Труды Русского энтомологического общества. С.-Петербург, 2006. Т. 77: 18–27.
Proceedings of the Russian Entomological Society. St. Petersburg, 2006. Vol. 77: 18–27.

A chromosomal analysis of the four British ladybirds of the subfamily Coccidulinae (Coleoptera: Coccinellidae)

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Хромосомный анализ четырех британских коровок подсемейства Coccidulinae (Coleoptera: Coccinellidae)

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Abstract. The karyotypes of the four British species of the ladybird subfamily Coccidulinae are described and illustrated. Both *Coccidula rufa* (Herbst) and *C. scutellata* (Herbst) have nine pairs of autosomes and sex chromosomes which are Xy_p (♂), XX (♀), with minor differences in their sequences of Relative Chromosome Lengths and in the C-banding patterns. *Rhyzobius chrysomeloides* (Herbst) has eight pairs of autosomes and sex chromosomes which are Xy_p (♂), XX (♀), but *Rh. litura* (F.) has seven pairs of autosomes and sex chromosomes which are Neo-XY (♂), neo-XX (♀). The small but clear differences between the karyotypes of the two *Coccidula* species are as expected for similar members of a small fairly uniform genus. However, the difference between the karyotypes of the two *Rhyzobius* species could not have been foreseen and is discussed.

Key words. Chromosomes, karyotypes, Coccinellidae, Coccidulinae, sex chromosomes.

Резюме. Приводятся описания и микрофотографии кариотипов 4 видов коровок подсемейства Coccidulinae из Великобритании. Кариотипы *Coccidula rufa* (Herbst) и *C. scutellata* (Herbst) включают 9 пар аутосом и половые хромосомы Xy_p (♂) и XX (♀). Между видами имеются незначительные различия в относительных размерах хромосом и в характере С-бэндинга. *Rhyzobius chrysomeloides* (Herbst) имеет 8 пар аутосом и половые хромосомы Xy_p (♂), XX (♀), тогда как у *Rh. litura* (F.) 7 пар аутосом и проловые хромосомы Neo-XY (♂) и neo-XX (♀). Небольшие, но отчетливые различия между кариотипами двух видов *Coccidula* выглядят естественными для внешне сходных видов небольшого и довольно однообразного рода. Разница же между кариотипами двух видов *Rhyzobius* неожиданна, и причины ее обсуждаются в статье.

Ключевые слова. Хромосомы, кариотипы, Coccinellidae, Coccidulinae, половые хромосомы.

Introduction

The system of individual research projects undertaken by final-year undergraduates in the School of Biological Sciences at Royal Holloway, University of London, provides an opportunity for limited and free-standing investigations into topics of interest to the supervising member of staff. The present work stems from one of these projects, by Rebecca Beauchamp, and it is a pleasure to be able to offer it as a

tribute to Gleb Sergeevich Medvedev. The recent discovery of a second species of the ladybird genus *Rhyzobius* Stephens, *Rh. chrysomeloides* (Herbst), in Britain (Hawkins, 2000, 2001), and its subsequent discovery on pine trees by the Windsor bypass (P.M. Hammond, pers. comm.) suggested the possibility of a chromosomal comparison of the two British *Rhyzobius* species. Following the classification followed by Majerus (1994), *Rhyzobius* belongs in the subfamily Coccidulinae, of which the only other British representatives are the two species of *Coccidula* Illiger, both of which are obtainable in the Surrey area. Inclusion of these two species in the project gives a total of four species, taxonomically reasonably isolated from other Coccinellidae, and hence forming a logical and manageable unit, with the opportunity of comparing the karyotypes of related species. Such comparisons have proved very useful in separating species of *Helophorus* (Hydrophiloidea) whose limits have been unclear (e.g. Angus, 1982), and have even showed that a very common and apparently highly distinctive dung beetle, *Aphodius fimetarius* (L.) (Aphodiidae), is actually two quite separate species (Wilson, 2001).

The objectives of the present study are:

1. To discover and illustrate the karyotypes of the four British species of Coccidulinae.
2. To investigate the C-banding properties of their chromosomes;
3. To investigate the similarities and differences between related species.

Chromosomal information on the Coccinellidae is reviewed by Smith, Virkki (1978). They give data for about 150 species (the approximate number stems from doubts about the taxonomic status of some forms). The species studied here are not included on the list, though one *Rhyzobius*, *Rh. ventralis* Erichson, from California, is included. Examination of the Zoological Record from 1978–2003 has revealed chromosomal data on fewer than 10 additional species, with nothing on either *Coccidula* or *Rhyzobius*. However, Gregory et al. (2003) gave genome-size estimates for *Rh. litura* (F.) and *C. rufa* (Herbst), based on image analysis of Feulgen-stained nuclei. They gave no karyological information.

The chromosomes of Coccinellidae show considerable diversity in both autosome numbers and sex chromosome systems. The basic arrangement in polyphagan Coleoptera is considered to be nine pairs of autosomes plus Xy_p sex chromosomes (Smith, 1950). Xy_p sex chromosomes refer to an arrangement whereby the y chromosome is very much smaller than the X, often appearing dot-like, and at first division of meiosis the sex chromosomes are held together by a cytoplasmic vesicle. This sex bivalent often looks like a small parcel (the y chromosome) suspended from a parachute (the X chromosome). John and Lewis (1960) regarded the cytoplasmic vesicle as a nucleolus, but later DNA analysis (Juan et al., 1993) has shown that in some cases no r-DNA (characteristic of nucleoli) is present. However, in Smith and Virkki's data, fewer than half the coccinellid species listed have this arrangement. About half the species listed are shown as having XY sex chromosomes (meaning the Y chromosome is nearly as large as the X), and nearly half of these have a neo-XY system, resulting from the fusion of an original X chromosome with an autosome.

Chromosomal investigations of Coccinellidae have revealed a number of interesting cases where geographically adjacent species occasionally hybridise. This is particularly well-studied in North American species of *Chilocorus* Leach (Smith, Virkki, 1978).

Material and Methods

The species studied, their localities of origin, and the number of specimens from which karyotypes were obtained, are given in Table 1.

Table 1. Ladybirds used for chromosome analysis.

Species	Localities	Number of analysed specimens
Coccidula rufa (Herbst)	Middlesex: Staines Moor	4
C. scutellata (Herbst)	Surrey: Epsom Common	3
Rhyzobius litura (F.)	Middlesex: Staines Moor	16
Rh. chrysomeloides (Herbst)	Berkshire: Windsor	10

Methods

A. Chromosome Preparation

The methods used for chromosome preparation were as modified by Angus (Angus, 1982; Shaarawi, Angus, 1991) based on the original acetic acid dissociation, air-drying method (Crozier, 1968) with Giemsa staining.

B. C-banding

Preparations were either treated straight from the unstained material after 2 days of drying, or from stained material of the same age. Immersion oil was removed with xylene followed by absolute ethanol. Then, preparations were destained by placing in 2 X SSC at 60 °C for 15 min. and rinsed in unbuffered distilled water before C-banding with saturated barium hydroxide for 5 min. at room temperature followed by SSC at 60 °C for 1 hour.

C. Curation of the specimens

Specimens from which chromosome preparations were obtained are housed in R.B. Angus' collection.

D. Microscopical examination and photography

The preparations were examined uncovered, using a photomicroscope. Slides were initially scanned under a 10× objective and nuclei of interest were further examined under a high power objective 40×. Photographs were taken using the oil immersion lens, 100×, and immersion oil was applied to the preparation. After sufficient photographs were taken and the film developed and checked, the slides were discarded.

E. Karyotype Preparation

The chromosomes on the photographs were cut out with scissors and then paired up and arranged as karyotypes. They were then scanned into a computer and subsequent processing was done using Adobe Photoshop.

F. Measurements and Calculations

The lengths of the chromosomes were measured (in millimetres) using a standard transparent ruler. Estimations were made if the chromosome was curved. The length of the short arm of the chromosome was also noted, for calculation of Centromere Index (CI). The images are at a magnification of 3000×, so 3 mm is the equivalent of 1 μm.

Two calculations were made. Relative Chromosome Length (RCL) is the length of each chromosome expressed as a percentage of the total haploid autosome length in the nucleus (Paris Conference, 1971). This was calculated by adding the lengths of all the autosomes in one nucleus together, then dividing by 2 because they are paired, to obtain the total haploid length. Then each chromosome length is divided by the total haploid length and multiplied by 100 to gain a percentage result. Centromere Index (CI) is the length of the shorter arm of a chromosome expressed as a percentage of the length of the chromosome (International Study Group, 1960). There is a problem inherent in this methodology, if the chromosome arms are of more or less equal length. Unless individual arms can be recognised by some feature other than their length, it is unlikely that the same arm will always appear the shorter, due to irregularities of chromosome condensation during mitotic prophase. Therefore in these cases the CI will appear lower than it should be. Centromere position may be conveniently classified into a number of categories, depending on where in the chromosome the centromere is. Based on Sumner (2003) these are: Metacentric – CI 46–50; Submetacentric – CI 26–45; Subacrocentric – CI 16–25; and Acrocentric – CI 3–15.

Variation of both RCL and CI stems mainly from irregular condensation of chromosomes, and the calculated results for a pair of homologous chromosomes may differ.

The results were analysed by t-test to give the 95 % confidence limits. The purpose of this analysis was to allow for variation due to irregularities of condensation. No attempt was made to detect minor chromosomal variations between individuals. Work on mammalian (human) chromosomes following G-banding treatments demonstrates that minor additions and deletions are not detectable from size measurement. Since the only variable being considered is irregular condensation of the chromosomes, multiple samples from different nuclei of the same individual beetle are true replicates, not pseudoreplicates.

Results

Mitotic chromosomes of the four species are shown in Figs 1 and 2. Meiotic preparations are shown in Fig. 3. Relative Chromosome Lengths are given in Table 2 and Centromere Indices in Table 3.

Table 2. RCL of *Coccidula* and *Rhyzobius*: Mean values, 95 % CI (t-test); N = sample size.

Chromosome	*Coccidula rufa*	*Coccidula scutellata*	*Rhyzobius chrysomeloides*	*Rhyzobius litura*
1	19.89 19.12–20.66 N = 18	15.66 15.32–16.00 N = 20	21.35 20.36–22.34 N = 15	28.61 27.97–29.24 N = 24
2	17.14 16.43–17.85 N = 18	14.29 13.86–14.72 N = 20	17.50 16.82–18.18 N=16	25.64 24.25–27.04 N = 24
3	14.61 14.08–15.15 N = 18	12.93 11.85–14.00 N = 20	14.35 13.77–14.93 N = 16	11.45 11.02–11.89 N = 24
4	12.17 11.79–12.55 N = 18	12.47 12.19–12.74 N = 20	12.31 11.92–12.71 N = 16	10.53 9.94–11.12 N = 24
5	10.50 9.94–11.06 N = 17	11.04 9.93–12.14 N = 20	10.43 9.86–11.01 N = 14	9.50 9.14–9.85 N = 24
6	8.97 8.55–9.40 N = 18	11.49 11.18–11.79 N = 20	10.09 9.66–10.52 N = 15	7.76 7.30–8.21 N = 24
7	7.73 7.39–8.06 N = 18	8.96 8.60–9.32 N = 20	8.96 8.53–9.39 N = 16	7.08 6.80–7.36 N = 24
8	5.02 4.59–5.44 N = 18	6.37 6.07–6.66 N = 20	5.53 5.15–5.90 N = 16	
9	4.12 3.77–4.48 N = 18	5.85 5.53–6.17 N = 20		
X	6.17 5.35–7.00 N = 13	5.87 5.22–6.51 N = 12	11.62 11.06–12.19 N = 13	53.26 51.03–55.94 N = 15
Y				22.59 22.32–23.86 N = 9

Table 3. CI of *Coccidula* and *Rhyzobius* chromosomes: Mean values, 95 % CI (t-test); N = sample size.

Chromosome	*Coccidula rufa*	*Coccidula scutel-lata*	*Rhyzobius chrysomeloides*	*Rhyzobius litura*
1	45.13 43.68–46.59 N = 18	47.25 46.03–48.47 N = 20	47.43 45.96–48.89 N = 15	40.91 39.49–42.33 N = 24
2	43.47 41.50–45.43 N = 18	45.06 43.46–46.66 N = 20	42.85 40.52–45.18 N=16	41.10 39.39–42.82 N = 24
3	39.21 37.26–41.17 N = 18	44.56 42.81–46.30 N = 20	41.38 39.68–43.07 N = 16	41.20 39.12–43.29 N = 24
4	40.64 37.99–43.30 N = 18	43.54 41.90–45.18 N = 20	37.37 35.07–39.67 N = 16	41.97 40.08–43.86 N = 24
5	46.25 42.18–50.31 N = 17	42.17 40.30–44.03 N = 20	38.77 35.38–42.17 N = 14	41.76 39.17–44.35 N = 24
6	43.99 41.99–46.0 N = 18	40.55 38.99–42.11 N = 20	32.14 28.60–35.68 N = 15	43.48 40.53–46.44 N = 24
7	38.6 36.09–41.11 N = 18	40.67 38.54–42.79 N = 20	34.18 31.71–36.64 N = 16	41.53 39.61–43.45 N = 24
8	43.82 40.63–47.02 N = 18	43.60 41.52–45.68 N = 20	33.24 29.96–36.52 N = 16	
9	36.53 33.77–39.29 N = 18	41.39 39.34–43.44 N = 20		
X	38.74 35.88–41.60 N = 13	39.99 36.12–43.87 N = 11	34.18 31.37–36.98 N = 13	46.51 44.68–48.33 N = 15
Y				12.00 10.14–13.86 N = 9

Coccidula rufa. Mitotic chromosomes: Fig. 1, a–c; Meiotic chromosomes (Metaphase 1): Fig. 3, a. 2N = 18 + Xy_p (♂), XX (♀).

The RCL values of the autosomes range from about 19 to 4, with marked decreases in size between autosomes 1 and 2 (RCLs about 20 and 17) and, more extreme, between autosomes 7 and 8 (RCLs about 8 and 5). The X chromosome (RCL about 6) is intermediate in size between autosomes 7 and 8. All the autosomes fall in the submetacentric range, though with some variation (Table 2). The y chromosome is dot-like. The X chromosome has a conspicuous secondary constriction, which may appear either open or closed (Fig. 1, b). C-banding (Fig. 1, c) shows moderate centromeric C-bands on autosomes 1 and 3, weaker ones on autosomes 2 and 6, a weak C-band on the X chromosome, and at most occasional hints of slight darkening of the centromeres of some other autosomes (1 replicate each of autosomes 5 and 7).

Coccidula scutellata. Mitotic chromosomes: Fig. 1, d–g. Meiotic chromosomes (Metaphase 1): Fig. 3, b. 2N = 18 + Xy_p (♂), XX (♀).

The RCL values range from about 16 to 6. There is an abrupt decrease in size between autosomes 7 and 8 (RCLs about 9 and 6). The X chromosome (RCL about 6) is intermediate in size between autosomes 7 and 8. Autosome 1 is metacentric, while all the other autosomes, and the X chromosome, fall in the submetacentric range. The y chromosome is very small, but appears about twice the size of that of *C. rufa*. C-banding (Fig. 1, g) shows strong centromeric C-bands on autosomes 5 and 8, and the X chromosome, and fairly strong ones on autosomes 1 and 9.

Rhyzobius chrysomeloides. Mitotic chromosomes: Fig. 2, a–f. Meiotic chromosomes (Metaphase 1) Fig. 3, c. 2N = 16 + Xy_p (♂), XX (♀).

The RCL values range from 21 to 6. The major decreases in size are between autosomes 1 and 2 (RCLs about 21 and 18), 2 and 3 (RCLs about 18 and 14), and 7 and 8 (RCLs about 9 and 6). The size of the X chromosome (RCL about 12) falls between autosomes 4 and 5. Autosome 1 is metacentric, but all the other autosomes, and the X-chromosome, are submetacentric. The y chromosome is very small, dot-like. C-banding (Fig. 2, b, d, f) appears variable. The male shown in Fig. 2, b has a very strong large double centromeric C-band on the X chromosome, a small but distinct one on autosome 1, and fainter bands on autosomes 2, 4, 5, and 7.The male shown in Fig. 2, d has the C-bands on autosomes 1 and 2 more or less as before, but the bands on autosomes 3–6 are much stronger, and that on autosome 7 appears somewhat stronger than in Fig. 2, b. One replicate of autosome 8 shows what appears to be a small centromeric C-band. The C-band on the X chromosome, however, is markedly different: the top section of the band, at the base of the short arm, is very boldly marked, but the lower section, on the long arm, is scarcely apparent. There is a slightly darker region in the appropriate part of the arm, and the total length of the arm as a proportion of the length of the chromosome, appears the same. The third C-banded preparation, the female shown in Fig. 2, e, has the C-banding of the autosomes similar to that shown in Fig. 2, d, but slightly fainter, and autosome 8 has no C-band. The C-band on the X chromosome, however, is very bold and of the same double form shown in Fig. 2, b. This preparation is also heterozygous for a deletion of the apical third of the short arm of autosome 1, and the position of this deletion site is apparent on some of the other figures (2, a and especially 2, d).

Rhyzobius litura. Mitotic chromosomes: Fig. 2, g–i. Meiotic chromosomes (Metaphase 1) Fig. 3, d, (Metaphase 2) Fig. 3, e, f. 2N = 14 + neo-XY (♂), XX (♀). The RCL values of the autosomes range from about 29 to 7, with the major decrease between autosomes 2 (RCL about 26) and 3 (RCL about 11). The neo-X chromosome (RCL about 53) is by far the largest in the nucleus, and the Neo-Y has an RCL of about 23, slightly shorter than autosome 2. All the autosomes are submetacentric, the X chromosome is metacentric, and the Y is acrocentric, and apparently corresponds with shorter arm of the X, with which it agrees in having a median constriction (Fig. 2, g). C-banding, though attempted several times, was unsuccessful, even though on one occasion preparations were made of both *Rhyzobius* species at the same time, and C-banding was subsequently attempted at the same time, successfully in the case of *Rh. chry-*

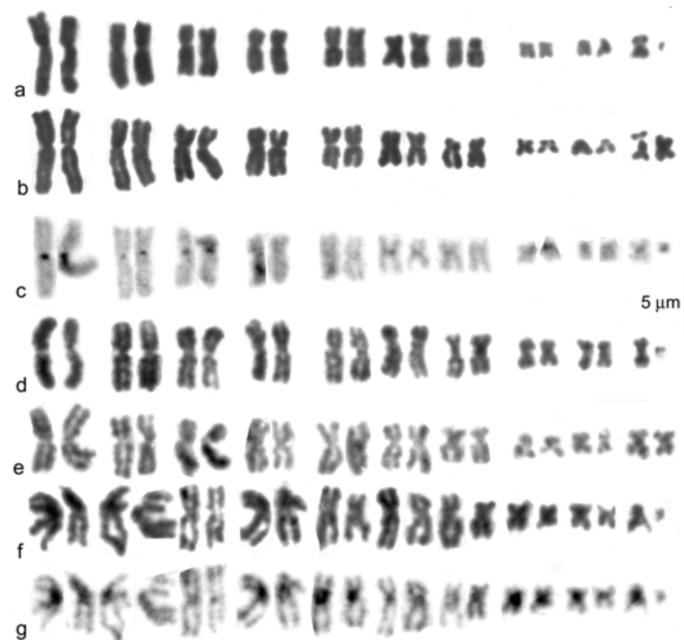

Fig. 1. Mitotic chromosomes of *Coccidula* species, arranged as karyotypes. a–c – *C. rufa* (Herbst): a – ♂, mid-gut; b – ♀, mid-gut; c – ♂, mid-gut, C-banded. d–g – *C. scutellata* (Herbst): d – ♂, testis; e – ♀, midgut; f, g – ♂, mid-gut: f – plain, g – C-banded.

someloides, but not *Rh. litura*. The preparation shown in Fig. 2, i has an apparent centromeric C-band on one replicate of autosome 2, but it is not evident on the other replicate, nor was it found in other nuclei. The X chromosome shows a pattern of faint bands, again not consistently produced in other nuclei. These bands appear to represent sections of the chromosome less affected by the C-banding protocol than other bits, but the patterns, even when produced, are not consistent.

Fig. 2. Mitotic chromosomes of *Rhyzobius* species, arranged as karyotypes. *Rh. chrysomeloides* (Herbst), ♂, testis, the same nucleus: a – plain, b – C-banded; c, d – *Rh. chrysomeloides*, ♂, mid-gut, the same nucleus, a different specimen from a & b: c – plain, d – C-banded; e – *Rh. chrysomeloides*, ♀, mid-gut, C-banded, 1 replicate of autosome 1 with part of the short arm missing; f – *Rh. chrysomeloides*, ♀, mid-gut, plain; g – *Rh. litura* (F.), ♂, mid-gut; h – *Rh. litura*, ♀, mid-gut; i – *Rh. litura*, ♀, mid-gut, treated for C-banding.

Discussion

The karyotypes of the four species studied here are all clearly different from one another, though those of the two *Coccidula* species are more similar to one another than to either of the *Rhyzobius* ones.

The differences between the *Coccidula* species are mainly concerned with the RCLs of the autosomes, with the longer autosomes relatively longer and the shorter ones relatively shorter, in *C. rufa* than in *C. scutellata*. Table 1 shows that in a number of cases the RCLs of equivalent autosomes of the two species are significantly different (95 % confidence values for two samples do not overlap). The autosomes involved are: 1, 2, 3, 6, 7, 8 and 9. Such differences between the karyotypes of two species can only arise as a result of multiple translocation of material between chromosomes. Since karyotypes heterozygous for translocations would be unable to pair up for first division of meiosis, individuals with such karyotypes would be sterile. Therefore such karyotype differences are very good evidence of the species difference between these two *Coccidula*.

The C-banding of the two species is different, as indicated in the Results section and the illustrations (Figs 1, c & 1, i), despite difficulties in obtaining banded preparations. Such differences are not in themselves evidence of species differences as some species have C-banding polymorphisms. However, no chromosomal polymorphism has been discovered in either of these species.

Both of the species have the supposed archetypal polyphagan karyotype of 9 pairs of autosomes plus sex chro-

mosomes comprising a normal X and a very small y, which associates with the X via a cytoplasmic vesicle during first division of meiosis (Xy_p).

The situation with the two *Rhyzobius* species is very different. The karyotype of *Rh. chrysomeloides* differs from those of the *Coccidula* species in having one fewer pair of autosomes. Unlike the two *Coccidula* species, *Rh. chrysomeloides* shows chromosome variation. The karyotype shown in Fig. 2, e is heterozygous for a deletion in the short arm, and, as noted in the Results, the site of this deletion is detectable in intact replicates of the chromosome. This may correspond to the fragile sites found in human and other chromosomes (Sumner, 2003). The chromosomes in two other nuclei from this specimen do not show the deletion, so it appears to represent a spontaneous event in a somatic (mid-gut) cell.

The variation shown by the C-banded preparations of *Rh. chrysomeloides* probably reflects, in part at least, difficulties with the technique. Fig. 2, b is from a testis cell, and R.B. Angus has found that testis often reacts differently from mid-gut to banding treatments. The general banding patterns shown in Figs 2, d and 2, e are broadly similar, except for the band at the base of the long arm of the X chromosome, which in Fig. 2, e is much weaker, if present at all, than in Figs 2, d and 2, b. Two further C-banded nuclei, from a different female, show the fully double-banded X chromosome. The variation in the lower part of this C-band may reflect genuine differences in the chromatin, but it is possible that there is a threshold effect whereby the chromatin sometimes shows the C-banding reaction, sometimes not. Thus Angus (1989) notes unreliability of telomeric C-bands in *Helophorus occidentalis*, where some cells ona C-banded slide showed the telomeric bands, while others did not, even though all the centromeric C-bands were well developed (R.B. Angus, pers. observ.).

The karyotypes of the two *Rhyzobius* species are totally different from one another. *Rh. chrysomeloides* has a fairly conventional karyotype. It differs from those of the *Coccidula* species in having one fewer pair of autosomes. If the chromosome formula of the *Coccidula* species ($2N = 18 + Xy_p$) is primitive, then the change to the $2N = 16 + Xy_p$ shown by *Rh. chrysomeloides* is most likely the result of fusion of a pair of autosomes. In *Rh. litura* there is a further reduction in the number of autosomes to 16, associated with the development of a neo-XY system of sex chromosomes. Neo-XY systems arise by the fusion of an original X chromosome with an autosome, to give neo-X. The unfused replicate of the auto some becomes neo-Y, and the XY bivalent at first division of meiosis is held together by chiasmata in the originally autosomal components. Such systems are not uncommon in Adephaga, where the norm is a sex chromosome system involving only X chromosomes – females XX, males X0. Development of neo-XY systems in beetle species where the norm is Xy_p is more complex: either both the X and the y chromosomes have to fuse with replicates of the same autosome, or the y chromosome mustbe lost. In some cases these small y chromosomes are heterochromatic [e.g. *Geotrupes mutator* Marsham and *Typhaeus typhoeus* (L.) (Wilson, Angus, 2004)] and thus carry no genes, but

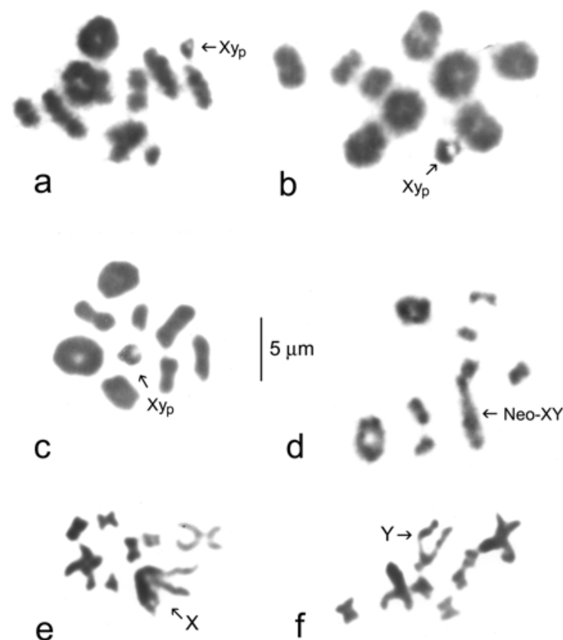

Fig. 3. Meiotic chromosomes of *Coccidula* and *Rhyzobius*. a–d – metaphase 1; e, f – metaphase 2: a – *Coccidula rufa* (Herbst); b – *C. scutellata*; c – *Rhyzobius chrysomeloides* (Herbst); d – *Rh. litura* (F.); e – *Rh. litura*, ♀-determining nucleus with X chromosome; f – *Rh. litura*, ♂-determining nucleus with Y chromosome.

merely serve as pairing partners for the X chromosomes. The Coccinellidae, though a polyphagan group with an ancestral Xy_p system, have numerous species with neo-XY sex chromosomes (Smith, Virkki, 1978). Nevertheless, the discovery of such different karyotypes in the two British species of *Rhyzobius* is unexpected.

Rhyzobius is a large genus, with 77 species listed by Korschefsky (1931). Nearly all are Australasian, and only two are European. At a first glance at their karyotypes, these two species do not appear to be particularly closely related. Nevertheless, they are the only two species known from Europe. There are one or two listed from the eastern Palaearctic (China), and Smith and Virkki give the karyotype of *Rh. ventralis*, a Californian species, as 2N = 17, 8 pairs of autosomes plus X0 (♂). This, at least, gives a theoretical starting point for the neo-XY system of *Rh. litura*. All that would be required is fusion of the X with an autosome.

The situation regarding the *Rhyzobius* karyotypes may be contrasted with that found in the *Coccidula* species, where the karyotypes are basically similar, with a number of clear species-specific differences only. Korschefsky (1931) lists only eight or nine species (the generic attribution of one species is queried), all from Eurasia (Palaearctic Realm) or North America (Nearctic Realm). The list of *Coccidula* species suggests a small, localised genus, probably not particularly old. *Rhyzobius*, on the other hand, is either polyphyletic (of multiple origins), perhaps with the Australasian species not closely related to the northern Hemisphere ones, and the small number of northern Hemisphere species either relicts of a once larger group, or disparate offshoots of the Australasian group.

One curious comparison of the situation encountered in this study of the karyotypes of the four British Coccidulinae may be made. Shaarawi and Angus (1991) investigated the karyotypes of five European species of the genus *Anacaena* Thomson (Coleoptera, Hydrophilidae). Three species have karyotypes of 2N = 16 + Xy_p, one has 2N = 14 + Xy_p, and one has 2N = 10 + Neo-Xy. These results more or less duplicate the finding reported here for a subfamily, within a single, morphologically rather homogeneous genus! Perhaps this should serve as a warning against attributing too much phylogenetic significance to karyotype differences.

Acknowledgements

We thank all who have helped in the carrying out of this project, including fellow students Antonios Konitsiotis and Constance Jeangirard for help with the collection of livestock, and Max Barclay of the Natural History Museum for information from the Coleoptorum Catalogus. We particularly thank Peter Hammond for drawing attention to the Windsor population of *Rhyzobius chrysomeloides*, which was the catalyst for the entire study.

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