Micron 40 (2009) 881–885

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Karyology of parthenogenetic weevils (Coleoptera, Curculionidae): Do meiotic prophase stages occur?

Maria Rożek [a], Dorota Lachowska [b,*], Milada Holecovà [c,d], Łukasz Kajtoch [a]

[a] Institute of Systematics and Evolution of Animals, Polish Academy of Science, Sławkowska 17, 31-016 Kraków, Poland
[b] Department of Entomology, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Kraków, Poland
[c] Department of Zoology, Comenius University, Mlynská dolina B-1, 842-15 Bratislava, Slovakia
[d] Institute of Environmental and Regional Development, University of Central Europe in Skalica, Král'ovská 386/11, 909-01 Skalica, Slovakia

ARTICLE INFO

Article history:
Received 11 May 2009
Received in revised form 10 June 2009
Accepted 10 June 2009

Keywords:
Asexuality
Curculionidae
Chromosomes
Meiosis
Parthenogenesis
Polyploidy

ABSTRACT

We investigated the cytological mechanism of parthenogenesis by analyzing the chromosomes in five weevil species. All examined species are polyploids, four of which: *Otiorhynchus ovatus*, *Simo variegates*, *Cathormiocerus aristatus*, and *Tropiphorus elevatus* possess three haploid sets of chromosomes ($3n = 33$), whereas the fifth, *Trachyphloeus parallelus*, is tetraploid with 44 chromosomes ($4n = 44$). The plates contained 27–31 chromosomes in triploid species and 38, 39, 41 and even 44 in tetraploid *T. parallelus*. In all species single clusters of metaphase plates with a haploid number of $n = 11$ were visible. Some oogonial cells showed nuclei configurations resembling the stages of diplotene and diakinesis. The spiralized chromosomes in these nuclei may have been connected by chiasmata resulting in rods figures and ring-shaped bivalents. Occurrence of the remnants of meiosis could suggest some degree of recombination in parthenogenetic lineages of weevils.

1. Introduction

Asexual reproduction and polyploidy are relatively rare in animals with chromosomal sex determination and always represent a derived condition. Asexual animals are commonly viewed as evolutionarily inferior and short-lived in an evolutionary sense (White, 1970; Bell, 1982). However, asexuality is most common among insects in which over 900 species are reported to be asexual or have asexual forms. Although many clonal insect species exist, little is known about the origin of parthenogenetic reproduction in this group (Normark, 2003). On a cytological basis, parthenogenesis can be divided into three main types: automictic (or meiotic), apomictic (or ameiotic), and generative or haploid parthenogenesis. In automictic parthenogenesis eggs are produced through meiosis, and the diploid chromosome number is restored by fusion of two of the haploid meiotic products (Suomalainen et al., 1987). Automixis has been considered to represent the first step in the evolution of parthenogenesis. In apomictic parthenogenesis, there is no recombination of alleles and the eggs develop only through mitoses that may show vestiges of meiosis (Saura et al., 1993). In this case heterozygosity steadily increases as a result of mutation and is maintained through generations (Suomalainen et al., 1987). In generative or haploid parthenogenesis, regular chromosome pairing and reduction takes place in the eggs. The eggs may develop either through fertilization or without it.

Parthenogenesis is often correlated with polyploidy. Polyploidy in animals is known to originate by failure of cell division during meiosis, failure of cell division after mitotic doubling, production of unreduced eggs and by hybridization between species (Otto and Whitton, 2000). Polyploidy increases the cell volume and also gives rise to alterations in general physiology. The sexuals and clones of closely related species often have different geographic distributions. Asexuals more commonly are found in higher altitudes or latitudes, on islands, in xeric, and marginal habitats as compared to sexual species. This distributional pattern has been termed geographical parthenogenesis and geographical polyploidy (Vandel, 1928; Suomalainen et al., 1987; Stenberg et al., 2003).

Among insects, the weevils of the subfamily Entiminae are well known for having an abundance of parthenogenetic lineages, many of them have been the objects of cytological investigations (Mikulska, 1953, 1960; Petryszak, 1972; Smith and Virkki, 1978; Takenouchi, 1983, 1986; Suomalainen et al., 1987; Holecová et al., 2008; Lachowska et al., 2008). A summary of parthenogenesis in weevils listed 75 cytologically defined parthenogenetic taxa within 52 morphologically defined species (Saura et al., 1993). All clonal weevils are known to have apomictic parthenogenesis in which the development of eggs occurs only through mitosis, the chromosomes do not conjugate and reduction does not take place (Saura et al., 1993), although an atypical course of apomictic oogenesis with

* Corresponding author. Tel.: +48 12 633 63 77 24 45.
E-mail address: dorota.lachowska-cierlik@uj.edu.pl (D. Lachowska).

0968-4328/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micron.2009.06.006

stages representing the remnants of meiosis was described for some species (Seiler, 1947; Mikulska, 1953; Lachowska et al., 2008).

The adoption of parthenogenetic reproduction by weevils is accompanied by a change in the mechanism of meiosis. Because there is no gene recombination, the progeny are genotypically similar to the mother and new forms may arise only through mutation. Almost all parthenogenetic forms of curculionids are polyploids, and triploidy is by far the most common level of ploidy.

The aim of this study was to examine the cytological mechanism of parthenogenesis in weevils and to describe the behaviour of chromosomes during individual stages of oogonial division based on observations of four triploid and one tetraploid species, as well as an analysis of the meiotic stages of prophase I in "rudimentary meiosis".

2. Materials and methods

The insects were collected in May–June 2007 and 2008 in the vicinity of Bratislava (SW Slovakia). The species were classified according to Alonso-Zarazaga and Lyal (1999) and Wanat and Mokrzycki (2005). The voucher specimens were deposited in the Institute of Systematics and Evolution of Animals PAS (Kraków, Poland).

Chromosome preparations were obtained from the germarial parts of telotrophic ovaries using two different methods:

(a) a modification of Imai's et al. (1977) method based on fixatives with a gradually rising concentration of acetic acid at a temperature of 32 °C. This method was also used in previous studies dealing with coleopteran cytogenetics (Rożek, 1994; Rożek and Lachowska, 2001; Lachowska et al., 2006; Holecová et al., 2008);
(b) the preliminary fixation was the same as in Imai's method, the second part of the method was modified by lengthening the time of gonad refixation in Carnoy's solution (3:1 96% ethanol:glacial acetic acid) in a thermostat at 34 °C for several hours (preferably until the next day). After gonad refixation, the karyological preparations were made according to the following methods:
    (1) parts of gonads were macerated in 45% acetic acid until a cell suspension was acquired, and then spread over a slide and dried on a warm plate at 45 °C;
    (2) the gonads were placed on a slide into a drop of 45% or 50% acetic acid, partitioned, squashed and frozen on dried ice.

C-Banding was performed using the procedure described by Sumner (1972) with some modifications: the slides were first treated with 0.2N HCl for 30 s. at room temperature, followed by thorough rinsing twice with distilled water and then slightly air-dried. Afterwards the slides were placed in a freshly prepared, saturated solution of barium hydroxide (3 g $Ba(OH)_2 \cdot 8H_2O$ in 60 ml of water) at room temperature for 1 min., rinsed with tap water, distilled water and incubated for 1 h in 2 × SSC at 50 °C and air-dried again. Then the slides were stained with 4% Giemsa in phosphate buffer (pH 6.8) for 15–20 min at room temperature. Oogonial cells of different stages of nuclei division were analyzed and photographed with a Nikon Eclipse 400 light microscope and CCD DS-U1 camera using the software NIS-Elements BR 2.30. The chromosome classification system followed Levan et al. (1964).

3. Results

The ovaries of many (no less then 20) females were examined due to a low ratio (index) of mitotic stages in oogenesis and synchronization of gonial division. All examined species are polyploid, four: *Otiorhynchus ovatus*, *Simo variegates*, *Cathormiocerus aristatus* and *Tropiphorus elevatus* possess three haploid sets of chromosomes ($3n = 33$), the fifth, *Trachyphloeus parallelus,* is tetraploid with 44 chromosomes ($4n = 44$). Mitotic metaphases exhibited a complete number of chromosomes, although in some plates a lack of a few chromosomes was observed. Such plates contained 27–31 chromosomes in triploid species and 38, 39, 41 in tetraploid *T. parallelus*. In all species single clusters of metaphase plates with haploid numbers $n = 11$ were visible. During oogonial division we observed many stages of mitotic prophase in which chromosome spiralization occurred (Fig. 1a–c). The most interesting phenomenon observed during oogenesis in *O. ovatus*, *C. aristatus* and *T. parallelus* was the division of nuclei in which stages could be discerned resembling the prophase of the first meiotic division. These probably represent primary oocytes in different stages of prophase I. The nuclei of these cells were smaller with highly condensed chromatin (more so than in mitotic prophase) in their central parts. This suggests that the synaptonemal complex occurs in these parthenogenetic species, a structure responsible for the connection of homologous chromosomes during zygotene and pachytene. Among numerous dividing oogonia, stages similar to diplotene and diakinesis were also observed. In such stages short, spiralized chromosomes were probably connected by chiasmata creating figures resembling rods and ring shaped bivalents (Fig. 1d–f).

The C-banding technique revealed constitutive heterochromatin around centromeres only in *O. ovatus* and *C. aristatus* (Fig. 2a and c). C-bands were not observed in the remaining species (Fig. 2b, d and e).

O. ovatus – $3n = 33$, $n = 11$; the karyotype is symmetric, containing metacentric chromosomes with slight differences in length. In mitotic metaphase constitutive heterochromatin is visible on all chromosomes located around centromeres, the first pair also possesses a short terminal C-band (Fig. 2a).

Simo variegates – $3n = 33$, $n = 11$; the karyotype is symmetric with slight differences in length, the last chromosome is clearly the shortest. The majority of chromosomes are metacentric, the second chromosome is subtelocentric, the next to the last chromosome is acrocentric. C-bands were not observed (Fig. 2b).

C. aristatus – $3n = 33$, $n = 11$; the 1st–8th chromosomes are metacentric and submetacentric with no differences in length, the three shortest pairs are acrocentric. C-Bands of almost the same size are located around the centromere on all chromosomes (Fig. 2c).

Trachyphloeus parallelus – $4n = 44$, $n = 11$; 10 chromosomes are metacentric and submetacentric, the last shortest chromosomes are acrocentric. The chromosomes probably possess a small amount of heterochromatin which is not detectable after C-banding (Fig. 2d).

T. elevatus – $3n = 33$, $n = 11$; the first chromosome is the longest and is metacentric, the remaining are smaller, metacentric and submetacentric with slight differences in length. The heterochromatin was not detectable after C-banding (Fig. 2e).

4. Discussion

All asexual weevils studied so far have been described as apomictic, lacking recombination with equationally divided chromosomes as in mitosis. Stenberg and Lundmark (2004) suggested that in weevils what appears to be mitosis could be meiosis II. Among the large number of mitotic prophases examined in three species, the chromosomes resemble the prophase of the first meiotic division. At this stage chromosomes are strongly contracted, short and thick and clearly deviate from mitotic chromosomes. Evidence of chromosomes forming figures of rings and rods suggests that recombination occurs in the parthenoge-