A CHROMOSOMAL ANALYSIS OF THE WEST
EUROPEAN SPECIES OF *APHODIUS* ILLIGER,
SUBGENUS *APHODIUS* S. STR.
(COLEOPTERA: APHODIIDAE)

Wilson, C. J. & R. B. Angus, 2004. Chromosomal analysis of the West European species of *Aphodius* Illiger, subgenus *Aphodius* s. str. (Coleoptera: Aphodiidae). – Tijdschrift voor Entomologie 147: 259–264, figs. 1–4, tables 1–2. [ISSN 0040-7496]. Published 1 December 2004. The karyotypes of the five West European species of *Aphodius* (*A.* str.) are described and illustrated. All have 9 pairs of autosomes plus Xy sex chromosomes. Two species, *A. fimetarius* (Linnaeus) and *A. foetidus* (Herbst) have long heterochromatic blocks on some chromosomes, while the other three species, *A. conjugatus* (Panzer), *A. foetens* (Fabricius), and *A. pedellus* (DeGeer) lack these segments. *A. pedellus* may have one or two B-chromosomes, while some southern populations of *A. foetidus* may have up to six B-chromosomes. It is suggested that the occurrence of B-chromosomes is the probably explanation of the differing chromosome numbers recorded by Yadav (1973) and Yadav et al. (1993) for *A. moestus* (Fabricius). The occurrence of *A. fimetarius* in North America is confirmed.

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In the course of a Ph.D. research programme on Scarabaeoid chromosomes, it was discovered that material regarded as *Aphodius fimetarius* (Linnaeus) in fact comprised two species, *A. fimetarius* and *A. pedellus* (DeGeer), which, while very similar morphologically, have very different karyotypes (Wilson 2001). The present paper reports the results of investigations of the chromosomes of the three remaining species of subgenus *Aphodius* s. str. and shows that while all five species have distinctive karyotypes, two basic karyotype patterns are present: in *A. fimetarius* and *A. foetidus* (Herbst) the X chromosome and at least one pair of autosomes have very long heterochromatic segments, while in *A. pedellus*, *A. conjugatus* (Panzer) and *A. foetens* (Fabricius) these long heterochromatic segments are absent.

MATERIAL AND METHODS

The material used for chromosome analysis is listed in table 1. In the case of *A. fimetarius* and *A. pedellus*, only material additional to that listed by Wilson

(2001) is given.

British localities are referred to their Watsonian Vice-Counties, as set out by Dandy (1969). Spanish localities are listed by Provincia.

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.

RESULTS

Aphodius conjugatus (figs. 1a, b)

Published information: none. $2N = 18 + Xy$. The X chromosome is about the same length as autosome 9 and the y is dot-like. The RCLs of the chromosomes range from about 16.5 to 6. Chromosome 3 is sub-

Table 1. Material used for chromosome analysis.

Species	Localities + total no. of specimens analysed.
<i>A. (Aphodius s. str.) conjugatus</i> (Panzer)	SPAIN. Segovia: La Salceda; Ávila: Sierra de Paramera. (5 specimens)
<i>A. (Aphodius s. str.) fimetarius</i> (Linnaeus)	ENGLAND. VC 9 Dorset: Wareham; VC 14 East Sussex: Camber; VC 15 East Kent: Deal; VC 17 Surrey: Box Hill; SPAIN. Segovia: La Salceda. USA. California. Marin County: Tocaloma. (70 specimens, 8 since Wilson 2001)
<i>A. (Aphodius s. str.) foetens</i> (Fabricius)	ENGLAND. VC 11 South Hants: New Forest, Denny Wood; VC 15 East Kent: Betteshanger; VC 17 Surrey: Pyrford; VC 22 Berks: Old Windsor. (7 specimens)
<i>A. (Aphodius s. str.) foetidus</i> (Herbst)	ENGLAND. VC 14 East Sussex: Rye; Camber; VC 15 East Kent: Betteshanger; VC 17 Surrey: Pyrford; Box Hill; VC 26 West Suffolk: Weather Heath S of Thetford; VC 28 West Norfolk: East Wretham Heath. SPAIN. Cáceres: Abadía; MA: Coín. CYPRUS. Pafos District: Anadhiou; Limassol District: Akrotiri, Fassouri. (44 specimens)
<i>A. (Aphodius s. str.) pedellus</i> (DeGeer)	ENGLAND. VC 9 Dorset: Wareham; VC 12 North Hants: Woolmer; VC 17: Box Hill; SPAIN. Cantabria: Puerto de Piedrasluengas; León: Sanabria. (52 specimens, 18 since Wilson 2001)

metacentric, with the short arm about half the length of the long arm, but the rest are all more or less metacentric. C-banding (fig. 1b) shows strong centromeric C-bands, but none extends far along the chromosome arms. The y chromosome appears entirely heterochromatic.

Aphodius foetens (figs. 1c-e)

Published information: $2N = 20$ (σ), 10 bivalents (Virkki 1951). $2N = 18 + Xy$. The RCLs of the autosomes range from about 15 to 8, decreasing evenly along the karyotype. The X chromosome is smaller still, RCL about 5, and the y chromosome is about half that size. Autosomes 1-8 are more or less metacentric, while autosome 9 and the X chromosome are acrocentric. C-banding (fig. 1e) shows all the autosomes, and the X chromosome, with heavy centromeric C-bands. The centromeric C-band of the y chromosome is also fairly large, accounting for about half the length of the chromosome, but the chromosome is acrocentric, with a short euchromatic arm.

Aphodius pedellus (figs. 1f, g, 3, 4b, c)

Published information: $2N = 18 + Xy$ (σ) + B-chromosomes, karyotype, C-banding (Wilson 2001); $2N = 20$ (σ), 9 bivalents + Xy (Virkki 1951) (as *A. fimetarius* – see Wilson 2001). $2N = 18 + Xy_p + B$ -chromosomes. The B-chromosomes are similar in size and shape to autosomes 6 and 7, but differ in that the distal ends of their arms are closely applied to one another and have weak C-bands in these regions. There is also a banding gap in the short arm. The only chro-

mosomal variation encountered since the publication of Wilson's 2001 paper was a triploid female (fig. 3) taken near Wareham. Only mid-gut cells were available for analysis in this specimen, and it is possible that other tissues may not have been triploid. Nevertheless, it is the only triploid material encountered. The specimen had a deformed (teratological) epipharynx, but a similar condition was present in a second (diploid) specimen, which appeared to have had difficulty in moulting from the pupa, in a total sample of nine beetles from this sample. Although a total of about 20 epipharynxes of *A. pedellus* and *A. fimetarius*, from various localities, has been examined, these are the only deformed specimens encountered. Deformed mouthparts of larvae are a characteristic symptom of poisoning by the helminthicide ivermectin (Sommer & Nielsen 1992, Sommer et al. 1993) and it seems possible that ivermectin poisoning may have been responsible for both the teratology and the triploidy in this sample. First metaphase of meiosis is shown in fig. 4b & c. The small Xy_p bivalent is arrowed. The preparation shown in fig. 4b has some spontaneous C-banding.

Aphodius fimetarius (figs. 2a, b)

Published information: $2N = 18 + Xy$, karyotypes, C-banding (Wilson 2001). $2N = 18 + Xy$. As explained by Wilson (2001), the karyotype is distinctive because of the long heterochromatic regions of autosome 2 and the X chromosome. The karyotype shown in fig. 1a & b is from the testis of an American specimen, the same nucleus shown both plain and C-banded. This is the first confirmed identification of *A. fimetarius* in North America. It also shows that in

Fig. 1. Mitotic chromosomes from mid-gut cells of *Aphodius* species, arranged as karyotypes. – a, b, *A. conjugatus*, male from Paramera, a plain, b the same nucleus, C-banded; c–e, *A. foetens*, c male from Pyrford, plain, d female from Betteshanger, plain, e male from Betteshanger, C-banded; f, g, *A. pedellus*, female from Purbeck, f plain, g C-banded.

testis preparations the heterochromatic segments cannot be detected without C-banding.

A. foetidus

(figs. 2c–h, 4a)

Published information: none. $2N = 18 + X_{y_p} + B$ -chromosomes. The karyotype of this species is distinctive because of the long heterochromatic regions of four pairs of autosomes and the X chromosome. The RCLs of the autosomes range from about 16 to 7, while

the X chromosome is the longest in the karyotype, with RCL about 19. The y chromosome is large and apparently entirely heterochromatic, not showing any distinct arms. C-banding (figs. 2c & h) shows autosomes 1, 2 and 7 to be largely heterochromatic, with small euchromatic regions on the distal portions of their short arms. The distal portion of the long arm of autosome 1 is heterochromatic and may appear as a satellite, separated from the rest of the arm by a clear gap. Autosome 6 appears to show regional variation. In the British specimen (fig. 2c) the distal part of the long arm is het-

Fig. 2. Mitotic chromosomes of *Aphodius* species, arranged as karyotypes. – a, b, *A. fimetarius*, testis, California, a plain, b the same nucleus, C-banded; c–h, *A. foetidus*: c, male, Rye Harbour, mid-gut, C-banded, with autosome 9 heterozygous; d, female, Camber, mid-gut, plain, with 1 replicate of autosome 7 lost, autosome 9 acrocentric and 1 B-chromosome; e, female, Abadía, mid-gut, plain, with autosome 9 heterozygous and 1 long and 2 short B-chromosomes; f, male, Anadhiou, mid-gut, plain, with autosome 9 heterozygous and 3 long B-chromosomes; g, male, Fassouri, mid-gut, plain, with autosome 9 acrocentric and 1 short B-chromosome; h male, Coín, testis, C-banded, with autosome 9 acrocentric and 6 B-chromosomes, 5 totally heterochromatic, 1 only partially so.

erohromatic and may separated from the rest of the chromosome by a gap (as in autosome 1), but in the Spanish specimen (fig. 2h) there is no distal heterochromatin and the chromosome merely has a very heavy centromeric C-band. The X chromosome is largely heterochromatic, with a short euchromatic area at the end of each arm. Autosomes 3, 4, 5, 8 and 9 have

strong but localised centromeric C-bands, smallest in pairs 4 and 9. Autosome 9 is polymorphic for a pericentric inversion, and may be either acrocentric or metacentric. The acrocentric form tends to appear smaller, RCL about 7 rather than 9, but there is no difference in the C-band sizes of the two forms. In preparations with more extended chromosomes (fig. 2f) the

Fig. 3. *Aphodius pedellus*, mid-gut cell from a triploid female from Wareham.

two forms appear the same length, suggesting that the apparent discrepancies in the lengths of the two forms result from differences in condensation. The numbers of specimens with the different forms of this chromosome are shown in table 2.

The sample from Camber is large enough for the frequencies of the three arrangements to be analysed for any departure from the Hardy-Weinberg equilibrium. The analysis is as follows: Sample size (N) = 19, giving 38 replicates of autosome 9. Frequency of acrocentric (p) = 0.605, frequency of metacentric (q) = 0.395. Expected occurrence of the three arrangements in a sample of 19 specimens: homozygous acrocentric (p^2N) = 6.93; heterozygote ($2pqN$) = 9.08; homozygous metacentric (q^2N) = 2.96. Observed occurrences: 6; 11; 2. A two-sample χ^2 indicates that the observed occurrences show no significant departure from the expected frequencies ($\chi^2 = 0.84$, degrees of freedom = 2, probability of null hypothesis = 0.66).

A conspicuous feature of the karyotype of *A. foetidus*, especially in southern material, is the pres-

ence of B-chromosomes. All four of the Cyprus specimens analysed, and the seven Spanish ones, had B-chromosomes, but only two of the 33 English specimens had B-chromosomes, one short B-chromosome in each case. These were from Camber, but 17 other specimens from this locality lacked the B-chromosomes. The B-chromosomes, in both Cyprus and Spain could be about the same size as the y chromosome (fig. 2g), in which case they cannot be distinguished from it, or about twice that size (fig. 2f & h). In some cases both sizes of B-chromosome are found in the same beetle (fig. 2e). Cyprus beetles had from one to three B-chromosomes, but Spanish specimens had from two to six. The B-chromosomes appear to be largely heterochromatic, but in one of the Spanish specimens (fig. 2h) one B-chromosome was only partly so. After C-banding it stained more darkly than the euchromatic regions of the autosomes and X chromosome, but not as heavily as their heterochromatic regions, or as the other five B-chromosomes. On the material available for study, the number of B-chromosomes appears to be the same in all cells of a beetle possessing them. First metaphase of meiosis is shown in fig. 4a. The X_y bivalent (arrowed) shows the long X chromosome in an almost ring-like formation. No preparations of meiosis were obtained from material with B-chromosomes.

DISCUSSION

The karyotypes of the five species of subgenus *Aphodius* s. str. reported here show two features of particular interest: the tendency of some species to develop very long heterochromatic segments on some of their chromosomes, and the presence of B-chromosomes, both heterochromatic and euchromatic.

The apparently sporadic occurrence of long heterochromatic bands in *Aphodius* chromosomes is a feature, which Wilson (2002) found on a number of occasions. A good example is provided by *A. (Liothorax) niger* Illiger (*A. niger* (Panzer) sensu auctt., see Krell et al. 2003) which has them, while *A. (L.) plagiatu* (Linnaeus) does not. Such heterochromatic segments tell nothing about the relatedness of different species, but they are very useful as recognition features of the karyotypes of different species. One of the most telling pieces of evidence in favour of the status of *A. fmetarius* and *A. pedellus* as separate species is the complete failure to find any karyotype combining features of the two, which would result from hybridisation.

The occurrence of B-chromosomes is not particularly unusual in Coleoptera, though numbers as high as six, as in Spanish *A. foetidus*, are unusual. However, even this number is exceeded by Greek material of *A. (Labarrus) lividus* (Olivier), where Angus et al. (in press) record nine heterochromatic B-chromosomes.

Table 2. Number of specimens of *Aphodius foetidus* with the various arrangements of autosome 9. The British localities from which more than one specimen was taken are listed separately, with the others included in "total British", and all the Cyprus material is entered together.

Locality	Acrocentric	Heterozygous	Metacentric
Camber	6	11	2
Rye	1		1
Pyrford	2	1	
Weather Heath	1	3	2
Total British	11	17	5
Abadía (Spain)	1	1	
Cofín (Spain)	1	4	
Cyprus	1	2	1

Fig. 4. Testis, first metaphase of meiosis. The Xy_p bivalent is arrowed. – a, *Aphodius foetidus* from Weather Heath; b, c, *A. pedellus*, b from Old Windsor, with some spontaneous C-banding, c from Purbeck.

B-chromosomes are discussed by Camacho et al. (2000). They are thought of as DNA parasites, of uncertain and probably varying origin. Generally they are inactivated genetically, and frequently become heterochromatic. The heterochromatic B-chromosomes of *A. foetidus* illustrate the final stage in this sequence of events, while the more or less euchromatic B-chromosomes of *A. pedellus* and the somewhat intermediate B-chromosome of *A. foetidus* may reflect earlier stages in the process.

It is worth noting that, with only one recorded exception, the karyotypes of *Aphodius* species comprise nine pairs of autosomes plus Xy sex chromosomes. The exception is *A. (Neocalaphodius) moestus* (Fabricius), for which Yadav (1973) recorded a diploid number of 22, with a meiotic formula of 10 bivalents plus Xy_p . However, later Yadav et al. (1993) recorded a diploid number of 20, 9 bivalents plus Xy_p for this species, and expressed surprise. The results reported here from different populations of *A. foetidus* suggest that in this case also B-chromosomes may be involved.

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