Mitotic and meiotic chromosomes in *Somatochlora metallica* (Cordulidae, Odonata). The absence of localized centromeres and inverted meiosis

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Nokkala, S., Laukkanen, A. and Nokkala, C. 2002. Mitotic and meiotic chromosomes in *Somatochlora metallica* (Cordulidae, Odonata). The absence of localized centromeres and inverted meiosis. — *Hereditas 136*: 7–12. Lund, Sweden. ISSN 0018-0661. Received May 25, 2001. Accepted November 11, 2001

Spermatogonial metaphase chromosomes were examined in two dragonfly species, *Somatochlora metallica* (Cordulidae) and *Aeshna grandis* (Aeshnidae), and the behaviour of male meiotic chromosomes was studied in *S. metallica*. Both in *S. metallica* and *A. grandis* the male mitotic metaphase chromosomes from cells treated with colchicine consisted of two equidistantly aligned chromatids, showing no primary constriction. In meiosis the chromosomes of *S. metallica* males showed telokinetic activity during the first meiotic division, and kinetic activity was restricted in the middle parts of chromosomes during the second division. The kinetic behaviour of the chromosomes both in mitosis and meiosis showed that they were holocentric. One chiasma arises interstitially in each bivalent in *S. metallica* male meiosis. The chiasmata retain their interstitial position at metaphase I and do not terminalize. At metaphase I bivalents co-orient with homologous telomere regions towards the opposite poles. Thus genuine dyads segregate at the first anaphase. Meiosis in these male dragonflies is thus pre-reductional or conventional, not post-reductional or inverted, as has been previously proposed.

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In conventional meiosis chromosomes pair and form bivalents. The homologous chromosomes segregate with dissociation of the bivalent in the first meiotic division and divide in the second meiotic division. However, the chromosomes of some insect and plant taxa have been suggested to undergo so called inverted or post-reductional meiosis, where the chromosomes of a bivalent divide equationally in the first meiotic division, and the resulting homologous daughter chromosomes segregate in the second meiotic division, which is therefore reductional (see WHITE 1973; NOKKALA and NOKKALA 1997).

Dragonflies (Odonata) is one of the first insect orders in which inverted or post-reductional meiosis has been suggested to occur. According to OKSALA (1943, 1945), Odonata chromosomes are metacentric. Males have one chiasma and females two chiasmata per bivalent. The chiasmata terminalize completely prior to first metaphase. OKSALA (1943, 1945) claimed that the homologous centromeres auto-orient at metaphase I and, consequently, the chromosomes divide at anaphase I. Chromatids derived from homologous chromosomes are claimed to show a double chromatid arrangement, held together by half a terminal chiasma. The centromeres of the double chromatids co-orient in the second meiotic metaphase and the chromatids segregate in the second anaphase.

More recent studies have suggested that Odonata chromosomes are of acrocentric nature (CHAUDURI and DAS GUPTA 1949; SESCHACHAR and BAGGA 1962). CUMMING (1964), on the other hand, has proposed that observations on the attachment of the spindle fibres to the chromosomes in mitosis and meiosis show Odonata chromosomes to be holocentric. Also KIAUTA (1969a,b) assumes that Odonata chromosomes are holocentric. Both CUMMING (1964) and KIAUTA (1969a,b) assume that chromosomes in dragonflies undergo inverted meiosis. Also MOLA (1995) has more recently strongly argued for the presence of post-reductional or inverted type of meiosis.

The kinetic nature of Odonata chromosomes is still a matter of debate. The reason for this disagreement has been the lack of reliable criteria for recognizing holocentric chromosomes. The present study has been prompted by developments in two fields. Firstly, it has been established that holocentric chromosomes display highly characteristic kinetic behaviour during meiosis (NOKKALA 1985; NOKKALA and NOKKALA 1997; PÉREZ et al. 1997) and hence these chromosomes can be reliably recognized. Secondly, chiasmata do not terminalize in meiosis, neither in chromosomes with localized centromere (review JONES 1987) nor in holocentric chromosomes (NOKKALA and NOKKALA 1997; PÉREZ et al. 1997). The

observation that chiasmata do not terminalize is crucial, since holocentric chromosomes can undergo inverted meiosis only if the chiasmata in bivalents are terminal or completely terminalized. Only in this case the whole chromosomes in a bivalent can divide equationally in the first meiotic division, while chromosomes segregate reductionally, if bivalents display chiasma(ta) in interstitial position (WHITE 1973; NOKKALA and NOKKALA 1997).

In the present study we have employed silver staining of axial structures of chromosomes to examine the presence or absence of the primary constriction in mitotic chromosomes of Odonata, and re-examined the behaviour of chromosomes in male meiosis, paying special attention to the location of chiasmata in the bivalents, the kinetic behaviour of chromosomes in meiotic divisions, and the type of reduction in male meiosis.

MATERIALS AND METHODS

Larvae of *Somatochlora metallica* v.d. Lind (Corduliidae) and *Aeshna grandis* L. (Aeshnidae) males were collected from a small river and pond in the vicinity of Turku in early June. The testes were dissected in insect Ringer's solution. Some of the testes were transferred to 0.05 % colchicine for 10–30 min followed by 4–6 min hypotonic treatment with 0.075 M KCl or 0.8 % trisodiumacitrate, while other testes were transferred directly into the hypotonic solution. The testes were fixed overnight at + 4° C in acetic-ethanol (1:9) fixative, which was replaced with a fresh fixative the next day.

The preparations were made according to the method of NOKKALA and NOKKALA (1983) and stained with 2 % Giemsa. Some of the slides were C-banded by 5 % $Ba(OH)_2$ pretreatment. The axial structures of mitotic chromosomes were stained with silver nitrate according to the method described previously by NOKKALA and NOKKALA (1986).

RESULTS

Mitosis

The chromosome number of both *Somatochlora metallica* and *Aeshna grandis* male is $2n = 25$ ($n = 12 + X$), which is in agreement with earlier studies (CUMMING 1964; CRUDEN 1968). The chromosomes are fairly similar both in size and shape. After colchicine treatment metaphase chromosomes consist of two chromatids aligned in parallel and there is no sign of primary constriction (Fig. 1). The chromosomes look, however, slightly diffuse after Giemsa staining and chromatids can be distinguished clearly only in few chromosomes. In order to obtain more detailed observations on the arrangement of chromatids within metaphase chromosomes, the axial structures of the chromosomes were stained with silver nitrate. Silver nitrate is known to stain the non-histone proteins of the axial structures of chromosomes (HOWELL and HSU 1979; NOKKALA and NOKKALA 1985). The chromatids can now be distinguished clearly in metaphase chromosomes. After colchicine treatment chromatids lie equidistantly from each other along their entire length. It is obvious that the chromosomes lack a primary constriction (Fig. 2).

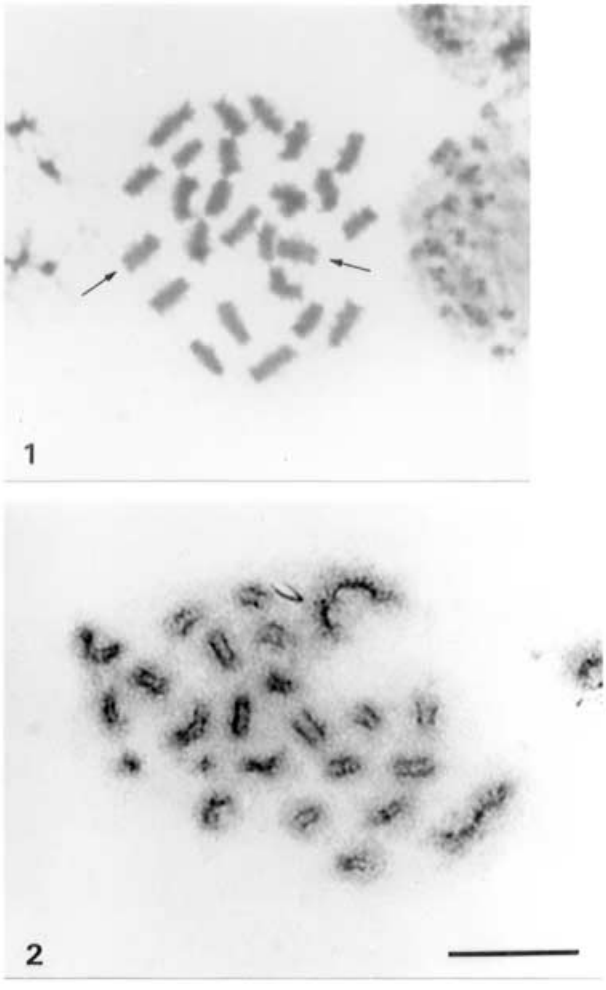

Fig. 1 and 2. Spermatogonial metaphase chromosomes. **Fig. 1.** Spermatogonial metaphase in *S. metallica*. The chromatids can be seen separately only in few chromosomes (arrowed). Giemsa staining. **Fig. 2.** Spermatogonial metaphase in *A. grandis*. The chromatids can be distinguished clearly. Primary constriction is absent. Silver-nitrate staining (bar = 10 μm).

The first meiotic division

Bivalents show a distinct bouquet orientation in pachytene cells. The univalent X-chromosome is located at the base of the bouquet, and it is positively heteropycnotic (Fig. 3). At late diplotene each bivalent shows only one chiasma located interstitially (Fig. 4). At diakinesis the chromosomes become shorter and more condensed (Fig. 5). At metaphase I the chromosomes are fairly short and thick. The spindle fibres pull the chromatids apart but still nonterminal chiasmata can be seen in bivalents (Fig. 6). The structure of the bivalents can be more clearly distinguished in cells treated with colchicine (Fig. 7). The colchicine treatment was so brief (10 min) that only cells at metaphase were subjected to it. Metaphase cells can still be recognized on the basis of the orientation of bivalents. Apparently, even being brief, the treatment with colchicine decreases the pulling force caused by the spindle fibres and hence the location of a chiasma within a bivalent is more readily seen. All chiasmata are clearly sub-terminal or medial. The bivalents are oriented with homologous telomeres (kinetic telomeres) towards opposite poles. The chromosomes in Fig. 3, 4 and 5 have been C-banded. The large heterochromatic blocks are mainly located at the telomeres of the chromosomes. Small dot-like heterochromatic blocks seen at subterminal positions in pachytene bivalents (Fig. 3) are not discerned in bivalents at later stages (Fig. 4 and 5).

At early anaphase I when the half-bivalents start to separate from each other (Fig. 8), the chromatids are joined together in the kinetic telomere and the half-bivalents move towards the poles with this telomere foremost (Fig. 9). Hence, during the first meiotic division the chromosomes are distinctly telokinetic.

The second meiotic division

At second prophase the chromatids of the half-bivalents are still joined to each other by the telomere left undevided in the first division. The chromatids are bent in the middle and thus the half-bivalents attain ε shape (Fig. 10). At second metaphase the half-bivalents are oriented on the division plane parallel to the axis of the spindle. The X-chromosome can often be seen outside the division plane (Fig. 11). The chromatids stain heavily and it is difficult to obtain a clear picture of how they are organized within the half-bivalents. From the structure of the half-bivalents at prophase II, however, it can be inferred that the chromatids are bent in such a way that both telomeres are located at the division plane, as has previously been observed by Oksala (1943) and Agopian and Mola (1988). This suggests that the half-bivalents are oriented to the poles the middle part of chromatids foremost, i.e. the chromatids are mediokinetic.

DISCUSSION

The kinetic organization of chromosomes

The following characteristics have usually been applied to recognize holocentric chromosomes (for review see, Ueshima 1979): Each chromosome orients with its long axis parallel to the division plane at mitotic metaphase, no primary constriction appears during mitosis, in mitotic anaphase chromatids separate by parallel disjunction, chiasmata are terminalized completely by late diakinesis, and chromosome fragments induced by X-radiation move from one cell generation to the next and are not lost.

All these criteria except that of chiasma terminalization are based on the assumption that spindle fibres are attached along the entire length of each chromatid. This, however, is not the case. For example, observations on hemipteran chromosomes show that the length of the kinetochore plate in mitotic chromosomes can vary from 4.2 % (Ruthman and Permantier 1973) up to 75 % (Comings and Okada 1972) of the entire length of the chromosome. Thus e.g. loss of small chromosome fragments during cell division does not necessarily reveal the actual kinetic organization of the chromosomes.

Chiasma terminalization cannot be used as a criterion of the kinetic organization of chromosomes. Chiasmata do not terminalize in species with localized centromeres (for review see Jones 1987). Complete terminalization of chiasmata is also absent in species with holocentric chromosomes (Nokkala and Nokkala 1997; Pérez et al. 1997)

In meiotic cells holocentric chromosomes behave in a highly characteristic way. In chiasmatic meiosis kinetic activity is restricted mainly to telomeres, i. e. bivalents orient towards the poles with their telomeres foremost (Piza 1958; Hughes-Schrader and Schrader 1961; Nokkala 1985; Nokkala and Nokkala 1997; Pérez et al. 1997). In addition, the telomeric region that is kinetically active during the first meiotic division is kinetically inactive during the second meiotic division. That is, the kinetically active region in chromosomes is changed between the two successive meiotic divisions (Nokkala 1985). Here we have shown that the mitotic chromosomes both in *S. metallica* and *A. grandis* lack primary constrictions. Moreover, meiotic chromosomes in *S. metallica* show telokinetic activity during the first meiotic division and the kinetic activity is restricted to the middle parts of chromatids in the second. These properties indicate that these Odonata species have holocentric chromosomes.

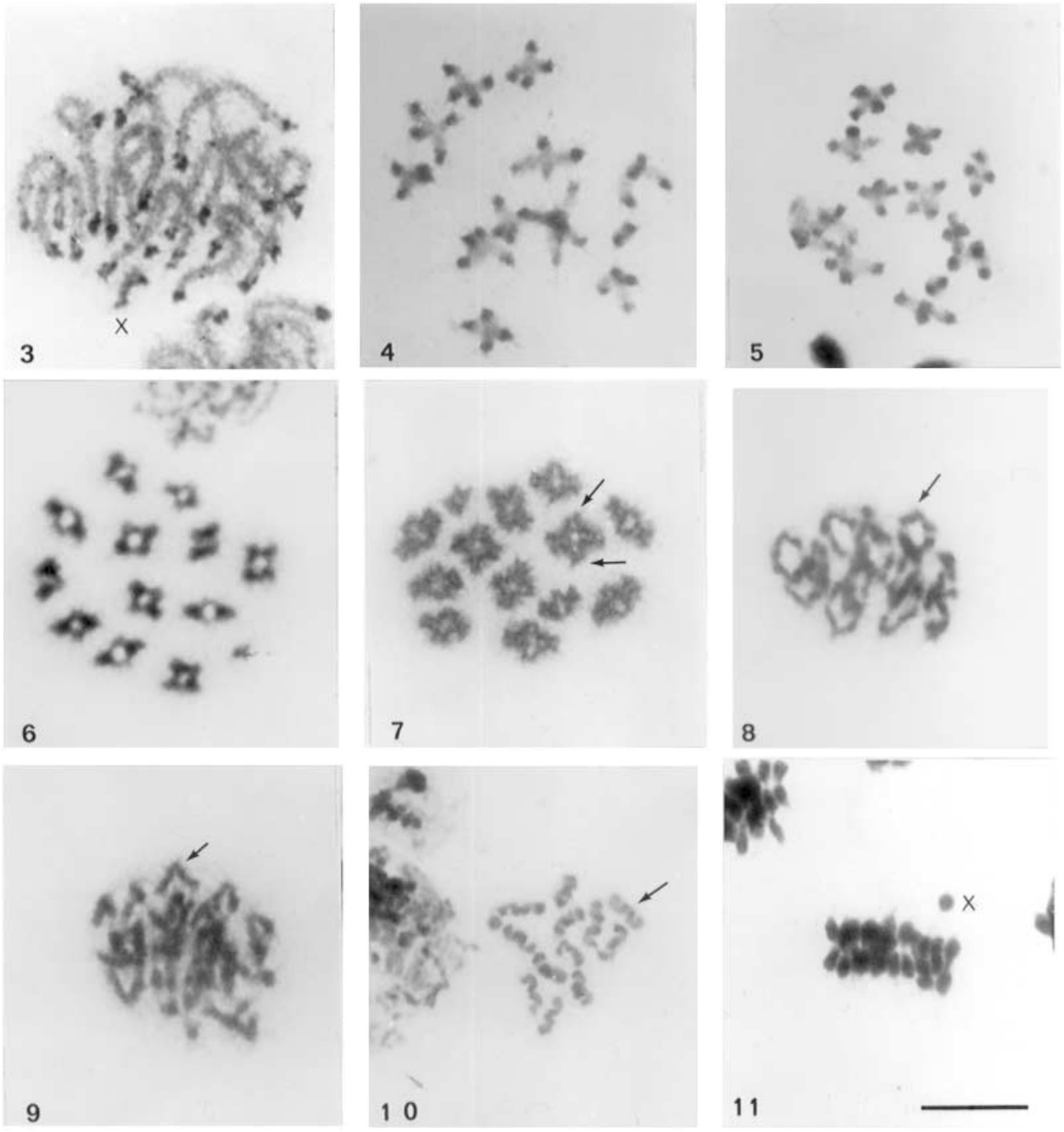

Fig. 3–11. *S. metallica* male meiosis. **Fig. 3.** Pachytene. The X-chromosome is positively heteropycnotic. C-banding. **Fig. 4.** Late diplotene. All bivalents display one medial chiasma. C-banding. **Fig. 5.** Diakinesis. **Fig. 6.** Metaphase I, chiasmata are clearly non-terminal. No colchicine treatment. **Fig. 7.** Metaphase I, chiasmata have retained their medial position. Colchicine treatment. Homologous telomeres (arrowed in one bivalent) oriented towards opposite poles. **Fig. 8.** Early anaphase I, bivalents are dissociating. Half-bivalents moving telomere foremost (one arroved) towards opposite poles. **Fig. 9.** Anaphase I. The dyads are held together by their telomeric region (one arrowed) and move towards the poles with one telomere foremost. **Fig. 10.** Prophase II. The chromatids of each dyad are bent in the middle and they are joined to each other via their telomeres (e.g. arrowed). **Fig. 11.** Metaphase II. The dyads are oriented on the division plane parallel to the axis of the spindle. The X-chromosome can be seen outside the division plane (bar = 10 μm).

Inverted meiotic sequence

In order to identify the type of reduction one has to recognize reliably if the chromosomes (half-bivalents) segregate or divide during the first meiotic division. This is, in turn, dependent on the orientation of chromosomes. In monocentric systems co-orientation or auto-orientation of centromeres of meiotic chromosomes are easily identified. The orientations of holocentric chromosomes are much more complicated, different alternatives are schematically presented in Fig. 12. According to WHITE (1973) only one chiasma is usually formed in these bivalents, and chiasmata are terminal or completely terminalized by metaphase I. Further he suggested that bivalents orient either equatorially or axially replacing the terms auto-orientation and co-orientation, respectively. Equatorial orientation of a bivalent (Fig. 12a) results in the division of half bivalents along their entire length producing double chromatids connected end-to-end by half a terminal chiasma (Fig. 12a′). On the other hand axial orientation of a bivalent (Fig. 12d) results in reductional separation of half bivalents, chromatids of which are held together by sister chromatid cohesion in the second meiotic division (Fig. 12d′). It is important to notice that the terms equatorial or axial cannot be applied if a chiasma in a bivalent is subterminal (Fig. 12b, c) or medial. However, in these situations homologous telomeres in bivalents orient towards opposite poles, that is they display co-orientation. To be logical, the terms equatorial orientation and axial orientation should be replaced with auto-orientation and co-orientation, respectively (NOKKALA and NOKKALA 1997). Then co-orientation of homologous telomeres in bivalents leads to pre-reductional separation of

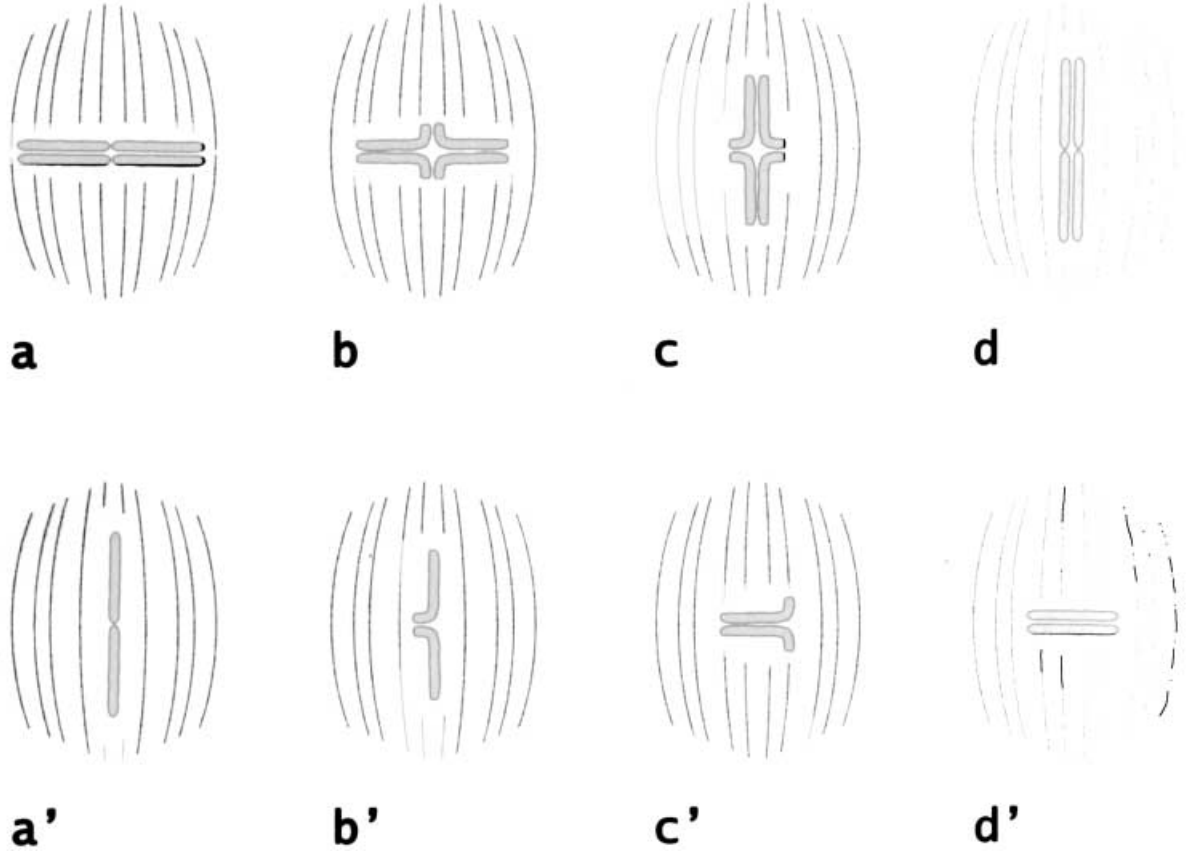

Fig. 12a–d′. Orientation types of holocentric chromosomes at metaphase I (a, b, c, d) and metaphase II (a′, b′, c′, d′). **a.** Auto-orientation or equatorial orientation (WHITE 1973) of a bivalent with terminal or completely terminalized chiasma results in the division of chromosomes in the bivalent, producing double chromatids connected by half a terminal chiasma. Chromatids originating from homologous chromosomes orient to opposite poles at metaphase II (**a′**), resulting in their segregation at anaphase II (post-reduction). **b.** and **c.** Two alternative orientations (co-orientation) of bivalent with subterminal chiasma. Homologous telomeres are oriented to opposite poles. The segregation of half-bivalents (pre-reduction) results in dyads within which the chromatids are held together by sister chromatid cohesion. The chromatids of dyads orient to opposite poles (auto-orientation) at metaphase II (**b′ and c′**) and divide at anaphase II. **d.** Co-orientation or axial orientation (WHITE 1973) of a bivalent with terminal or completely terminalized chiasma. The segregation of half-bivalents results in dyads that auto-orient at metaphase II (**d′**) and divide at anaphase II.

half-bivalents (Fig. 12b, c, d) in the first meiotic division, i.e. in all these cases some part of chromosomes are left undivided in the first division and, hence, the integrity of a dyad is maintained by sister chromatid cohesion (Fig. 12b′, c′, d′). In this study, we have shown that in *S. metallica* males the chiasmata form interstitially in bivalents and do not terminalize. Homologous telomeres orient towards opposite poles (co-orient) during the first meiotic division, indicating that male meiosis is pre-reductional for autosomes.

Earlier observations indicating chiasma terminalization (OKSALA 1943, 1945, 1948; CHAUDURI and DAS GUPTA 1949; CUMMING 1964; KIAUTA 1967, 1969a,b; CRUDEN 1968; AGOPIAN and MOLA 1988) can be attributed to so-called pseudo-terminalization (JONES 1978). The chromosomes are highly condensed at metaphase I, and it is extremely difficult to observe the short unterminalized part that is easily absorbed into the bivalent structure and chiasmata, actually subterminal, appear terminal or terminalized.

Previously, it has been shown that inverted meiosis is absent both in male and female meiosis in certain Heteroptera. In these insects bivalents can show spatially two different kinds of orientations, but both of them represent co-orientation (NOKKALA and NOKKALA 1997). In addition, although the plant species in the genus *Luzula* have often been cited as typical examples for post-reduction, they display pre-reduction. The whole concept of post-reduction in *Luzula* is based on erroneous interpretation of the arrangements of the chromatids in the half-bivalents (NOKKALA and NOKKALA 1997). Taken together these observations suggest that post-reductional type of meiosis is either non-existent or quite exceptional in nature. It might be confined only to the behaviour of achiasmatic sex chromosomes in some insect taxa.

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