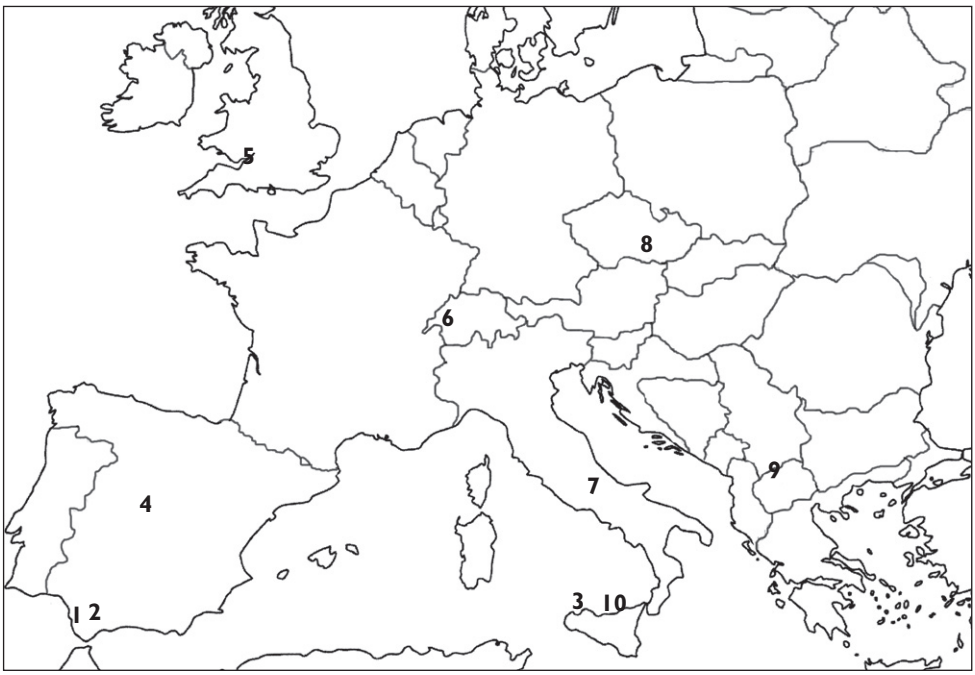

Figure 1. Map showing the collection sites of the material used in this paper. See Table 1 for explanation of the numbers, and note that neighbouring sites may share the same number.

haploid autosome length in RCL calculations follows the procedure used with human chromosomes (Paris Conference, 1971) and means that, although the X and y chromosomes can have calculated RCL values, it is the RCL values of the autosomes that should add up to 100. The CI calculations, again following the Paris Conference, are the basis of morphological classification of the chromosomes. Based on Sumner (2003), these are: metacentric, CI 50–46; submetacentric, CI 46–26; subacrocentric, CI 25–15; and acrocentric (including telocentric), CI less than 15.The beetles from which the preparations were obtained were card-mounted and are in R. B. Angus' collection.

The fossil material was photographed using a Zeiss photomicroscope with oblique surface illumination from a standard bench light. The elytra were very crumpled and only small portions were in focus at any one time. However, the resolution was good and the resulting photographs are sufficient to show the diagnostic features.

Results

Copris hispanus hispanus and C. h. cavolinii

2n = 16 + Xy (♂), 16 + XX (♀).

Plain (Giemsa stained) and C-banded karyotypes are shown in Fig. 2, while RCL and CI data are shown in Table 2, where values showing differences between the two forms significant at the 95% level are indicated by yellow highlight. For practical reasons (difficulty of accurate measurement) CI values below 15 are listed as acrocentric, without further analysis. Points to note are the RCL differences in autosomes 3, 6 and 7, and the y chromosome, and the CI differences in autosomes 4 and 7. Autosome 6 of *C. h. hispanus* appears to show a pericentric inversion in the specimen illustrated, but not in the other analysed material. In both forms the heterochromatic blocks are very small and confined to the centromere region, so differences in the RCLs of the chromosomes are almost certainly due to translocation.

Onthophagus fracticornis and O. massai

2n + 18 + Xy (♂), 18 + XX (♀).

Plain and C-banded karyotypes are shown in Fig. 3, while RCL and CI data are shown in Tables 3 and 4. In the tables instances where the values for chromosomes from different populations of *O. fracticornis* differ at the 95% significance level are indicated by yellow highlight, while cases in which the chromosomes of *O. massai* differ from those of *O. fracticornis* are indicated by green highlight. Within *O. fracticornis* the most

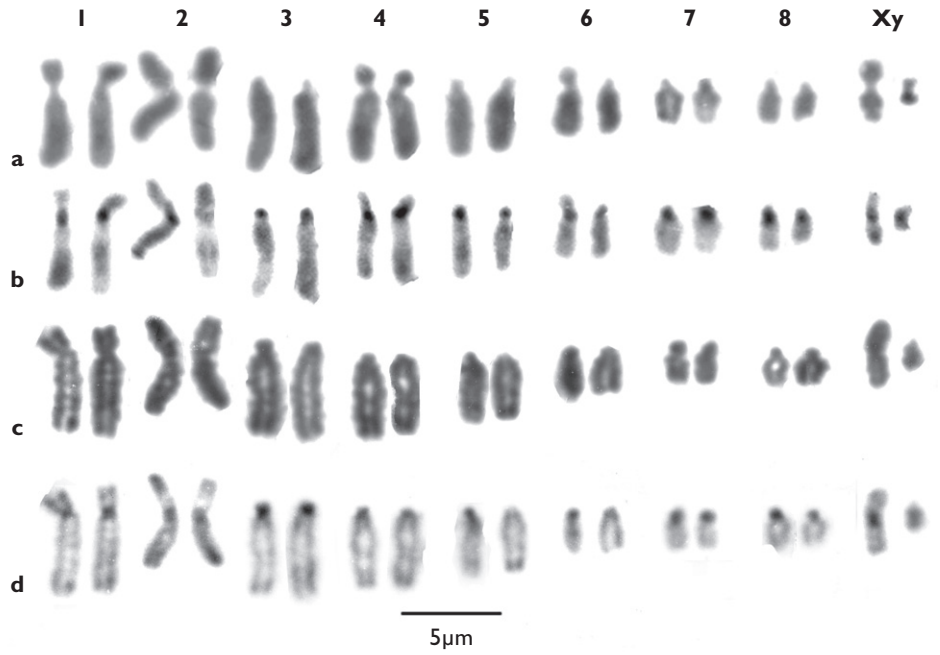

Figure 2. Mitotic chromosomes of *Copris hispanus hispanus* (**a**, **b**) and *C. h. cavolinii* (**c**, **d**) arranged as karyotypes. **a**, **c**, plain, **b**, **d**, the same nuclei C-banded.

notable size differences are shown by autosomes 5 and 9 of Spanish material (Fig. 3a, b), and the y chromosome of the Italian material (Fig. 3k, l). The large Italian y chromosome is very striking in all preparations, resulting in no clear size difference between it and autosome 9. C-bands are heavy on all the autosomes and in some preparations small intercalated C-bands may be present, possibly sites of nucleolus organisers. The C-band of the y chromosome is clearly weaker than that of autosome 9. CI differences are shown by the y chromosome, with a fairly median (metacentric) centromere in Macedonian (Fig. 3 e–h) and Italian material (Fig. 3k, l) – and the single Swiss specimen (not shown here), and the subterminal (subacrocentric) centromere of Spanish and English material (Fig. 3a–d). In one Macedonian specimen (Fig. 3g, h) autosome 5 is heterozygous for a pericentric inversion, and this specimen also has one B-chromosome.

The chromosomes of *O. massai* (Fig. 3 m, n) show an extensive suite of RCL differences from those of *O. fracticornis* (Fig. 3a–l). Thus the RCL values for autosomes 1, 5, 8 and 9 are different at the 95% level. The y chromosome is as large as that of Italian *O. fracticornis*, but differs from it in having a subterminal centromere. There is also an extensive suite of CI differences, involving autosomes 1, 5 and 7–9, as well as the X chromosome. The subterminal centromere of the X chromosome is very distinctive.

Table 2. *Copris hispanus* and *C. h. cavolinii*, chromosome parameters. Mean, 95% confidence intervals by t-test, number of chromosomes analysed. Significant differences are indicated by gray background.

Chromosome	RCL		CI	
	Copris hispanus hispanus	***C. hispanus cavolinii***	***Copris hispanus hispanus***	***C. hispanus cavolinii***
1	18.54 18.14–18.93 N = 38	18.97 18.61–19.33 N = 44	submetacentric 29.01 27.47–30.56 N = 28	submetacentric 28.48 26.89–30.08 N = 36
2	16.81 16.44–17.17 N = 38	17.07 16.75–17.39 N = 44	submetacentric 40.60 38.43–42.77 N = 28	submetacentric 41.43 40.26–42.60 N = 36
3	15.20 14.75–15.65 N = 38	16.41 16.09–16.72 N = 42	acrocentric N = 28	acrocentric N = 36
4	14.79 14.43–15.14 N = 38	13.96 13.70–14.22 N = 44	subacrocentric 24.40 22.91–25.89 N = 28	acrocentric N = 36
5	12.09 11.71–12.48 N = 38	11.96 11.66–12.29 N = 44	acrocentric N = 28	acrocentric N = 36
6	9.50 9.15–9.87 N = 38	8.33 7.97–9.02 N = 44	acrocentric N = 28	acrocentric N = 36
7	6.69 6.42–6.95 N = 38	7.41 7.07–8.59 N = 44	acrocentric N = 28	subacrocentric 17.73 16.46–18.99 N = 36
8	6.29 6.05–6.52 N = 38	6.59 6.38–6.79 N = 44	subacrocentric 17.55 16.58–18.52 N = 28	subacrocentric 17.65 16.66–18.63 N = 36
X	10.07 9.69–10.46 N = 19	10.75 10.30–11.21 N = 26	submetacentric 44.24 40.16–48.32 N = 14	metacentric 48.04 46.23–49.85 N = 22
y	4.56 4.17–4.94 N = 19	5.59 5.26–5.92 N = 18	metacentric 46.74 44.05–49.44 N = 14	submetacentric 40.06 35.11–45.02 N = 14

Discussion

Copris hispanus hispanus and C. h. cavolinii

The RCL differences between the karyotypes of these two beetles, taken in combination with their very small heterochromatic regions, similar in size in the two taxa, suggest very strongly that the karyotypes differ as a result of translocation of material

Figure 3. Mitotic chromosomes of *Onthophagus fracticornis* (**a** – **l**) and *O. massai* (**m**, **n**), arranged as karyotypes. **a**, **c**, **e**, **g**, **i**, **k**, **m**, plain, **b**, **d**, **f**, **h**, **j**, **l**, **n**, the same nuclei C-banded. **a**, **b**, Spain; **c**, **d**, England; **e**, **f**, Macedonia, Šar Planina; **g**, **h**, Macedonia, Mavrovo National Park, with one B-chromosome and autosome 5 heterozygous for a pericentric inversion; **i**, **j**, Czech Republic; **k**, **l**, Italy; **m**, **n**, Sicily, Piano Zucchi.

Table 3. *Onthophagus fracticornis* and *O. massai*, Relative Chromosome Length. Mean, 95% confidence intervals by t-test, number of chromosomes analysed. Significant differences between populations of *O. fracticornis* are indicated by light gray background, and those between *O. fracticornis* and *O. massai* by dark gray background.

Chromosome	Spain	England	Macedonia	Czech Rep.	Italy	*O. massai*
1	16.06 15.54– 16.58 N = 16	15.96 15.41– 16.50 N = 16	16.05 15.59– 16. 51 N = 26	15.72 15.28– 16.17 N = 18	14.98 14.40– 15.55 N = 16	14.93 14.60– 15.26 N = 28
2	14.13 13.86– 14.39 N = 16	13.95 13.58– 14.32 N = 16	14.26 13.84– 14.68 N = 26	13.54 13.07– 14.02 N = 18	14.14 13.68– 14.59 N = 16	13.70 13.46– 13.94 N = 28
3	12.88 12.48– 13.28 N = 16	13.16 12.79– 13.53 N = 16	13.20 12.86– 13.55 N = 26	12.64 12.17– 13.11 N = 18	13.24 12.89– 13.59 N = 16	12.66 12.35– 12.96 N = 28
4	11.49 11.07– 11.90 N = 16	11.95 11.53– 12.36 N = 16	12.48 12.17– 12.80 N = 26	11.88 11.42– 12.34 N = 18	11.75 11.35– 12.15 N = 16	11.54 11.24– 11.83 N = 28
5	10.98 10.64– 11.33 N = 16	12.76 12.38– 13.13 N = 16	12.12 11.73– 12.50 N = 26	12.29 11.79– 12.80 N = 18	11.66 11.50– 11.81 N = 16	10.86 10.62– 11.11 N = 28
6	10.47 10.10– 10.85 N = 16	9.89 9.56– 10.22 N = 16	10.44 10.18– 10.70 N = 26	10.15 9.54– 10.76 N = 18	10.18 9.72– 10.64 N = 16	9.99 9.74– 10.24 N = 28
7	8.77 8.45–9.09 N = 16	8.61 8.35–8.88 N = 16	8.73 8.40–9.06 N = 26	9.04 8.59–9.49 N = 18	9.44 9.12–9.76 N = 16	9.48 9.20–9.76 N = 28
8	7.81 7.61–8.02 N = 16	7.41 7.08–7.73 N = 16	7.78 7.41–8.16 N = 26	8.03 7.62–8.44 N = 18	7.73 7.43–8.03 N = 16	8.66 8.45–8.87 N = 28
9	7.39 7.09–7.68 N = 16	6.24 5.93–6.56 N = 16	6.68 6.40–6.95 N = 26	6.59 6.30–6.88 N = 18	6.86 6.54–7.18 N = 16	8.14 7.90–8.38 N = 28
X	13.89 13.16– 14.62 N = 8	15.03 13.33– 16.72 N = 8	14.42 12.57– 16.28 N = 13	14.86 14.23– 15.50 N = 18	14.10 13.06– 15.14 N = 8	14.48 13.09– 15.87 N = 21
y	4.86 4.40–5.31 N = 8	4.46 4.44–5.08 N = 8	5.12 4.61–5.62 N = 13		6.25 5.92–6.58 N = 8	6.40 4.67–8.13 N = 7

Table 4. *Onthophagus fracticornis* and *O. massai*, Centromere Index. Mean, 95% confidence intervals by t-test, number of chromosomes analysed. Significant differences between populations of *O. fracticornis* are indicated by light gray background, and those between *O. fracticornis* and *O. massai* by dark gray background.

Chromosome	Spain	England	Macedonia	Czech Rep.	Italy	*O. massai*
1	submetacentric 40.82 39.08–42.55 N = 16	submetacentric 41.03 39.55–42.51 N = 16	submetacentric 38.12 34.87–41.37 N = 26	submetacentric 39.11 37.13–41.08 N = 18	submetacentric 39.59 37.8–41.39 N = 16	submetacentric 44.27 43.08–45.47 N = 27
2	submetacentric 45.17 43.42–46.92 N = 16	submetacentric 45.36 43.56–47.15 N = 16	metacentric 46.50 44.90–48.11 N = 26	submetacentric 43.23 38.25–48.21 N = 18	submetacentric 44.42 42.5–46.34 N = 16	submetacentric 45.97 42.64–49.29 N = 28
3	submetacentric 44.76 42.71–46.83 N = 16	metacentric 46.09 44.42–47.77 N = 16	submetacentric 44.70 42.72–46.68 N = 26	metacentric 47.57 45.86–49.28 N = 18	metacentric 46.71 45.67–47.75 N = 16	metacentric 46.76 45.67–47.85 N = 28
4	metacentric 46.60 45.52–47.68 N = 16	submetacentric 45.99 44.37–47.61 N = 16	metacentric 48.76 47.53–49.99 N = 26	submetacentric 44.53 42.23–46.82 N = 18	submetacentric 44.08 41.34–46.81 N = 16	metacentric 47.40 45.53–49.27 N = 28
5	submetacentric 28.96 27.41–30.51 N = 16	submetacentric 31.41 29.93–32.88 N = 16	submetacentric 31.81 29.40–34.22 N = 26	submetacentric 32.07 29.89–34.24 N = 18	submetacentric 34.29 32.39–36.18 N = 16	metacentric 48.56 47.06–50.06 N = 28
6	metacentric 46.28 44.62–47.93 N = 16	submetacentric 43.93 40.80–47. 05 N = 16	submetacentric 44.66 43.19–46.13 N = 26	submetacentric 45.37 43.15–47.58 N = 18	metacentric 46.31 44.16–48.46 N = 16	metacentric 48.16 46.70 -= 49.63 N = 28
7	submetacentric 41.09 37.75–44.44 N = 16	submetacentric 41.21 39.63–43.61 N = 16	submetacentric 41.52 39.88–43.15 N = 26	submetacentric 43.64 42.19–45.10 N = 18	submetacentric 43.06 40.59–45.53 N = 16	metacentric 47.13 45.24–49.01 N = 28
8	submetacentric 40.08 38.03–42.13 N = 16	submetacentric 41.57 39.53–43.61 N = 16	submetacentric 40.50 38.94–42.06 N = 26	submetacentric 42.52 39.93–45.10 N = 18	submetacentric 42.23 40.46–43.99 N = 16	metacentric 46.94 45.30–48.57 N = 28
9	submetacentric 43.08 41.34–44.83 N = 16	submetacentric 44.55 43.25–45.85 N = 16	submetacentric 45.92 44.08–47.76 N = 26	metacentric 47.14 44.77–49.51 N = 18	submetacentric 42.45 40.21–44.70 N = 16	metacentric 49.88 48.31–51.44 N = 28
X	submetacentric 42.75 41.05–44.45 N = 8	submetacentric 44.24 40.80–47.68 N = 8	submetacentric 44.68 42.60–46.76 N = 12	submetacentric 43.46 41.78–45.14 N = 18	submetacentric 44.58 41.94–47.21 N = 8	submetacentric 35.40 33.51–37.28 N = 21
y	subacrocentric 25.92 21.68–30.17 N = 8	submetacentric 33.08 27.85–38.31 N = 8	submetacentric 43.09 39.82–46.36 N = 12		submetacentric 44.66 41.86–47.46 N = 8	subacrocentric 24.20 20.04–28.36 N = 7

between non-homologous chromosomes, and thus provide good evidence that they should be considered as separate species, *Copris hispanus* and *C. cavolinii*.

Copris cavolinii was described, as a species, by Petagna (1792) on the basis of material from the Naples area, but most subsequent works, including the Catalogue of Palaearctic Coleoptera (Löbl et al., 2006a) place it as a subspecies of *C. hispanus.*

The morphological and geographical characteristics of *C. hispanus* and *C. cavolinii* (which she regarded as a subspecies of *C. hispanus*) were discussed in depth by Rommel (1965). She figured details of the cephalic horns and pronotal carinae, but not the genitalia. She gave the distribution of *C. hispanus* as extending from southern France, Corsica and Sardinia, via the Iberian Peninsula to North Africa, where its range is shown as extending as far as Egypt. No local variation was noted. *C. cavolinii* was noted from Italy, the Balkans, Turkey, Israel, and extending eastwards through northern Iran to the former Middle Asian republics of the USSR. She distinguished three forms. The western form occurs in Italy and the former Yugoslavia, and Albania, the eastern form in Greece (including Crete), Turkey, Cyprus and Israel, and the northeastern form occurs in middle Asia. Dellacasa (1968) reviewed the morphological characteristics of *C. hispanus* and *C. cavolinii* and, although he left them as subspecies, pointed out that *C. cavolinii* was in fact very distinct.

As far as *C. cavolinii* is concerned, it is important to note that our data refer only to Italian (Sicilian) material, and therefore to Rommel's western form. In the case of *C. hispanus*, the situation at first appears more straightforward as no geographical variation was noted. However Ebied et al. (2000) record and figure a completely different karyotype, with 20 mainly metacentric chromosomes, from Egyptian *C. hispanus*. This is so different from those reported here that it cannot refer to *C. hispanus*, and means that, unless their material is misidentified, the species passing as *C. hispanus* in Egypt is something entirely different.

It would be useful to study material from a wider area. The *C. hispanus* localities lie at the apices of an equilateral triangle with 40 km sides, but at least Spanish material must be considered typical of *C. hispanus*. The two Sicilian localities are only about 16 km apart, but nevertheless the results from all the material are consistent so that there is no reason to doubt their validity. The main unanswered question is whether study of material from a wider area, especially of the different forms of *C. cavolinii*, would reveal the presence of other species. This question is given added weight by the differences between some of the populations of *O. fracticornis*, to be discussed next.

Onthophagus fracticornis and O. massai

In the case of *O. fracticornis*, in contrast to those of the two *Copris* species, material from populations over a wide area of Europe has been studied. While, in terms of RCL at least, this has revealed a considerable level of stability, the Spanish material, in particular, shows some significant differences: autosome 5 (recognisable in all populations because of its low CI) is significantly smaller than in other populations,

while autosome 9 is significantly larger. One effect of this is that in some preparations autosome 5 actually appears shorter than autosome 6. The centromeric C-bands of Spanish material appear similar in size to those of other populations, so it is difficult not to believe that some interchromosomal translocation of material has taken place. It would therefore seem logical to suggest that there is a prima facie case for regarding the Spanish material as representing a separate species. However, we have detected no morphological difference between Spanish and other material, so that for the moment it seems prudent to leave it as *O. fracticornis*, but note the problem. At this stage it is interesting to note that Angus (2008) found that autosome 5 of Spanish *O. opacicollis* was significantly larger than that of Sardinian and Cyprus material. It may be appear a curious coincidence that this same chromosome is involved in both cases, but there is a simple explanation: autosome 5 in all the species concerned has a distinctly lower CI than those of autosomes 4 and 6, so autosome 5 is clearly recognisable. This raises the question as to whether this autosome is homologous in all the species, and whether the observed differences in its length are the only ones involved. As mentioned in the introduction, only translocational differences resulting in obvious changes to the length of a chromosome can be detected – and one requirement for this is that the chromosome concerned is itself clearly identifiable. Altering the RCLs of metacentric autosomes occupying adjacent positions in a karyotype might simply reverse the order in which they were placed, without this being apparent.

Apart from the Spanish situation, the Italian material has a significantly larger y chromosome than those of other populations (only females were available from the Czech material, so we have no data on its y chromosome), meaning that, without C-banding, which shows the small heterochromatic block on the y, it could be difficult to distinguish from autosome 9. It is difficult to assess the significance of this larger y chromosome. The sex chromosomes of most Polyphaga pair via a cytoplasmic vesicle (the parachute or Xy_p association, cf. Smith and Virkki, 1978), and the small y chromosomes of these *Onthophagus* species suggest that they are likely to follow this pattern. This would mean that no impaired meiosis need be involved in hybrids, and we have no idea what, if any, extra genes the Italian y chromosome may be carrying.

When the CI data are considered, the only variation is found in the y chromosome, more or less metacentric in Macedonian and Italian material, as well as the single Swiss example, but subacrocentric in English and Spanish material. This is of no taxonomic significance as it would not affect the Xy_p pairing at meiosis. It is worth noting that the sequence of CI values along the karyotype of Spanish material does not differ from those of the other populations, suggesting that the amount of chromosomal difference between Spanish and other material is small.

Comparison of the karyotypes of *O. fracticornis* and *O. massai* reveals a very different situation, with four of the nine pairs of autosomes of *O. massai* having significantly different RCLs from their apparent counterparts in *O. fracticornis*. When the CIs are compared, five pairs of autosomes appear different, as does the X chromosome. This degree of difference is clearly far more than that shown by populations of *O. fracticornis* and vindicates the placing *O. massai* as a separate species.

Onthophagus massai was described, as a distinct species, by Baraud (1975) on the basis of material from the Piano Battaglia in the mountains of the Parco delle Madonie in northern Sicily. We were unable to find it on the Piano Battaglia in early November 2008, but it was present on the Piano Zucchi slightly lower down the same mountains. The species status of *O. massai* was denied by Palestrini (1981), who placed it as a synonym of *O. fracticornis*. Baraud (1992) reasserted its species status and reviewed the morphological distinctions, especially as regards the sculpture of the elytral striae, between it and *O. fracticornis*. Subsequent authors (e.g. Carpaneto and Piatella (1995), Sparacio (1995), Pesarini (2004) and Lapiana and Sparacio (2006)) have followed Baraud's assessment, and this view is maintained in the Palaearctic Catalogue of Löbl et al. (2006b). Thus our chromosomal data are in agreement with the current taxonomic consensus.

Onthophagus massai as a Pleistocene fossil in England

As mentioned in the introduction, *O. massai* has been recorded as a fossil from the Last (Eemian or Ipswichian) Interglacial in England. Coope (2001) reviewed its occurrences and showed that it occurs, sometimes abundantly, in deposits of that interglacial, but not in the immediately preceding one.

Validation of *O. massai* as a species separate from *O. fracticornis*, rather than as a local variant of it, means that there is no theoretical difficulty with its fossil distribution, since many species of beetle have altered their geographical ranges on a dramatic scale in response to the glacial/interglacial oscillations (Coope, 2001). Nevertheless the occurrence in Britain of what is now an endemic confined to a small region of northern Sicily is so unexpected that it requires special verification. It is therefore appropriate to consider the characters on which the identification was and is based. Russell Coope first encountered this species in material from Trafalgar Square (see Franks et al., 1958), where at least 49 individual specimens were represented. The material clearly belonged to the *O. fracticornis* group on details of the head and pronotum, and the strength of the basal portion of the cephalic horn of the males was too great for either *O. opacicollis* or *O. similis*. The specimens appeared consistently small and dark when compared with *O. fracticornis*, but did match a small series of *O. massai*. The fossil pronota had the punctures large, especially towards the basolateral edge, matching the *O. massai*, but not the *O. fracticornis* available for study, and the basal portion of the cephalic horns of the males seemed more like the *O. massai* than the *O. fracticornis*. Coope also felt that the puncturation of the elytral interstices was somehow coarser in the fossils and in *O. massai* than in *O. fracticornis*, but at the time he did not know that this elytral puncturation character was the one Baraud now uses (e.g. Baraud, 1992) to key out *O. massai*. Much of the Trafalgar Square fossil material is now in a parlous state having been dry-mounted on cards for many years, but in some cases the elytral puncturation is adequately preserved. It should at this stage be noted that in Pleistocene fossil material the lipid components of the cuticle are lost and the structure tends to collapse on drying. However, as well as the Trafalgar Square fossil material, we have been able to study

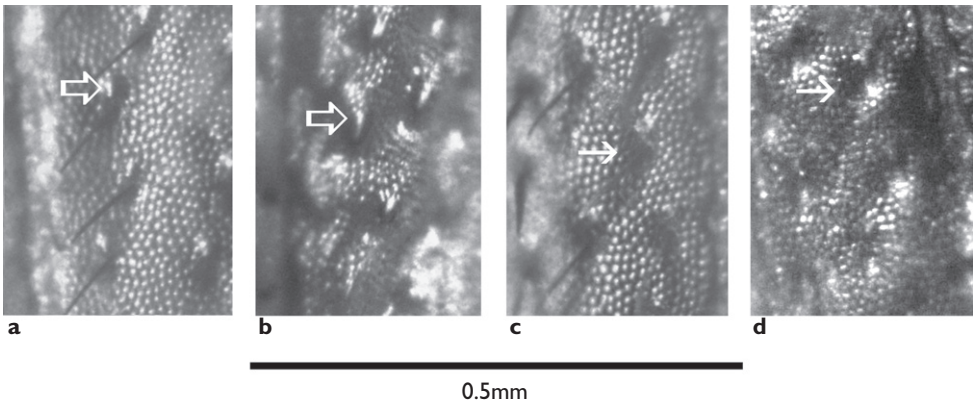

Figure 4. Elytral sculpture of *Onthophagus fracticornis* (**a**, **b**) and *O. massai* (**c**, **d**), to show the prominent presetal granules of the interstices in *O. fracticornis* (white-bordered black arrow) and the prominent perisetal punctures in *O. massai* (white arrow). a, modern, Šar Planina, Macedonia; b, Bronze Age, Wilsford, Wiltshire, England, age about 4000 years; c, modern, Parco dei Nebrodi, Sicily; d, Last Interglacial, Trafalgar Square, London, age about 120,000 years.

fossil *O. fracticornis* from Bronze Age deposits (aged about 4000 years) from Wilsford in Wiltshire, England (see Osborne, 1969). This material, though much younger than that from Trafalgar Square, shows a similar fragility due to loss of the lipid components.

Details of modern and fossil elytra of *O. fracticornis* and *O. massai* are shown in Fig. 4. The presetal granules of *O. fracticornis* (indicated by white-bordered black arrows) are very clear, and partial collapse of the fossil material serves merely to enhance them. In the case of the *O. massai* these granules are less conspicuous, but the perisetal punctures (indicated by white arrows) are clear and distinct in both the modern and fossil material. The fact that this character, unknown to Russell Coope when he originally studied the material, leads to the same identification, gives ample support for the recognition of *O. massai* as a Pleistocene fossil, and graphically illustrates how modern restricted distributions may not reflect the former ranges of the species concerned.

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References

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

Angus RB (1989) Towards an atlas of *Helophorus* chromosomes. Balfour-Browne Club Newsletter 44: 13–23.

Angus RB (2008) A chromosomal analysis of the *Onthophagus similis-opacicollis-fracticornis* species group (Coleoptera: Scarabaeidae). Tijdschrift voor Entomologie 151: 235–244.

Angus RB, Brown RE, Bryant LJ (2000) Chromosomes and identification of the sibling species *Pterostichus nigrita* (Paykull) and *P. rhaeticus* Heer (Coleoptera: Carabidae). Systematic Entomology 25: 325–337.

Angus RB, Wilson CJ, Mann DJ (2007) A chromosomal analysis of 15 species of Gymno pleurini, Scarabaeini and Coprini (Coleoptera: Scarabaeidae). Tijdschrift voor Entomologie 150: 201–211.

Aradottir GI, Angus RB (2004) A chromosomal analysis of some water beetle species recently transferred from *Agabus* Leach to *Ilybius* Erichson, with particular reference to the variation in chromosome number shown by *I. montanus* Stephens (Coleoptera: Dytiscidae). Hereditas 140: 185–192.

Baraud J (1975) *Onthophagus massai,* nouvelle espèce paléarctique (Coleoptera Scarabaeoidea). Bulletin de la Société Linnéenne de Lyon 44: 292–295.

Baraud J (1992) Coléoptères Scarabaeoidea d'Europe. Faune de France et Régions Limitrophes 78: ix+856 pp, 11 plates. Fédération Française des Sociétés Naturelles, Paris.

Carpaneto GM, Piatella E (1995) Coleoptera Polyphaga 5 (Lucanoidea, Scarabaeoidea). In: Minelli A, Ruffo S, La Posta S (Eds) Checklist delle Specie della Fauna Italiana 50: 1–18.

Coope GR (2001) Biostratigraphical distinction of interglacial coleopteran assemblages from southern Britain attributed to Oxygen Isotope stages 5e and 7. Quaternary Science Reviews 20: 1717–1722.

Dellacasa G (1968) Sulle sottospecie del *Copris* (*s. str.*) *hispanus* nell'ambito della fauna italiana (Coleoptera Scarabaeidae). Bollettino della Società Entomologica Italiana 98: 135–142.

Ebied AM, Mostafa FM, Yaseen AE, Kawashti LS (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.

Franks JW, Sutcliffe AJ, Kerney MP, Coope GR (1958) Haunt of elephant and rhinoceros: the Trafalgar Square 100,000 years ago – new discoveries. Illustrated London News (1958): 1101–1103.

Lapiana F, Sparacio I (2006) I Coleotteri Lamellicorni delle Madonie (Coleoptera Lucanoidea et Scarabaeoidea). Naturalista Siciliano 30: 227–292.

Löbl I, Krell F-T, Král D (2006a) Coprini. In: Löbl I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera. Vol. 3. Apollo Books, Stenstrup, 151–154.

Löbl I, Krell F-T, Ziani S, Král D (2006b) Onthophagini. In: Löbl I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera. Vol. 3. Apollo Books. Stenstrup, 159–176.

Martín-Piera F, Boto L (1999) A reappraisal of the '*fracticornis-opacicollis*' taxonomic complex of the genus *Onthophagus* Latreille using allozymes (Coleoptera, Scarabaeoidea). Journal of Zoological Systematics and Evolutionary Research 37: 75–84.

Osborne PJ (1969) An insect fauna of Late Bronze Age from Wilsford, Wiltshire. Journal of Animal Ecology 38: 555–566.

Palestrini C (1981) *Onthophagus fracticornis* (Preyssl.) e *O. similis* (Scriba): status tassonomico e considerazioni zoogeografiche. Bollettino del Museo di Zoologia dell'Università di Torino 2: 13–24.

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

Pesarini C (2004) Insetti della fauna Italiana, Coleotteri Lamellicorni. Natura 93: 1–132.

Petagna V (1792) Institutiones Entomologicae 1: 140. Napoli.

Rommel E (1965) Systematische und tiergeographische Untersuchungen an dem Mondhornkäfer *Copris hispanus* (L.) (Coleoptera, Scarabaeidae). Entomologische Abhandlungen, Staatliches Museum für Tierkunde in Dresden 31: 57–71.

Salamanna G (1972) Aspetti della cariologia degli Scarabaeidae. Atti del Congresso Nazionale di Entomologia 1971: 313–324.

Shaarawi FA, Angus RB (1991) A chromosomal investigation of five European species of *Anacaena* Thomson (Coleoptera: Hydrophilidae). Entomologica Scandinavica 21 (1990): 415–426.

Smith SG, Virkki N (1978) Animal Cytogenetics 3: Insecta 5: Coleoptera. Gebrüder Borntraeger, Berlin, Stuttgart, x+366 pp.

Sparacio I (1995) Coleotteri di Sicilia. Parte prima. L'Epos, 240 pp., pls.

Sumner AT (2003) Chromosomes: organization and function. Blackwell, Oxford, 287 pp.

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

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