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THE KARYOLOGY OF *TISBE RELUCTANS* (COPEPODA, HARPACTICOIDA) *

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INTRODUCTION

For many years, genetic investigations have been carried out on different species of the genus *Tisbe*, but the karyology of this genus has been neglected; in fact there is only a report about the haploid number of chromosomes in *Tisbe reticulata* (BOCQUET 1951). The recent discovery of two sibling species: *T. reluctantans* and *T. persimilis* (VOLKMANN-ROCCO and FAVA 1969), which are very similar in morphology and life-cycle but are reproductively isolated, raised the question of whether the complete intersterility between them could be cytologically explained. With this preliminary investigation of the karyology of *Tisbe reluctantans* we intend to collect information that could be useful for distinguishing the two sibling species *reluctans* and *persimilis* from each other on a cytological basis.

MATERIAL AND METHODS

The species *reluctans* of the genus *Tisbe* (Copepoda, Harpacticoida) is quite difficult to find in the field. The animals employed for this research are the offspring of a few females carrying egg sacs, collected in the lagoon of Venice near Chioggia in 1963 by Dr. I. LAZZARETTO, and since then raised in our laboratory, employing the usual methods (BATTAGLIA 1959).

Chromosome preparations were made as follows: the animals were fixed and stained in toto in acetic orcein 50%; oocytes and spermatocytes were then extracted from 20 females and 5 males respectively by disrupting them with a tungsten needle shortly before squashing; squashing was very gentle. Mounting was performed as elsewhere described (COLOMBERA 1970). About 10 egg sacs

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were treated the same way after separation from the mother. These temporary squash preparations were sometimes made permanent ones as elsewhere described (COLOMBERA 1970) More than 50 sets of pachytene chromosomes, extracted from 5 females and about 40 sets of metaphase I chromosomes, extracted from 6 females were examined. Leptotene and post diffuse stage chromosomes are more frequently found than the chromosomes above mentioned.

All microphotographs were taken using a Zeiss phase contrast microscope. Drawings were made using an Abbe camera lucida.

OBSERVATIONS

Mitotic chromosomes.

The diploid number $2n=24$, has regularly been found in about 15 dividing eggs and young embryos. All the chromosomes appear to be metacentric or submetacentric (Fig. 1) and their length varies gradually so that it is not possible to group them into classes according to the size. Neither allocyclic behaviour nor differentiated stainability of chromosomes were observed, and therefore all chromosomes are considered to be autosomes.

In the embryonic mitosis, chromosomes are split into two chromatids from prophase on. The number of chromosomes in the zygotes, at least in the few nuclei observed, proves the homogametic condition of *Tisbe reluctans*.

Meiotic chromosomes.

Leptotene chromosomes are randomly distributed in the big nucleus of the developing oocyte, already characterized by a dark cytoplasm; these chromosomes look like thin single threads with numerous positive heterochromatic dots.

Subsequently the homologues pair up so closely that it is not possible to distinguish one from the other (Fig. 2) In this stage, which we consider as a pachytene, homologues do not separate even under drastic squashing. Therefore possible cross connections or chiasma-like formations between them cannot be observed. It is only in this stage that homologous chromosomes are associated closely enough to form chiasmata. Occasionally, chromosomes appears to be diffused and are lightly stained (Fig. 3). Homologous chromosomes separate and chiasma like formations are visible (Fig. 3 see arrows). We cannot say whether they disappear completely during this diffuse stage. After the diffuse stage the chromosomes are longer than during pachytene and homologues are distantly paired: in few cases and in single points chromosomes appear double (Fig. 4 see arrow). At this stage the chromosome ends are positively heteropycnotic; homologues are often more closely associated at their ends, seemingly by a chromatin bridge.

A drastic squash is able to spread the chromosomes over a large surface (Fig. 4) but homologues remain close and parallel to each other.

Then the chromosomes shorten rapidly, become deeply and homogeneously stained (Fig. 5) and lie near to the equatorial plane of the spindle (Fig. 5). Consequently at metaphase I, homologous chromosomes are parallel to each other, one group « below » and one « above » the equatorial plane of the spindle. Metaphase I chromosomes are clearly and completely formed by two distinct chromatids. The number of chromosomes in oocyte metaphase I is 24, as in mitotic metaphases.

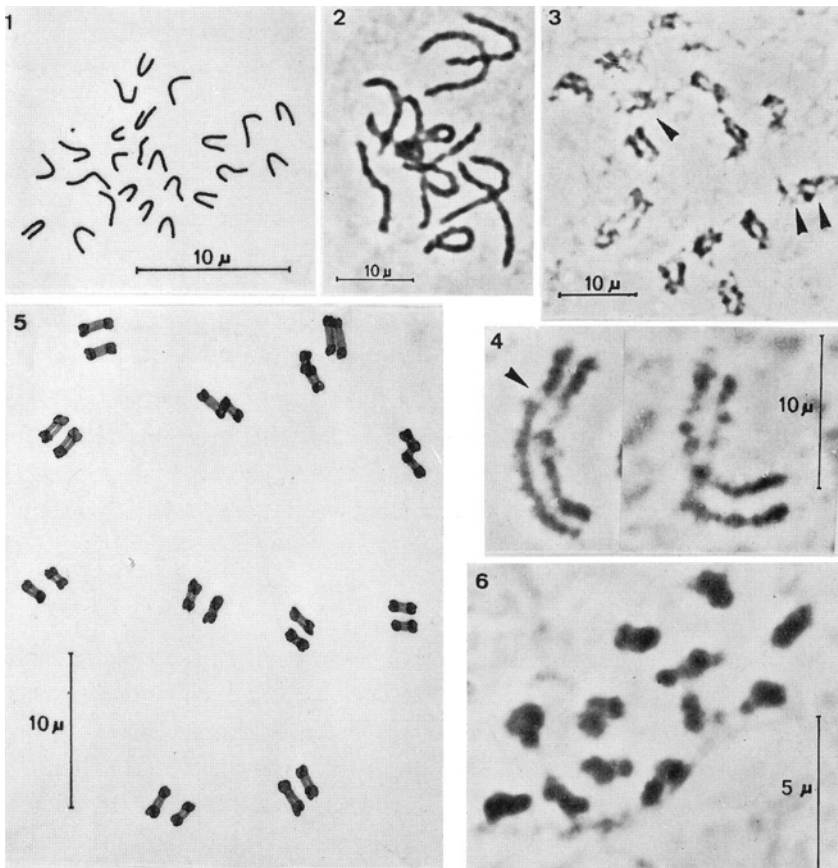


Fig. 1. — Metaphase chromosomes $2n=24$ in young embryos of *Tisbe reluctans*.
 Fig. 2. — Pachitene chromosomes in the female.
 Fig. 3. — Meiotic chromosomes of the female in the diffuse stage.
 Fig. 4. — The distantly paired homologous chromosomes at the post-diffuse stage.
 Fig. 5. — Metaphase I chromosomes. Note the distant parallel pairing between homologues.
 Fig. 6. — Chiasmatic bivalents in the male *T. reluctans*.

Only a few observations have been made on chromosomes of *T. reluctantans* in spermatogenesis. In a heavily squashed preparation (Fig. 6), it can be seen that homologous chromosomes are extremely contracted and intimately paired and the presence of chiasmata can be deduced from their morphology. At least in the few cases observed, the haploid number 12 was established and the absence of allocyclic behaviour of chromosomes indicates chromosomal homogamety.

DISCUSSION

The karyology of the genus *Tisbe*, as far the species *reluctans* is concerned, is very similar to that of other Harpacticoida (AR-RUSHDI 1963). In particular the distant parallel pairing of oocyte chromosomes during post-diffuse stage and metaphase I appears the same in *Tisbe reluctantans* as in the genus *Tigriopus* (AR-RUSHDI 1963). Differences are found in comparison with other species, where telosyndetic association and consequent circular arrangement of heterologous chromosomes occur at metaphase I (MATSCHEK 1910; HEBERER 1932, 1938; AR-RUSHDI 1963).

Other parameters, such as the morphology of mitotic chromosomes, suggest a close relationship between *Tisbe* and *Tigriopus* at a karyological level.

The hypothesis that distant parallel pairing and consequent achiasmatic meiosis may indicate a step toward a chromosome sex-determination mechanism (HALDANE 1922) is supported by the presence in Copepods of species without differentiated sex-chromosomes (AR-RUSHDI 1963) and species with differentiated sex-chromosomes (BEERMAN 1954; RÜSCH 1960). In this respect the genera *Thisbe*, and *Tigriopus* (AR-RUSHDI 1963) seem to be primitive within Copepods.

On the other hand, the presence of distant pairing at metaphase I in the female is by no means a proof that the meiosis I of *reluctans* is achiasmatic: in fact prior to the diffuse stage homologous chromosomes are closely associated and during the end of the diffuse stage, when homologues separate, chiasmata seem to occur. Of course only experiments of classical genetics could prove that such chiasmata are the morphological evidence of a crossing over. Therefore our research opens the question as to whether oocyte meiosis in Copepods is really achiasmatic or whether chiasmata disappear precociously after the diffuse stage prior to metaphase I.

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SUMMARY

The diploid chromosome number, $2n=24$, has been established, in both sexes of the harpacticoid copepod *Tisbe reluctantans*. The chromosomes are metacentric or submetacentric and differentiated sex chromosomes are not present. In the male chiasmatic bivalents form at metaphase I. In the female, during the first meiotic division and before the diffuse stage, homologous chromosomes are closely associated so that chiasmata may occur. After the diffuse stage, the homologues separate but remain parallel and juxtaposed to each other till metaphase I. The chromosomes seem to have doubled into two chromatids during the post-diffuse stage.