

POPULATIONAL STUDY OF CHROMOSOME POLYMORPHISM IN *Zonotrichia capensis* (EMBERIZIDAE, PASSERIFORMES, AVES)

Edislane Barreiros de Souza and Edmundo José de Lucca

ABSTRACT

The karyotypes of 328 specimens of *Zonotrichia capensis* from four regions, three in the state of Paraná and one in the state of São Paulo (Brazil), were studied. This species presents a chromosomal polymorphism due to pericentric inversion, involving the 3rd and 5th chromosome pairs. Chromosome 3 exists in the 3st and 3sm conditions and chromosome 5, in 5st and 5^m conditions. Mitotic metaphase spreads were obtained from bone-marrow material of colchicine-treated animals.

At all collection sites the frequency of chromosome 5^m was considerably lower than that of the inverted chromosome 3sm.

The present work tried to establish a correlation between the frequencies of the 3rd and 5th chromosome pairs with the climatical and geographical parameters at the collecting regions.

Data show that the frequency of chromosome 5^m is higher in regions with low temperature and high altitude.

INTRODUCTION

The majority of chromosome rearrangements remain in the populations as polymorphisms, without development of reproductive isolation. The polymorphic variations are widely spread in animal populations and are adaptative in most cases.

Chromosome polymorphisms due to pericentric inversions have been identified among several bird species (Thornycroft, 1966, 1975; Hammar, 1970; Shields, 1973; Hammar and Herlin, 1975; Ansari and Kaul, 1979; Bass, 1979; Ray-Chaudhuri, 1976).

Thornycroft (1966) described the occurrence of chromosome polymorphism in *Zonotrichia albicollis*, involving the chromosomes of the pairs 2 and 3, both submetacentric, that through pericentric inversion originated a metacentric and an acrocentric chromosome, respectively. In 1975, Thornycroft found a correlation between the chromosome polymorphism and the lateral head plumage color.

In *Z. capensis*, Lucca and Rocha (1985) observed the presence of a chromosome polymorphism due to pericentric inversion involving pairs 3 and 5, both subtelocentric that, in this way, originated the chromosomes 3, submetacentric, and 5, metacentric. Handford (1983) analyzed the skeletal characteristics of 1,554 specimens of *Z. capensis* from 450 museum collections throughout South America. He found that birds in the Tropical zone had beaks of larger size and volume than those from Patagônia. Animals with longer wings are found at high latitudes and altitudes in temperate climates. However, up to now, no studies have been carried out thus far on *Zonotrichia capensis* to correlate phenotypic alterations with chromosome polymorphism.

Despite the fact that several reports of naturally occurring inversion polymorphisms in birds are on record, only a few are well documented (Shields, 1973, Thornycroft, 1975).

Our major concerns here are: a) a description of chromosomal polymorphism in the species *Zonotrichia capensis* in different regions and b) to establish a correlation between different cytotypes and geographic and climatic variations.

MATERIAL AND METHODS

The chromosomal complements of 328 specimens of the species *Zonotrichia capensis* (Emberizidae, Passeriformes, Aves), commonly known as the crown sparrow, from four different regions of Brazil, were analyzed.

In the state of Paraná, specimens were collected in the following regions: Marilândia do Sul, Cadeado Mountain (100), Londrina (100) and Paiquerê (28). One hundred specimens were collected in Botucatu, state of São Paulo.

Mitotic metaphase spreads were obtained from bone marrow material using hypotonic and colchicine pretreatment, according to the method of Lee and Elder (1980) with some modifications. Cells were stained with 5% Giemsa in phosphate buffer, pH 6.8, and analysed with a photomicroscope. The χ^2 method of Pearson was used for the statistical analysis. As this method is based on a continuous distribution, the correction factor of Yates was applied (Stansfield, 1985).

RESULTS

The presence of polymorphism in two different pairs of chromosomes makes of nine different chromosome arrangements possible. Among the 328 specimens analysed in the four different regions eight karyotypes were identified (Table I). No animal was detected carrying the $3^{st} 3^{st} 5^{sm} 5^{sm}$ cytotype (Figure 1).

Table I - Number of specimens analysed of the species *Zonotrichia capensis* and different chromosome arrangements observed in four different regions, one in the state of São Paulo (SP) and three in the state of Paraná (PR).

Region	Botucatu-SP		Marilândia do Sul-PR		Londrina-PR		Paiquerê-PR		Total
Chromosome arrangement	Male	Female	Male	Female	Male	Female	Male	Female	
$3^{st} 3^{st} 5^{st} 5^{st}$	13	04	08	06	25	06	09	-	71
$3^{st} 3^{st} 5^{st} 5^{sm}$	07	01	09	02	01	-	01	-	21
$3^{st} 3^{st} 5^{sm} 5^{sm}$	-	-	-	-	-	-	-	-	-
$3^{st} 3^{sm} 5^{st} 5^{st}$	24	03	25	10	29	08	14	-	113
$3^{st} 3^{sm} 5^{st} 5^{sm}$	03	01	13	01	07	02	01	-	28
$3^{st} 3^{sm} 5^{sm} 5^{sm}$	02	02	-	-	-	-	01	-	05
$3^{sm} 3^{sm} 5^{st} 5^{st}$	25	10	15	05	14	07	-	-	76
$3^{sm} 3^{sm} 5^{st} 5^{sm}$	04	01	05	01	01	-	01	-	13
$3^{sm} 3^{sm} 5^{sm} 5^{sm}$	-	-	-	-	-	-	01	-	01
Sub Total	78	22	75	25	77	23	28	-	328
Total	100		100		100		28		328

Table II shows the frequencies of chromosomes 3^{st} , 3^{sm} , 5^{st} and 5^{sm} for the animals as a whole. The expected frequencies for the nine possible rearrangements were calculated from the chromosome frequencies (Tables III and IV).

In Marilândia do Sul, Londrina and Paiquerê the expected values for the 3^{rd} chromosome pair arrangements were not different from those observed ($p > 0.10$; $\alpha = 0.05$). In Botucatu, the expected values differ significantly from the observed ones ($0.001 > p > 0.01$; $\alpha = 0.05$) (Table III). The expected values for the 5^{th} chromosome

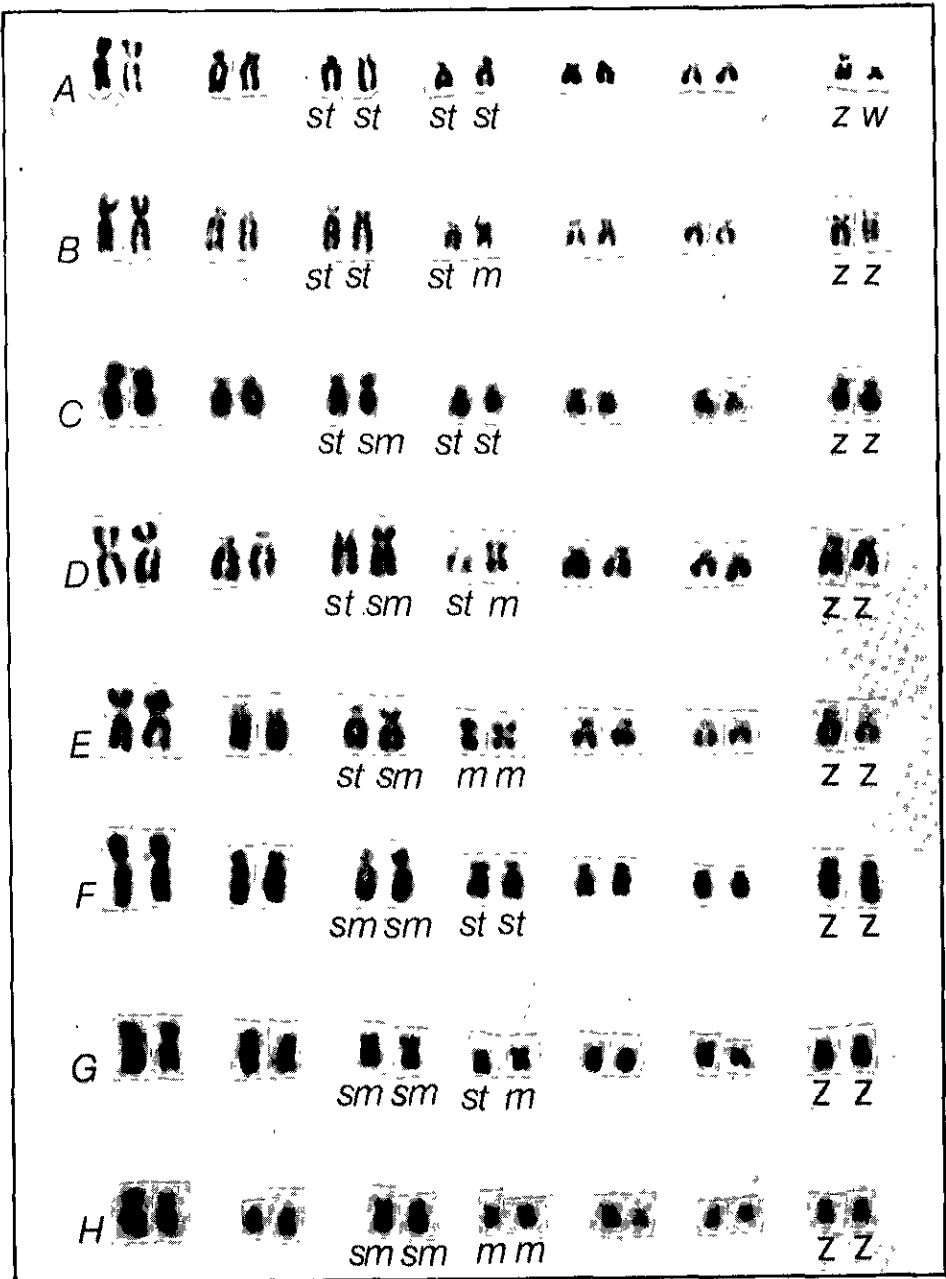


Figure 1 - Eight different cytotypes observed in male and female specimens of *Zonotrichia capensis*. (st = subtelocentric; sm = submetacentric; m = metacentric).

pair arrangements differ significantly from the observed values in Marilândia do Sul and Paiquerê ($p < 0.05$; $\alpha = 0.05$). Otherwise, in Botucatu and Londrina, in no case did the expected values differ significantly from the observed values ($p > 0.05$; $\alpha = 0.05$) (Tables IV).

Table II - Frequencies of chromosomes 3st, 3sm, 5st and 5^m among 328 specimens of the specie *Zonotrichia capensis* from four regions.

Region Chromosome	Botucatu-SP	Marilândia do Sul-PR	Londrina-PR	Paiquerê-PR
	*n = 100	*n = 100	*n = 100	*n = 100
3 st	0,425	0,495	0,550	0,643
3 sm	0,575	0,505	0,450	0,357
5 st	0,875	0,845	0,945	0,875
5 ^m	0,125	0,155	0,055	0,125

*n = number of animals captured.

DISCUSSION

In the family Emberizidae, reports of chromosome polymorphism due to pericentric inversion are frequent. Chromosomes 3 and 5 in *Z. capensis* (Lucca and Rocha, 1985) and chromosomes 2 and 3 in *Z. albicollis* (Thornycroft, 1966) are involved. In *Junco*, chromosomes 2 and 5 in five different species (Shields, 1973) and chromosome 5, in *Cardinalis cardinalis* (Bass, 1979) are involved. According to Bulatova (1981), the various genera in the family are considerably similar in karyotype and the few differences observed in chromosome morphology are due to rearrangements of the pericentric inversion type. The chromosome inversions that involve chromosomes 2 (or 3) and/or 5, in *Zonotrichia*, *Junco* and *Cardinalis* seem to involve the same chromosomes and confers to them the same morphology. However, at present, there are not sufficient data to conclude that the inversions observed in the three genera are due to the presence of the same inversion in a common ancestor or, alternatively, that, they occurred repeatedly in each genus. According to Challey (1983) ancestral forms of *Z. capensis* were already present in South America at least seventy million years ago. If we consider that the inversions present in the genera

Table III - Frequencies for the three chromosome arrangements of the 3rd chromosome pair among 328 specimens of the species *Zonotrichia capensis* from São Paulo and Paraná States (Brazil).

Chromosome arrangement	Botucatu-SP			Marilândia do Sul-PR			Londrina-PR			Paiquerê-PR		
	Frequency	χ^2 (*)		Frequency	χ^2 (*)		Frequency	χ^2 (*)		Frequency	χ^2 (*)	
	obs.	exp.		obs.	exp.		obs.	exp.		obs.	exp.	
3 st 3 rd	25	18.063	2.294	25	24.502	0.01 x 10 ⁻⁶	32	30.250	0.052	10	11.576	0.372
3 st 3 sm	35	48.875	4.228	49	49.995	0.004	46	49.500	0.323	16	12.855	0.544
3 sm 3 sm	40	33.062	1.254	26	25.503	0.3 x 10 ⁻⁶	22	20.250	0.077	02	3.568	1.199
Total	100	100.000	7.776 (**)	100	100.000	0.004	100	100.000	0.452	28	28.000	2.115

(*) Applied the correction factor of Yates applied.

(**) 0.001 > p > 0.01

Table IV - Frequencies for the three chromosome arrangements of the 5th chromosome pair among 328 specimens of the species *Zonotrichia capensis* from São Paulo (SP) and Paraná (PR) States (Brazil).

Chromosome arrangement	Botucatu-SP			Marilândia do Sul-PR			Londrina-PR			Paiquerê-PR		
	Frequency obs.	exp.	χ^2 (*)	Frequency obs.	exp.	χ^2 (*)	Frequency obs.	exp.	χ^2 (*)	Frequency obs.	exp.	χ^2 (*)
5 st 5 st	79	76.563	0.049	69	71.403	0.118	89	89.303	0.007	23	12.438	0.053
5 st 5 ^m	17	21.875	1.321	31	26.195	0.707	11	10.395	0.001	03	6.125	2.145
5 ^m 5 ^m	04	1.562	2.404	-	2.402	3.506	-	0.302	2.129	02	0.437	2.586
Total	100	100.000	3.774	100	100.000	4.331 (**)	100	100.000	2.137	28	28.000	4.784 (**)

(*) Applied the corrective factor of Yates.

(**) $0.01 > p > 0.05$.

Zonotrichia, *Junco* and *Cardinalis* are due to their presence in a common ancestor, there was sufficient time for mutations peculiar to each one. Therefore it would not be surprising if the correlations between phenotype and cytotype in one genus or species did not correspond to those in the different species.

Few studies have been carried out thus far to correlate phenotypic, biochemical or behavioral traits with chromosome polymorphism.

In *Z. albicollis*, birds with an inversion on the 2nd pair show changes in plumage color, skeletal size and song pattern and preferentially mate with birds of the opposite phenotype (Thornecroft, 1975).

Rising and Shields (1980) correlated 24 skeletal traits of *Z. albicollis* and *Junco hyemalis* with chromosomal polymorphism existing in these species. They noticed that in *Z. albicollis*, males bearing chromosome 2 in the inverted form are larger than males not bearing such an inversion. In *J. hyemalis*, no correlation was observed between skeletal size and chromosome polymorphism. In both species, however, there was a correlation between beak dimensions and feather and tail length and the different cytotypes.

No studies have been carried out thus far on *Zonotrichia capensis* to correlate phenotypic alterations with chromosome polymorphism.

Rocha (1987) analysed the karyotype of 60 specimens of *Z. capensis* from Botucatu, state of São Paulo, Brazil, and detected only 8 specimens carrying the 3st 3st 5st 5st combination. Of the 52 animals bearing the inversion, those with a karyotype heterozygous for one or both pairs were more frequent (63.5%) than those with a homozygous karyotype (36.5%). Considering all the specimens, however, heterozygotes were only 55% of the population. Of the 328 *Z. capensis* specimens investigated here, 21.65% did not show an inversion involving the 3rd and/or 5th pairs. Of the 257 specimens bearing the inversion, those with a karyotype heterozygous for one or both pairs (Figure 1) were more frequent (70.04%) than those with a homozygous karyotype (29.96%).

Of the 100 *Z. capensis* specimens investigated here, in the region of Botucatu, 48% had a karyotype heterozygous for one or both pairs (Table I). Rocha (1987) detected 55% heterozygotes but, considering all 328 specimens, 54.9% were heterozygotes (Figure 1 and Table I).

In the genus *Zonotrichia*, in which chromosomal polymorphism is present, the species are distributed throughout the American continent, from the Arctic to the Antarctic. *Z. capensis* occurs from Mexico to the southern most part of Chile and Argentina and subspecies occupy regions that vary in altitude from sea level to approximately 4500 m.

The 328 specimens of *Z. capensis* were collected between 1986 and 1988 and from the chromosome frequencies it was possible to calculate the expected frequen-

cies for the possible chromosome arrangements of the 3rd or 5th pairs in every year and compare them with climatic conditions of each region.

In the region of Botucatu, state of São Paulo, a significant difference was detected only in the frequency of the arrangements of the 3rd pair. No significant difference was detected, for the same arrangements, in the other regions (Table III). Otherwise, in Marilândia do Sul and Paiquerê, state of Paraná, a significant difference was detected only in the frequency of the arrangements of the 5th pair (Table IV). No significant difference was observed for the arrangements of the 3rd and 5th pairs of the 100 specimens from Londrina, state of Paraná (Table III and IV). The maintenance of different karyotypes in the population suggests that these may confer different adaptative values and may therefore represent different responses of the organism to environmental conditions.

The city of Botucatu, state of São Paulo, with 786 meters of altitude, a humid sub tropical climate, with dry winters (Cfa) showed variations in the rainfall indicator between 1986 and 1987. There was an increase of the precipitation. During this period a significant difference was detected only in the frequency of the arrangements of the 3rd chromosome pair.

Marilândia do Sul, state of Paraná, is 1020 m above sea level, with a humid subtropical climate and cold winters (Cfb). As pointed out, a significant difference was detected in this region only in the frequency of the arrangements of the 5th pair.

Londrina, state of Paraná, is 586 m above sea level, with a humid subtropical climate and dry winters (Cfa). There was an increase in precipitation from 1986 to 1987. No significant difference was observed for the arrangements of the 3rd and 5th chromosome pairs, despite the fact that a significant difference for the arrangements of the 5th chromosome pair was observed in 1986.

In Paiquerê, state of Paraná, 30 Km from Londrina, a significant difference was detected only in the frequency of the arrangements of the 5th pair, in 28 specimens analysed.

Out of the eight cytotypes found in the 328 specimens of *Z. capensis* the 3st 3sm 5st 5st combination was the most frequent (34.45%) and the 3sm 3sm 5^m 5^m the least frequent (0.31%). In the region of Londrina only 1% showed a 3st 3st 5st 5^m combination. The same cytotype appeared at a frequency of 3.6% in Paiquerê and 8.2% in Botucatu. The 3sm 3sm 5st 5^m combination showed the following frequencies: 1/100 in Londrina, 1/28 in Paiquerê, 6/100 in Marilândia do Sul and 5/100 in Botucatu (Table I and Figure 2). The cytotype 3st 3sm 5st 5^m showed a low frequency in Botucatu, around 4%, and the cytotype 3st 3sm 5^m 5^m in Paiquerê represented 3.6%. The cytotype 3st 3st 5st 5st and 3st 3sm 5st 5st appeared more frequently in all regions (Table I and Figure 2).

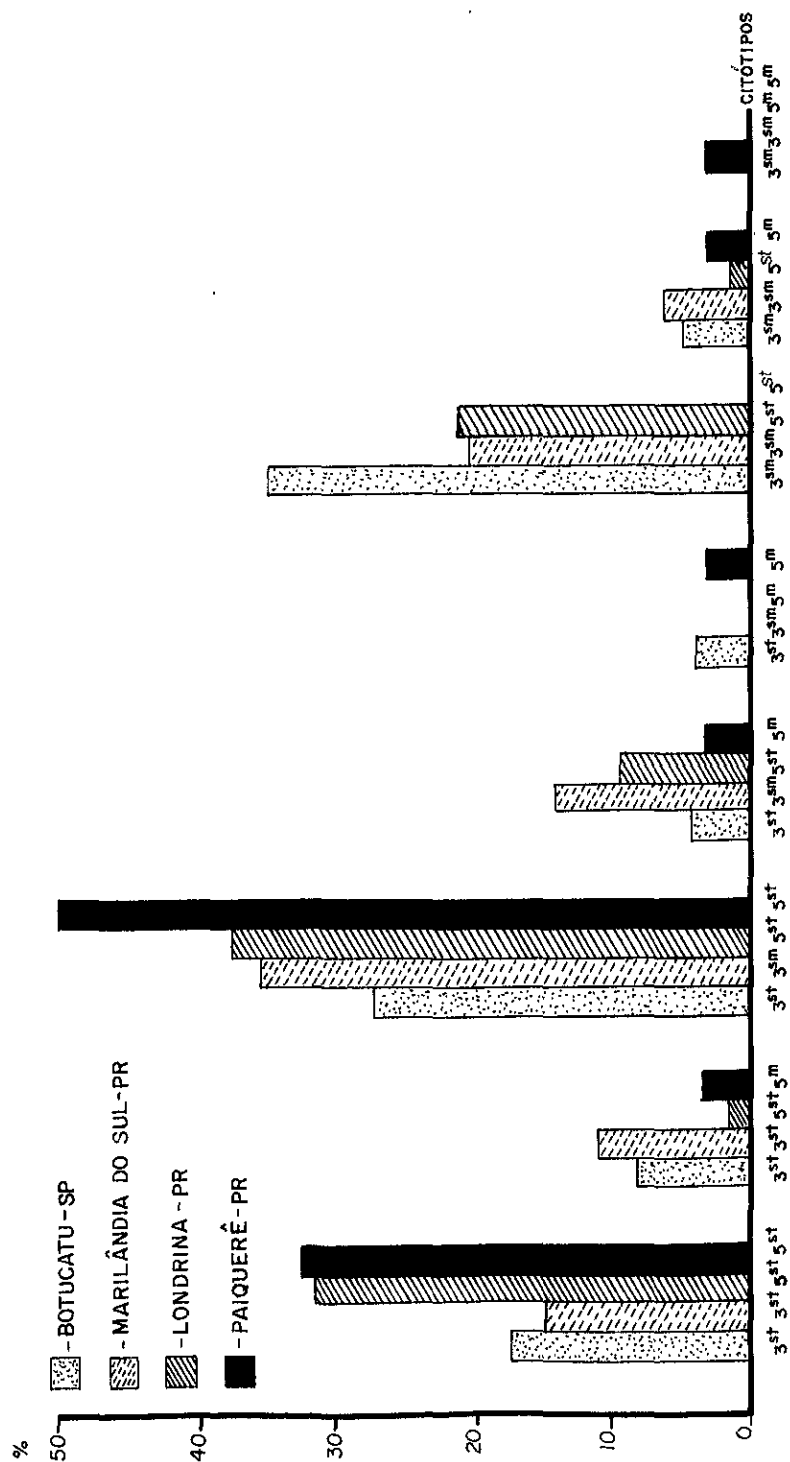


Figure 2 - Frequency of the eight different cytotypes found in the species *Zonotrichia capensis* from São Paulo and Paraná states (Brazil).

Seven cytotypes were identified in the 60 animals analysed by Rocha (1987). The most frequent one was $3^{sm} 3^{sm} 5^{st} 5^{st}$ (32%) and the least frequent was $3^{st} 3^{sm} 5^{m} 5^{m}$ (5%).

Recent studies by Carvalho and Erdtmann (1987) and Carvalho (1989) have indicated that in the state of Rio Grande do Sul the most frequent cytotype was $3^{sm} 3^{sm} 5^{st} 5^{m}$ (38.2%). The same cytotype was detected only in 6% of the 328 specimens analysed in the present study. Another cytotype very frequent in Rio Grande do Sul was $3^{sm} 3^{sm} 5^{m} 5^{m}$ (29.9%) and it was observed in only one specimen with the same cytotype in the present study (Figure 3). The cytotype $3^{st} 3^{st} 5^{m} 5^{m}$ (Figure 3) was the least frequent (3.6%) found by Carvalho and Erdtmann (1987) and Carvalho (1989). In the present study no animal carrying this cytotype was detected.

With respect to the frequency of the chromosomes involved in the inversion, it was observed that chromosome 5^m was found to be considerably less frequent than chromosome 3^{sm} (Table II). The difference observed between the frequencies of these chromosomes may possibly be due to the "founder effect", as stated by Rocha (1987). Carvalho and Erdtmann (1987) and Carvalho (1989) have indicated that chromosome 5^m has a frequency of more than 55% in the southern regions of Brazil.

In the present study, the frequency of chromosome 3^{sm} in Marilândia do Sul, Londrina and Paiquerê (state of Paraná) was slightly lower than that observed in Botucatu. The same was not true for the 5^m chromosome: the frequency of this chromosome in Marilândia do Sul was slightly higher than that observed in Botucatu (state of São Paulo), Londrina and Paiquerê (state of Paraná).

Carvalho and Erdtmann (1987) and Carvalho (1989) stated that there is a positive correlation between an increase in frequency of chromosome 5^m and high latitudes. In Marilândia do Sul (annual average temperature of 18°C and 1020 m above sea level), the frequency of chromosome 5^m was higher than that observed in the other regions. It appears that there is a positive correlation between the increased frequency of chromosome 5^m and low temperatures. Animals with 5^m chromosomes are more frequent at high latitudes and altitudes. Handford (1983), who was unaware of the chromosome polymorphism of this species, analysed the skeletal characteristics of 1,554 specimens of *Z. capensis* from 450 museum collections throughout South America and found that beaks of larger size and volume are preferentially detected in the Tropical zone, while the smallest beaks are detected in the region of Patagonia. Animals with longer wings are found at high latitudes and altitudes in temperate climates. Other skeletal characteristics showed latitudinal clines. It would be highly informative to correlate phenotypic alterations with chromosome polymorphism in *Z. capensis*, particularly the ones involving chromosome 5^m .

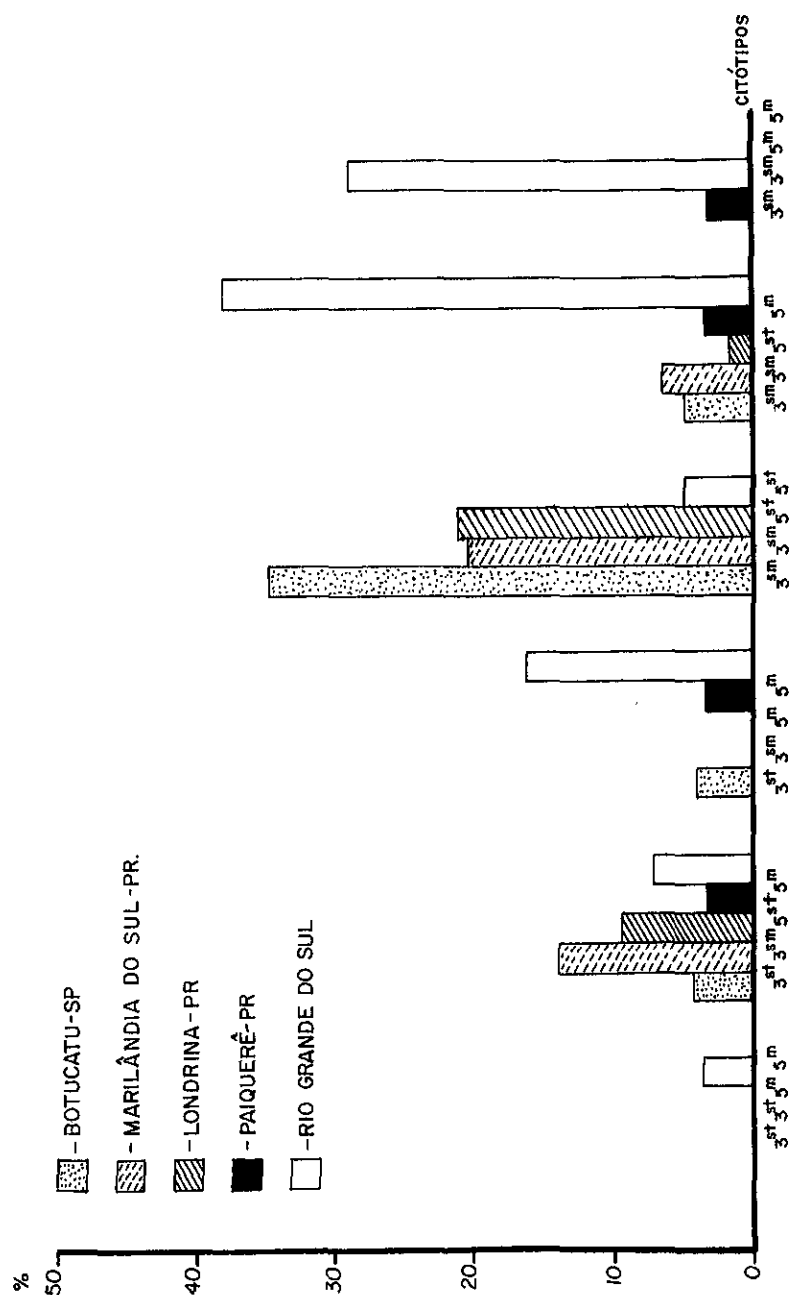


Figure 3 - Comparative frequency of six different cytotypes found in the species *Zonotrichia capensis* from São Paulo, Paraná and Rio Grande do Sul States (Brazil).

A clear picture of the significance of this polymorphism in *Z. capensis* will emerge only after a thorough investigation, involving an extensive inter and intra-population biochemical, behavioral, morphologic and karyotypic data, including banding patterns.

ACKNOWLEDGMENTS

The authors are indebted to Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Grant 405375/87.5).

Publication supported by FAPESP.

RESUMO

No presente trabalho foram analisados os cariótipos de 328 exemplares de *Zonotrichia capensis*, provenientes de quatro regiões, sendo três do estado do Paraná e uma do estado de São Paulo. Esta espécie apresenta um polimorfismo cromossômico por inversão pericêntrica, envolvendo o 3º e o 5º pares cromossômicos. O cromossomo 3 existe na condição 3st e 3sm e o cromossomo 5, na forma 5st e 5^m. As preparações mitóticas foram obtidas a partir da suspensão de células da medula óssea dos ossos longos de animais pré-colchicinizados.

Nas regiões onde foram realizadas as coletas, o cromossomo invertido do 5º par é consideravelmente menos freqüente que o cromossomo invertido do 3º par.

O presente trabalho procurou estabelecer uma correlação das freqüências dos cromossomos do 3º e 5º pares, com parâmetros climáticos e geográficos nas regiões de coleta.

Nossos dados mostram que a freqüência do cromossomo 5^m é mais alta nas regiões cuja temperatura é baixa e de maior altitude.

REFERENCES

- Ansari, H.A. and Kaul, D. (1979). Inversion polymorphism in common pigeon, *Theron phoenicoptera* (Latham) (Aves). *J. Genet.* 54: 197-202.
- Bass, R. (1979). Chromosomal polymorphism in *Cardinalis cardinalis*. *Can. J. Genet. Cytol.* 21: 549-553.
- Bulatova, N.S. (1981). A comparative karyological study of passerine birds. *Acta Sc. Nat. Brno.* 15: 1-44.
- Carvalho, M.U.P. (1989). Estudos citogenéticos na família Fringillidae (Passeriformes, Aves). Masters Thesis. Universidade Federal do Rio Grande do Sul.
- Carvalho, M.U.P. and Erdtmann, B. (1987). Ocorrência de polimorfismo cromossômico em tico-tico (*Zonotrichia capensis*) nos estados de Espírito Santos e Rio Grande do Sul. *Resumos da 39ª Reunião Anual da SBPC*, Brasília.
- Challey, L.C. (1983). La Biogeografía desde mi punto de vista. *Atas del congres. Latinoamericano Zool.* 1: XVI 784p, Caracas.

- Hammar, B. (1970). The karyotypes of thirty-one birds. *Hereditas* 65: 29-58.
- Hammar, B. and Herlin, B. (1975). Karyotypes of four birds species of the order Passeriformes. *Hereditas* 80: 177-184.
- Handford, P. (1983). Continental patterns of morphological variation in a South American Sparrow. *Evolution* 37: 920-930.
- Lee, M.R. and Elder, F.F. (1980). Yeast stimulation of bone marrow mitosis for cytogenetic investigations. *Cytogen. Cell Genet.* 26: 36-40.
- Lucca, E.J. de and Rocha, G.T. (1985). Chromosomal polymorphism in *Zonotrichia capensis* (Passeriformes, Aves). *Rev. Brasil. Genet.* VIII: 71-78.
- Ray-Chaudhuri, R. (1976). Cytotaxonomy and chromosome evolution in Passeriformes (Aves). *Z. Zool. Syst. Evol.* 14: 299-320.
- Rising, J.D. and Shields, G.F. (1980). Chromosomal and morphological correlates in two new world sparrows (Emberizidae). *Evolution* 34: 654-662.
- Rocha, G.T. (1987). Estudo do complemento cromossômico e da região organizadora de nucléolo em algumas espécies de Aves. Masters Thesis, UNESP, Botucatu.
- Shields, G.F. (1973). Chromosomal polymorphism in common to several species of *Junco* (Aves). *Can. J. Genet. Cytol.* 15: 461-471.
- Stansfield, W.D. (1985). *Genetica*. MacGraw-Hill do Brasil Ltda., 515 p.
- Thornycroft, H.B. (1966). Chromosomal polymorphism in the white-throated Sparrow, *Zonotrichia albicollis*. *Science* 154: 1571-1572.
- Thornycroft, H.B. (1975). A cytogenetic study of the white-throated Sparrow. *Zonotrichia albicollis*. *Evolution* 29: 611-621.

(Received December 5, 1989)