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**Karyological differences between the sibling species
Tisbe reluctantans and Tisbe persimilis (Copepoda,
Harpacticoida)**

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Zoologia. — *Karyological differences between the sibling species Tisbe reluctans and Tisbe persimilis (Copepoda, Harpacticoida)*. Nota di IVANA LAZZARETTO-COLOMBERA (*), presentata (**) dal Corrisp. B. BATTAGLIA.

RIASSUNTO. — Il confronto cariologico tra le due specie gemelle *Tisbe reluctans* e *Tisbe persimilis* ha messo in luce l'esistenza di differenze nella lunghezza dei cromosomi, nella posizione del centromero e nella morfologia dei bivalenti della meiosi spermatocitaria.

La differenza di lunghezza rilevata tra cromosomi mitotici e meiotici viene interpretata come un fenomeno di riduzione di cromatina.

The high morphological uniformity of the species belonging to the genus *Tisbe*, proved by the presence of several sibling groups (Volkman-Rocco and Fava, 1969 [14]; Volkman-Rocco, 1971 [10]; 1972 [11]; Volkman-Rocco and Battaglia, 1972 [15]; Volkman-Rocco, 1973 [12]; 1975 [13]), makes any phylogenetic study of this genus very difficult. Therefore two different approaches have been started: the first considering karyological characters, the second comparing the electrophoretic patterns of some enzymes (Bisol and Battaglia, 1973 [3]; Bisol, 1976 [4]).

The presence of different chromosome numbers in *Tisbe* species belonging to the same sibling groups (Lazzaretto-Colombera, in prep. [9]) suggests that karyological parameters might be useful in distinguishing species otherwise very similar to each other. For instance the use of karyological parameters might be very useful in those extreme cases of morphological kinship such as the case of *Tisbe reluctans* and *T. persimilis* (Volkman-Rocco and Fava, 1969 [14]).

Unfortunately *T. reluctans* and *T. persimilis* possess the same chromosome numbers (Lazzaretto-Colombera, 1976 [8]) and therefore other karyological characters such as chromosome morphology, length and dynamics have been examined.

Moreover the correlations already individuated in some marine invertebrates between evolutionary trends and karyotypes (Colombera and Lazzaretto-Colombera, 1978 [6]) lead us to believe that at this level it could be possible to find a clue to the phylogenesis in *Tisbe*.

MATERIALS AND METHODS

The specimens employed in this research were from populations reared in our laboratory, employing the same standard method for each (Battaglia, 1970 [1]). The cultures of *T. persimilis* came from collections from the

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port of Malta and the specimens of *T. reluctantans* came from samples collected in the Lagoon of Venice, near Chioggia.

Chromosome preparations were made employing several males and females of each species, following the method described elsewhere (Colombera and Lazzaretto-Colombera, 1972 [5]). For observations and photos a Zeiss phase-contrast microscope was used. The length of chromosomes of both species were measured on 15 pachytene plates of oocytes chosen from the best.

OBSERVATIONS

Tisbe persimilis: meiotic chromosomes of females. The first stage observed is characterized by the presence of thin threads randomly distributed and overlapping each other in the big nucleus of the oocyte. Later on the homologous chromosomes appear arranged in parallel pairs. These look like double threads with numerous positive heterochromatic dots and with the positively heteropycnotic ends (Pl. I, 1), no cross-connections or chiasmata are seen between the homologous pairs. At this stage, regarded as a pachytene, the homologous pairs are of large dimensions, easily detected and do not tend to adhere together and therefore they are suitable for measuring the total chromosomal length. The measurements of chromosomes (Pl. I, 7) given in Table I were taken on 15 pachytene plates. After this stage the chromosome elements appear to be diffuse and lightly stained (Pl. I, 2). Homologous chromosomes separate and occasionally chiasma-like formations are recognisable (Pl. I, 2 see arrow). After this diffuse stage the chromosomes become gradually visible showing homologous distantly paired (Pl. I, 3). They are longer than during the previous stages and show numerous positive heterochromatic dots. The chromosome ends are often associated and positively heteropycnotic. The chromosomes then condense and look like more deeply stained, short, small sticks with enlarged ends. Contraction of chromosomes continues without interruption to form, at MI, two dense elements clearly formed by sister chromatids (Pl. I, 4).

Tisbe persimilis: meiotic chromosome of male. Observation of dissected testes yielded the analysis of few pachytene (Pl. I, 5) and MI plates. The bivalents of MI plates are extremely contracted and deeply stained (Pl. I, 5); from the analysis of ten plates three chromosomes are clearly smaller than the others and they are clearly distinguished by their roundish shape (Pl. I, 6, see arrows). All the chromosomal elements appear randomly distributed.

Tisbe persimilis: mitotic chromosomes. Metaphase stages of the first few cleavage division were observed in 10 blastomers of cleaving eggs (Pl. I, 7). In all the cells here analysed the number did not deviate from 24 implying no numerical heterogamety in either sex of this species. Neither allocyclic behaviour nor differential staining could be attributed to any chromosomes therefore supporting the heterogametic conditions of this species. All chromosomes are seen to be metacentric or submetacentric and their length

reduces gradually from larger elements to smaller ones as seen in the karyogram presented in Pl. I, 9. This karyogram was obtained from studies of 6 metaphase plates in cleaving eggs.

Tisbe reluctans. The course of meiosis and mitosis in the species *T. reluctans* is reported in a previous paper (Colombera and Lazzaretto-Colombera, 1972 [5]). The measurements of total length of chromosomes (Pl. I, 8) given in Table I were performed using 15 pachytene plates of oocyte chromosomes. The karyogram reported in Pl. I, 9 was obtained from observations of 5 metaphase plates in cleaving eggs.

DISCUSSION

This analysis supports the view that the karyology of the genus *Tisbe*, very peculiar for the distant parallel pairing of oocyte chromosomes at metaphase I, is consistently uniform. The presence of homologous chromosomes closely associated in early prophase and of chiasmata-like formations in late prophase of *T. reluctans* (Colombera and Lazzaretto-Colombera, 1972 [5]), and *T. persimilis* after the diffuse stage, gives some evidence against the hypothesis, suggested by Heberer (1938 [7]), of the occurrence of achiasmatic oocytes during meiosis.

Although a consistent chromosome affinity has been evidenced between these two sibling species, two interesting differences can be pointed out:

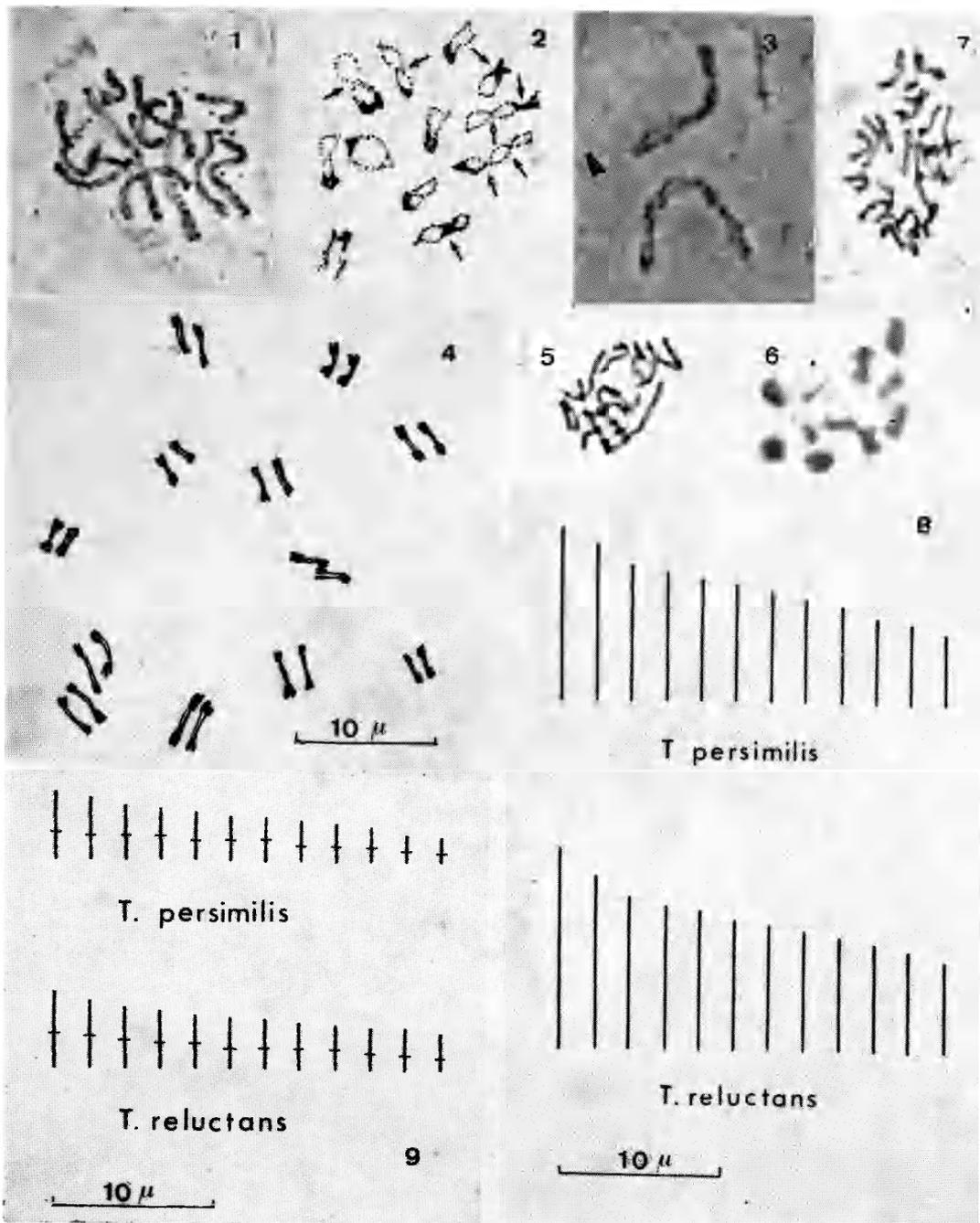
1) The chromosome length of *Tisbe reluctans* is significantly greater than that of *Tisbe persimilis*, as indicated by the length of pachytene chromosomes;

2) An unexpected consistent difference has been detected in the morphology of male bivalents. In *Tisbe persimilis* there are three elements clearly differentiated by their small dimensions which cannot appear in *Tisbe reluctans*. Moreover, whereas the chromosomes of *Tisbe persimilis* are condensed to small, roundish bodies, the bivalents of *Tisbe reluctans* show a structure seemingly complicated by the presence of several chiasmata.

The comparison between mitotic chromosomes of Pl. I, 9 indicates a slight difference in the position of centromeres which, on the whole, are located in a medial zone; other differences cannot be evaluated because of the smaller number of observations.

The seemingly higher DNA content in pachytene than in mitotic chromosomes, as can be deduced from chromosome lengths (Pl. I, 8, 9) might be the expression of a phenomenon of chromatin reduction already observed in Copepods (Beermann, 1959 [2]). If shorter chromosomes indicate a lower DNA content *T. persimilis* might be a more recent form than *T. reluctans* species as supported by the peculiarity of its male bivalents.

It is interesting to point out that a preliminary comparison between biochemical characters indicates a difference in the enzymatic systems of these two species (Bisol e Battaglia, 1973 [3]; Bisol 1976 [4]).



LEGENDS: 1) Pachytene chromosomes in the female of *Tisbe persimilis*; 2) Meiotic chromosomes of the female in the diffuse stage; 3) Homologous chromosomes at the post-diffuse stage in the female; 4) Bivalents at early metaphase I in the female. Note the distant parallel pairing between homologues; 5) Pachytene chromosomes in the male; 6) Bivalents at metaphase I in the male; 7) Metaphase chromosomes in cleaving eggs; 8) Karyograms of the species *Tisbe persimilis* and *T. reluctant* obtained from pachytene chromosomes in oocytes; 9) Karyograms of the species *Tisbe persimilis* and *T. reluctant* obtained from metaphase chromosomes of cleaving eggs.