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Chromosome Numbers, C-banding Patterns and Sperm of Some Ladybird Species from Central Europe (Coleoptera, Coccinellidae)

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C-banded karyotypes were studied in the males of seven ladybird species from the subfamily Coccinellinae, viz. Adonia variegata (Goeze), Typhaspid sedecimpunctata (L), Coccinella septempunctata (L), Culvia decempunctata (L), Culvia quatuordecimpunctata (L), Propylea quatuordecimpunctata (L), Phylosbora vigintidupunctata (L). All the species, with the exception of Typhaspid sedecimpunctata (L), possess 2n = 20, n = 9+Xy. Typhaspid sedecimpunctata (L), however, has 2n = 24, n = 11+Xy. The examined karyotypes show only a paracentromeric position of constitutive heterochromatin in all autosomes and the sex chromosome X. while the y heterochromosome is dot-like and wholly euchromatic. Successive stages of spermiogenesis were analysed.

Key words: Coleoptera, Coccinellidae, chromosome number, C-bands, karyotype, spermiogenesis.

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The order Coleoptera comprises approximately 350 000 described species (LAWRENCE 1982), but karyological information concerning the families is highly fragmented, and covers only 3 000 species (SMITH & VIRKKI 1978; SHARMA et al. 1980; SERRANO & YADAV 1984; PETITPIERRE 1988; PETITPIERRE et al. 1988; GILL et al. 1990; GALIAN & MOORE 1994; LACHOWSKA et al. 1998). The chromosome numbers vary widely, ranging from 2n = 4 (FERREIRA et al. 1984) to 2n = 69 (SERRANO 1981). There is certainly a need for study of a greater number of species for a better understanding of this variability (FERREIRA & MESA 1977; MARTINS 1994; MAFFEI et al. 2000). The basic beetle karyotype, probably the ancestral one, has been reported to consist of nine pairs of autosomes and the X and Y sex chromosomes, which associate in a "parachute" configuration during metaphase I (SMITH 1950; SMITH & VIRKKI 1978). Cytogenetic analyses of Coleoptera have mostly been performed during male meiosis because of the difficulty in obtaining mitotic metaphase chromosomes (SMITH & VIRKKI 1978).

The family Coccinellidae is a medium-sized, economically very important group of beetles comprising about 5000 species (RICHARDS & DAVIES 1979). Many ladybird species have been found to be efficient predators of aphids, scales, and lepidopteran eggs (DE BACH 1964; GORDON 1985). The members of this family are also known to exhibit chromosomal polymorphism. So far, about 200 species from that family (4%) have been studied cytologically, while basic chromosomal data concerning ladybirds have been reported from Europe and America (SMITH 1960; ENNIS 1974; SMITH & VIRKKI 1978; LYAPUNOVA et al. 1984; MAFFEI et al. 2000), China (LI 1940), Japan (YOSIDA 1944, 1948, 1949, 1952; TAKEUCHI 1976) and India (SHARMA et al. 1959; AGARWAL 1961; SMITH 1965; MANNA & LAHIRI 1972; DUA & KACKER 1975; KACKER 1973; MITAL et al. 1989; YADAV & PILAI 1979; YADAV et al. 1991; YADAV & GAHLAWAT 1993, 1994).

Earlier cytogenetic studies on ladybirds were carried out on the levels of α and β karyology (WHITE 1978). Banded karyotypes of Coccinellidae are almost unknown, with the exception of the

The aim of the present work was to describe the karyotypes and C-banding pattern in seven ladybird species: *Adonia variegata* (Goeze), *Tythispis sedecimpunctata* (L.), *Coccinella septempunctata* (L.), *Calvia decemguttata* (L.), *Calvia quatuordecimpunctata* (L.), *Propylea quatuordecimpunctata* (L.), *Phyllobora vigintiduopunctata* (L.).

**Material and Methods**

For the cytogenetic study, male adults of coccinellid species were collected in forest ecosystems in the vicinity of Bratislava (SW Slovakia) and Kraków (S Poland) in May 2001.

The gonads of males were dissected and used as material for squashes. The former were fixed according to the method described by ROZEK (1994) with minor modification (ROZEK 1998a, b; ROZEK et al. 1999; ROZEK & HOLECOVA 2000; ROZEK & LACHOWSKA 2001). C-bands were determined using modifications of the procedure as described by ROZEK (2000). Dry slides were stained with 4% Giemsa for 10 to 20 min. Observations of chromosomes, spermogenesis, and photomicrographs were made with a Jenaval light microscope (C. Zeiss, Jena).

**Results and Discussion**

*Adonia variegata* (Goeze) 2n=20, n♂=9+XYp

Only meiotic stages were observed in the preparations. During the metaphase I it was possible to distinguish the sex bivalent forming a typical parachute. The metaphase II plates confirmed the presence of 7 pairs of subtelocentric and 2 pairs of metacentric autosomes, the metacentric X and the dot-shaped y chromosome (Figs 1 & 2).

*Tythispis sedecimpunctata* (L.) 2n=24, n♂=11+XY

Eleven autosomal bivalents and the sex heterochromosomes X and y were clearly recognizable during the male metaphase I. The parachute association of the sex heterovalent was not observed in the preparations. The metaphase II plates confirmed the presence of metacentric and submetacentric autosomes (Figs 3 & 4).

*Coccinella septempunctata* (L.) 2n=20, n♂=9+XYp

This species was earlier investigated by Indian (SHARMA et al. 1959; AGARWAL 1961; MANNA & LAHIRI 1972), British (JOHN & LEWIS 1960), and Russian (LYAPUNOVA et al. 1984) karyologists. The present investigations on the material from Central Europe confirm the numerical data of other authors. Substantial differences were noticed in the karyotypic details. AGARWAL (1961) reported 5acrocentrics, two pairs of autosomes and the X-chromosome, in specimens from India. LYAPUNOVA et al. (1984) observed one pair of metacentric, seven pairs of submetacentric, one pair of subtelocentric autosomes, the submetacentric X and dot-shaped y forming a typical parachute. Six pairs of subtelocentric and three pairs of submetacentric autosomes were observed in the present material from Bratislava (Slovakia) (Fig. 5). The sex heterovalent of the parachute type was visible during the diplotene and metaphase I. The morphology of individual chromosomes and localization of centromeres was clearly visible in mitotic metaphase and during the metaphase II (Figs 5, 6, 7 & 8).

*Calvia decemguttata* (L.) 2n=20, n♂=9+XYp

Nine autosomal bivalents, and the sex heterochromosomes X and y, forming the typical parachute, were observed in the male metaphase I (Fig. 9).

*Calvia quatuordecimpunctata* (L.) 2n=20, n♂=9+XYp

Meiotic metaphase I and II plates were exclusively observed in the preparations. Chromosomes were metacentric and submetacentric. The sex heterovalent Xyp was recognizable during metaphase I (Figs 10 & 11).

*Propylea quatuordecimpunctata* (L.) 2n=20, n♂=9+XYp

The mitotic plates confirmed the ancestral beetle karyotype consisting of metacentric and submetacentric autosomes, the submetacentric X and the dot-shaped y which was the smallest element of the set (Fig. 12). The sex heterovalent of the parachute type was visible during the metaphase I (Fig. 13). The present data on the morphology of the examined karyotype confirm and complete its earlier description given by LYAPUNOVA et al. (1984).
Figs 1-18. Figs 1-2. Meiotic chromosomes of *Adonia variegata* (Goeze). Fig. 1. Metaphase I. Fig. 2. Metaphase II. Figs 3-4. Meiotic chromosomes of *Tetraspis sedecimpunctata* (L.). Fig. 3. Metaphase I. Fig. 4. Metaphase II. Figs 5-8. Mitotic and meiotic chromosomes of *Coccinella septempunctata* (L.). Fig. 5. C-banded karyotype. Fig. 6. Dihoton. Fig. 7. Metaphase I. Fig. 8. Metaphase II. Fig. 9. Metaphase I of *Calyx decemguttata* (L.). Fig. 10-11. Meiotic chromosomes of *Calyx* *quatuordecimguttata* (L.). Fig. 10. Metaphase I. Fig. 11. Metaphase II. Figs 12-13. Mitotic and meiotic chromosomes of *Propylea quatuordecimguttata* (L.). Fig. 12. C-banded karyotype. Fig. 13. Metaphase I. Fig. 14. Diakinesis of *Phyllobora vigintiduopunctata* (L.). Arrows indicate the sex bivalent of the parachute type during the first meiotic division. Bar equals 5 μm. Figs 15-18. Spermiogenesis in lady-birds. Fig. 15-16. Elongated spermatids in *Phyllobora vigintiduopunctata* (L.). Fig. 17. Spermatoecyst – syncytial cyst with young spermatids in which the sperm heads become embedded and the bundle of flagella in *Coccinella septempunctata* (L.). Fig. 18. Mature spermatozoa in *Coccinella septempunctata* (L.). Bar equals 10 μm.
Phyllobora vigintiduopunctata (L.) 2n=20, nσ=9+XYp

Only diakineses of the first meiotic division were available in this hitherto uninvestigated species for study. Nine autosomal bivalents and the sex parachute XYp, were clearly visible (Fig. 14).

The present paper deals with the karyotypic details and meiotic behaviour in seven ladybird species. Five of them had never been studied from the karyological point of view. Although the diploid chromosome number of Coccinellidae ranges from 2n=12 in Mulsantina hudsonica Csy. to 2n=28 in Dralia pactoralis Muls. (SMITH & VIRKKI 1978; YADAV & GAHLAWAT 1994), this beetle family is characterized by the possession of ancestral polyphagous karyotype 2n=20, nσ=9+XYp occurring in approximately more than 40% of species examined cytologically (MANNA & LAHIRI 1972; TAKENOUCHI 1976; SMITH & VIRKKI 1978; LYAPUNOVA et al. 1984; YADAV & GAHLAWAT 1994; MAFFEI et al. 2000). Six species under the present investigation also depicted this karyotype. Tytaphis sedecimpunctata (L.) with 2n=24, however, deviates from the modal number. The male sex chromosome mechanism XYp is the most common system described in 101 ladybird species (including the present study).

The C-banding pattern in all examined species indicated that constitutive heterochromatin had a paracentromeric location in all autosomes and the sex chromosome X while the y was dot-like and wholly euchromatic. Paracentromeric location of C-bands is characteristic of the majority of insects (IMAI 1991) and was also confirmed in Coccinellidae (ENNIS 1974; MAFFEI et al. 2000 and the present investigation).

The spermigenesis consists of a series of morphological and cytological modifications of the spermatids. It begins after the telophase stage of the second meiotic division and is complete with the formation of spermatozoa. The slides stained by the C-banding method facilitated observation of the successive stages of spermigenesis in the analysed species. The early spermatids form synctial groups. The spermatocyte survives the metamorphosis of the spermatids to spermatozoa, producing a large cap cell in which the sperm heads become embedded, and a thin cytoplasmatic sheath, enveloping the bundle of flagella. In the next phase the spermatids are elongated and the flagellum is many times longer than the head (Figs 15, 16 & 17). The mature spermatozoa are of the modified type, thread-like in shape (Fig. 18).

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Karyological Studies on some Coccinellidae


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