

Estimated frequency of B-chromosomes and population density of *Astyanax scabripinnis paranae* in a small stream

Fábio Porto-Foresti¹, Claudio Oliveira¹, Edson Luis Maistro² and Fausto Foresti¹

ABSTRACT

A study was made of the frequency of B-chromosomes and the population density of *Astyanax scabripinnis paranae* (Pisces, Characidae, Tetragonopterinae) from three stretches of the Cascatinha stream (Botucatu, SP). In the first stretch the population was estimated to be about 212 individuals and among 35 karyotyped specimens, 23 carried one macro B-chromosome; in the second stretch the population was estimated to be about 650 individuals and among 20 specimens karyotyped, two possessed one macro B-chromosome; in the third stretch the population was estimated to be about 107 individuals and among 10 specimens karyotyped, one carried one macro B-chromosome. A significant difference was observed in the frequency of macro B-chromosomes in females (57%) and males (8.7%) ($P = 0.0001$). These data suggest that the B-chromosome frequency and the populational density are not directly related. The hypothesis of the existence of some adaptive effect conferred by the B-chromosomes to the specimens from the first stretch of the Cascatinha stream is presented and discussed.

INTRODUCTION

The species *Astyanax scabripinnis* presents several interesting peculiarities for evolutionary studies, i.e., a large number of subspecies described (Fowler, 1948), geographic distribution restricted to the headwaters of small streams (Gomes and Azevedo, 1960), phenotypic plasticity (Moreira-Filho and Bertollo, 1991; Maistro, 1991), and high chromosomal diversity (see references in Souza *et al.*, 1995). Moreover, some local populations have a high incidence of individuals carrying macro B-chromosomes (Salvador and Moreira-Filho, 1992; Maistro *et al.*, 1994; Vicente *et al.*, 1996; among others).

Many aspects related to B-chromosomes have been intensively investigated in the last years (Beuckeboom, 1994). Moreover, several theoretical studies have shown that the probability of the fixation of chromosomal rearrangements is positively correlated with the adaptive value that they confer to a local population, and with the population structure where they occur (Lande, 1985). The main objective of the present investigation was to study the relationship between the population density of *A. s. paranae* and the frequency of occurrence of macro B-chromosomes in local populations of *A. s. paranae* from three stretches of a small stream.

MATERIAL AND METHODS

Cytogenetic studies were conducted on samples of *Astyanax scabripinnis paranae* (Pisces, Characidae, Tetragonopterinae) collected from three stretches of the

¹ Departamento de Morfologia, Instituto de Biociências, UNESP, Campus de Botucatu, 18618-000 Botucatu, SP, Brasil and Centro de Aqüicultura da UNESP. Send correspondence to C.O.

² Departamento de Biologia, Instituto de Ciências Químicas e Farmacêuticas, Universidade de Alfenas, 37130-000 Alfenas, MG, Brasil.

Cascatinha stream (S 22°53'30" W 48°28'36"), a small tributary of the Tietê River, Botucatu, SP, Brazil (Figure 1) from April to May, 1995. The first stretch, about 300 m long, is located about 880 m above sea level and comprises the headwaters. This habitat is characterized by a slight downward steep, a small volume of water, sandy bottom, and little stream-side vegetation. The second stretch, about 500 m long, is located about 860 m above sea level and comprises the medium course of the stream. In this segment, the habitat is characterized by a marked downward steep, the volume of water increases, the bottom is generally stony, and a lot of stream side vegetation, with many trees can be found; this stretch ends in a small lake. The third stretch, about 800 m long, is located about 820 m above sea level and comprises the final course of the stream before it discharges into the river. The habitat in this stream segment is characterized by a small downward steep, a large volume of water, sandy bottom, and by the occurrence of some stream-side grass vegetation.

The karyotypic study was conducted on 65 specimens (42 females and 23 males). Chromosome spreads and the staining technique were performed as described by Foresti *et al.* (1993). The fishes used in cytogenetic analysis were identified and deposited in the fish collection of the Laboratory of Fish Biology, UNESP, Botucatu, SP, Brazil.

The analyses of population density of *A. s. paranae* were performed according to the multiple census method, described by Schumacher and Eschmeyer (1943 as cited by Krebs, 1989). During the first collect all specimens collected were marked with India ink, which was injected in the dorsal part of the fish body (red color for the first stretch, black for the second stretch and green for the third stretch), and immediately returned to the water. In regular intervals of 5 days, new captures were performed in all stretches of the stream. The number of previously marked individuals was determined and the specimens without identification were marked and returned to the same position in the stream. This procedure was repeated seven times in the first stretch and six times in the second and third stretches resulting in 45 specimens marked in the first stretch, 131 specimens marked in the second stretch, and 65 specimens marked in the third stretch. We used India ink for

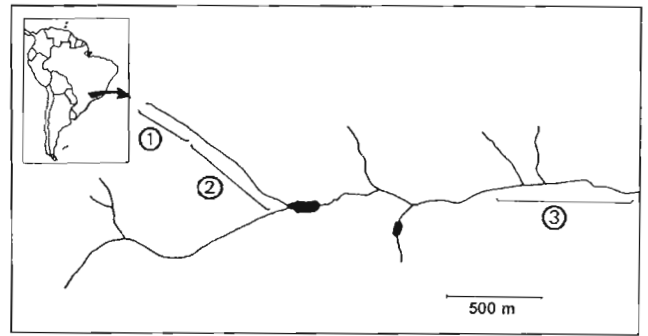


Figure 1 - Collection sites of *Astyanax scabripinnis paranae* in the Cascatinha stream (S 22°53'30" W 48°28'36"), Botucatu, SP, Brazil: 1, first stretch (headwater region); 2, second stretch (middle course); 3, third stretch (final course).

marking the fishes because it does little damage to the fish and because it facilitated identification. According to previous experiments conducted in our laboratory, the marks remain perfectly readable for about three months (Porto-Foresti, personal communication).

Fisher's exact test (Zar, 1984) was used for comparison of the frequency of occurrence of B chromosomes between males and females.

RESULTS AND DISCUSSION

The cytogenetic analysis revealed a diploid number of $2n = 50$ chromosomes in 39 animals of both sexes ($8m + 22sm + 10st + 10a$). The other 26 individuals presented $2n = 51$ chromosomes, the extra chromosome being a macro B-chromosome (Figure 2 and Table I). This karyotype and the presence of a macro B chromosome were previously related in a study performed in this local population by Maistro *et al.* (1994). The present analysis showed that the frequency of B chro-

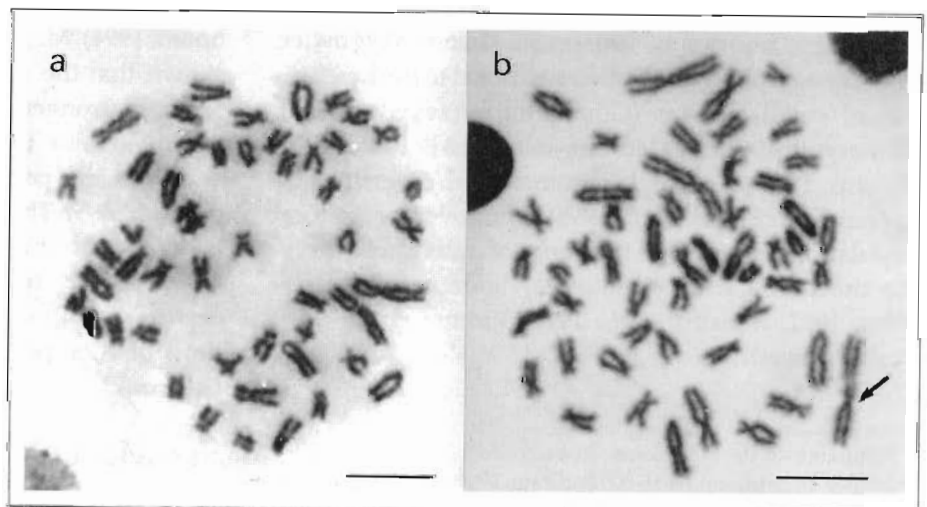


Figure 2 - Somatic metaphases of *Astyanax scabripinnis paranae* from the Cascatinha stream with $2n = 50$ chromosomes (a) and $2n = 51$ chromosomes (b) stained with Giemsa. The arrow indicates the B-chromosome. Bar = 10 μ m.

Table I - Occurrence of specimens of *Astyanax scabripinnis paranae* without ($2n = 50$) and with ($2n = 51$) B-chromosomes in the Cascatinha stream.

Locality	Specimens with $2n = 50$ (♀/♂)	Specimens with $2n = 51$ (♀/♂)
First stretch	04/08	21/02
Second stretch	09/09	02/00
Third stretch	05/04	01/00

mosomes in specimens from this stream is about 40% (Table I). The frequency of B chromosomes in fish populations ranges from 3.6% in *Characidium cf. zebra* to 100% in *Moenkhausia sanctafilomenae* (see references in Salvador and Moreira-Filho, 1992). The observed frequency of 40% in *A. s. paranae* is within this distribution interval.

Karyotypic analyses performed in local populations of *A. scabripinnis* have shown that usually only one macro B-chromosome is found in each fish as was observed in the present investigation (see references in Maistro *et al.*, 1994). However, the occurrence of two macro B-chromosomes was also reported for three other populations of *A. scabripinnis* (Vicente, 1994; Néó and Moreira-Filho, 1995).

A significative difference was observed in the frequency of macro B-chromosomes in females (57.1%) and males (8.7%) of *A. s. paranae* from Cascatinha ($P = 0.0001$). Similar differences were also observed in several local populations of *A. scabripinnis* with similar macro B-chromosome (Salvador and Moreira-Filho, 1992; Maistro *et al.*, 1992; Maistro *et al.*, 1994; Migozuchi and Santos, 1995; Néó and Moreira-Filho, 1995; Vicente *et al.*, 1996). Moreover, in a study of reproductive biology of *A. scabripinnis* from a natural population from Fazzari stream (São Carlos, SP, Brazil), a higher proportion of females (63%) than males was observed (Barbieri, 1992).

A possible explanation for the higher frequency of B-chromosomes in females could be the occurrence of gynogenesis in this species (Vicente *et al.*, 1996); thus, spermatozoon could induce the embryonic development of a diploid egg without contribution in the future chromosome constitution of the progeny. However, in a population of *A. scabripinnis* from the Jucu River, which presents a system of medium-sized B-chromosomes, only males carried B-chromosomes (Rocon-Stange and Almeida-Toledo, 1993). Unfortunately, the sex ratio of this population is not known. The results cited above permit to suggest an alternative hypothesis to explain the excessive occurrence of B-chromosomes in one sex, that is the B-chromosomes could be related with the production of some directional effect in the gonadal development.

The application of multiple census method showed that during the period analyzed, about 212 fishes inhabited the first stretch of the Cascatinha stream (the 95% confidence limits on estimated population size were 99 and 983); about 650 fishes inhabited the second stretch (the 95% confidence limits on estimated population size were 339 and 1.420), and about 107 fishes inhabited the third stretch (the 95% confidence limits on estimated population size were 67 and 191). No one fish was found out of its original place of capture, suggesting that during that period of the year there was no movement of fishes between these stretches of the stream. However, considering that the captures were performed in a short period of time, it is not possible to know if some movement occurs in other periods of the year. Although in the literature no information could be found about population density of *A. scabripinnis*, the data obtained showed that the presence of a larger fish number in the first and second stretches are in accordance with observations of Gomes and Azevedo (1960) that this species is best adapted to the headwaters of streams or small rivers.

Among 35 specimens karyotyped in the first stretch, 23 had B-chromosomes; in the second stretch B-chromosomes were found in two among 20 karyotyped animals; and in the third stretch B-chromosomes were found in one among 10 karyotyped specimens. All frequencies calculated were accepted as representatives of the real frequency of B chromosomes in each population, but the small sample sizes in the second and third stretches could result in sampling errors. Similar results were obtained by Néó and Moreira-Filho (1995) in a study performed in *Astyanax scabripinnis* from the Ribeirão Grande stream where the frequency of B chromosomes ranged from 65.8% in a stretch located 1920 m above sea level to zero in a stretch located 700 m above sea level.

A comparative analysis of B-chromosome frequency and populational density data shows that these two parameters are not directly related; thus the frequency of B-chromosomes in fish of the second and third stretch is identical, in spite of the fact that these populations present a very different number of individuals. The high frequency of B-chromosomes found in fish from the first stretch of the Cascatinha stream could be maintained by genetic drift or, alternatively, be related to an adaptive effect conferred by the presence of B-chromosomes and specific ecological characteristics found in this habitat.

Experiments conducted with rye showed that under conditions of increasing stress and mortality, selection favors plants without Bs (Rees and Hutchinson, 1973). Considering the observations of

Gomes and Azevedo (1960) that *A. scabripinnis* has a limited distribution due to their specific environmental requirements, and the population data obtained in the present investigation, we suggest that local populations in the second and third stretches of Cascatinha stream may be suffering a selective pressure against fish with B-chromosomes. Alternatively, B-chromosomes found in *A. s. paranae* could confer a selective advantage to fishes living in the first stretch, with no advantage for fish from the other stretches.

ACKNOWLEDGMENTS

The authors are grateful to PhD. Francisco Langeani Filho for the identification of the specimens and to Mr. Renato Devidé for technical assistance. Funds supporting this study were provided by FUNDUNESP, CNPq, FAPESP and CAUNESP. Publication supported by FAPESP.

RESUMO

Um estudo comparativo foi realizado em exemplares de *Astyanax scabripinnis paranae*, envolvendo a frequência de indivíduos portadores de cromossomos B e a densidade populacional desta espécie em três trechos consecutivos do córrego da Cascatinha (Botucatu, SP). No primeiro trecho, a população foi estimada em cerca de 212 indivíduos e, entre os 35 espécimes cariotipados, 23 (65,7%) apresentaram um macrocromossomo B; no segundo trecho, a população foi estimada em cerca de 650 indivíduos e, entre os 20 espécimes cariotipados, 2 (10%) apresentaram um macrocromossomo B; no terceiro trecho, a população foi estimada em cerca de 107 indivíduos e, entre os 10 espécimes cariotipados, 1 (10%) apresentou um macrocromossomo B. Uma diferença significativa foi observada na frequência do cromossomo B presente nas fêmeas (57,1%) em relação aos machos (8,7%) ($P = 0,0001$). Esses dados sugerem que a frequência de cromossomos B e a densidade populacional não estão diretamente relacionadas. A hipótese de ocorrência de algum efeito adaptativo conferido pelos cromossomos B aos peixes do primeiro trecho do córrego da Cascatinha é apresentada e discutida.

REFERENCES

- Barbieri, G.** (1992). Biologia de *Astyanax scabripinnis paranae* (Characiformes, Characidae) do Ribeirão do Fazzari, São Carlos, Estado de São Paulo. I. Estrutura Populacional e Crescimento. *Rev. Bras. Biol.* 52: 579-588.
- Beuckeboom, L.M.** (1994). Bewildering Bs: an impression of the 1st B-chromosome conference. *Heredity* 73: 328-336.
- Foresti, F., Oliveira, C. and Almeida-Toledo, L.F.** (1993). A method for chromosome preparations from large fish specimens using *in vitro* short-term treatment with colchicine. *Experientia* 49: 810-813.
- Fowler, H.W.** (1948). Os peixes de água-doce do Brasil. *Arq. Zool.* 6: 1-204.
- Gomes, A.L. and Azevedo, P.** (1960). Os peixes de Monte Alegre do Sul, Estado de São Paulo. *Papéis Avulsos do Departamento de Zoologia, IB-USP* 14: 133-151.
- Krebs, C.J.** (1989). *Ecological Methodology*. Harper Collins Publishers Inc., New York, NY.
- Lande, R.** (1985). The fixation of chromosomal rearrangements in a subdivided population with local extinction and colonization. *Heredity* 54: 323-332.
- Maistro, E.L.** (1991). Caracterização citogenética e morfológica de populações de *Astyanax scabripinnis paranae* (Pisces, Characidae) das bacias dos rios Tietê e Parapanema. Master's thesis, UNESP, Botucatu, SP.
- Maistro, E.L., Foresti, F., Oliveira, C. and Almeida-Toledo, L.F.** (1992). Occurrence of macro B-chromosomes in *Astyanax scabripinnis paranae* (Pisces, Characiformes, Characidae). *Genética* 87: 101-106.
- Maistro, E.L., Foresti, F. and Oliveira, C.** (1994). New occurrence of a macro B-chromosome in *Astyanax scabripinnis paranae* (Pisces, Characiformes, Characidae). *Rev. Bras. Genet.* 17: 153-156.
- Migozuchi, S.M.H.M. and Santos, I.C.M.** (1995). Citogenética de *Astyanax scabripinnis* das bacias do rio Ivaí e Pirapó. *Anais do 41º Congresso Nacional de Genética*, pp. 452.
- Moreira-Filho, O. and Bertollo, L.A.C.** (1991). *Astyanax scabripinnis* (Pisces, Characidae): a species complex. *Rev. Bras. Genet.* 14: 331-357.
- Néo, D.M. and Moreira-Filho, O.** (1995). Diferenças morfológicas dos cromossomos B em *Astyanax scabripinnis* (Pisces, Characidae). *Anais do 41º Congresso Nacional de Genética*, pp. 452.
- Rees, H. and Hutchinson, J.** (1973). Nuclear DNA variation due to B-chromosomes. *Cold Spring Harb. Symp. Quant. Biol.* 38: 175-182.
- Rocon-Stange, E.A.R. and Almeida-Toledo, L.F.** (1993). Supernumerary B-chromosomes restricted to males in *Astyanax scabripinnis* (Pisces, Characiformes). *Rev. Bras. Genet.* 16: 601-615.
- Salvador, L.B. and Moreira-Filho, O.** (1992). B-chromosomes in *Astyanax scabripinnis* (Pisces, Characidae). *Heredity* 69: 50-56.
- Souza, I.L., Moreira-Filho, O. and Bertollo, L.A.C.** (1995). Cytogenetic diversity in the *Astyanax scabripinnis* (Pisces, Characidae) complex. II. Different cytotypes living in sympatry. *Cytologia* 60: 273-281.
- Vicente, V.E.** (1994). Estudo do cromossomo B em três populações de *Astyanax scabripinnis* (Pisces, Characidae) Master's thesis, UFSCAR, São Carlos, SP.
- Vicente, V.E., Moreira-Filho, O. and Camacho, J.P.M.** (1996). Sex-ratio distortion associated with the presence of a B chromosome in *Astyanax scabripinnis* (Teleostei, Characidae). *Cytogenet. Cell Genet.* 74: 70-75.
- Zar, J.H.** (1984). *Biostatistical Analysis*. 2nd edn. Prentice-Hall Inc., Englewood Cliffs.

(Received July 26, 1996)