KARYOLOGY AND SEX DETERMINATION OF ORIBATID MITES

BY M. HEETHOFF[1], P. BERGMANN[1], ROY A. NORTON[2]

(Accepted April 2006)

HOLOKINETIC
CHROMOSOMES
KARYOTYPE
ACARI
ORIBATIDA
INVERTED MEIOSIS
ARCHEGOZETES
LONGISETOSUS

SUMMARY: In a recent publication, ZHANG, FU & WANG (2004) reported a karyotype analysis of two species of brachypyline oribatid mites: *Brasilobates spinosus* Fujita (Protoribatidae, or Haplozetidae) and *Galumna longiporosa* Choi (Galumnidae).[3] After studying what they referred to as oocytes, the authors concluded that both species have an XX:XO sex determining mechanism and that the karyotype is 2n=16 for male *B. spinosus* and 2n=19 for male *G. longiporosa*. Chromosomes were reported to be monocentric.

However, we believe that some of the applied methods and interpretations are questionable, and the authors overlooked important past studies of oribatid mite karyology. Our purpose is to briefly discuss these issues and to highlight the rather dramatic contradictions with published literature. As an example, we present the first data on the karyotype of *Archegozetes longisetosus* Aoki (Trhypochthoniidae), and discuss why the conclusions of ZHANG *et al*. cannot represent the general state of cytogenetics in Oribatida.

Editorial note: In a recent article published in Acarologia, a study reported questionable data on the karyotype of Oribatida. These data might spread erroneous interpretation, considering the data published in various articles, or on which is based the problematic of present research. The editor proposed to open the columns of the journal and to bring in a short''review'' a stone in order to give a comprehensive interpretation in oribatid karyology and sex determinism.

Materials and Methods

ZHANG *et al*. (2004) reported that they collected eggs after oviposition, treated them with 0.01% colchicines for 30 min, and then crushed the eggs with glass-sticks.

Colchicine treatment is a classical method to inhibit spindle formation and thereby induce non-disjunction of chromosomes; however, their method could have had no such result, since the vitelline membrane of oribatid mite eggs is compact, lacks channels (WITALINSKI, 1986) and is impermeable to

1. Abteilung für Evolutionsbiologie der Invertebraten Universität Tübingen Auf der Morgenstelle 28E 72076 Tübingen Germany Corresponding author Heethoff@gmx.de

2. SUNY College of Environmental Science & Forestry 1 Forestry Drive Syracuse, New York USA, 13210

3. The authors misspelled *Galumna longiporosa* as "*G. longoporosa'',* and Subias (2004) made two judgments that affect these names: a) *G. longiporosa* Choi is a junior homonym of *Galumna longiporosa* Fujikawa, and was replaced by *Galumna reiterata* Subias; b) *Brasilobates spinosus* was recombined to *Protoribates* (*Trianungius*) *sponosus* (Fujita).

aqueous fixatives (Aeschliman & Hess, 1984). The egg shells (chorion) either have to be removed (Thomas & Telford, 1999; Walzl & Gutweniger, 2002) or be microwave-treated (Walzl, 1993) to enable fixatives to enter the egg and come in contact with the embryo.

In the next step, Zhang *et al*. (2004) claimed to have collected oocytes from the crushed eggs by centrifugation, but this seems unlikely. Embryonic development is the least investigated field of acarology (Walzl et al., 2004), but it is known that early cleavage occurs inside the oviducts (Aeschliman & Hess, 1984; Thomas & Telford, 1999; Walzl et al., 2004) and that the embryos of oribatid mites are at a rather late stage of development when oviposition takes place (Telford & Thomas, 1998). Taberly (1987) also showed from two oribatid mites species, that the embryo starts its development after vitellogenesis, within the oviducts. Therefore, oocytes are present only in the ovary of the female and the proximal part of the oviducts, but not in eggs after oviposition.

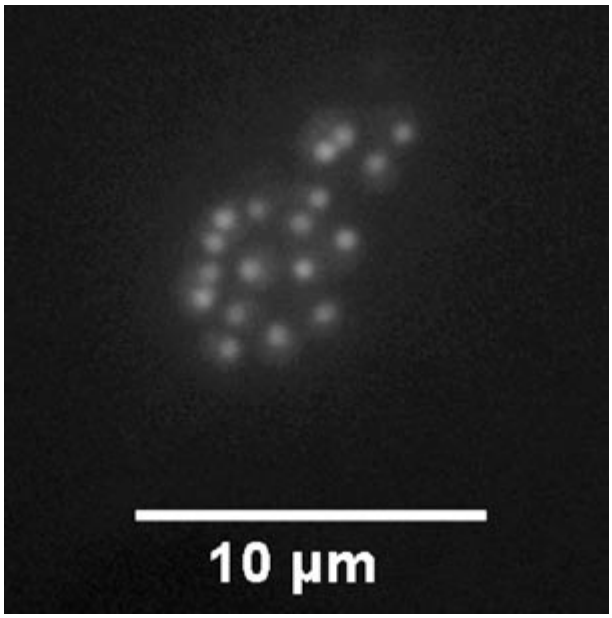

Fig. 1. — Gray-scaled micrograph of DAPI-stained metaphase chromosomes of *Archegozetes longisetosus* ran. The average size of the chromosomes is 0.6 µm.

Monocentric chromosomes

Zhang *et al*. (2004) provided two micrographs that purportedly showed chromosomes of the studied species, and statements in the text indicated that these were thought to be from males. The objects have an elongated appearance and were described as monocentric chromosomes having different localisations of the centromere; these were represented schematically in a separate figure.

In strong contrast, all literature descriptions of oribatid mite chromosomes, and indeed those from all studied members of the mite order Acariformes (Actinotrichida), are consistent with being holokinetic (Wrensch *et al.,* 1994 and many included references; Izraylevich et al., 1995). Holokinetic chromosomes are small (0.5-2 µm, Fig. 1), stain uniformly along their whole length, lack a centromere and can be found in a wide variety of animals and plants (Wrensch *et al*., 1994; Sumner, 2003). Zhang *et al*. (2004) made no reference to this conflicting literature, or to the unique nature of their interpretation. Unfortunately, no scale bars were included in the figures, nor was the absolute size of the structures mentioned in the text. Whether the micrographs represent a repeatable pattern is unknown, because the authors did not indicate if more than one egg per species was studied; the crushing method can generate much cell debris, and the quality of their micrographs seems insufficient to differentiate between such particles and chromosomes.

Karyotypes of oribatid mites

Zhang *et al*. (2004) stated that the karyotype is 2n=16 for male *B. spinosus* and 2n=19 for male *G. longiporosa*, but this leaves significant questions unanswered. First, they did not indicate how they differentiated female from male eggs. We are unaware of any way to determine the sex of an embryo within the egg-shell, and if sex was inferred only from their karyotype interpretation, this would seem to represent circular logic. Second, how did they arrive at the karyotypes? We cannot recreate the interpreted chromosome-numbers by counting the particles shown in the two micrographs.

In general, oribatid mites have a diploid chromosome number of 2n=18 (Oliver, 1977; Norton *et al.,*

1993 and included references) with only some exceptions having 2n=16 (Taberly, 1958; Helle *et al.,* 1984). A diploid chromosome number of 18 has also been reported for *Galumna* sp. (Sokolov, 1954).

Sex determination

Zhang *et al.* (2004) cited a study of Chen & Meng (1990), published in Chinese, which purportedly indicated that "... most species of Oribatida presented the XX:XO sex-determining mechanism, and that few species have the X-Y sex-determining mechanism". Accordingly, Zhang *et al*. stated "The two species of Oribatida have the same sex-determining mechanism (XX:XO)". In fact, there is no information in the Chen & Meng (1990) paper about sex determining mechanisms in oribatid mites; instead, the discussion of XX:XO and X-Y sex determination relates to Astigmata rather than Oribatida.

Diplodiploidy, without distinct sex chromosomes, appears to be ancestral in the Acari and is predominant, if not exclusive, in oribatid mites (Norton *et al*., 1993; Wrensch *et al.,* 1994). Using the egg-squash technique, Helle *et al.* (1984) inferred that some oribatid mite species are haplodiploid, but this conclusion was questioned by Norton *et al.* (1993) who noted a total absence of evidence for arrhenotokous reproduction, and suggested parahaploidy might explain these unusual results. Therefore, the sex-determination mechanism in oribatid mites remains unknown (Oliver, 1983; Norton *et al*., 1993; Wrensch *et al.,* 1994) due to the fact that male and female karyotypes contain the same number and kind of chromosomes (Sokolov, 1954). XX:XO and XX:XY sex determination mechanisms have been described for some species of astigmatic mites (reviewed by Norton *et al*., 1993). However, diplodiploid genetic systems (2n=14) with unknown sex determination mechanism have since been reported for the Astigmata (Izraylevich *et al*., 1995).

Chromosomes of *Archegozetes longisetosus* ran

The thelytokous oribatid mite species *Archegozetes longisetosus* is rapidly becoming a model chelicerate organism. A laboratory strain was established in 1993, based on a single gravid female for which the designation "*A. longisetosus* ran" was suggested (Heethoff *et al*., in press). Many aspects of the development and morphology of *Archegozetes longisetosus* ran are well studied but not the cytological basis of its parthenogenesis. Here, we present—for the first time—the chromosome morphology of *A. longisetosus* ran (Fig. 1).

Eggs were sampled after oviposition, mechanically cleaned with a brush and placed on an object slide in 0.005% w/v hypotonic colchicines solution (1% sodium citrate). The chorion was removed using sharpened tungsten needles (Norton & Sanders, 1985). After 35 minutes of incubation, the cells were homogenised and fixed using the protocol described by Imai *et al.* (1977). Chromosomes were stained with DAPI (0.2 mg/ml) and observed on a Leica CTR 5000 fluorescence microscope.

Mitotic figures of metaphase plates clearly show small, holokinetic chromosomes. Chromosomes cannot be differentiated, and the staining is uniform. The karyotype seems to show a diploid number of 18, typical of nearly all studied oribatid mites, but verification is in progress.

Outlook

Chromosomal morphology and behaviour are significant parameters influencing the developmental and evolutionary biology of oribatid mites. Most important, meiosis can differ dramatically between organisms with holokinetic and monocentric chromosomes. Unlike monocentric systems, cells with holokinetic chromosomes can effectively reverse the sequence of reductional and equational divisions (inverted meiosis; Wrensch *et al*., 1994). While the order of divisions does not theoretically affect haploid meiotic products (gametes) in sexual species, it has overwhelming significance if meiosis is followed by an immediate reconstitution of diploidy, as occurs in automictic diplodiploid parthenogens, i.e. parthenogenetic species that incorporate meiosis in egg production. For example, the fusion of the egg pronucleus with a second polar nucleus (i.e. fusion of

second-division haploid sister nuclei, a mechanism called terminal fusion), would result in homozygosity if meiosis were "normal", but would conserve all maternal genotypes if meiosis were "inverted". The latter result would mimic the mitotic production of parthenogenetic eggs, or the rare mechanism of central fusion under a normal meiotic order (in central fusion the egg pronucleus fuses with a second division product of the first polar nucleus; SUOMALAINEN *et al*., 1987).

For *Platynothrus peltifer* and *Trhypochthonius tectorum*, the only two parthenogenetic oribatid mites that are well-studied cytologically, TABERLY (1987) reported a chromosome behaviour that can be interpreted as terminal fusion automixis. This should lead to homozygosity if meiosis occurs in its normal sequence, but PALMER & NORTON (1992) reported fixed heterozygosity in studied populations of *Platynothrus peltifer* and in other parthenogenetic oribatid mite species, including *Archegozetes longisetosus*. These results can be explained without contradiction only if inverted meiosis is the mechanism underlying reproduction. However, inverted meiosis is not possible with monocentric chromosomes (WRENCH *et al*. 1994). Inverted meiosis can be only inferred, since the kinetics of chromosomes during meiotic divisions in oribatid mites remains to be demonstrated, but clearly the presence or absence of a centromere in their chromosomes has a strong effect on theories about development and evolution. Probably neither of the species studied by ZHANG *et al*. (2004) is parthenogenetic, but the identification of centromeres must be considered suspicious.

Also puzzling is the sex determination mechanism of oribatid mites. Most bisexual oribatid mite species show a balanced sex ratio, but species proven to be parthenogenetic have a strongly biased sex ratio with infrequent and sterile males (PALMER & NORTON, 1992). In diplodiploid systems with sex chromosomes, the sex ratio will usually be balanced (FISHER, 1930) but can be influenced by factors such as temperature (Ewert et al., 1994) or by hormonal or pheromonal determination (WHITE, 1973). Due to the infrequent occurrence and the sterility of males in many parthenogenetic oribatid mites, these species represent interesting model systems to study sex determination of oribatid mites. Possibly, the production of males can be induced in some experimental way, thereby uncovering this mechanism.

REFERENCES

AESCHLIMAN, A., HESS, E. (1984). — What is our current knowledge of acarine embryology? In: Griffiths, D. A., Bowman, C. E. (eds.) Acarology VI, Vol. 1. Ellis Horwood Ltd., Chichester, England. pp. 90-99.

CHEN, C., MENG, Y. (1990). — The chromosomes of species of Acari. KunChong ZhiShi 3: 188-192. (In Chinese).

EWERT, M. A., JACKSON, D. R., NELSON, C. E. (1994). — Patterns of the temperature-dependant sex determination in turtles. J. Exp. Zool. 270: 3-15.

FISHER, R. A. (1930). — The Genetical Theory of Sexual Selection. Clarendon Press.

HEETHOFF, M., LAUMANN, M., BERGMANN, P. (2006). — Adding to the reproductive biology of the parthenogenetic oribatid mite *Archegozetes longisetosus* (Acari, Oribatida, Trhypochthoniidae). Turk. J. Zool., in press.

HELLE, W., BOLLAND, H. R., JEURISSEN, S. H. M., VAN SEVENTER, G. A. (1984). — Chromosome data on the Actinedida, Tarsonemida and Oribatida. In: Griffiths, D. A., Bowman, C. E. (eds.) Acarology VI, Vol. 1. Ellis Horwood Ltd., Chichester, England. pp. 449-454.

IMAI, H. T., CROZIER, R. H., TAYLOR, R. W. (1977). — Karyotype evolution in Australian ants. Chromosoma 59: 341-393.

IZRAYLEVICH, S., GERSON, U., WYSOKI, M. (1995). — Karyotype and the sex determining mechanism of the mite *Hemisarcoptes coccophagus* Meyer (Acariformes: Astigmata: Hemisarcptidae). Internat. J. Acarol. 21(4): 229-232.

NORTON, R. A., SANDERS, F. (1985). — Super micro-needles for manipulation and dissecting soil invertebrates. Quest. Entomol. 21: 673-674.

NORTON, R. A., KETHLEY, J. B., JOHNSTON, D. E., OCONNOR, B. M. (1993). — Phylogenetic perspectives on genetic systems and reproductive modes of mites. In: Wrensch, D., Ebbert, M. (eds.) Evolution and Diversity of Sex Ratio in Insects and Mites. Chapman & Hall, New York. pp. 8-99.

OLIVER JR., J. H. (1977). — Cytogenetics of mites and ticks. Ann. Rev. Entomol. 22: 407-429.

OLIVER Jr., J. H. (1983). — Chromosomes, genetic variance and reproductive strategies among mites and ticks. Bull. Entomol. Soc. Am. 29: 8-17.

PALMER, S. C. & NORTON, R. A. (1992). — Genetic diversity in thelytokous oribatid mites (Acari; Acariformes; Desmonomata). Biochem. Syst. Ecol. 20: 219-231.

Sokolov, I. I. (1954). — The chromosome complex of mites and its importance for systematics and phylogeny. Tr. O. Estestvoispyt. 72: 124-159.

Subias, L.S. (2004). — Listado sistemático, sinonímico y biogeográfico de los Ácaros Oribátidos (Acarifomes, Oribatida) del mundo (1748-2002). Graellsia 60: 3-305.

Sumner, A. T. (2003). — Chromosomes - Organization and Function. Blackwell Science Ltd., Malden, USA.

Suomalainen, E. Saura, A., Lokki, J. (1987). — Cytology and Evolution in Parthenogenesis. CRC Press, Florida, USA.

Taberly, G. (1958). — Les nombres chromosomique chez quelque espèces d'Oribates (Acariens). C. R. Acad. Sci. 246: 3284-3285.

Taberly, G. 1987. — Recherches sur la parthéogenèse thélytoque de deux espèces d'acariens oribatides : *Trhypochthonius tectorum* (Berlese) et *Platynothrus peltifer* (Koch). III. Etude anatomique, histologique et cytologique des femelles parthénogenétiques. Acarologia 28: 389-403.

Telford, M. J., Thomas, R. H. (1998). — Expression of homeobox genes shows chelicerate arthropods retain their deuterocerebral segment. Proc. Natl. Acad. Sci. USA 95 : 10671-10675.

Thomas, R. H., Telford, M. J. (1999). — Appendage development in embryos of the oribatid mite *Archegozetes longisetosus* (Acari, Oribatei, Trhypochthoniidae). Acta Zool. 80: 193-200.

Walzl, M. G. (1993). — Microwave-enhanced osmium tetroxide fixation and processing of mite embryos for scanning electron microscopy. Europ. J. Morphol. 31: 151-155.

Walzl, M. G., Gutweniger, A. (2002). — A simple preparation technique for transmission electron microscopic investigations of acarine eggs. Abh. Ber. Naturkunde Görlitz 74: 3-7.

Walzl, M. G., Gutweniger, A., Wernsdorf, P. (2004). — Embryology of mites: new techniques yield new findings. Phytophagy XIV: 163-181.

White, M. J. D. (1973). — Animal Cytology and Evolution. Cambridge University Press, Cambridge.

Witalinski, W. (1986). — Egg-shells in mites. Cell Tissue Res. 244: 209-214.

Wrensch, D., Kethley, J. B., Norton, R. A. (1994). — Cytogenetics of holokinetic chromosomes and inverted meiosis: keys to the evolutionary success of mites, with generalizations on eukaryotes. In: Houck, M. A. (ed.) Mites: Ecoplogical and Evolutionary Analyses of Life-History Patterns. Chapman & Hall, New York. pp. 282-343.

Zhang, P., Fu, R., Wang, Y. (2004). — Karyotype analysis of two species of Oribatida (Acari). Acarologia 44(3-4): 213-234.