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SUPERNUMERARY CHROMOSOMES, ROBERTSONIAN REARRANGEMENT AND MULTIPLE NORs IN *CORYDORAS AENEUS* (PISCES, SILURIFORMES, CALLICHTHYIDAE)

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SUMMARY — Cytogenetic studies in four samples of *Corydoras aeneus* from tributaries of the Tietê River in the upper Paraná System, Brazil (rivers Araquá, Corumbataí, Capivara and Alambari) evidenced a diploid number of $2n=60$ and $2n=61$ plus a variable number of 0 to 3 small supernumeraries or B chromosomes. The variability in the A complement was due to the occurrence of a Robertsonian rearrangement. The supernumerary chromosomes were almost totally C-band positive. The analysis of male meiotic cells showed the occurrence of 29 bivalents and one trivalent in the individuals heterozygous for the Robertsonian rearrangement and of univalents in the individuals with supernumerary chromosomes. Four chromosome pairs are involved in nucleolar organization. All the 4 samples presented supernumerary chromosomes, but the Robertsonian rearrangement was restricted to the rivers Araquá and Corumbataí.

INTRODUCTION

The development of cytogenetic techniques and their application to the study of fish populations have disclosed the occurrence of extensive chromosome variability and in many cases this variability was due to the occurrence of Robertsonian rearrangements and of supernumerary or B chromosomes.

Robertsonian polymorphism, which may result from centric fusions and dissociations, is a relatively rare phenomenon requiring special preconditions for persistence in natural populations (WHITE 1973). Nevertheless, polymorphism of this nature appears to be widespread in the salmonid fishes (HARTLEY and HORNE 1984). Robertsonian polymorphism was also reported in some species of other groups of fishes, but is not very common (ALMEIDA TOLEDO *et al.* 1985; THODE *et al.* 1985; GILES *et al.* 1985).

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B chromosomes are widely distributed in plants and animals (JONES and REES 1982). The karyotype of a large number of species of fishes has already been analyzed (about 1,300 species, according to KIRPICHNIKOV 1981) and the occurrence of supernumerary chromosome is restricted to a few species. Supernumerary chromosomes have been reported in *Prochilodus scrofa* (PAULS and BERTOLLO 1983), *P. cearensis* (PAULS 1985), *Rhamdia* sp. (HOCHBERG *et al.* 1985), *Bergiaria westermani* (DIAS and FORESTI 1985) and *Moenkhausia sanctaefilomenae* (FORESTI *et al.* 1987); in other cases reported (see TAYLOR 1967; FALCÃO *et al.* 1984 and VENERE and GALETTI Jr. 1985), the B condition of the extra chromosome described is not conclusive. A review about B chromosomes in fishes has been published by FORESTI *et al.* (1987).

The present paper reports the karyotype and the localization of constitutive heterochromatin and Nucleolus Organizer Regions (NORs) of *Corydoras aeneus*, and describes the occurrence of a Robertsonian rearrangement, of supernumary chromosomes and of multiple NORs. These data are part of a cytogenetic survey of the genus *Corydoras*.

MATERIALS AND METHODS

33 specimens of *Corydoras aeneus* were collected from four small tributaries of the Tietê River (SP, Brazil). Table 1 lists the collection sites and the number and sex of the specimens investigated at each location. The animals were kept in well-aerated aquaria. The technique of COLE and LEAVENS (1971) for increasing the number and quality of mitotic figures, was used with slight modifications: the animals received an injection (1 ml/100 mg body weight) of a yeast suspension (0.5 g yeast, 0.5 g glucose and 7 ml H_2O) which had been previously incubated at 40° C for 20 minutes. The specimens were killed 48 or 72 h later.

Chromosome preparations were made according to the procedure described by FORESTI *et al.* (1981). To avoid excessive chromosome condensation, colchicine concentration was reduced to 0.05% (1 ml/100 g body weight) and the animals were killed after 50 minutes.

The material was submitted to C-banding by the method of SUMMER (1972) and to silver staining of the Nucleolus Organizer Regions, by the method of HOWELL and BLACK (1980). Chromosome morphology was identified on the basis of arm ratios, as proposed by LEVAN *et al.* (1964).

RESULTS

Corydoras aeneus from the Tietê River Basin presented diploid numbers $2n=60$, $2n=61$, $2n=62$, and $2n=63$. Seven distinct karyotypes were found. The specimens with $2n=60$ present a basic karyotype with 13 pairs of metacentrics, 13 pairs of submetacentrics and 4 pairs of subtelocentrics (Fig. 1). Chromosome difference between sexes were not found.

Of the 33 animals sampled, 21 presented the basic karyotype $2n=60$; whereas the other 12 specimens were variants (Table 1). Five of these variants



Fig. 1. — Karyotype of a female *Corydoras aeneus* with $2n=60$. Bar corresponding to 10 µm.

presented only one of the metacentrics in pair 1; with the homologue being replaced by two acrocentrics (Fig. 2a): A trivalent was observed at metaphase I in the meiotic cells of males with this karyotype (Fig. 5b). Individuals homozygous for two pairs of acrocentrics were not observed.

The other 7 variant individuals presented pair 1 formed by two metacentrics and 1 to 3 additional submetacentrics or subtelocentrics. These chromosomes were usually observed as univalents in meiotic cells either at pachytene or at metaphase I (Fig. 5a) and were characterized as supernumeraries.

The two types of chromosomal variations (supernumeraries and Robertsonian rearrangement) were found in association, so that four combinations were observed (Fig. 2).

The C-banding pattern of one specimen with 2 supernumeraries is presented in Fig. 3. C-band positive regions are present on the short arm of at least 6 chromosome pairs or are restricted to the distal portion of the short arm as in pair 2, which is one of the NOR-bearing pairs. Pair 1 has a very conspicuous pericentromeric C-positive block. Pairsi28 and 29, two small subtelocentrics, also have pericentromeric blocks. The supernumerary chromosomes are almost entirely heterochromatic. The two small acrocentrics and the related odd metacentric present C-positive pericentromeric blocks in the individuals heterozygous for the Robertsonian rearrangement (Fig. 3).

TABLE 1 - *Chromosome number variation in «Corydoras aeneus».*

Locality	Fish no. and sex	Chromosomes numbers							No. of cells conted	2*n*	R	B
		58	59	60	61	62	63	64				
Araquá River	302 ♀		3	65					68	60		0
	304 ♀	2	1	61					64	60		0
	305 ♀	3	3	13	54				73	61	+	0
	306 ♀	2	6	71					79	60		0
	308 ♂		2	15	9	16	22	3	67	63	+	2
	309 ♂	2	3	9	27	3	5		49	61		1
	314 ♀	4	6	56					66	60		0
	384 ♀		2	11	59				72	61	+	0
	385 ♀	1	4	57					62	60		0
	403 ♂		3	59	2				64	60		0
	417 ♂			3	34	1			38	61	+	0
	418 ♀		5	59					64	60		0
	421 ♀		4	35					39	60		0
	422 ♂		1	5	26				32	61		1
	423 ♀		3	26	2				31	60		0
Corumbataí River	316 ♂			1	3	67			71	62		2
	317 ♀	3	3	25	1				32	60		0
	319 ♀		5	10	3	18			36	62	+	1
Capivara River	321 ♀	1	6	59					66	60		0
	324 ♀		5	17					22	60		0
	326 ♀		5	65	2				72	60		0
	332 ♀		5	59	1				65	60		0
	333 ♀		2	8	13	4	14		41	63		3
	335 ♀	2	3	26	1				32	60		0
	336 ♀	1			5	47	1		54	62		2
	432 ♀		3	26	1				30	60		0
	433 ♂		2	28	1				31	60		0
	434 ♂		1	29					30	60		0
Alambari River	441 ♂			2	5	58			65	62		2
	442 ♂			1	2	59			62	62		2
	443 ♀		1	38					39	60		0
	463 ♀	2	3	12					17	60		0
	464 ♂		1	10					11	60		0

Note: R - Robertsonian rearrangement; B - Supernumerary chromosomes.

Fig. 2. — Karyotypes of the variant types of *Corydoras aeneus*. A) female specimen with $2n = 61$, with a Robertsonian rearrangement. b) male specimen with $2n = 61$, with a B chromosome. c) female specimen with $2n = 62$, with a Robertsonian rearrangement and a B chromosome. d) male specimen with 2 Bs. e) male specimen with $2n = 63$, with a Robertsonian rearrangement and 2 Bs. f) female specimen with $2n = 63$, with 3 Bs. Bar corresponding to 10 μm.

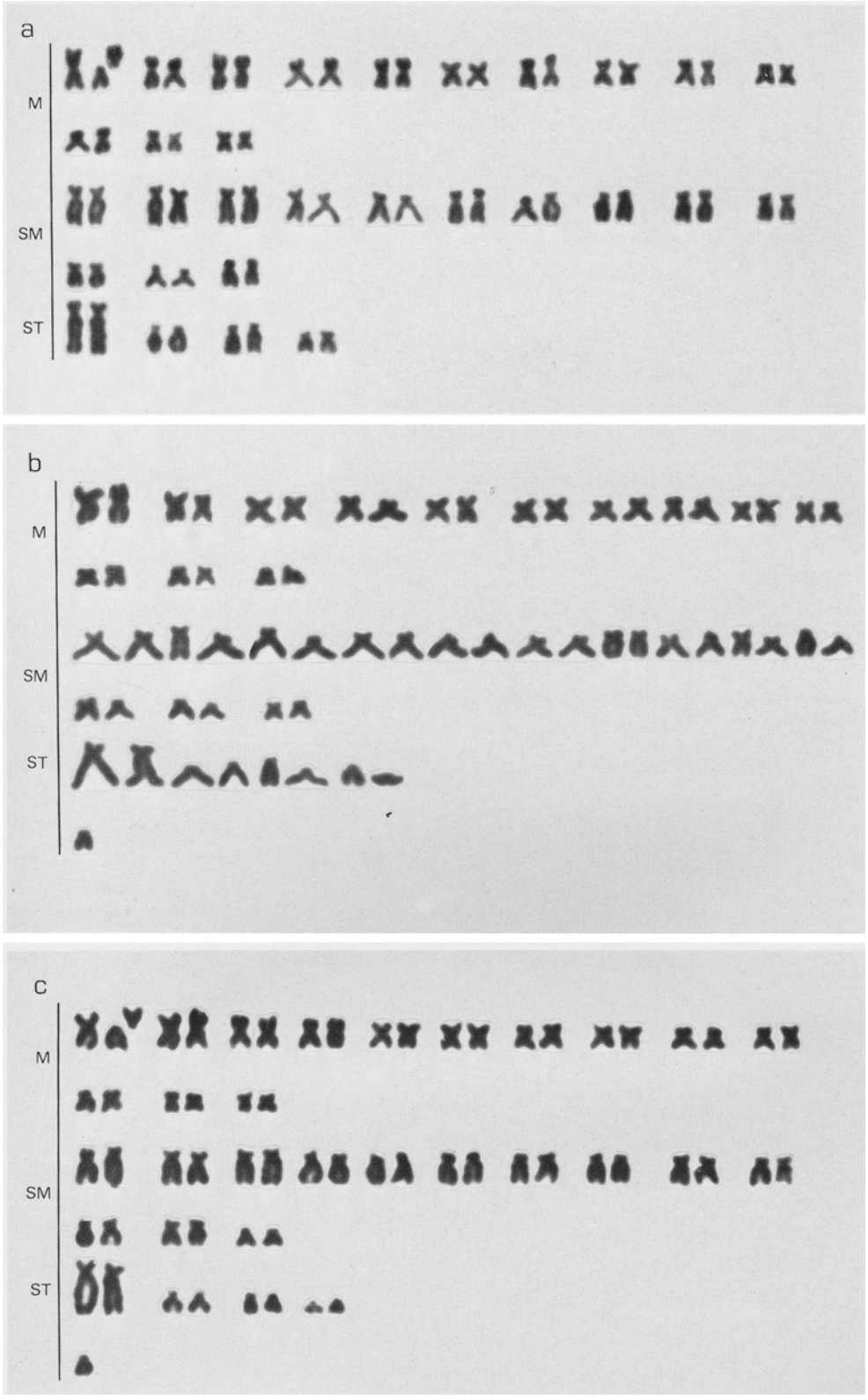
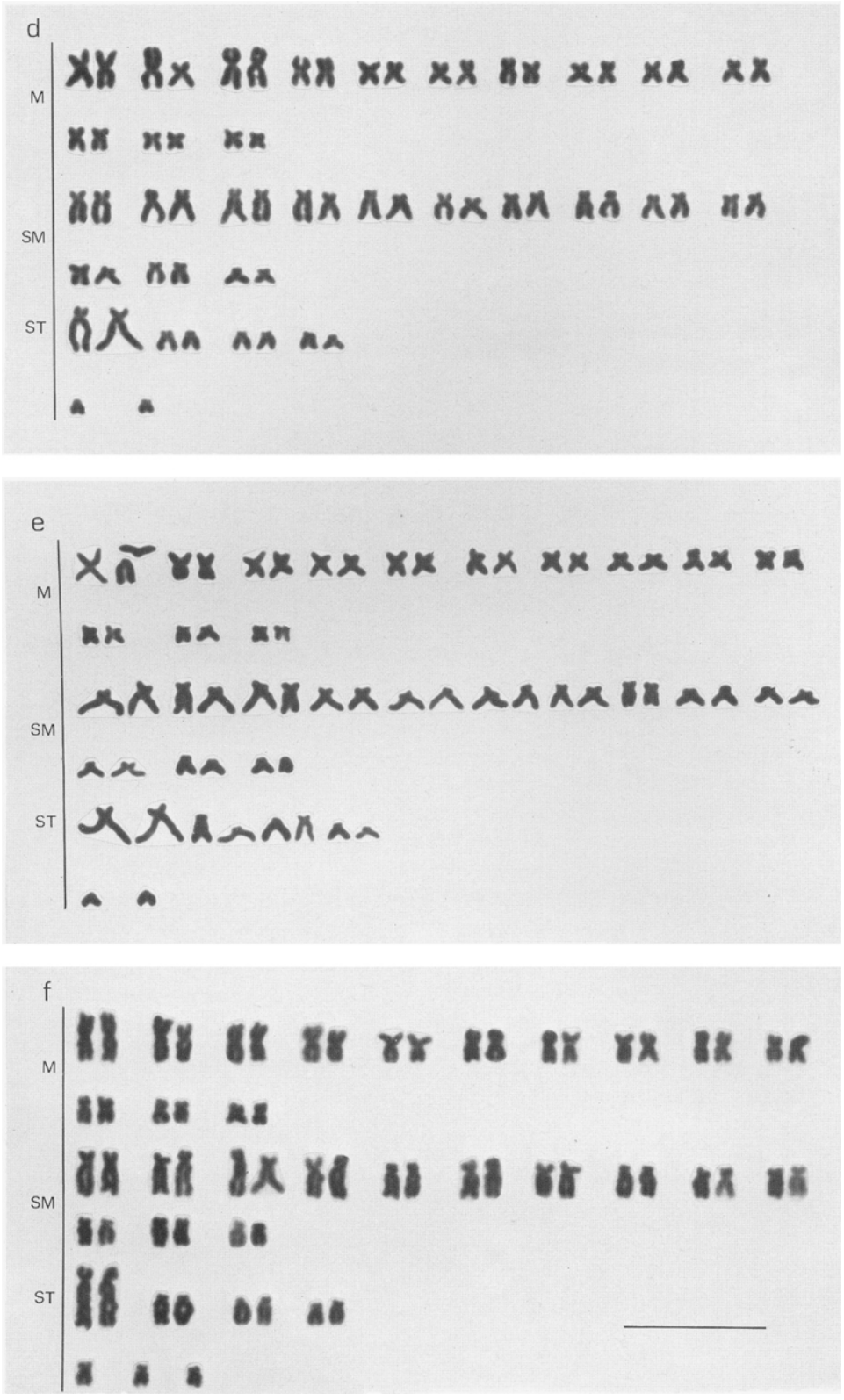

a
M
SM
ST
b
M
SM
ST
c
M
SM
ST

Fig. 2B



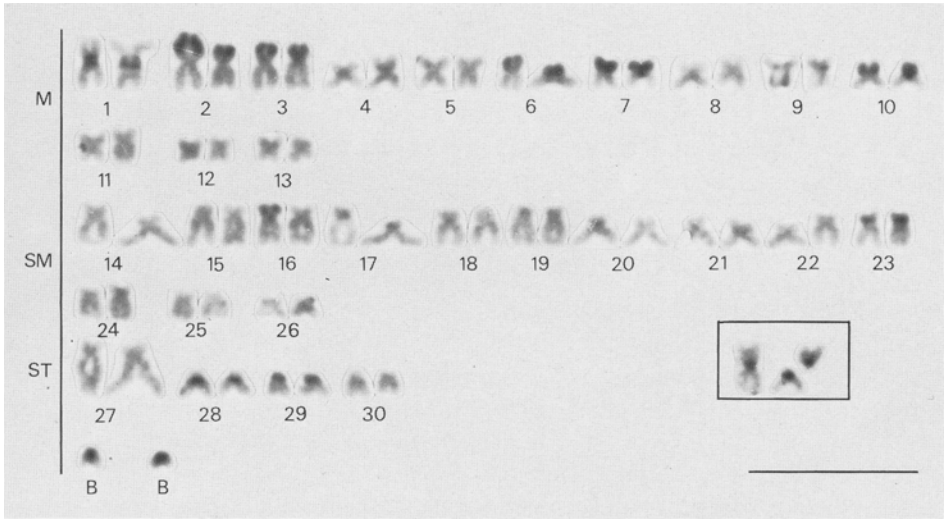


Fig. 3. — C-banded karyotype of a male specimen of *Corydoras aeneus* with $2n = 62$, with 2 Bs. Bs are almost totally heterochromatic. Notice large pericentric C-positive blocks in pair 1. In the insert, pair 1 of one individual heterozygous for the Robetsonian rearrangement. Bar corresponding to 10 μm.

Nucleolus Organizer Regions (NORs) were observed in 4 chromosome pairs (pairs 2, 6, 7 and 10) but they were never found to be active simultaneously (Fig. 4). The NORs are C-banding-positive and show high polymorphism of size, as can be observed in pair 2 in Figs. 2d and 3.

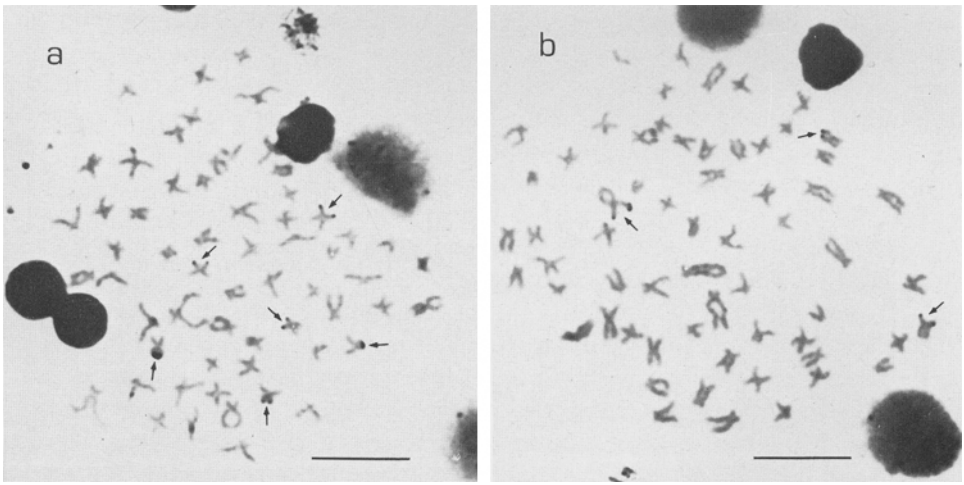


Fig. 4. — Ag-stained metaphases of *Corydoras aeneus*. Arrows indicate the marked chromosomes. Bar corresponding to 10 μm.

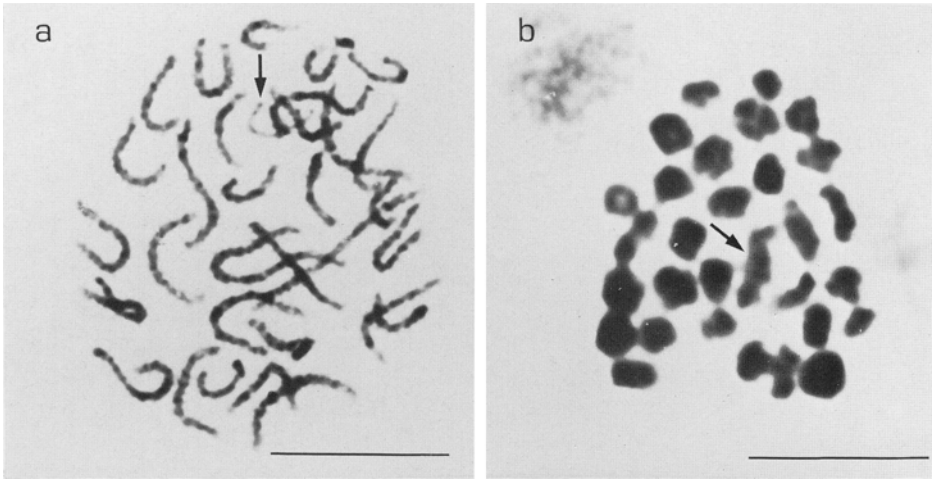


Fig. 5. — Meiotic cells of *Corydoras aeneus*. a) pachytene of a $2n = 62$ individual with 2 Bs; arrow indicates a univalent. b) metaphase I of a $2n = 61$ specimen with a Robertsonian rearrangement; the arrow indicate a probable trivalent. Bar corresponding to 10 μm.

DISCUSSION

The diploid number of *C. aeneus* has been reported to be $2n = \pm 132$ (SCHELL *et al.* 1972) and the haploid number, $n = 60$ (HINEGARDNER and ROSEN 1972). The origin of the specimens analyzed in both cases was unknown. The specimens of *C. aeneus* from the Tietê River Basin, analyzed in the present study, have a basic diploid number $2n = 60$ and $2n = 61$ plus 0 to 3 supernumeraries. Differences between our data and those from the literature may be ascribed to misidentification of the specimens in previous studies, or to the existence of actual differences between populations, indicating the possible occurrence of a diplo-tetraploid system. Further studies with specimens of known origin are needed to test this hypothesis.

The karyotypic variability in *C. aeneus* from the Tietê River Basin is due to the occurrence of a Robertsonian rearrangement and the presence of a system of supernumerary chromosomes.

The occurrence of two acrocentrics associated with the absence of one homologue in a metacentric chromosome pair is considered as the occurrence of a Robertsonian rearrangement. In the present case, this assumption is reinforced by the correspondence of the C-banding patterns of the metacentric and the two acrocentrics, and the finding of a trivalent in the meiotic cells. On the other hand, individuals with the Robertsonian rearrangement presented a very sharp mode of chromosome number in metaphase cell counts, in strong contrast to those individuals with supernumerary chromosomes (Table 1).

In our samples of *C. aeneus* in which the Robertsonian rearrangement was detected, 5 out of a total of 18 animals were heterozygous for par 1; homozygotes for two pairs of acrocentrics were not found. This could indicate that possibly, the mechanism that occurred to give origin to this karyotype was a centric fission of one of the homologues of an ancestral individual with pair one formed by two metacentrics. However the origin of this polymorphism by fusion of two acrocentrics can not be discarded.

Very little is known about the significance of Robertsonian, polymorphism in fish populations. The data obtained for *Spicara flexuosa* ($2n = 44$, 45, 46) with a Robertsonian polymorphism (VASILIEV 1980) have shown the occurrence of selective pressure against forms with 45 and 46 chromosomes. This selection seems no to be constant and is more effective during the first year of life. The Robertsonian polymorphism is maintained when older individuals are crossed with younger ones.

The extra chromosomes found either in the presence or in the absence of the Robertsonian rearrangement may be caracterized as supernumerary or B chromosomes because they present numerical variation, are almost entirely heterochromatic and are visualized as univalents in the germ cells. Individuals presenting these chromosomes are sometimes mosaics for diploid number, probably due to mitotic non-disjunction (Table 1) and these conditions are characteristic of B chromosomes.

The supernumerary chromosomes were present in all samples analyzed, but the Robertsonian rearrangement was apparently restricted to pupulations of the Araquá and Corumbataí rivers. This may suggest a possible isolation between these populations from those from the Capivara and Alambari rivers.

The findings of multiple NORs in *C. aeneus* and in other species of the genus *Corydoras* which we are studying seems to indicate that this is a characteristic of this genus. As rule, fishes present only a pair of NORs and only the Characidae have been characterized until now as a group with multiple NORs (ALMEIDA TOLEDO and FORESTI 1985).

Although the nucleolar chromosomes were identified in *C. aeneus*, as belonging to four pairs, eight NORs were never detected simultaneously. This could be due either to the absence of ribosomal cistrons in one of the homologues or to regulation mechanisms. The inter and intraindividual variability in activity as well as size differences between homologous NORs confirms the previous evidence of the existence of extensive NOR polymorphism in fishes (FORESTI *et al*, 1981).

Further detailed analysis is necessary to understand the possible evolutive significance of B chromosomes and the Robertsonian rearrangement in this species.

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