C-BANDING KARYOTYPE AND MALE MEIOSIS OF *ACROPHYLLA WUELFINGI* (REDTENBACHER) (PHASMATODEA: PHASMATIDAE)

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Abstract

The C-Banded karyotype and the male meiosis of the Australian species *Acrophylla wuelfingi* are described and compared with other members of the subfamily Phasmatinae. The chromosomes of *A. wuelfingi* are rather large and their number is 2n ♂ = 45, XO, 2n ♀ = 46, XX; the metacentric X being the largest element of the set. Karyotype analysis shows a predominance of heterobrachial chromosomes and centromeric heterochromatin bands or blocks in all elements. Male meiosis is chiasmate, with mainly terminal chiasmata.

Introduction

In the Phasmatodea, many speciation instances seem to be correlated with extensive chromosome changes but chromosome variability usually appears too high to allow cytotaxonomic comparisons between members of different genera (Hughes-Schrader 1959; Craddock 1972; Scali 1972; White 1976; Marescalchi *et al.* 1985). However karyotype differentiation appears to be meaningful in closely related species (Craddock 1975; Goday *et al.* 1982; Nascetti *et al.* 1982; Scali 1982; Marescalchi *et al.* 1985). The most important contribution to phasmid cytotaxonomy is that given on Australian groups by Craddock (1972, 1975).

This paper gives the chromosome number, the C-Banded karyotype and the main features of male meiosis of the giant species *Acrophylla wuelfingi* (Redtenbacher), in order to contribute further to chromosomal knowledge of the Australian Phasmatidae.

Materials and methods

Adult specimens of *A. wuelfingi* (2 ♂, 2 ♀) were obtained from the London Zoo Insect House and maintained on a fresh bramble diet in a wide, well ventilated cage.

Testes and ovaries were dissected from CO_2-anaesthetised individuals 1 h after injection of 0.05% colchicine and used immediately for chromosome preparations according to Crozier's technique (1968). About half of the slides were used for C-Banding, according to Sumner's (1972) method. All slides were stained with a 2% Giemsa solution in Sörensen buffer at pH 7 for 15 min.

For chromosome typing we followed the criteria and terms suggested by Levan *et al.* (1964). Measurements for chromosomes pairing were taken from both enlarged prints and "camera lucida" drawings of C-metaphases.

Results

The chromosome number of *A. wuelfingi* is 2n = 45 ♂, 46 ♀, with an XO/XX sex-chromosome mechanism (Figs 1, 2). The chromosomes are rather large compared with other phasmid species, the largest ranging from 12 to 15μ in our colchicine treated squashes. The female karyotype (Fig. 2) shows the following composition: 2 pairs of large metacentrics, the largest being the X chromosomes; 3 pairs of medium-sized metacentrics; 4 pairs of medium-sized subacrocentrics, the largest bearing a satellite; 2 pairs of small metacentrics; 6 pairs of small subacrocentrics and 6 pairs of small telocentrics.

In both sexes, C-Banding invariably revealed the presence of a heterochromatic centromeric band or mass in all elements. In the various metaphase plates the C-heterochromatin proved to be nearly constant in size and shape for a given chromosome pair; on the other hand clear differences were sometimes noticed between pairs. Therefore the C-Banding pattern helped us in assessing some of the otherwise uncertain chromosome pairings, especially within the same plate (see chromosome pairs 7 to 9). In most mitoses and in all meiotic prophase 1st divisions of the male germ line of the 2 specimens, the X chromosome showed a tandemly repeated centromere along the chromosome axis (Fig. 1, arrow).

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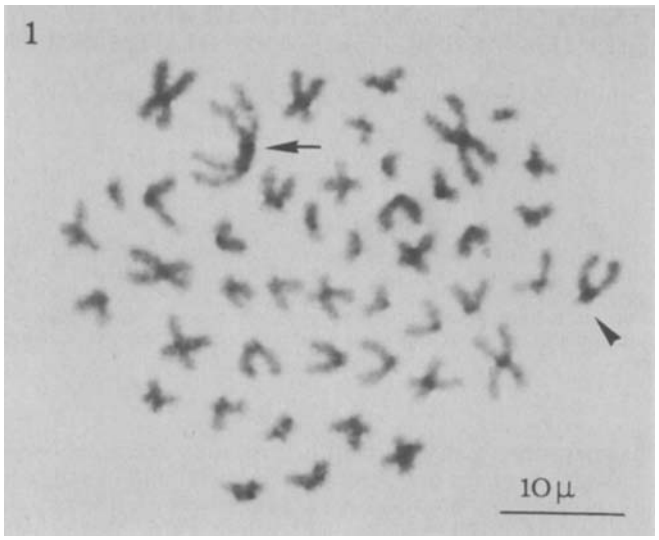

FIG. 1—Spermatogonial C-metaphase plate of *A. wuelfingi* showing the 45 chromosome set of the male. Arrow points the metacentric X chromosome with the apparently double centromere; arrowhead, a satellite-bearing chromosome. Giemsa stain.

In our slides it was not possible to follow the full characteristic sequence of male prophase I at the "pre-metaphase stretch" stage, as described by Hughes-Schrader (1947); this was probably due to colchicine treatment. Nuclear features, however, corresponded well to those found in other Australian species (Craddock 1972). The meiotic prophase of *A. wuelfingi* showed the characteristic post-diplotene appearance, with rather thin and long bivalents, often with the usual fuzzy outline (Fig. 3), very different from the thick, sharp bivalents of the immediately following metaphase (Fig. 4). It was apparent that the single X chromosome is completely heteropycnotic during prophase I and showed the chromatids and the centromeric region undivided until the end of metaphase I; in contrast, from rather early in the diplotene stage the autosomes already had split chromatids and centromeres.

Meiosis was chiasmate, with chiasma frequency only slightly exceeding one per bivalent. Chiasmata generally showed a terminal position. Table 1 gives their frequency and distribution for 10 spermatocytes at pre-metaphase. Two kinds of metaphase II plates were found, with 23 or 22 double-stranded chromosomes, respectively; the X chromosome must therefore migrate undivided to one pole at metaphase I.

Table 1. Chiasma number and position in 10 spermatocytes at the pre-metaphase stage for *Acrophylla wuelfingi*

Cell number	Total chiasmata	Chiasma position		
		distal	intermediate	proximal
1	23	19	4	0
2	22	17	5	0
3	23	19	3	1
4	23	16	5	2
5	24	17	7	0
6	24	21	3	0
7	22	17	2	3
8	23	21	1	1
9	22	19	1	2
10	24	21	0	3

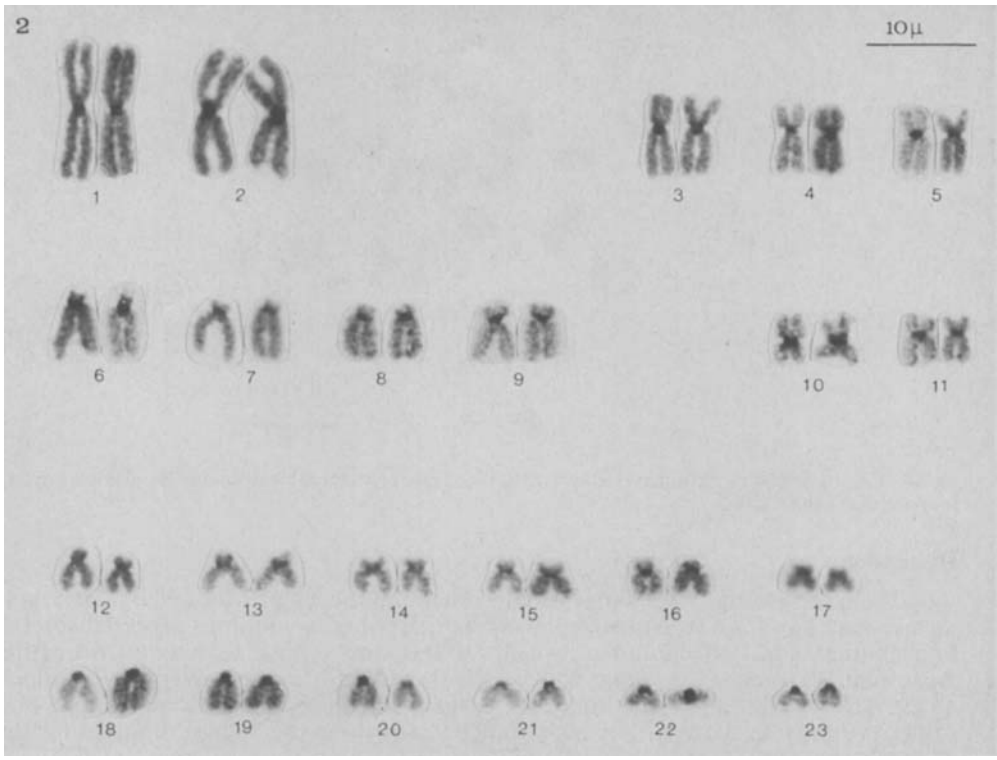

FIG. 2—The 46 chromosomes of the female karyotype of *A. wuelfingi* derived from a mitotic C-banded metaphase plate of a follicular cell; some pairings assigned on the basis of C-heterochromatin size and shape. Giemsa stain.

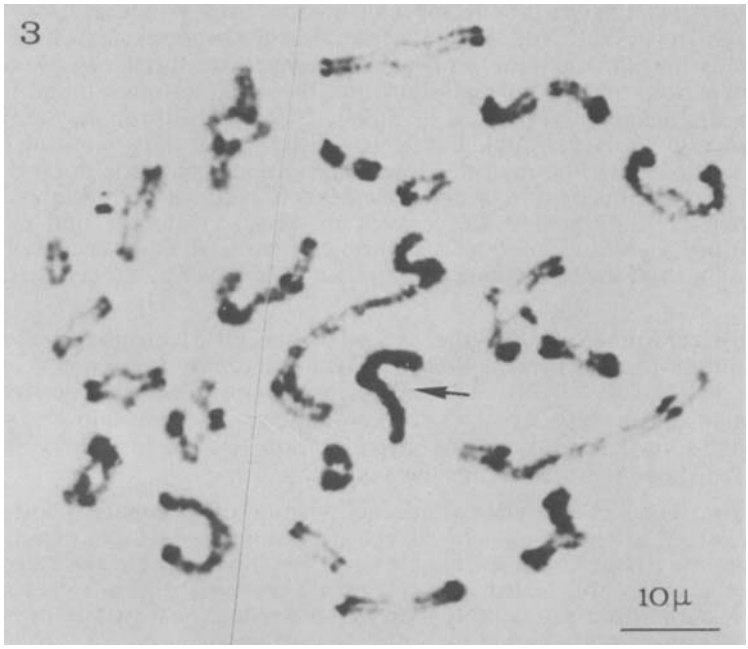

FIG. 3—Male meiotic prophase I at the pre-metaphase. Note the strongly heterochromatic X chromosome (arrow), the thin appearance and fuzzy outline of most bivalents, and the split centromere of the homologous chromosomes. Giemsa stain.

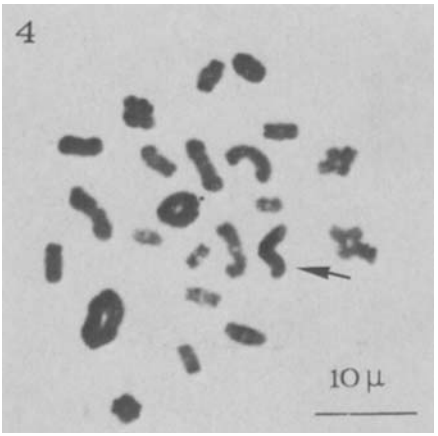

FIG. 4—Late male meiotic metaphase I: the sharp outline of the contracted bivalents and X univalent (arrow) is apparent. Giemsa stain.

Discussion

The large average size of the chromosomes and the length attained by the largest metacentric pairs are in agreement with the karyological findings reported for the Phasmatinae and Podacanthinae, which are the most typical representatives of the Australian Phasmatodea, being widely distributed and largely endemic (Craddock 1972). The number and structure of *A. wuelfingi* chromosomes give indications of a rather primitive karyotype. The high number—equalling the highest reported for the Australian phasmids—and the predominance of heterobrachial elements are in favour of a conservative chromosome condition. Also, we may recall that in the Phasmatodea an XO/XX sex-chromosome mechanism and a large metacentric X appear to be ancestral karyotype traits (Craddock 1972, 1975; White 1976).

Craddock (1972) gives the chromosome number of the congeneric species *A. titan* (Macleay) as 2n = 35 ♂, 36 ♀. Such a large difference between species, which on the basis of body morphology, are thought to be congeneric species, is not common in other orthopteroid insects, but well illustrates the situation often found in Phasmatodea (Hughes-Schrader 1959; Scali 1972; White 1976). Unfortunately the karyotype of *A. titan* is not reported; it could have given us the opportunity of testing the general hypothesis that in the Phasmatodea a reduction in chromosome number is mainly achieved by centric fusion of telocentric elements (Craddock 1975; Marescalchi *et al.* 1985). However additional evidence such as genetic distances and egg chorion differentiations should be obtained before one can definitely accept the present taxonomic status of the two species (Mazzini and Scali 1983 a, b; Nascetti and Bullini 1983).

Double centromeres along the axis of metacentric chromosomes have been reported and thoroughly investigated in the orthopteran *Neopodismopsis abdominalis* (Thomas) by Moens (1978). These had been interpreted as derived from a Robertsonian centric fusion of two acrocentric chromosomes, and electron microscope findings further support this interpretation. For *A. wuelfingi* no data are available to explain this X chromosome feature.

The low frequency and mainly terminal position of chiasmata reported here for male *A. wuelfingi* are typical of most Phasmatodea. It would seem possible that the "pre-metaphase stretch" is, at least in part, responsible for such a generally occurring feature in specimens not treated with colchicine. We think it of interest to note that, while the X chromosome invariably follows a pre-reductional pattern of meiosis, for the autosomal bivalents, which at early diplotene show well-separated chromatids and split centromeres, a post-reductional segregation cannot be excluded.

The sharp blocks of C-heterochromatin and the relative large number and size of chromosomes are important features of this phasmatine karyotype. These karyological traits suggest that the species has a large amount of DNA. The more specialised Ramulini and Bacillini so far analysed have much smaller chromosomes and apparently less C-heterochromatin (Mosti and Scali 1975; Goday *et al.* 1982; Scali *et al.* unpubl. data). This would support the idea of a general trend of genome size evolution as suggested by Hinegardner (1976).

References

CRADDOCK, E. M. (1972)—Chromosomal diversity in the Australian Phasmatodea. *Aust. J. Zool.* **20:** 445-462.

CRADDOCK, E. M. (1975)—Intraspecific karyotypic differentiation in the Australian Phasmatid *Didymuria violescens* (Leach). *Chromosoma (Berlin)* **53:** 1-24.

CROZIER, R. H. (1968)—An acetic acid dissociation, air-drying technique for insect chromosomes, with aceto-lactic orcein staining. *Stain Technol. (Geneva)* **43:** 171-173.

GODAY, C., BIANCHI BULLINI, A. P., NASCETTI, G. and BULLINI, L. (1982)—Chromosome studies on *Bacillus atticus*, *Bacillus rossius* and their hybrids (Cheleutoptera, Bacillidae). Atti della Accademia Nazionale dei Lincei. Rendiconti. *Classe di Scienze Fisiche Matematiche e Naturali* **71:** 126-133.

HINEGARDNER, R. (1976)—Evolution of genome size. *In* Ayala, F. J. (Ed.) *Molecular evolution*: pp. 179-199. Sinauer Associated Inc.: Massachusetts.

HUGHES-SCHRADER, S. (1947)—The "pre-metaphase stretch" and kinetochore orientation in Phasmids. *Chromosoma (Berlin)* **3:** 1-21.

HUGHES-SCHRADER, S. (1959)—On the cytotaxonomy of phasmids (Phasmatodea). *Chromosoma (Berlin)* **10:** 268-277.

LEVAN, A., FREDGA, K. and SANDBERG, A. A. (1964)—Nomenclature for centromeric position on chromosomes. *Hereditas (Lund)* **52:** 210-220.

MARESCALCHI, O., CORNI, M. G. and SCALI, V. (1985)—I cromosomi di tre specie di *Bacillus* (Insecta Phasmatodea) del Mediterraneo orientale. Atti XIV Congresso Nazionale Italiano di Entomologia, Palermo-Erice-Bagheria: 201-207, Tipografia Bonfardino.

MAZZINI, M. and SCALI, V. (1983 a)—Le uova dei Phasmatodea al microscopio elettronico a scansione: loro valore tassonomico. Atti XII Congresso Nazionale Italiano di Entomologia, Roma, **II:** 425-431, Tipografia M. Sticca.

MAZZINI, M. and SCALI, V. (1983 b)—Le uova di quattro specie di Ramulini (Phasmatodea, Heteronemiidae) della Somalia al microscopio elettronico a scansione. Atti XIII Congresso Nazionale Italiano di Entomologia, Sestriere-Torino: 89-96, Tipografia Grafital.

MOENS, P. B. (1978)—Kinetochores of grasshoppers with Robertsonian chromosome fusions. *Chromosoma (Berlin)* **67:** 41-54.

MOSTI, P. and SCALI, V. (1975)—Osservazioni sul corredo cromosomico di *Bacillus rossius* (Insecta, Cheleutoptera). Atti della Accademia Nazionale dei Lincei. Rendiconti. *Classe di Scienze Fisiche Matematiche e Naturali* **LIX:** 493-498.

NASCETTI, G., BIANCHI BULLINI, A. P. and BULLINI, L. (1982)—Ricerche elettroforetiche e cariologiche su un fasmide partenogenetico di origine ibrida, *Bacillus whitei*, e i suoi progenitori bisessuati, *B. rossius* e *B. grandii*. Boll. di Zool. **49** (supplemento): 133.

NASCETTI, G. and BULLINI, L. (1983)—Differenziamento genetico e speciazione in Fasmidi dei generi *Bacillus* e *Clonopsis* (Cheleutoptera, Bacillidae). Atti XII Congresso Nazionale Italiano di Entomologia, Roma, **II:** 215-223, Tipografia M. Sticca.

SCALI, V. (1972)—Problemi di citotassonomia nei Cheleutoptera Crampton 1915. Atti IX Congresso Nazionale Italiano di Entomologia, Siena: 285-293, Tipografia Bertelli & Piccardi.

SCALI, V. (1982)—Evolutionary biology and speciation of the stick insect *Bacillus rossius* (Insecta Phasmatodea). *In* Barigozzi, C. (Ed.), *Mechanisms of speciation*, pp. 393-410. Alan R. Liss Inc.: New York.

SUMNER, A. T. (1972)—A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* **75:** 304-306.

WHITE, M. J. D. (1976)—Insecta 2. *In* John, B. (Ed.), *Animal cytogenetics 3*, pp 1-75. Gebruder Borntraeger: Berlin.

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