Some remarks on the cytogenetics of oribatid mites

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Abstract

The behaviour of the meiotic segregations of the parthenogenetic oribatid mite *Archegozetes longisetosus* Aoki were investigated in maturing oocytes. Data about the meiotic mechanisms in parthenogenetic oribatid mites are very sparse, although the mechanism of reproduction is fundamental for the restoration of diploidy and has different consequences for the genotype of the offspring. For our analyses we used a combination of traditional methods and the novel technique of synchrotron X-ray holotomography. We describe the points in maturation at which the meiotic segregations occur and the positions of the oocytes at this time in the female genital tract. As the position of the nuclei in the first segregation is located beneath the cell membrane and in the second one central in the cell, we believe to have evidence for an inverted meiotic sequence.

Keywords: Parthenogenesis, holokinetic chromosomes, inverted meiosis, *Archegozetes longisetosus*, synchrotron X-ray micro-holotomography

Zusammenfassung

In reifenden Oozyten der parthenogenetischen Hornmilbe *Archegozetes longisetosus* Aoki werden die meiotischen Teilungen untersucht. Die Datengrundlage über meiotische Prozesse bei parthenogenetischen Oribatiden ist sehr spärlich, obwohl der Reproduktionsmechanismus fundamental für die Wiederherstellung der Diploidie ist und unterschiedliche Konsequenzen für den Genotyp des Nachwuchses mit sich bringt. Für unsere Untersuchungen benutzten wir eine Kombination aus traditionellen Methoden und Synchrotron Röntgen-Holotomographie. Wir beschreiben die Zeitpunkte und Positionen der meiotischen Teilungen im weiblichen Genitalsystem. Da die erste meiotische Teilung direkt unterhalb der Zellmembran stattfindet, während die zweite zentral in der Zelle stattfindet, meinen wir Hinweise auf eine invertierte meiotische Teilung zu finden.

1. Introduction

The cellular mechanism of meiosis forms the basis of sexual reproduction, whereas asexual reproduction is mostly linked with mitosis. But also a certain type of parthenogenesis includes meiosis as basic process: automixis (Bell 1982, Suomalainen et al. 1987, Hughes 1989, Heethoff 2003). Automictic organisms restore diploidy by fusion of the egg and one of the polar nuclei, which is possible by a number of alternative mechanisms. For example, by duplication of the haploid egg and fusion of these two products to give rise to a complete homozygous diploid embryo, as some crustaceans and insects do (Suomalainen et al. 1987). Also, diploidy can be rebuilt by central fusion. In this mechanism the two central polar nuclei

fuse, restoring the heterozygous state of the mother depending on the segregation of chromosomes in meiosis II. Central fusion is known from some insect species (Suomalainen et al. 1987). A third mechanism is terminal fusion, the fusion of the egg with the second polar nucleus, which leads to complete homozygosity. Some species of nematodes, lumbricids, crustaceans, insects and mites have realised terminal fusion (Suomalainen et al. 1987, Heethoff 2003).

There are a lot more mechanisms for automicts to restore diploidy, many of them incompletely known, but as the eggs of automictic parthenogenetic species have undergone meiosis, they differ considerably from apomicts and asexuals in genetic and evolutionary aspects (Heethoff 2003).

For studying parthenogenesis, oribatid mites (Acari, Oribatida) have become an important group (Norton et al. 1993, Schaefer et al. 2006, Domes et al. 2007, Heethoff et al. 2007b, Laumann et al. 2007, Heethoff et al. in press) since they include species-rich clusters of exclusively thelytokous taxa (Palmer & Norton 1992, Maraun et al. 2004). Recent molecular works have shown that speciation events happened in these parthenogenetic oribatid mite clusters (Maraun et al. 2004, Heethoff et al. 2007b, Laumann et al. 2007). Due to biogeographic evidence it was concluded that these taxa are more than 100 million years old (Heethoff et al. 2007b) and that some taxa even predate the break up of Pangaea (Hammer & Wallwork 1979). This implies that parthenogenesis is not necessarily an evolutionary dead end. And an impressing case displaying this is a group of oribatid mites which indeed re-evolved sex from a parthenogenetic ancestor, a spectacular case of breaking Dollo's law (Domes et al. 2007).

A specific cellular mechanism should be fundamental for the radiation of parthenogenetic oribatid mite species. Only one cytological study of meiosis in parthenogenetic oribatid mites is available (Taberly 1987). Here, automictic thelytoky (including the meiotic production of haploid gametes) and terminal fusion (fusion of the egg pronucleus with the second polar body) were proposed as the cellular mode of reproduction. This mechanism restores diploidy and maintains homozygosity (Suomalainen et al. 1987). Contradictory to these findings, Palmer & Norton (1992) indicated fixed heterozygosity and absence of recombination for parthenogenetic oribatid mites using isozyme techniques. Molecular analyses of nuclear genes also indicated that nuclear recombination is absent in parthenogenetic oribatid mites (Schaefer et al. 2006). However, these incompatible findings can be in unison, if the sequence of reductional and equational divisions in meiosis is inverted. This idea of inverted meiosis, based on the presence of holokinetic chromosomes, as mode of reproduction for parthenogenetic oribatid mites was established by Wrensch et al. (1994).

Holocentric chromosomes have no localised centromer, the microtubules attach over the whole length of the chromosome. These holocentric chromosomes are termed holokinetic, as the kinetochore is the functional part in the movement of the chromosome, not the centromere per se (John 1990, Wrensch et al. 1994, Dernburg 2001, Heethoff 2003).

As the idea of inverted meiosis is based exclusively on theoretical considerations, empirical data of meiotic cleavages in parthenogenetic oribatid mites are urgently needed.

We used X-ray synchrotron micro-holotomography and semi-thin sectioning to study characteristics of the meiotic disjunctions in the parthenogenetic oribatid mite *Archegozetes longisetosus* Aoki, 1965. We show the place and the point in time of the meiotic segregations in the ovary and we trace the fate of the first polar body.

2. Materials and methods

All specimens used in this study belong to the lineage *Archegozetes longisetosus* ran (Heethoff et al. 2007a). This species is rapidly becoming a model organism for a wide spectrum of biological traits in chelicerate animals (Telford & Thomas 1998, Thomas 2002, Heethoff et al. 2007a). Laboratory conditions of the culture are described in Heethoff et al. (2007a).

2.1. Light microscopy

The fixation and processing steps were carried out in a fume cupboard at room temperature unless otherwise noted. Adult specimens were collected alive, and killed and fixed by incubation in 2.5 % glutaraledyde in 0.1 M cacodylate buffer (pH 7.2) for at least 24 h at 4° C. The samples were washed three times in 0.1 M cacodylate buffer (pH 7.2). Specimens were dehydrated through graded concentrations of 70 to 100 % ethanol (5 % steps) for 10 min per step; each step was repeated three times. The 100 % ethanol step was done at 4 °C overnight. The dehydrated material was transferred to propylene oxide for 60 min and embedded in 1:1, 1:3, 1:7 propylene oxide low-viscosity Agar Resin LV (Plano GmbH, Germany) by incubation for 1 h per step. The specimens were finally incubated in pure resin at 4° C overnight and polymerised by incubation at 60 °C for 2 days. Semi-thin sections (2 μm) were cut using a diamond knife and a Leica Ultracut UCT. These sections were stained with 1 % toluidine blue (Richardson et al. 1960), mounted in Entellan® (Merck KGaA, Germany) and observed under a Zeiss Axioplan microscope with a Zeiss MrC5 digital camera.

2.2. X-ray synchrotron microtomography

Adult specimens were collected alive, killed and fixed by incubation in a 6:3:1 mixture of 80 % ethanol, 38 % formaldehyde and 100 % acetic acid for at least 24 h.

The samples were washed and dehydrated as indicated above for light microscopy. The dehydrated specimens were critical point dried with CO_2 (CPD 020, Balzers AG, Liechtenstein), glued with the posterior end of the opisthosoma onto the tips of plastic pins (3.0 mm diameter) and stored in an exsiccator. X-ray synchrotron microtomography was accomplished at the European Synchrotron Radiation Facility (ESRF, Grenoble: beamline ID 19, experiment SC2127) at an energy level of 20.5 keV. The effective pixel size was 0.7 μm and the detector-to-sample distance was 10, 20 and 45 mm. Over the 180° sample rotation, 1300 projections were recorded (ESRF FreLoN camera) with an exposure time of 0.35 s each. Details about this novel technique and case studies of microarthropods are provided in Cloetens et al. (2006), Betz et al. (2007) and Heethoff & Cloetens (this issue). Separation and visualisation of individual structures, such as single cells, was accomplished by using the visualisation software tool amira™ (Mercury Computer Systems, Germany) on a 64-bit Dual-Opteron computer system.

3. Results

The reproductive system of *Archegozetes longisetosus* comprises an unpaired ovary, located posterioventral to the ovipositor, and paired oviducts which emanate from the ovary, form an s-curve and pass in an arc through the body. The oviducts merge anterior to the ovipositor. As a more precise description of the female reproduction system of *A. longisetosus* is introduced by Bergmann et al. in this issue, we will not go into further detail. Consequently, we use the terminology for the different parts of the female genital tract suggested by Bergmann et al. in this issue.

Oocytes are cuboid in form and constantly growing after the disconnection from the rhodoid of the ovary. The nuclei of this previtellogenetic oocytes measure 10 to 20 μm in diameter. In the majority of cases nuclei are in a central position and typically lobed (Fig. 1). As the growing oocytes arrive in the proximal, rostrad-oriented part of the meros of the ovary, the nucleus migrates to the cell membrane and the first meiotic division takes place (Fig. 2). The oocyte nucleus segregates and provides the egg nucleus and the first polar body. The oocyte nucleus migrates into a central position in the cell. Both show nucleoli and completed nuclear membranes. At this stage of maturation the growing oocyte is located in the first bend of an s-curve of the meros (Fig. 3).
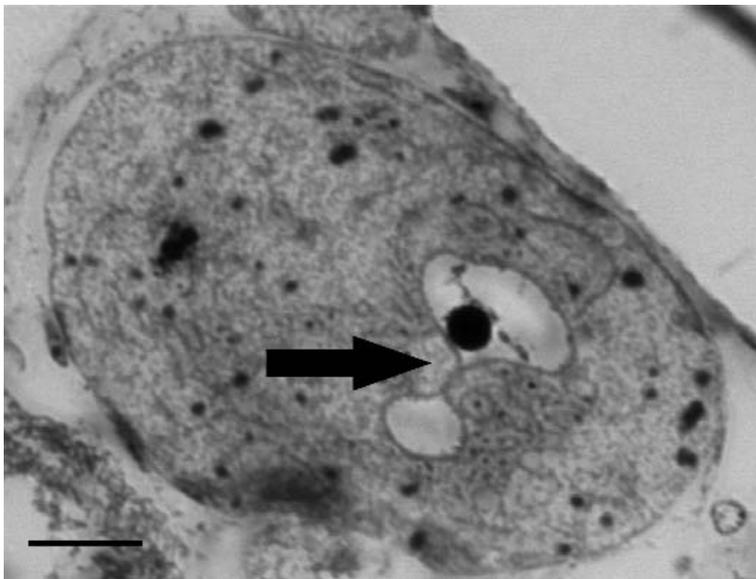
Fig. 1   Semi-thin section showing the lobed nucleus in the centre of a growing oocyte. One nucleolus is visible in the larger nuclear lobe. Both lobes are connected by a cytoplasm-nucleoplasm-cytoplasm sheet (arrow). Bar: 10 μm.

In the following growing process of the oocyte the nuclei remain in central position. The second meiotic division takes place in the distal, anad-oriented part of the meros of the ovary (Fig. 4). The nuclear membrane is disintegrated and the chromosomes are condensed. Here the growing oocyte is located in the second bend of the s-curve of the meros (Fig. 3). Presently after the second meiotic division the growing oocytes are covered with a compact vitelline envelope and further development of the embryo begins.
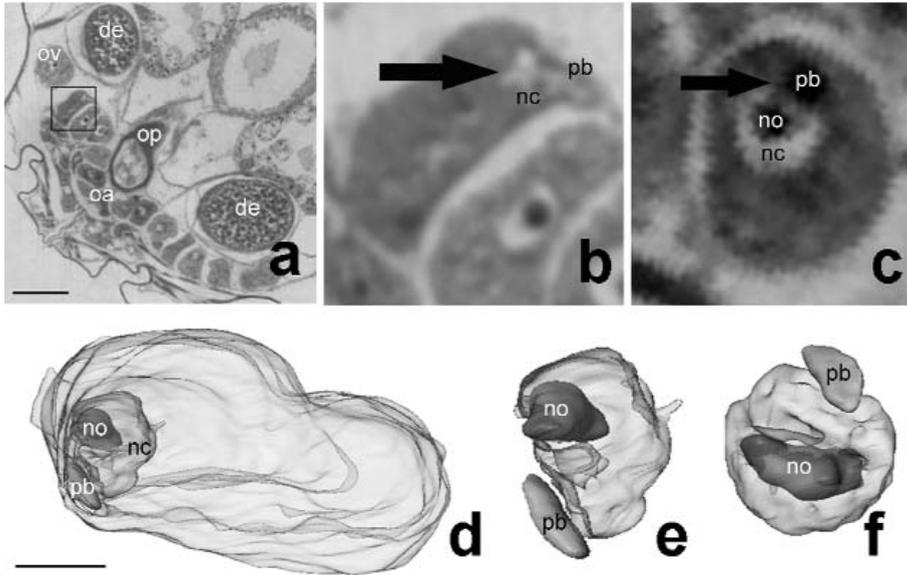
Fig. 2   Holotomographic slices (a to c) and 3D-reconstruction (d to f) of a growing oocyte in meiosis I. Disjunction has taken place, noticeable is the difference in size between the two nuclei. (a) transversal holotomographic slice of the reproductive system showing the ovary (oa), an oocyte in vitellogenesis (ov), two developing eggs (de) and the ovipositor (op). The frame is indicating the detail scaled up in (b) and (c). Bar: 50 μm. (b) transversal holotomographic slice showing the nucleus of the oocyte (nc) and the putative polar body (pb; indicated by the arrow). (c) sagittal holotomographic slice of the same cell, the arrow is indicating the putative polar body (pb), indicating the spatial separation of nucleus and pb. (d) 3D-reconstruction in sagittal view of the growing oocyte. Nucleus (nc), nucleolus (no), polar body (pb). Bar: 10 μm. If the cell membrane of the growing oocyte is removed, in sagittal view (e) and transversal view (f) the spatial separation of the nucleus and the putative polar body is obvious.
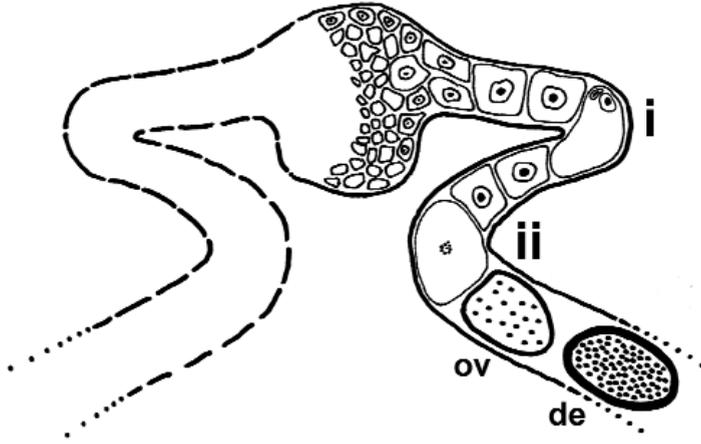
Fig. 3   Schematic diagram of the topology of the ovary showing the unpaired rhodoid and the paired meros of the ovary up to the periphery of the oviduct with an oocyte in vitellogenesis (ov) and a developing egg (de). (i) first bend of the s-curve in which the first meiotic division is taking place. (ii) second bend of the s-curve, the meiotic segregation is completed.
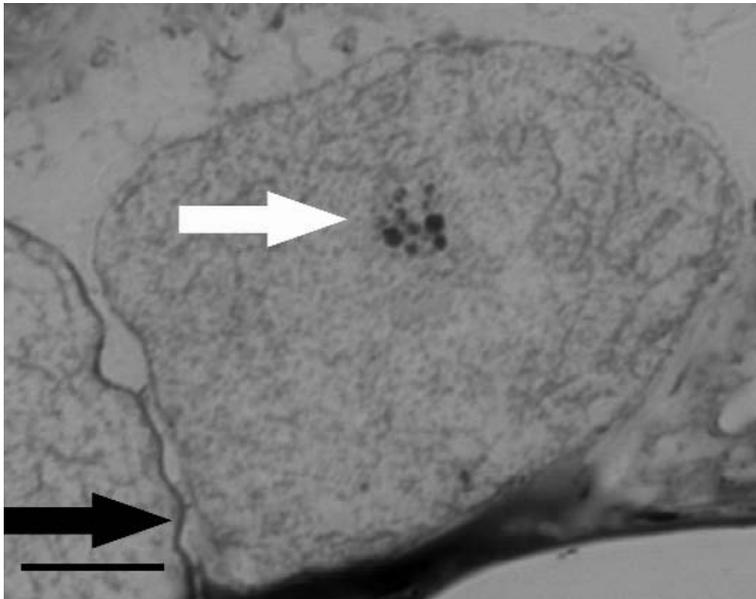
Fig. 4   Semi-thin section showing a growing oocyte in metaphase II, the nuclear membrane is disintegrated; chromosomes are condensed (white arrow). The second meiotic disjunction occurs in the centre of the cell, in contrast to the first meiotic segregation. The black arrow is indicating the onset of the production of the vitelline membrane. Bar: 10 μm.

4. Discussion

The essential premise to accomplish an inverted sequence of meiotic divisions are holokinetic chromosomes (Wrensch et al. 1994), where kinetochore activity is diffused along the entire length of the chromosome. A number of oribatid mite taxa have been analysed, but yet no specific peculiarities in the gross morphology of the chromosomes were found, especially no primary and secondary constrictions, suggesting the oribatid mite species examined do not have a localised centromere (Helle et al. 1984). *Archegozetes longisetosus* also possess holokinetic chromosomes (Heethoff et al. 2006), but the time and mode of reconstitution of diploidy have not been precisely documented yet. Our analysis showed foremost that nuclei in growing oocytes are typically lobed, as are the nuclei of the first blastomeres in acarid mites (Fig. 1, Walzl et al. 2004). The formation of these cytoplasm-nucleoplasm-cytoplasm sheets results in an increase in the area of chromatin in contact with the cytoplasm and hence the availability of the DNA molecules to cytoplasmic factors (Haynes & Davies 1973). Considering their presence in certain oocytes, they are due to the increased growth of the oocyte.

In the proximal, rostrad-oriented part of the meros of the ovary we located what we suppose to represent the nucleus and the first polar body in the first meiotic division. In comparable analysis of Walzl et al. (2004) with astigmatid mites the polar body was also located beneath the cell membrane in vitellogenesis. However, Walzl et al. (2004) showed the polar body after the second meiotic segregation. This discrepancy remains to be clarified in detail.

The actual process of chromosome disjunction has not been detected as such yet, but the cell is elongated as preparation for division and a new membrane surrounds both nucleus and the putative polar body. As this is characteristic for Telophase I, we would expect cytokinesis to complete the creation of two daughter cells. Given the parietal position of both nuclei in the cell a division is rather unlikely. Furthermore we believe the chromosomes of the first polar body to be expelled from the remainder of the oocyte, as was shown for a prostigmatid mite (Feiertag-Koppen 1976). Due to the presumed degeneration of the polar body, diploidy can only be restored by terminal fusion of both sets of chromosomes after meiosis II.

The second meiotic division in the oocyte proceeds in the distal, anad-oriented part of the meros of the ovary. Meiosis is completed before the oocytes are covered with a compact vitelline envelope, as it is also known for prostigmatid and acarid mites (Heinemann & Hughes 1970, Walzl et al. 2004). In contrast to metaphase I, the metaphase II figure is situated near the centre of the ooplasm. This indicates a cytokinesis at telophase II. As diploidy is to be restored, both nuclei have to fuse again after the second meiotic segregation. Unfortunately, figures of telophase II are not available but it seems apparent to accomplish a complete or incomplete meiosis II.

The effect of a terminal fusion automixis with the precondition of inverted meiosis on the embryo would be the same genetic constitution (except for crossing-over regions) as the mother, possibly characterised as functional apomixis (Heethoff 2003).

If *A. longisetosus* exhibits an incomplete meiosis II, the constraints of automixis and apomixis will not only blur in genetic constitution but also in cellular mechanisms. In acarid mites, oogenesis in a thelytokous strain is accomplished by apomixis: during oogenesis pairing of homologous chromosomes does not take place and there is only one pseudo-maturation division (Heinemann & Hughes 1969).

However, with the data available, the assumption of an inverted meiosis with terminal fusion automixis as a cellular mechanism of parthenogenesis can be further supported but not proved without doubt, observations about the chromosomal behaviour in the first and second meiotic division have to be clarified in detail.

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