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**Karyological comparison among three species of the
genus *Tigriopus* (Copepoda, Harpacticoida)**

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Zoologia. — *Karyological comparison among three species of the genus Tigriopus (Copepoda, Harpacticoida)*. Nota di IVANA LAZZARETTO e ANGELO LIBERTINI, presentata (*) dal Corrisp. B. BATTAGLIA.

RIASSUNTO. — Vengono confrontati i cariotipi delle specie: *Tigriopus brevicornis*, proveniente da Tayvallich (Scozia), *T. californicus* di S. Francisco (California) e *T. fulvus* di Livorno, ottenuti da preparati di mitosi embrionali allestiti con una nuova tecnica. Delle prime due specie era noto da tempo il numero di cromosomi e le caratteristiche della meiosi ma non era stato fatto nessun tentativo per individuare eventuali differenze interspecifiche nel cariotipo; la specie *Tigriopus fulvus* viene studiata invece per la prima volta. È stato confermato il corredo $2n = 24$ sia per *Tigriopus brevicornis* che per *T. californicus*, e nella specie *T. fulvus* è stata accertata la presenza di $2n = 24$ e l'assenza di cromosomi sessuali; quest'ultima caratteristica è comune a tutte le altre specie finora studiate. Il confronto dei cariotipi mette in evidenza le seguenti caratteristiche: *Tigriopus fulvus* presenta 9 coppie di cromosomi metacentrici e 3 di submetacentrici; in una delle coppie di media grandezza si nota sporadicamente la presenza di un satellite. *T. californicus* è caratterizzato da 10 coppie di cromosomi metacentrici e 2 submetacentrici; nella coppia più grande è talvolta rilevabile la presenza di un satellite. *T. brevicornis* si distingue dai precedenti perché possiede 12 coppie di elementi metacentrici nel più grande dei quali è sempre presente un grosso satellite; questa specie si differenzerebbe anche per le maggiori dimensioni dei cromosomi. La presenza di queste caratteristiche permette di assegnare un cariotipo specie-specifico che differenzia queste tre specie anche dalle due popolazioni sub-antartiche di Crozet e Kerguelen avallando i risultati delle prove biologiche e biochimiche in base alle quali si proponeva di confermare come buone specie le popolazioni di Tayvallich, S. Francisco e Livorno e di riconoscere come nuove specie le popolazioni di Crozet e Kerguelen.

According to Lang's revision (1948 [9]), in the genus *Tigriopus* only the following species were included: *Tigriopus brevicornis*, which inhabits the European coasts, both Mediterranean and Atlantic, and those of West Africa, and *Tigriopus californicus* which can be found in the Pacific Ocean, in New Zealand, in the Sub-Antarctic area and in East Africa. The discovery of some new species and the confirmation of others previously described have nevertheless more enriched the composition of this genus in the last twenty years and some researches now taking place are revealing the necessity of further modifications.

The harpacticoid copepods of the genus *Tigriopus* are easily reared and crossed in the laboratory; because of this characteristic it's possible, in order to settle taxonomic problems, to carry out breeding experiments as well as to utilize the classic morphological criteria. This kind of approach, which was

(*) Nella seduta del 15 giugno 1984.

previously used by Bozic (1960 [5]), has been reconsidered in our laboratory and accompanied by biochemical research aiming to determine the degree of genetic affinity between different geographic populations. The results of a conspicuous series of cross-breeding among populations of both the Mediterranean and Atlantic coasts of Europe, of the East coasts of North America and the Sub-Antarctic area of Crozet and Kerguelen have revealed reproductive barriers between populations which have been attributed to the same species; the biochemical data have confirmed these results revealing great genetic distances between these populations (Battaglia, 1982 [3]; Battaglia *et al.* in press [4]). In particular the attribution of all populations of the Mediterranean area to *Tigriopus fulvus* species as proposed by Bozic (1960 [5]), is put to question. By cross testing it has been proved that the populations of the Tyrrhenian area are completely isolated from a reproductive point of view from those of the Adriatic; the biochemical tests have put in evidence a great genetic distance between these two populations. No similar situations have shown up in the North European or Californian coast populations attributed respectively to the species *Tigriopus brevicornis* and *T. californicus*.

The analysis of the karyotype of all the populations, examined by means of biological and biochemical tests has been started in order to individuate possible interspecific differences at the cytological level which could justify the reproductive isolation.

In this paper are reported the results concerning the species *Tigriopus brevicornis* and *T. californicus* and the populations coming from Livorno defined as *T. fulvus* (Livorno). In the first two species the chromosome number and the dynamics of meiosis were analyzed (Ar-Rushdi, 1963 [1]); instead *T. fulvus* (Livorno) is studied for the first time. Also included is a description of the technique used for chromosome preparation which is a modification of the standard air drying technique used on other classes of animals (Kligermann and Bloom, 1977 [8]).

MATERIAL AND METHODS

All specimens employed were taken from populations reared in our laboratory, following standard culture methods. (Battaglia, 1970 [2]). These populations originate from samples collected in Livorno (*Tigriopus fulvus*), S. Francisco, California (*T. californicus*) and Tayvallich, Scotland (*T. brevicornis*).

Chromosome preparations were made by exposing several ovigerous females of each species to a solution of 10^{-4} M colchicine for 30 minutes which were then transferred to distilled water for 50-60 minutes. Later on the egg sacs were separated from the mother and placed in acetic acid at 60% for a few minutes. Then the brood sacs were gently minced with a tungsten needle so forming a suspension of cells. This suspension was thereafter dropped with a micropipette on to carefully washed slides; then the drops were quickly removed and the preparations were made permanent by staining in 10% buffer solution of Giemsa for thirty minutes.

Observations and photos were made with the aid of a Zeiss photomicroscope III at 1250 magnification.

RESULTS

The chromosomes of the three species have been studied in c-metaphase plates of dividing eggs.

The diploid chromosome number of the species *Tigriopus fulvus* (Livorno) corresponds to $2n = 24$ (Table I); from the analysis of seven meiotic metaphase plates the haploid number results $n = 12$. From the study of the best plates it appears that the karyotype of this species consists of 9 pairs of metacentric chromosomes and 3 pairs of sub-metacentric ones in the largest of which the position of the centromere is more eccentric (Plate I, fig. 5, 6). In a median pair a satellite sometimes appears. The chromosomes decrease gradually in size and therefore it is impossible to group them in different classes of length. The diploid chromosome number in the species *Tigriopus californicus* has been confirmed to be 24 (Table I); from the best plates it has been possible to ascertain that the karyotype of this species consists of ten pairs of metacentric chromosomes and two submetacentric ones (Plate I, fig. 3, 4). The largest pair sometimes reveals the presence of a satellite. All elements decrease gradually in size. It has been confirmed that in *Tigriopus brevicornis* the diploid value is $2n = 24$ (Table I); this species possesses the most symmetric karyotype, in fact all their chromosomes are metacentric (Plate I, fig. 1, 2). In the largest pair a satellite is regularly evident. The size of the chromosomes of this species results larger than those of the other two species; therefore a further verification of this difference is necessary. Also in this species the chromosome size decrease gradually. Neither heteromorphic elements nor allocyclic behaviour of chromosomes was observed in any species, confirming the absence of differentiating sex-chromosomes as verified in all species of this genus studied so far.

DISCUSSION

The karyological comparison carried out on three species of the genus *Tigriopus* has above all verified the same diploid number 24 in all the three species.

As far as the chromosomal morphology is concerned these species differ from each other by some characteristics: *Tigriopus brevicornis* possesses a karyotype composed of twelve metacentric elements, of which the largest regularly presents a satellite; *T. californicus* possesses two submetacentric and ten metacentric chromosomes and a satellite can be sometimes observed on the largest element; *T. fulvus* (Livorno) presents the karyotype with the highest number of sub-metacentric chromosomes: three out of twelve; however in some cases the presence of a satellite localized in a median pair can also be seen in this species. It has been noted that the secondary constrictions which are the meta-

phasic morphological expression of the nucleolar organizer, can show a high variability not only among individuals of the same species but also among different cells of the same individual. Therefore the differences observed in the location and in the regularity of the presence of the satellite indicate some diversity among the three species that should be more closely studied.

Moreover the karyotypes of the *Tigriopus californicus* and *T. fulvus* (Livorno) species differ also from those ascertained in the sub-antarctic populations of Crozet and Kerguelen: the first characterized by the presence of $2n = 22$ and of one chromosome clearly longer than the others; all the elements of this species are metacentric like those of the species coming from Kerguelen which possesses $2n = 24$; no satellite has been noted in these species. These results confirm that the two sub-antarctic species do not belong to the *Tigriopus californicus* species as proposed by Lang 1948 [9]). Also Brady's hypothesis (1875 [7]) ascribing these populations to the *Tigriopus fulvus* species could be rejected. It could be interesting to test the hypothesis advanced by Bradford (1967 [6]), according to which the inhabitants of the Kerguelen region belong to the *Tigriopus angulatus* species. A karyological analysis of the latter species could reveal whether its karyotype corresponds to that of the Crozet or the Kerguelen island species.

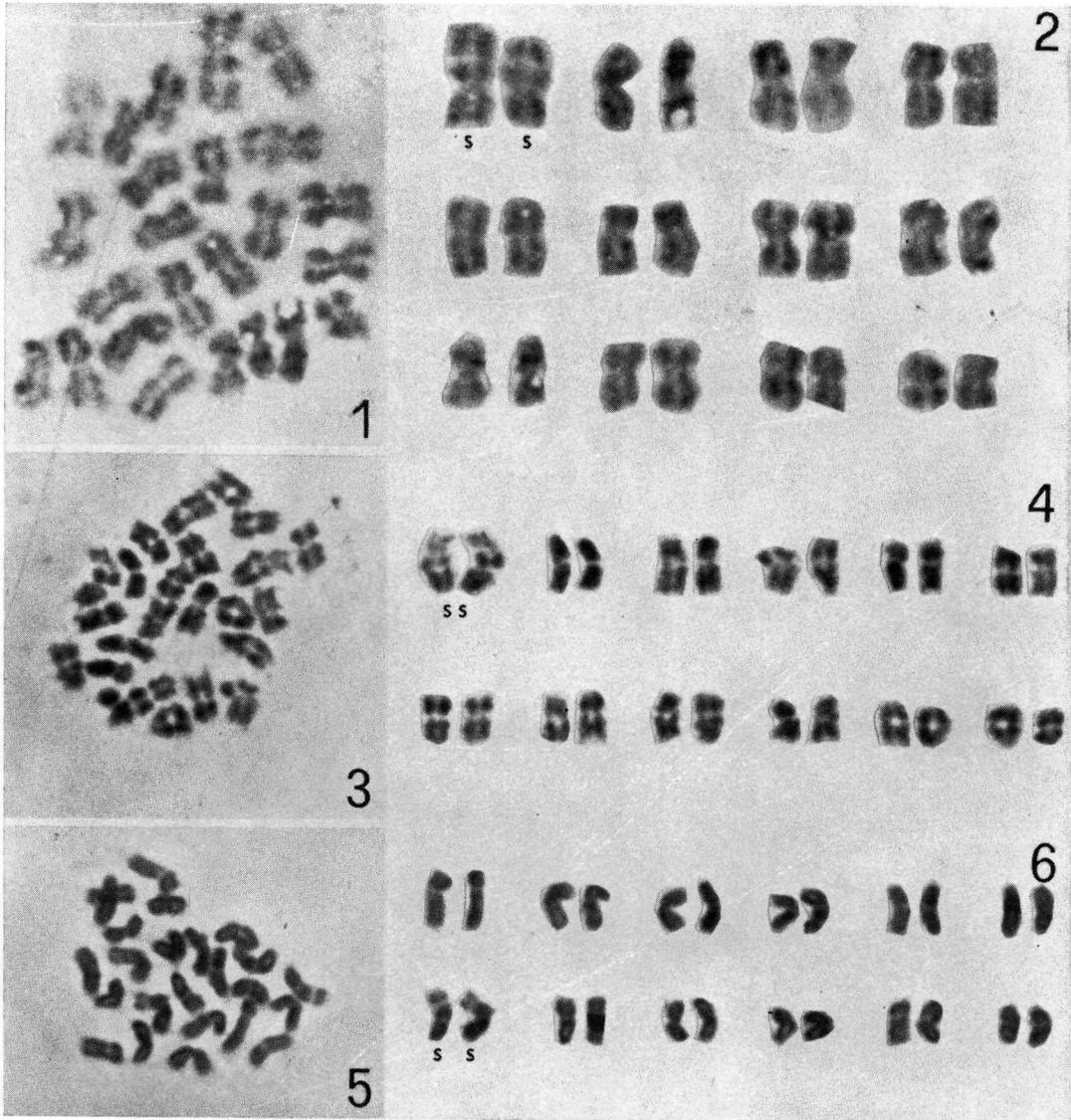
As far as it is known we believe that, as observed in the genus *Tisbe*, also in the genus *Tigriopus* there is a trend to maintain the chromosome number, and that the chromosomal evolution could occur mainly by means of rearrangements of chromosomal arms.

The analysis of the dynamics of meiosis in all different species might indicate if the peculiar arrangements of bivalents at metaphase I (Ar-Rushdi, 1963 [1]) can be considered an interspecific character suitable for classification. This examination should also include the three species studied by Ar-Rushdi (1963 [1]) because this Author states that «for the completion of the study of the meiotic cycle, material additional was collected at Fiascherino and Porto Venere in North Western Italy, where the second meiotic division was investigated». Consequently, in our opinion, these latest investigations could have been carried out on individuals belonging to *Tigriopus fulvus* (Livorno).

TABLE I

Distribution of mitotic chromosome counts for three species of the genus Tigriopus.

	Number of cells with diploid chromosome number of							Total no. of cells examined
	20	21	22	23	24	25	26	
<i>Tigriopus fulvus</i>	3		2	5	48	4	3	65
<i>Tigriopus californicus</i>	1	1	2	5	35	2		46
<i>Tigriopus brevicornis</i>		5	3	13	68	1		90



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EXPLANATION OF PLATE I

PLATE I

- 1) *Tigriopus brevicornis*, photomicrograph of metaphase chromosomes in cleaving zygotes ($\times 2400$).
- 2) *Tigriopus brevicornis*, karyotype obtained from the photomicrograph of the figure 1.
- 3) *Tigriopus californicus*, photomicrograph of metaphase chromosomes in cleaving zygotes ($\times 2400$).
- 4) *Tigriopus californicus*, karyotype obtained from the photomicrograph of the figure 3.
- 5) *Tigriopus fulvus*, photomicrograph of metaphase chromosomes in cleaving zygotes ($\times 2400$).
- 6) *Tigriopus fulvus*, karyotype obtained from the photomicrograph of the figure 5.
The *s* indicates the presence of a satellite.