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First evidence of achiasmatic male meiosis in the water bears *Richtersius coronifer* and *Macrobiotus richtersi* (Eutardigrada, Macrobiotidae)

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Chromosome behaviour during male meioses has been studied in two bisexual amphimictic populations of two tardigrade species, namely *Richtersius coronifer* and *Macrobiotus richtersi* (Eutardigrada, Macrobiotidae). Both bisexual populations exhibit a diploid chromosome number 2n = 12 and no sex chromosomes were identified. DAPI staining and C-banding data indicate that all chromosomes of the bisexual population of *R. coronifer* are acrocentric. In both species, at male meiotic prophase, all six bivalent homologous chromosomes are aligned side by side along their length and show no evidence of chiasmata. However, in the oocytes of both species a chiasma is generally present in each bivalent at diplotene stage. Lack of recombination is previously unknown in tardigrades, but is a well known phenomenon in many other metazoans where it is always restricted to the heterogametic sex. In tardigrades there is no evidence of heterochromosomes, but it does not mean that in tardigrades, the heterogametic sex does not exist. The adaptive and evolutionary significance of achiasmatic meiosis is discussed.

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Many species of tardigrades exist as a number of populations which are morphologically very similar to each other but which differ in sex ratio (bisexual or unisexual populations), ploidy (di-, tri-, or tetraploidy) and mode of reproduction (amphimixis or parthenogenesis). Parthenogenesis is a frequently occurring reproductive mode in tardigrades. It is often apomictic, less frequently automictic, the latter with or without genetic recombination (BERTOLANI 2001; REBECCHI et al. 2003). Heterogony has never been observed. The bisexual amphimictic populations are always diploid, whereas the parthenogenetic ones are often polyploid (tri- and tetraploid) and less frequently diploid (BERTOLANI 1994; REBECCHI et al. 2003). In diploid strains, the karyotype has very low variability both in chromosome number (n = 5 and n = 6) and in chromosome shape and size (BERTOLANI 1982; REBECCHI et al. 2002). Heterochromosomes have never been identified. To date, the karyological analysis of germ cell maturation in tardigrades has mainly been focused on the oocytes and very little on the male germ cells, in which only mitotic and meiotic metaphases were observed (BERTOLANI 1975, 1994; REBECCHI et al. 2002). Male meiotic metaphases showed very condensed bivalents attached to one another to form a five-six-pointed star (BERTOLANI 1975; REBECCHI et al. 2002). Consequently, karyotype and chromosome behaviour during male meioses have in this paper been subjected to a deeper analysis, based on observations in two bisexual populations belonging to two different eutardigrade species.

MATERIAL AND METHODS

Sexually mature males and some females of a bisexual population of *Richtersius coronifer* (Richters, 1903) and sexually mature males of a bisexual population of *Macrobiotus richtersi* Murray, 1911 were examined. Both species belong to Macrobiotidae (Eutardigrada). The bisexual population of *R. coronifer* was collected from a moss growing on beech-tree-trunks in a locality near Lago di Pratignano (1480 m a. s. l., northern Apennines, Italy), whilst the bisexual population of *M. richtersi* was collected from hazel leaf litter at Formigine (80 m a. s. l., Modena, Italy).

Chromosome preparations were performed by means of air-drying technique according to REBECCHI et al. (2002). Slides were stained either by conventional Giemsa solution (5 % in 0.01 M phosphate buffer, pH 7.2 for 20 min), or by DAPI (4′,6-diamidino-2-phenylindole) and/or by CMA_3 (Chromomycin A_3) according to SCHWEIZER (1976). Other slides were C-banded according to SUMNER (1972) and stained with Giemsa, or sequentially stained with methyl green, DAPI and CMA_3, according to REBECCHI et al. (2002). Some animals were stained in toto with a drop of lactic acetic orcein. Slides were exam-

ined with a Leitz DM RB microscope or with a Zeiss Axioplan fluorescence microscope connected to digital cameras Polaroid DMC Ie Low Light Kit and Spot (Diagnostic Instruments) respectively.

RESULTS

Richtersius coronifer has a karyotype of 2n = 12 chromosomes which are very similar to each other in shape (roundish or lightly rod-shaped) and size (1.4 μm in length) in mitoses of both males and females (Fig. 1a). No sex chromosomes were observed. Prophases I and metaphases I of spermatocytes exhibit 6 bivalents. A primary constriction was not visible either with Giemsa or fluorochromes staining alone, whereas C-banded prophases I, made visible with Giemsa, showed a more heterochromatic region on one telomere of each bivalent (Fig. 1c). Prophases were found in different condensation stages (Fig. 1b–j). In early condensation stage, highly uncoiled

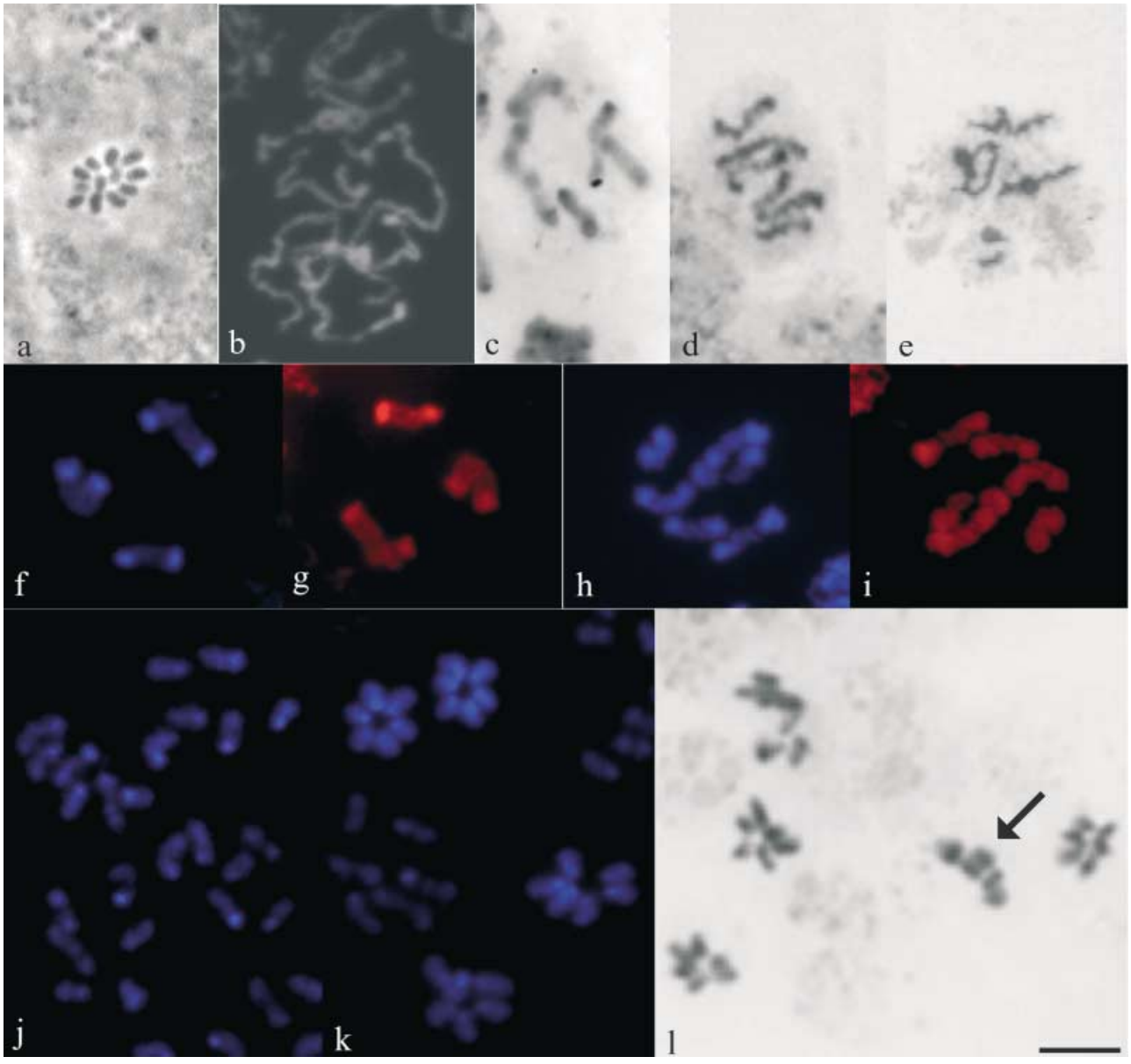

Fig. 1a–l. Mitotic and meiotic chromosomes in *Richtersius coronifer*. **a** Twelve mitotic chromosomes in a female gonad. **b** Early condensation stage of spermatocyte. **c-j** Mid-late condensation stages, note the bivalents without chiasmata. **c** C-banded bivalents. **e** Connected homologous forming a circular feature. **d** and **f-j** Bivalents with two (**d** and **f-i**) or one only (**j**) more highly stained telomere, and the same regions DAPI (**f** and **h**) and CMA_3 (**g** and **i**) positive; note that in **f** and **g** only three bivalents are shown. **k** and **l** Metaphases I in polar view; in **l** a metaphase in side view is present (arrow). Lactic acetic orcein (**a**), Giemsa (**c-e** and **l**), DAPI (**b, f, h, j** and **k**) and CMA_3 (**g** and **i**). Bar: 5 μm.

bivalents could be seen (Fig. 1b). In mid-late condensation stage the two telomeres of each bivalent, or sometimes only one, often appeared more intensely stained than the other regions (Fig. 1d and f–j). At these stages the bivalents consist of parallel-aligned homologous chromosomes (Fig. 1f and g) and none of the six bivalents showed chiasmata (Fig. 1c, d and f–j). In only two prophases, of 125 observed, the two homologous appeared partly overlapped or, in one case, connected to form a circular feature (Fig. 1e). At metaphase I, in polar view, the six bivalents showed a radial arrangement on the equatorial plate to form a six-point star (Fig. 1k and l); they were very coiled and the homologous chromosomes of each bivalent were not distinguishable. In side view, each homologous chromosome was recognisable (but not the single sister chromatids) and oriented with its longer axis parallel to the equatorial plate (Fig. 1l). After DAPI staining, the telomere of each bivalent towards the centre of the plate sometimes fluoresced more strongly than the other (Fig. 1k).

In male meioses of *Macrobiotus richtersi*, the six prophasic bivalents looked homogeneous when Giemsa stained, whereas, sometimes, DAPI stained spermatocyte prophases revealed an evident fluorescence at the telomeres of each of the six bivalents (Fig. 2a and b). The two homologous of each bivalent were very close to each other. In spermatocyte prophases, none of the six bivalents showed chiasmata (Fig. 2a and b). In metaphase I, in side view, the bivalents were disposed as in the former species, with the homologous chromosomes of each bivalent aligned side by side and facing the opposite poles on the equatorial plate, whereas in polar view, the radial arrangement of the very condensed bivalents looked like a six-point star. Single chromatids and centromeres were not visible.

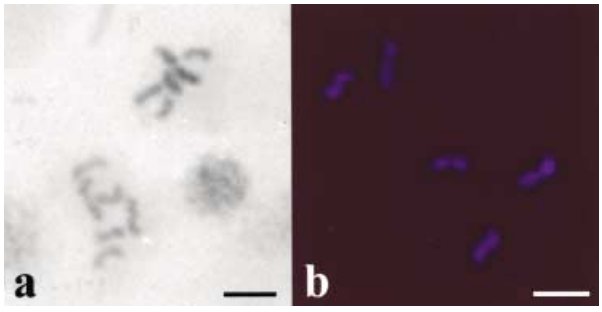

Fig. 2. Prophase spermatocytes in *Macrobiotus richtersi*: bivalents without chiasmata; note that in **b** only five bivalents are shown. Giemsa (**a**), DAPI (**b**). Bar: 5 μm.

DISCUSSION

Previous studies have shown that populations of *Richtersius coronifer* differ in reproductive mode (Rebecchi et al. 2003). In fact, these authors found that one Italian and one Swedish population were automictic, whereas another Italian population, discussed in this paper, was amphimictic. All these populations, irrespective of their reproductive mode, were diploid with the same chromosome number, $2n = 12$ (Rebecchi et al. 2003). *Macrobiotus richtersi*, on the other hand, had a diploid ($2n = 12$) amphimictic population and a triploid apomictic one characterised by 17-18 univalents in the oocytes (Rebecchi et al. 2002). In both cytotypes of *M. richtersi* (bisexual and unisexual) all chromosomes were acrocentric (Rebecchi et al. 2002). DAPI staining and C-banding data suggest that in bisexual amphimictic population of *R. coronifer* also, all chromosomes are acrocentric. The greater intensity staining on the two telomeres of each bivalent at early prophase is due to a different degree of coiling in the various parts of the chromosome, whereas the presence of only one more highly stained telomere at late prophase, when the coiling is almost complete, must be interpreted as an AT-rich region of each bivalent.

A chiasma was identified in each oocytes bivalent of the two automictic populations of *R. coronifer* (Rebecchi et al. 2003). Moreover, in the oocytes of the amphimictic populations of both *M. richtersi* and *R. coronifer*, a chiasma was generally present in each bivalent at diplotene (Rebecchi et al. 2002, 2003), whereas chiasmata were absent in the spermatocyte prophases of both species. The case of Fig. 1e may be due to an achiasmatic meiosis of collochore type. It is very unlikely that it represents a chiasmatic meiosis because not all the bivalents show circular or cross shape. Achiasmatic male meiosis of collochore type has been found in *Drosophila melanogaster* (Cooper 1964) and in the heteropteran Miridae (Nokkala and Nokkala 1986).

This is the first report of the absence of recombination in tardigrades males although it is a well known phenomenon in many other invertebrates such as molluscs, oligochaetes, crustaceans, scorpions, mites and insects (White 1973; Bell 1982; Nokkala and Grozeva 2000). The achiasmatic meiosis is always restricted to the heterogametic sex (White 1973; Bell 1982; Nokkala and Grozeva 2000). In particular, in one species of mites, in one genus of Mecoptera (Ullerich 1961), in many genera of mantids (Orthoptera), in many families of dipterans, including the very well known *Drosophila melanogaster* (reviewed by White 1973; Bell 1982), in coleopterans (Serrano 1981, Virkki and San-

TIAGO-BLAY 1996), and in several families of Heteroptera (reviewed by KUZNETSOVA and MARYANSKA-NADACHOWSKA 2000; NOKKALA and GROZEVA 2000) it occurs only in males. In Copepoda, Lepidoptera and Trichoptera achiasmatic meiosis occurs in females, the heterogametic sex (SUOMALAINEN 1966; SUOMALAINEN et al. 1973; WHITE 1973; BELL 1982; TRAUT and CLARKE 1996). An exception to the correlation of achiasmatic meiosis-heterogametic sex occurs in certain hermaphroditic (and therefore without heterogametic sex) enchytraeid worms with achiasmatic meioses in both oogenesis and spermatogenesis (CHRISTENSEN 1961). In bisexual gonochoric tardigrades there is no evidence of heterochromosomes (REBECCHI et al. 2002, 2003), but it does not mean that in tardigrades, the heterogametic sex does not exist. NOKKALA and NOKKALA (1983) proposed a connection between restriction of achiasmatic meiosis to the heterogametic sex and the nature of the control of the meiotic mechanism. Probable controlling elements of the meiotic mechanism are localised in the X-chromosome, therefore homogamety leads to normal chiasmatic meiosis and heterogamety to achiasmatic meiosis.

The adaptive significance of achiasmatic meiosis is not yet clear. WHITE (1973) proposed two alternative theoretical explanations: i) a selection for a low level of recombination ii) a facilitation of paracentric inversion heterozygosity avoiding the homozygosity of lethal alleles. The former explanation was later sustained by SERRANO (1981). Nevertheless, these two hypotheses are not supported by cytological data. According to NOKKALA and NOKKALA (1983), one more likely explanation might be that achiasmatic meiosis per se does not have adaptive value, but it can be considered as one of the mechanisms by which regular segregation of homologous chromosomes is achieved, the reduction of recombination being only a secondary effect. In tardigrades, the selection for a low level of recombination might represent a more likely explanation, since in both tardigrade species studied the absence of chiasmata in male germ cells and a very low frequency of chiasmata in female oocytes were observed. The absence of male genetic recombination represents a further explanation of heterozygote deficiency and deviation from Hardy-Weinberg equilibrium frequencies observed in the bisexual population of *R. coronifer*; in which males are heterozygous (REBECCHI et al. 2003). Heterozygote deficiency was previously explained by hypothesising a genetic drift or inbreeding (REBECCHI et al. 2003). The presence of automictic parthenogenesis may, however, explain the almost complete absence of heterozygosity observed in the two automictic populations of *R. coronifer*. In fact, automixis will produce heterozygote deficiencies and, in the long run, completely homozygous lineages (REBECCHI et al. 2003).

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