PŘÍRODOVĚDECKÁ FAKULTA

JIHOČESKÉ UNVERZITY V ČESKÝCH BUDĚJOVICÍCH

Rigorózní práce

Codling moth cytogenetics: karyotype, chromosomal location of rDNA, and molecular differentiation of sex chromosomes

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Školitel: Prof. RNDr. František Marec, CSc.

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Annotation:

In this study, a detailed karyotype analysis was performed in the codling moth, *Cydia pomonella*, the key pest of pomme fruit in the temperate regions of the world. Besides chromosome number, distribution of ribosomal genes was determined using fluorescence *in situ* hybridization (FISH) with codling moth 18S rDNA probe. Sex chromosomes were molecularly differentiated by genomic *in situ* hybridization (GISH) and the gross DNA composition of the heterochromatic W-chromosome was assessed by comparative genomic hybridization (CGH).

Stanovisko spoluautorů:

Potvrzuji, že Petr Nguyen přispěl významnou měrou ke vzniku předložené práce.

František Marec

Prohlášení

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Codling moth cytogenetics: karyotype, chromosomal location of rDNA, and molecular differentiation of sex chromosomes

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Abstract: We performed a detailed karyotype analysis in the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), the key pest of pome fruit in the temperate regions of the world. The codling moth karyotype consisted of 2*n* = 56 chromosomes of a holokinetic type. The chromosomes were classified into 5 groups according to their sizes: extra large (3 pairs), large (3 pairs), medium (15 pairs), small (5 pairs), and dot-like (2 pairs). In pachytene nuclei of both sexes, a curious NOR (nucleolar organizer region) bivalent was observed. It carried 2 nucleoli, each associated with one end of the bivalent. FISH with an 18S ribosomal DNA probe confirmed the presence of 2 clusters of rRNA genes at the opposite ends of the bivalent. In accordance with this finding, 2 homologous NOR chromosomes were identified in mitotic metaphase, each showing hybridization signals at both ends. In highly polyploid somatic nuclei, females showed a large heterochromatin body, the so-called sex chromatin or W chromatin. The heterochromatin body was absent in male nuclei, indicating a WZ/ZZ (female/male) sex chromosome system. In keeping with the sex chromatin status, pachytene oocytes showed a sex chromosome bivalent (WZ) that was easily discernible by its heterochromatic W thread. To study molecular differentiation of the sex chromosomes, we employed genomic in situ hybridization (GISH) and comparative genomic hybridization (CGH). GISH detected the W chromosome by strong binding of the Cy3-labelled, female-derived DNA probe. With CGH, both the Cy3-labelled female-derived probe and Fluor-X labelled male-derived probe evenly bound to the W chromosome. This suggested that the W chromosome is predominantly composed of repetitive DNA sequences occurring scattered in other chromosomes but accumulated in the W chromosome. The demonstrated ways of W chromosome identification will facilitate the development of genetic sexing strains desirable for pest control using the sterile insect technique.