

**A chromosomal analysis of eight species of *Aphodius* Illiger,
subgenera *Agiolinus* Schmidt, *Agrilinus* Mulsant & Rey and
Planolinus Mulsant & Rey (Coleoptera: Aphodiidae)**

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**Хромосомный анализ 8 видов рода *Aphodius* Illiger
из подродов *Agiolinus* Schmidt, *Agrilinus* Mulsant & Rey и
Planolinus Mulsant & Rey (Coleoptera: Aphodiidae)**

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Abstract. An account is given of the karyotypes of eight *Aphodius* species. All show clear inter-species differences, and their C-banding patterns illustrate the range of types found within *Aphodius*.

Key words. Chromosomes, karyotypes, C-banding, Aphodiidae, Scarabaeoidea.

Резюме. Представлен обзор кариотипов 8 видов рода *Aphodius*. Все кариотипы обладают отчетливыми видовыми особенностями, а их С-бэндинг иллюстрирует широту изменчивости этого параметра, характерную для рода *Aphodius*.

Ключевые слова. Хромосомы, кариотипы, С-бэндинг, Aphodiidae, Scarabaeoidea.

Introduction

The work reported here is largely based on part of a thesis submitted for the degree of Ph.D. in 2002 by Christine Wilson, supervised by Robert Angus. Working with Christine gave Robert Angus the opportunity to resume an interest in a group of beetles which has held a lifetime fascination, and the invitation to offer the present chapter as part of a tribute to Gleb Sergeevich Medvedev seems particularly apt in view of the eminence of his father as a researcher on Scarabaeoidea.

The subgenera of *Aphodius* can reasonably be described as being in a state of flux. The elevation of the subgenera to full generic rank by Dellacasa et al. (2000) does not appear to have gained wide acceptance, while recent DNA-based studies cast doubt on the integrity of some of the subgenera as well as on the phylogenetic relationships between them (Maté, 2003; Cabrero-Sañudo, Zardoya, 2004). There is thus no overriding taxonomic reason for the choice of subgenera to be included in this chapter – they merely represent a convenient number of small to medium-sized, mainly black, *Aphodius* species whose karyotypes illustrate much of the range of variation shown by *Aphodius* species as a whole (Wilson, 2001, 2002; Wilson, Angus, 2003, 2004a, 2004b, 2005; Angus et al., 2004; Maté, Angus, 2005).

Material and Methods

The material used in this study is listed in Table. Nomenclature is after Baraud (1992), except that, following the application by Krell (2004) to the International Commission on Zoological Nomenclature, *A. fasciatus* (Olivier) is used instead of *A. uliginosus* (Hardy).

Table. Material used for chromosome analysis

Species	Localities (No. of specimens analysed)
<i>Aphodius (Agiolinus) lapponum</i> Gyllenhal	ENGLAND. Cumbria: High Street (3 specimens)
<i>A. (Agrilinus) ater</i> (DeGeer)	ENGLAND. Devon: Ilfracombe; East Sussex: Rye; Kent: Betteshanger; Surrey: Box Hill SCOTLAND. Angus: Edzell (8 specimens)
<i>A. (Agrilinus) constans</i> Duftschmid	ENGLAND. Hampshire: New Forest, Bolderwood Hill SPAIN. Cantábria: Puerto de Piedrasluengas (2 specimens)
<i>A. (Agrilinus) nemoralis</i> Erichson	ENGLAND. Cumbria: Finsthwaite. (3 specimens)
<i>A. (Agrilinus) rufus</i> Moll	ENGLAND. Hampshire: Romsey; Kent: Betteshanger; Surrey: Box Hill; Berkshire: Old Windsor; Suffolk: Weather Heath S of Thetford (13 specimens)
<i>A. (Planolinus) borealis</i> Gyllenhal	ENGLAND. Berkshire: Windsor Deer Park (3 specimens)
<i>A. (Planolinus) fasciatus</i> (Olivier)	ENGLAND. Worcestershire: Wyre Forest (3 specimens)
<i>A. (Planolinus) vittatus mundus</i> Reitter	CYPRUS. Limassol District: Akrotiri, Fassouri (7 specimens)

Methods of chromosome preparation, photography and analysis are as described by Beauchamp and Angus in this volume. The specimens from which preparations were obtained are housed in the authors' collections.

Results

Subgenus *Agiolinus* Schmidt

A. lapponum. Fig. 1, a–c. Published information: none. $2N = 18 + Xy$. The autosomes decrease in RCL fairly evenly from about 16 to 8 along the karyotype. Autosomes 1–5 are submetacentric, while the rest are more or less metacentric. The X chromosome is metacentric, about as long as autosome 5 (RCL about 12). The y chromosome is fairly large (RCL about 5.5). C-banding (Fig. 1, c) shows strong but localised centromeric C-bands on all the autosomes, while the X chromosome appears almost entirely heterochromatic, with a more intense double C-band on the short arm and the distal part of the long arm euchromatic. The y chromosome is acrocentric with a distinct centromeric C-band. The C-banding is very clear in Fig. 1, c, which is an incomplete karyotype, and is less clearly evident in the complete karyotype shown in Fig. 1, b.

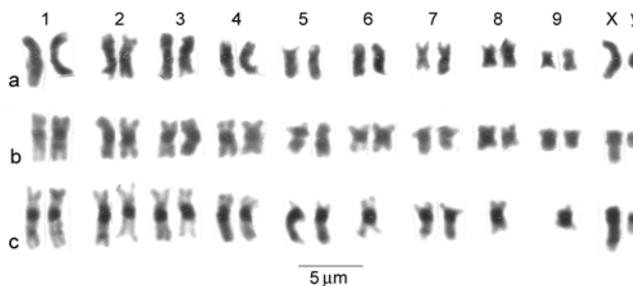


Fig. 1. *Aphodius*, mitotic chromosomes of subgenus *Agiolinus*. *A. lapponum* Gyll., ♂, mid-gut, High Street: a – plain; b – weakly C-banded, from the same specimen; c – incomplete, clearly C-banded, from a different specimen.

Subgenus *Agrilinus* Mulsant & Rey

A. ater. Fig. 2, a–c. Published information: $2N = 20$ (♂) (Virkki, 1951). $2N = 18 + Xy$. A somewhat complex karyotype, with long weakly heterochromatic (C-banding) regions in autosomes 1 and 5, and the X chromosome, and a length polymorphism in autosome 1. Autosomes 1 and 6, and the X chromosome are clearly submetacentric, with the short arm either half the length of the long arm (the X chromosome, autosome 6 and the short form of autosome 1), or a third of its length (the long form of autosome 1). The remaining autosomes are metacentric and the y chromosome is acrocentric. The RCL of the long form of autosome 1 is about 16, of the short form about 13. The RCLs of the remaining autosomes decrease from about 14 (autosome 2) to 5, while the RCL of the X chromosome is about 15. The y chromosome is fairly large, RCL about 6. C-banding is well developed in the karyotype shown in Fig. 2, b, while in Fig. 2, c it is partly lost through more severe treatment, though in this case the regions immediately adjacent to the centromeres have retained their C-bands. The long C-bands on autosome 1 and the X chromosome are situated mainly on the long arm, and the size polymorphism of autosome 1 results from a difference in the size of the C-band. The long C-band on autosome 5 is placed symmetrically about the centromere. All the material analysed was heterozygous for the size polymorphism in autosome 1.



Fig. 2. *Aphodius*, mitotic chromosomes of *Agrilinus* species. a – *A. ater* (DeGeer), ♀, mid-gut, Ilfracombe; b – *A. ater*, ♀, mid-gut, Bette-shanger, C-banded; c – *A. ater*, ♂, mid-gut, Ilfracombe, C-banded; d, e – *A. constans* Duft., ♂, testis, Piedrasluengas; f, g – *A. constans*, ♀, mid-gut, New Forest (f – weakly C-banded, g – strongly C-banded); h–k – *A. nemoralis* Er., ♂, mid-gut, Finsthwaite (h – plain, i – the same nucleus C-banded; j – plain; k – the same nucleus C-banded); l – *A. nemoralis*, Finsthwaite, ♀, mid-gut, C-banded; m, n – *A. rufus* Moll, ♂, mid-gut, Box Hill (m – plain, n – C-banded, the same nucleus); o – *A. rufus*, ♂, mid-gut, Box Hill; p – *A. rufus*, ♀, mid-gut, Romsey, weakly C-banded.

A. constans. Fig. 2, d–g. Published information: none. $2N = 18 + Xy$. Autosomes 1–7 are metacentric, 8 is almost acrocentric and 9 is entirely so. Their RCLs range from about 17 to about 6, with the decrease along the karyotype fairly even. The X chromosome, also metacentric, is about as large as autosome 1. The y chromosome is small, less than half the length of the *A. ater* y, and too small to measure. The autosomes have heavy centromeric C-bands (Fig. 2, g), with these distinctly smaller in autosomes 8 and 9. The X chromosome has a very long C-band, placed symmetrically about the centromere and occupying about half the length of each arm. No C-banded preparation of a male karyotype is available.

A. nemoralis. Fig. 2, h–l. Published information: none. $2N = 18 + XX$ (♀), $Xy?$ (♂). The uncertainty about the male sex chromosomes stems from the fact that only two (almost) complete karyotypes were obtained from the single male which yielded chromosomes, and these comprised 19 chromosomes, none of which resembled the small y chromosome found in *A. constans*. The two most likely explanations

are that 1: the small y chromosome was lost from these preparations – though no such chromosome was found in a further three more incomplete karyotypes; or 2: the y chromosome is rather larger, comparable with autosomes 8 or 9, and that the preparations lacked one chromosome from this group. A third possibility is that there is no y chromosome in *A. nemoralis*, but this seems unlikely as no other *Aphodius* species is known to have an X0 sex chromosome system. More material is clearly needed, but *A. nemoralis* is rare and localised in England (the only records are for Cumbria – Angus, 1964, 1965, 1987). It is less rare in the Scottish Highlands – even further away from London, where the present work was done!

The RCLs of the autosomes range from about 19 to 7 and the RCL of the X chromosome is about 7. Autosomes 1–3 and the X chromosome are submetacentric, autosomes 4–7 are metacentric, while autosomes 8 and 9 are subacrocentric. All the chromosomes have distinct centromeric C-bands, and autosome 5 and the X chromosome have additional C-bands on one arm (the long arm of X). The long arms of autosomes 1 and 2 have the chromatids tending to lie closely appressed, and may be weakly C-banding, as in autosome 1 and the X chromosome of *A. ater*.

A. rufus. Figs. 2, m–p; 3, a, b. Published information: $2N = 20$ (♂), 10 bivalents (Virkki, 1951). $2N = 18 + Xy_p$. A very distinctive karyotype, with autosomes 3–6 and 8 and 9 with long heterochromatic arms. The RCLs of the autosomes range from about 15 to 7.5, and the X chromosome is similar in size to the smallest autosomes. The y chromosome has a length polymorphism, and may be either as long as the X chromosome (Fig. 2, m, n), or about half that length (Fig. 2, o). The long form of the y appears to be the result of an attached satellite, separated from the rest of the chromosome by a narrow gap (Fig. 2, m), and was encountered in only 1 of the 7 males from which successful preparations were obtained. C-banding (Fig. 2, n, p) shows long C-bands symmetrically placed about the centromere on autosomes 1, 2 and 7, and occupying about a third of the length of the arms. The long C-bands of autosomes 3–6, and 8 and 9 occupy the whole of the long arms, but in autosome 4 there is a secondary constriction, which may open as a gap in the arm. Fig. 2, m, n, shows plain and C-banded karyotypes assembled from the same nucleus. This gives a clear demonstration of the value of C-banding in pairing up homologous chromosomes in a karyotype. The X chromosome has a small centromeric C-band and the y chromosome, in both of its forms, is entirely heterochromatic. Fig. 3, b shows first metaphase of meiosis from the male with the long y chromosome. The X and y chromosomes are clearly in a parachute association, but are about the same size as one another and the cytoplasmic vesicle is somewhat stained. Fig. 3, a, from a male with the short y chromosome, the sex bivalent, shows that the y chromosome, while still quite large, is distinctly smaller than the X. The cytoplasmic vesicle is scarcely stained in this preparation.

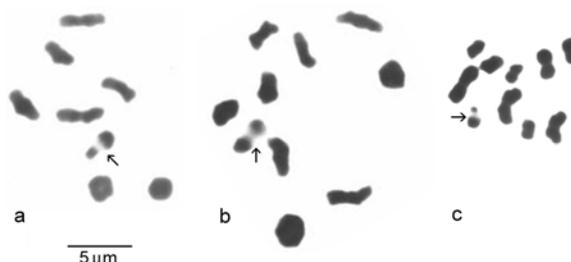


Fig. 3. *Aphodius*, first metaphase of meiosis from testis. a, b – *A. rufus* Moll from Box Hill (a – with the short y chromosome, b – with the long y chromosome); c – *A. borealis* Gyll. from Windsor Deer Park. The Xy_p bivalent is arrowed.

Subgenus *Planolinus* Mulsant & Rey

A. borealis. Figs 3, c; 4, a–d. Published information: none. $2N = 18 + Xy_p$. The RCLs of the autosomes range from about 16 to 6.5, and the X chromosome (RCL about 6) is about the same size as autosome 9. The y chromosome is dot-like, about half the length of the X. Autosomes 1–3 are more or less submetacentric, with a secondary constriction in the short arm of autosome 2. Autosomes 4–7 are more subacrocentric, while autosome 8 and the X chromosome are acrocentric. Autosome 9 is a small metacentric. C-banding (Fig. 4, c) shows distinct localised centromeric C-bands on all the autosomes and the X chromosome, while the y appears entirely heterochromatic. First metaphase of meiosis (Fig. 3, c) shows the sex bivalent with the typical Xy_p configuration.

A. fasciatus. Fig. 4, e–h. Published information: none. $2N = 18 + Xy$. The RCLs of the autosomes range from about 16 to 7, and the X chromosome (RCL about 10) is about as long as autosome 6. The y chromosome is a small acrocentric, RCL about 5. Autosomes 1–7 and the X chromosome are more or less metacentric, autosome 8 is subacrocentric, and autosome 9 is acrocentric. C-banding (Fig. 4, f, h) shows all the chromosomes with distinct centromeric C-bands, and the X chromosome has an additional C-band at the base of the long arm, possibly associated with a secondary constriction. The secondary constrictions on the long arms of autosomes 3 and 8 show as C-bands in Fig. 4, h, but not at all in Fig. 4, f. This is not unusual and is a threshold effect of the C-banding technique when applied to secondary constrictions.

A. vittatus mundus. Fig. 4, i–k. Published information: none. $2N = 18 + Xy$. The RCLs of the autosomes range from about 17 to 6.5, and the X chromosome is about the same length as autosome 9. The y chromosome is very small, dot-like. Autosomes 1–5 and 8 are more or less metacentric. Autosomes 6, 7 and 9, and the X chromosome are submetacentric to subacrocentric and have secondary constrictions on

their short arms, while autosome 4 has a secondary constriction on its long arm. C-banding (Fig. 4, k) shows all the autosomes and the X chromosome to have small but strong centromeric C-bands, and the secondary constrictions are more weakly C-banded.



Fig. 4. *Aphodius*, mitotic chromosomes of *Planolinus*. a – *A. borealis* Gyll., ♂, testis, Windsor Deer Park; b, c – *A. borealis*, ♂, mid-gut, Windsor Deer Park (b – plain, c – C-banded, from the same specimen); d, *A. borealis*, ♀, mid-gut, Windsor Deer Park; e, f – *A. fasciatus* (Ol.), ♂, mid-gut, Wyre Forest (e – plain, f – C-banded, from the same specimen); g – *A. fasciatus*, ♀, mid-gut, Wyre Forest; h – *A. fasciatus*, ♀, mid-gut, Wyre Forest, C-banded; i – *A. vittatus mundus* Reitt., ♂, mid-gut, Fassouri; j – *A. v. mundus*, ♀, mid-gut, Fassouri; k – *A. v. mundus*, ♂, mid-gut, Fassouri, C-banded.

Discussion

As mentioned in the introduction, the karyotypes of the eight species discussed here illustrate much of the variation shown by *Aphodius* as a whole. Among the autosomes, long heterochromatic chromosome arms are shown by *A. lapponum* and *A. rufus*, while *A. ater* and *A. nemoralis* show long arms which are rather weakly heterochromatic. This character is unusual in the *Aphodius* species studied to date (Wilson, 2002), and has been found only in these two *Agrilinus* species. The centromere positions of the autosomes range from metacentric to acrocentric, and the range of RCLs is normal for the genus. The X chromosomes range from acrocentric to metacentric, and from being the largest in the nucleus (*A. ater*) to the smallest (*A. borealis* and *A. vittatus mundus*). The size variation shown by the y chromosome is notable. In three of the six species whose y chromosomes are known, the y chromosome is more or less dot-like, and meiosis of one of the species (*A. borealis*) shows a typical Xyp configuration. In the other species the y chromosome is larger, and in one, *A. rufus*, there is a size polymorphism, with the longer form of the y being as long as the X chromosome. In both forms the meiotic sex bivalent is a normal Xy_p.

The karyotypes of all the species are distinctive, and this is a consistent feature of the *Aphodius* species studied. Karyotype differences have led to the recognition of separate, sometimes new, species, whose existence had been unsuspected. Notable examples are *A. fimetarius* (L.) and *A. pedellus* (DeGeer) (Wilson, 2001) and *A. niger* Illiger and *A. wilsonae* Maté & Angus (Maté, Angus, 2005). In both cases DNA differences support the conclusions drawn from the karyotypes (Maté, 2003). The situation regarding *A. vittatus mundus* is of particular interest. Current taxonomic thinking places this as an Eastern Mediterranean subspecies of the American *A. vittatus* Say, with two further subspecies, *A. v. sellatus* Mannerheim in Eastern Siberia and *A. v. tjanshanicus* Balthasar in Middle Asia. Maté (2003) reports DNA differences between American *A. v. vittatus* and Cyprus *A. v. mundus* of similar magnitude to those between *A. niger* and *A. wilsonae*, but also to those between geographically separated populations of the same species, e.g. English and Spanish material of *A. pedellus*. Clearly, karyotype data on the other subspecies of *A. vittatus* would be of great interest.

Acknowledgements

We thank the School of Biological Sciences, Royal Holloway, for facilities to carry out this research, and Jason Maté for identifying *A. vittatus mundus*.

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