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Structural chromosomal alterations in *Hyla albopunctata* and *Aplastodiscus perviridis* (Anura, Hylidae) from the Morro do Ferro region of Minas Gerais

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ABSTRACT

Metaphase chromosomes were analyzed in *Aplastodiscus perviridis* from the locations: Morro do Ferro (MG), Serra da Bocaina (SP), Botucatu (SP), and in *Hyla albopunctata* found at: Morro do Ferro, Botucatu, and Campinas (SP). A significant quantity of metaphases with structural alterations were found in the Morro do Ferro population. These alterations were classified as: decondensation, condensation, breaks, gaps, deletions and acentric fragments. Breaks, deletions and fragments were the most frequent alterations and a great number of these occurred near centromeric heterochromatin. The frequency of altered metaphases varied among individuals in all populations studied. The majority of the alterations can probably be attributed to the high level of natural radiation at Morro do Ferro.

INTRODUCTION

The Morro do Ferro, a large hill located near the city of Poços de Caldas in the State of Minas Gerais (MG), Brazil, has high levels of environmental gamma radiation due to the presence of abnormally high concentrations of thorium and traces of uranium in the local soil and rocks. At the foot of the southeastern slope, the plant and animal community is exposed to external gamma radiation levels that range from 0.1 to 3.2 mR/h (Roser and Cullen, 1962; Drew and Eisenbud, 1970). The maximum values obtained for ^{226}Ra and ^{210}Pb in some vegetables (beans, carrot, potatoes and corn) cultivated in farms around the Poços de Caldas Plateau was one order of magnitude greater than in regions with normal levels of radiation (Vasconcelos *et al*., 1986). A fern, *Adantun lorentzii*, growing on the Morro do Ferro was radioactive due to the absorption of ^{228}Ra from soil, a nuclide in the ^{232}Th decay chain (Eisenbud *et al*., 1984).

Induced ionizing radiation can produce genetic and chromosomic mutations. However, the effect of natural radiation in biological systems is not very well known. There has been no structural chromosomal analysis of the Anura population from that region. Twenty-seven species of Anura have been identified on Morro do Ferro (Cardoso *et al*., 1989).

MATERIAL AND METHODS

Metaphase chromosomes of 30 males, adult specimens of *A. perviridis* and 28 of *H. albopunctata*

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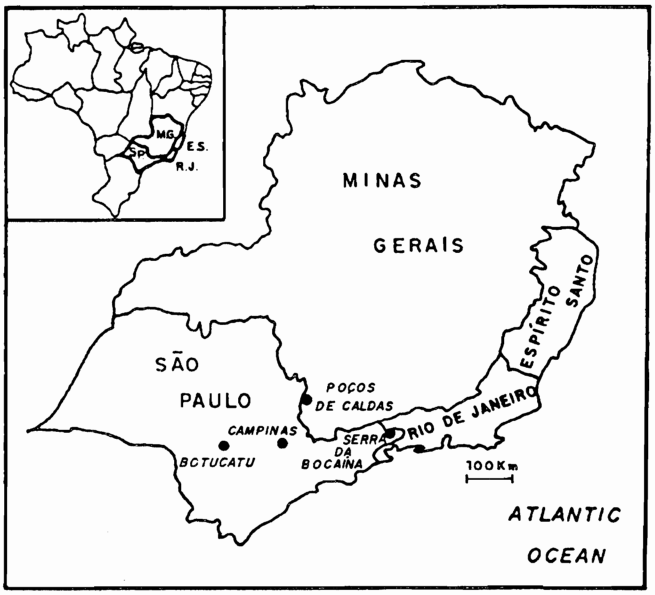
were analyzed. They were collected, from november to february, in the Morro do Ferro (MG) region and also in Botucatu, Serra da Bocaina and Campinas (all localities in São Paulo state), where the level of natural radiation is considered normal. These localities are shown in the map below.



Map indicating the localities where animals were collected.

All animals received an intraperitoneal injection of 1% colchicine (0.02 mg/g) 4 h before they were sacrificed. The metaphases were obtained by the following techniques: 1. Conventional squashing method (Ohno *et al.*, 1964) of intestine, spleen, oesophagus and testes fragments; 2. Intestinal epitelium cell suspension (King and Rofer, 1976); and 3. Bone marrow (Schmid, 1978). Slides were treated with 0.1 N HCl at 60^{o}C for 5-10 minutes, then stained in a 2% Giemsa solution pH 6.8 for 30 minutes. All metaphases of all slides of each animal were analyzed. *H. albopunctata* has a diploid complement of 2n=22 chromosomes (Beçak, 1968) and *A. perviridis*, 2n=24 (Feitosa *et al.*, unpublished results).

RESULTS

Chromosomal alterations appearing in *A. perviridis* and *H. albopunctata* were characterized as: deletions (Figures 1 and 6), gaps (Figures 1, 2 and 5), break (Figures 2 and 3), condensation (Figures 7-9), decondensation (Figures 3, 6, 7, 9 and 10) and acentric fragments (Figures 2 and 6). Breaks were considered to be chromosome sections that are longer than one chromatid width or fragments that are removed from the chromosomic axis. The gaps are pieces of chromosome smaller than one chromatid width (Brogger, 1974).

The numbers of alterations obtained for each type described above are shown in Table I. The sample from Morro do Ferro, in both species, presented a higher number of alterations than those from the other localities, Serra da Bocaina, Botucatu and Campinas. Deletions, fragments, breaks and condensations were the most frequent alterations. A great number of breaks and deletions occurred near the centromere (Table I). The samples from Campinas presented somewhat more alterations than those from the other control regions, but the difference was not statistically significant.

Differences in the frequency of altered metaphases among individuals were found in all populations studied (Tables II and III). All control samples maintained frequencies below 5% except for one animal (identification number: ZUEC 7578) from Campinas, whose frequency of altered metaphases was about 15%. Because of this specimen, the total frequency of altered metaphases in Campinas samples was higher than expected (Tables II and III).

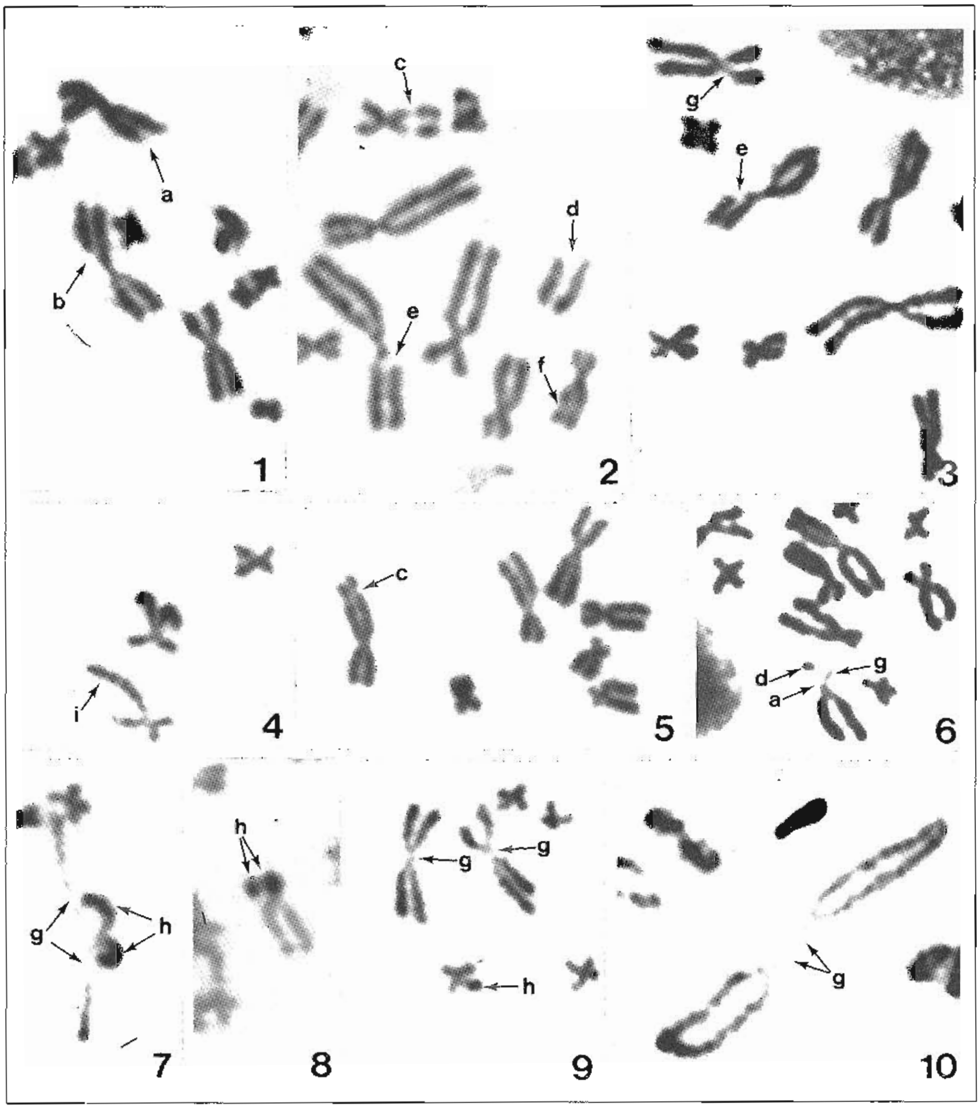
Structural alterations were not detected in meiotic chromosomes of either species, but mitotic metaphases of the testes with many alterations are included in the total number of metaphases analyzed (Tables II and III). Metaphases with two or more types of alterations also were found in all populations analyzed.

The results obtained for germinative and somatic altered mitotic metaphases for each species were statistically compared by variance analysis and by the Scheffé test (Table IV). The number of metaphases with one and two or more structural alterations were found to be highly significant (at the 1% level) in the specimens collected at Morro do Ferro, compared to the controls.

DISCUSSION

Somatic and germinative cells of *A. perviridis* and *H. albopunctata* from the radioactive area presented significantly more chromosome structural alterations than those from other regions, where the radiation level is considered to be normal.

We considered that at least part of the high number of chromosome alterations detected in the animals from Morro do Ferro is due to natural radiation, since it is known that high levels of ionizing radiation can promote chromosomic and genic mutations



Figures 1-10 - Mitotic chromosomes of *A. perviridis* (1, 3, 4 and 5) and *H. albopunctata* (2, 6, 7, 8, 9 and 10). Note: deletion (a), chromatidic and isochromatidic gaps (b, c), acentric fragments (d), chromatidic and isochromatidic breaks (e, f), decondensation (g), condensation (h) and deletions/adition (i). (1-3, 6, 8 and 10: 2200x; 4, 5, 7 and 9: 2600x).

Table I - Types and numbers of structural chromosomal alterations in *Aplastodiscus perviridis* and *Hyla albopunctata* from Morro do Ferro (MF), Serra da Bocaina (SB), Botucatu (BT) and Campinas (CP).

Species	Locality	N	Number of metaphases analyzed	Type of alterations									Total number of alterations
				B		G		DL	FR	DC	CD	D	
				C	IC	C	IC						
A. perviridis	MF	10	2367	20	11	6	4	140	123	0	57	31	392
	SB	10	2149	1	0	0	0	6	4	0	10	1	22
	BT	10	2649	0	0	1	0	0	13	0	0	0	14
H. albopunctata	MF	10	2201	32	1	0	1	170	53	1	79	24	361
	CP	09	2271	11	8	0	0	91	41	0	23	17	191
	BT	09	2736	1	0	0	0	22	1	0	12	0	36

N = number of individuals; B = breaks; G = gaps; C = condensation; IC = isochromatid-type aberrations; DL = deletions; FR = fragments; DC = dicentric chromosomes; CD = condensation; D = decondensation.

Table II - Chromosomal analysis of *Aplastodiscus perviridis* from Morro do Ferro (MF), Botucatu (BT) and Serra da Bocaina (SB).

Locality	ZUEC identification codes	Number of meiotic metaphases analyzed in testes	Number of mitotic metaphases analyzed: Total – In testes	Number of mitotic metaphases analyzed: Total – In other tissues	Number of mitotic metaphases analyzed: With alterations – In testes	Number of mitotic metaphases analyzed: With alterations – In other tissues	Total number of metaphases analyzed	Frequency of alterations (%)
MF	7535	149	20	122	15	15	291	10.3
	7536	111	43	60	6	10	214	7.5
	7537	65	56	50	4	7	171	6.4
	7544	63	30	92	10	18	185	15.1
	7545	41	64	50	6	28	155	21.9
	7547	47	48	56	4	8	151	7.9
	7548	68	86	25	12	9	179	11.7
	7549	105	175	20	11	2	300	4.3
	7550	40	50	274	10	35	364	12.4
	7579	93	169	95	25	10	357	9.8
	Total	782	741	844	103	142	2367	10.4
BT	7543	23	50	57	0	3	130	2.3
	7551	29	72	68	0	0	169	0.0
	7552	111	46	92	0	3	249	1.2
	7553	35	45	52	0	0	162	0.0
	7554	37	31	75	0	2	143	1.4
	7555	44	55	69	2	0	168	1.2
	7556	62	105	85	0	0	252	0.0
	7557	483	126	117	0	4	726	0.5
	7558	236	42	88	2	3	366	1.4
	7574	98	112	74	0	0	284	0.0
	Total	1158	714	777	4	15	2649	0.7
SB	7542	6	42	58	0	2	106	1.9
	7564	189	281	33	5	1	503	1.2
	7565	15	76	56	0	1	147	0.7
	7566	32	80	33	1	1	151	1.3
	7567	218	88	20	3	0	326	0.9
	7568	30	70	51	0	0	151	0.0
	7570	65	50	60	0	1	175	0.6
	7571	60	72	30	0	0	162	0.0
	7572	36	75	43	1	1	154	1.3
	7573	100	124	50	0	0	274	0.0
	Total	757	958	434	10	7	2149	0.8

ZUEC = Natural History Museum of the State University of Campinas. The animals used in this research were catalogued and preserved in the ZUEC collection.

(Savage, 1989). Up to now, the effect of natural radiation in that region has only been studied in meiotic chromosomes of scorpions, *Tityus bahiensis*, from Morro do Ferro (Takahashi, 1976) and in this case an increase in chromosome breaks and atypical chromosome pairing was detected.

Different frequencies of alterations among individuals can probably be related to genotypic differences inherent to each animal and/or to the differences in radiation exposure, since it is not possible to determine the exact ages of the animals, and consequently the total dose received before capture.

Table III - Chromosomal analysis of *Hyla albopunctata* from Morro do Ferro (MF), Botucatu (BT) and Campinas (CP).

Locality	ZUEC identification codes	Number of meiotic metaphases analyzed in testes	Number of mitotic metaphases analyzed				Total number of metaphases analyzed	Frequency of alterations (%)
			Total		With alterations			
			In testes	In other tissues	In testes	In other tissues		
MF	19.01*	48	64	87	11	11	199	11.0
	19.02*	67	70	51	15	4	188	10.1
	7546	112	57	86	15	20	255	13.7
	19.12*	194	144	37	28	3	375	8.3
	19.13*	66	56	64	10	12	186	11.8
	19.20*	112	98	22	8	9	232	7.3
	7580	84	42	72	3	14	198	8.6
	7581	49	44	77	5	32	170	21.8
	19.40*	54	118	35	8	7	207	7.2
	19.41*	56	50	85	11	5	191	8.4
	Total	842	743	616	114	117	2201	10.5
BT	7538	202	82	28	0	0	312	0.0
	7539	107	70	32	0	1	209	0.5
	7540	151	88	20	0	1	259	0.4
	7541	44	20	96	0	1	160	0.6
	19.09*	161	207	20	3	0	388	0.8
	19.10*	76	109	35	0	1	220	0.4
	7569	43	156	24	13	0	273	4.7
	19.31*	249	170	26	0	0	445	0.0
	19.32*	126	314	30	2	1	470	0.6
	Total	1209	1216	311	18	5	2736	0.9
CP	7559	165	143	16	9	0	324	2.8
	7560	207	130	29	0	1	366	0.3
	7561	74	97	33	5	0	204	2.4
	7562	35	88	52	6	1	175	4.0
	7563	121	120	15	10	1	256	4.3
	7575	86	92	24	0	0	202	0.0
	7576	175	46	66	7	0	287	2.4
	7577	96	90	49	0	1	235	0.4
	7578	90	48	84	0	33	222	14.9
	Total	1049	854	368	37	37	2271	3.3

ZUEC = Natural History Museum of the State University of Campinas.
* = No ZUEC numbers. These animals were discarded because they were used to obtain bone marrow.

However, since they are captured only during the reproductive season, and these amphibians reach reproductive maturity after 18 months, we can say that this is the shortest period of exposure for the animals we analyzed. Nevertheless, even with controlled experimental conditions and ages, individual variation can also be found. For example, it occurs in *Triatoma infestans* exposed to artificial radiation (Alvares-Garcia, 1988) and to starvation (Andrade and Mello, 1987). Therefore, a genotypic difference should be considered.

We also found that the frequency of altered metaphases was greater in the intestine and testis than in other organs analyzed. This was expected since cellular radiosensitivity appears to depend mainly on

Table IV - Variance analysis (F) for comparison of the number of altered mitotic metaphases of *Hyla albopunctata* and *Aplastodiscus perviridis* from different localities. MF = Morro do Ferro; SB = Serra da Bocaina; BT = Botucatu; CP = Campinas. (The data were normalized by Fisher's angular transformation = arc sen x).

Species	Tissues	Number of alterations per metaphases	$\overline{X} \pm S$				F
			MF	SB	BT	CP	
A. perviridis	other tissues	1	0.346 ± 0.133	0.072 ± 0.077	0.065 ± 0.072	---	26.73** > 5.49 = $F_{2.27}$ (0.99)
	others tissues	≥ 2	0.265 ± 0.101	0.031 ± 0.065	0.068 ± 0.072	---	24.09** > 5.49 = $F_{2.27}$ (0.99)
	testes	1	0.280 ± 0.123	0.027 ± 0.058	0.029 ± 0.061	---	28.80** > 5.49 = $F_{2.27}$ (0.99)
	testes	≥ 2	0.314 ± 0.183	0.039 ± 0.053	0.029 ± 0.061	---	19.43** > 5.49 = $F_{2.27}$ (0.99)
H. albopunctata	others tissues	1	0.116 ± 0.064	---	0.008 ± 0.013	0.027 ± 0.076	9.43** > 5.57 = $F_{2.25}$ (0.99)
	others tissues	≥ 2	0.273 ± 0.118	---	0.045 ± 0.090	0.112 ± 0.153	8.76** > 5.57 = $F_{2.25}$ (0.99)
	testes	1	0.270 ± 0.054	---	0.047 ± 0.084	0.091 ± 0.112	18.51** > 5.57 = $F_{2.25}$ (0.99)
	testes	≥ 2	0.279 ± 0.106	---	0.024 ± 0.054	0.122 ± 0.122	16.25** > 5.57 = $F_{2.25}$ (0.99)

** = Highly significant (P 0.01 level).

cells in a proliferative state and on the cell cycle phase encountered (Grosch and Hopwood, 1979; Rojas and Denekamp, 1989).

In testes, the alterations were found only in spermatogonial cells. The cells are very sensitive to radiation (Grosch and Hopwood, 1979). On the other hand, it has been found that different phases of mouse spermatocytes are moderately to highly resistant to radiation (Searle, 1974; Grosch and Hopwood, 1979).

In this work, a significant number of breaks were found in somatic and spermatogonial cells, especially adjacent to the centromeric region where heterochromatic blocks occur. Fragile sites have been identified in different chromosomal regions, such as the heterochromatic region, in studies of spontaneous (Dutrillaux *et al.*, 1977; Kato, 1979) and induced translocations (Cerrano and Wolff, 1975). The breaks are considered as a primary lesion from which many others are derived (Savage, 1989).

The presence of a more or less compact chromosome region suggests that radiation can affect the degree of chromatin condensation. An apparent unravelling of heterochromatin has also been found in *T. infestans* under stress conditions (Alvares-Garcia, 1988; Andrade and Mello, 1987; Mello, 1989; Dantas and Mello, 1992). The authors suggested that the loosely arranged chromatin may represent an attempt to activate silent genes. In Anura there is evidence of euchromatin transformation into heterochromatin (King, 1980), related to this group's evolution. In Holarctic *Hyla* the most common types of chromosomic changes are additions/deletions of heterochromatin and rare translocations. It is significant that most functions attributed to constitutive heterochromatin are associat- ed with chromosome evolution (Anderson, 1991). Chromosomal evolution within the Hylidae has included at least one shift in active NOR location and modification of C-band heterochromatin (Anderson, 1991; King, 1991).

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RESUMO

Aplastodiscus perviridis provenientes do Morro do Ferro (MG), da Serra da Bocaina (SP) e de Botucatu (SP), e *Hyla albopunctata* do Morro do Ferro, de Botucatu e de Campinas (SP) foram analisadas quanto a estrutura dos cromossomos metafásicos.

Foi encontrada uma quantidade significativa de metáfases com alterações cromossômicas nas populações do Morro do Ferro se comparadas às de outras localidades. Essas alterações foram classificadas como: descondensações, condensações, quebras, falhas, deleções e fragmentos acêntricos. As alterações mais freqüentes foram as quebras, as deleções e os fragmentos acêntricos. Parte destas ocorrem próximas à região centromérica. A freqüência de metáfases alteradas é variável entre os indivíduos em todas as populações estudadas.

A maioria dessas alterações podem ser provavelmente atribuídas ao alto nível de radiação natural do Morro do Ferro.

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