GEOGRAPHICAL KARYOTYPIC VARIATIONS AND CHROMOSOME BANDING PATTERNS IN TROPIDURUS HISPIDUS (SAURIA, IGUANIDAE) FROM BRAZIL

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SUMMARY — Karyotypic data are presented for Tropidurus hispidus (Sauria, Iguanidae) collected at six different localities in Brazil. The specimens from all the populations share the same basic 2n = 36 karyotype characterized by 12 macrochromosomes and 24 microchromosomes. The X is an acrocentric microchromosome and the Y, a minute microchromosome. The mechanism of sex determination is of XX:XY type. Geographical karyotypic variations related to the presence or the absence of a prominent secondary constriction in one pair of macrochromosomes and to different sites of the nucleolar organizer regions (NORs) were found. Chromosome polymorphisms related to the size of the secondary constriction, the differential activity of NORs and the amount of constitutive heterochromatin were observed in specimens from some populations. G- and R-banding patterns were obtained for the first time in T. hispidus after «in vivo» treatment with 5-bromodeoxyuridine (BrdU).

INTRODUCTION

The genus Tropidurus comprises a group of species, the torquatus group, which are characterized by the presence of keeled and imbricate dorsal scales and absence of a middorsal crest. Until 1978, this group included T. torquatus, T. hispidus and T. hygomi which were considered as three different species or were classified as subspecies of one species. An extensive taxonomic review of these lizards inhabiting the Southern regions of the Amazon river was then carried out by one of us (M. T. Rodrigues), who was able to recognize 12 different species within the torquatus group: T. hispidus, T. itambere, T. torquatus, T. hygomi, T. cocoebensis, T. erythrocephalus, T. oreadicus, T. insulanus, T. mucuiensis, T. etheridgei, T. montanus and T. psammonastes (RODRIGUES 1984, 1987 and RODRIGUES et al. 1987).

Cytogenetic investigations of the species belonging to the torquatus group
were started with the study of two of them (KASAIARA et al. 1983), which are currently recognized as *T. hispidus* and *T. itambere*. These species showed differences in the amount and distribution of constitutive heterochromatin and in the mechanisms of sex determination which are of the XX:XY and X₁X₁X₂Y: X₁X₁X₂X₂ types, respectively (KASAIARA et al. 1983).

This paper reports the chromosome variability found in specimens of *T. hispidus* collected at six different localities in Brazil and the characterization of the autosomal heteromorphisms present in some individuals. Cytogenetic analysis was performed by G-, R- and C-banding techniques and silver staining of nucleolus organizer regions (NORs). Reproducible G- and R-banding patterns were obtained along the metaphase chromosomes with incorporation of 5-bromodeoxyuridine (BrdU) into the replicating DNA by «in vivo» treatment.

**MATERIAL AND METHODS**

*Tropidurus hispidus* is a heliophilous lizard occurring in several types of open formation habitats in South America, specially in the caatinga area (xerophytic morphoclimatic domain, according to AB’SABER, 1977). This species has a wide and continous distribution in Northeastern and Eastern Brazil (Fig. 1) and some relictual populations are found in open areas North of the Amazon (RODRIGUES 1984, 1987). The localities and the specimens of the present study are listed in Table 1. The specimens are deposited in the Museu de Zoologia, Universidade de São Paulo, Brazil (MZUSP).

About 48 h before sacrifice, the specimens were injected intraperitoneally with an aqueous solution of Fleischmann yeast plus dextrose (COLE and LEAVENS 1971) and incubated at 30°C (PECCINI-SEALE and PROTA-PESSOA 1974). Most specimens were also injected intraperitoneally with a solution of 5-bromodeoxyuridine and 5-fluorodeoxyuridine (10 mg BrdU and 0.5 mg FudR in 2 ml 0.9% NaCl solution), about 0.1 ml/10 g body weight, 9 to 28 h before they were killed (SCHEMPP and SCHMID 1981). A 0.1% colchicine solution was then injected intraperitoneally (0.1 ml/10 g of body weight) and the animal was killed 2 or 5 h later.

Mitotic chromosomes were obtained from bone marrow, intestine, liver and kidney. Meiotic preparations from males were also made. C-banding was done by the method of SUMNER (1972) and NOR staining by the technique of HOWELL and BLACK (1980). The G- and R-banding patterns for the specimens injected with BrdU/FudR were obtained by the technique of DUTRILLAUX and COUTURIER (1981) with the chromosomes being differentially stained with 33258 Hoechst-Giemsa (FPG staining) or acridine orange.

**RESULTS**

All the specimens of *T. hispidus* presented a diploid number of \(2n = 36\) and the same basic karyotype with 12 metacentric or submetacentric macrochromosomes and 24 microchromosomes (Figs. 2a and 2b). The mechanism of
sex determination is of the XX:XY type, the Y being a minute microchromosome present only in male cells and the X an acrocentric microchromosome not identified morphologically.

Some differences were noticed among the specimens of \textit{T. hispidus} from the six populations studies, so that two distinct karyotypes could be established. Karyotype A is found in specimens from João Pessoa, Santo Amaro das Brotas and Raso da Catarina. Karyotype B occurs in the specimens collected at Santo Inácio, Morro do Chapéu and Mucujê. For the specimens from Grão Mogol, which were previously analyzed by Kasahara \textit{et al.} (1983), a third karyotype was found and designated Karyotype C (Fig. 2c).
TABLE 1 — Locality, sample size, sex and MZUSP number of the specimens of *Tropidurus hispidus* (*2n = 36*) collected in Brazil.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Sample size and sex</th>
<th>Specimen number</th>
<th>Reference</th>
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<tr>
<td></td>
<td>males</td>
<td>females</td>
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Karyotype A of *T. hispidus*.

This karyotype is characterized by a prominent secondary constriction at the distal end of the long arm of macrochromosome pair 2 (Fig. 2a). All the 11 specimens with Karyotype A have NORs restricted to this secondary constriction (Figs. 3a, 3b, 3c and 3e). The precise localization of NORs is clearly seen in some metaphases just in the end of the secondary constriction region (Fig. 3b).

One male specimen from João Pessoa (MZUSP 65001) presented only one active NOR per cell in half of the 50 metaphases analyzed by the Ag-staining technique (Fig. 3b). The remaining metaphases had two NORs which were of different sizes (Fig. 3c).
Fig. 2. — Karyotypes of *T. hispidus* (*2n = 36*) obtained by Giemsa staining. 

*a.* Male Karyotype A with a prominent secondary constriction in pair 2; 

*b.* Male Karyotype B with a slight secondary constriction in pair 2; 

*c.* Male Karyotype C with no secondary constriction in pair 2. 

Fig. 3. — Metaphase with NORs in pair 2 (arrows) in *T. hispidus* having Karyotype A; b-e. Pair 2 of the specimens from João Pessoa (MZUSP 65001): b. one NOR, c. NORs of different sizes; d-e. Pair 2 of the specimen from Raso da Catarina (MZUSP 63558): d. Giemsa staining showing heteromorphism in the secondary constriction, e. NORs of different sizes. Bar = 10 μm.

One male from Raso da Catarina (MZUSP 63558) was heteromorphic for chromosome pair 2, with the secondary constriction being not always evident in one of the homologous chromosomes (Fig. 3d). AG-staining showed that both chromosomes have NORs which are also of different sizes (Fig. 3e). In the chromosome with an evident secondary constriction the NOR appears to be larger than that present in the other homologue.

Two C-band patterns were observed in the specimens with Karyotype A
Fig. 4. — Different C-banding patterns in T. hispidus (2n = 36) having Karyotype A. a. C-banding for the specimens from Santo Amaro das Brotas and Raso da Catarina; b. C-banding for the specimens from João Pessoa. Bar = 10 μm.

(Fig. 4a and 4b). C-bands are restricted to the centromeric regions of pair 5 and in the larger microchromosomes, but the specimens from Raso da Catarina and Santo Amaro das Brotas had also an evident centromeric C-band in pair 2 (Fig. 4a), while the specimens from João Pessoa showed C-bands bordering the secondary constriction of this chromosome (Fig. 4b). Telomeric C-bands were observed in some chromosomes (Fig. 4b) but were not evident in all metaphases.

R- and G-banding patterns produced by BrdU incorporation allowed the precise identification of the macrochromosome pairs (Fig. 5a and 5b). In R-
banded metaphases, the distal end of pair 2, including the secondary constriction, appears as a less intensely staining region (Fig. 5a), even in the specimens from Santo Amaro das Brotas where this region is not C-positive stained. The larger microchromosomes present also a pale area at the centromeric region which corresponds to the late replicating constitutive heterochromatin (Fig. 5a). In G-banded metaphases, these microchromosomes have a reverse pattern with the pericentromeric region staining as in the C-banding pattern (Fig. 5b). In two specimens (MZUSP 63558 and MZUSP 65000) with Karyotype A, metaphases with sister chromatid differentiation were produced as a result of BrdU incorporation during two DNA replication cycles (Fig. 6). After 28 hours
of BrdU treatment, one of the specimens (MZUSP 63558) showed a mean number of 4.53 sister chromatid exchanges (SCEs) varying from 1 to 9 per metaphase in the macrochromosomes of a sample of 30 cells analyzed. SCEs were also observed in some microchromosomes.

Meiotic analysis of male specimens with Karyotype A revealed 18 bivalents at diplotene and metaphase I cells (Fig. 7a). One of the small bivalents is formed by microchromosomes of different sizes which were interpreted to be the X and Y chromosomes. Metaphase II cells showed 6 macrochromosomes and 12 microchromosomes, including the X or the Y chromosome.

Karyotype B of «T. hispidus».

Karyotype B differs from Karyotype A by the presence of a slight secondary constriction at the distal end in the long arm of pair 2 (Fig. 2b). This secondary constriction is variable in size and may appear to be completely missing in some cells or to be as large as in Karyotype A within the same individual (Figs. 8a and 8b).
Fig. 7. — Meiotic cells in T. hispidus (2n = 36) having Karyotype A. a. Diplotene with 18 bivalents, including the XY sex bivalent (arrow); b. Metaphase II with 6 macrochromosomes and 12 microchromosomes. Bar = 10 μm.
Contrary to Karyotype A, the secondary constriction of pair 2 in Karyotype B is never stained by the NOR procedures. In the specimens with Karyotype B, the NORs are located in a pair of microchromosomes which frequently appear to be in close association (Fig. 9).

The C-banding pattern of Karyotype B is similar to that of Karyotype A by presenting centromeric C-bands in pair 5 and in the larger microchromosoma-

Fig. 8. — Pair 2 in *T. hispidus* (*2n* = 36) from Morro do Chapéu (MZUSP 63561) having Karyotype B. *a-b*. Giemsa staining showing variable sizes of the secondary constriction; *c*. C-banding; *d*. Late-replicating region at the distal end after BrdU incorporation and FPG staining.

Fig. 9. — NORs in two microchromosomes (arrows) in *T. hispidus* (*2n* = 36) having Karyotype B. Bar = 10 μm.
Fig. 10. — a. C-banding in a female specimen of *T. hispidus* (2n = 36) from Santo Inácio (MZUSP 65006) having Karyotype B and showing an additional heterochromatin region in one of the homologue of pair 4; b. Giemsa staining; c. C-banding; d. R-banding after BrdU incorporation and acridine orange staining. Bar = 10 μm.

mes (Fig. 10a). Pair 2, however, did not show C-bands either in the centromeric region or at the distal end of the long arm (Figs. 8c and 10a). One female from Santo Inácio (MZUSP 65006) had a heteromorphic pair 4, with one of the homologues showing an enlarged long arm (Fig. 10b) which appears to be C-positive stained (Figs. 10a and 10c). The remaining four individuals from this locality as well as the two from Morro do Chapéu and that from Mucujé, all presenting Karyotype B, have a pair 4 of normal size (Fig. 2b).

The R- and G-banding patterns of the specimens with karyotype B are similar to those of Karyotype A specimens. The constitutive heterochromatin of the large microchromosomes as well as the distal end of pair 2 is clearly late replicating (Fig. 8d), although this region does not show evident C-positive staining (Fig. 8c). As expected, the additional C-positive region in one of the chromosomes of pair 4 in the female from Santo Inácio is also late replicating and stains red with acridine orange (Fig. 10d).
Meiotic analysis of male specimens revealed the same result described for the males with Karyotype A, confirming the occurrence of the sex determination mechanism of the XX:XY type in *T. hispidus*.

**DISCUSSION**

*Geographical variations in karyotype.*

All of the 19 specimens of *T. hispidus* described in the present study, and collected at João Pessoa, Santo Amaro das Brotas, Raso da Catarina, Santo Inácio, Morro do Chapéu and Mucujê, and the 8 previously reported by Kasahara *et al.* (1983), from Grão Mogol, have the same basic karyotype, with \(2n = 36\) comprising 12 macrochromosomes and 24 microchromosomes. The males have a heteromorphic pair including a minute Y chromosome not shown by the female specimens, and the mechanism of sex determination is clearly of the XX:XY type.

Some of the karyotypic variants found in *T. hispidus* seem to be characteristic for specimens from certain localities, suggesting an intraspecific geographical chromosome variations in this lizard species. Taking into account the secondary constriction of pair 2 and the NOR localization, three different karyotypes could be easily recognized. Karyotype A with a prominent secondary constriction which is always NOR stained characterizes the specimens from João Pessoa, Santo Amaro das Brotas and Raso da Catarina. Karyotype B with a less conspicuous secondary constriction in pair 2 and active NORs in a pair of microchromosomes was found in the specimens collected at Santo Inácio, Morro do Chapéu and Mucujê. In Karyotype C, which is characteristic of the specimens from Grão Mogol, no secondary constriction was detectable in pair 2 and NORs were also restricted to a pair of microchromosomes.

At present, no correlation can be established between this karyotypic pattern and the morphological traits of the animals. Rodrigues (1987) showed that the populations of *T. hispidus* from the South to the Amazon river have remarkably homogeneous morphology. Association with altitude is possible although not very strong: Karyotypes B and C are limited to mountainous areas near or above 900 meters of altitude, which are characterized by rocky habitats; Karyotype A occurs in lowlands but João Pessoa and Santo Amaro das Brotas are on the coastal plain and the specimens were found in white sand habitats (restingas) while Raso da Catarina is a yellow sand area with caatinga vegetation, in the interior of the State of Bahia.

*Chromosomal polymorphisms.*

The karyotypic diversity found in some specimens of *T. hispidus* shows the polymorphic nature of the populations of this lizard species. The chromosomal
polymorphisms are related to the size of secondary constriction, to the differential activity of NORs and to the amount of constitutive heterochromatin.

The small size of the secondary constriction in one of the chromosomes of pair 2 in the specimen from Raso da Catarina (MZUSP 63558) is not apparently related to a deficiency in rRNA gene sequences. In this chromosome the NOR appears to be of normal size while the homologue has a NOR of increased size. A similar NOR heteromorphism was observed in the specimen from João Pessoa (MZUSP 65001) but in this case, half of the metaphases analyzed presented NORs of different sizes and the remaining cells had only one active NOR per cell which was of large size. It seems therefore that the intrapopulational NOR polymorphisms reported in the present study are related to a differential transcriptional activity of rRNA genes rather than to an increase or the absence of the ribosomal genes, as described for fish (Foresiti et al. 1981) and rodent (Souza and Yonenaga-Yassuda 1982; Yonenaga-Yassuda et al. 1985) species.

The heteromorphism of pair 4 in the specimen from Santo Inácio (MZUSP 63007) is due to an increase in the amount of constitutive heterochromatin in one of the chromosome arms. Chromosome polymorphisms related to variable amounts of C-bands are not rare, but a large additional block of constitutive heterochromatin on the autosome arms, as found in the present study, appears to occur less frequently. A similar heteromorphism of pair 4 was described in the karyotype of T. torquatus from São Paulo, Brazil, studied only by Giemsa staining (Bečak et al. 1972).

**Banding patterns by BrdU incorporation.**

Reproducible R- and G-banding patterns along metaphase chromosomes could be demonstrated for the first time in T. hispidus by BrdU incorporation. Each pair of macrochromosomes was precisely identified and the banding patterns observed in both Karyotypes A and B were essentially the same. G-banded metaphases can be obtained when BrdU is available only during the early S phase at the time of R-band replication. With in vivo treatment we must assume that BrdU is no longer available during the remaining replication phase, perhaps owing to BrdU degradation in the live organism.

Chromosome banding by BrdU incorporation in DNA is useful for the identification of the late-replicating regions of the karyotype, such as the distal end of the long arm of pair 2 in T. hispidus or the additional C-positive region in chromosome 4. For some specimens, different patterns of incorporation were obtained due to the asynchrony of the cell population. For example, the presence of BrdU during the late S phase produced a typical pattern where only the heterochromatic regions could be identified as pale bands (Fig. 5a), whereas incorporation during two replication cycles produced some metaphases with sister chromatid differentiation (Fig. 6).
Chromosome banding after «in vivo» treatment with BrdU was previously described by SHEMPP and SCHMID (1981) for several species of Amphibia. The banding procedures based on the incorporation of DNA base analogues are advantageous for lower vertebrates for which it is not easy to obtain G-, Q- or R-bands by the usual techniques. Undoubtedly, the new banding techniques for lizards need to be improved for application to the comparative analysis of karyotypes.

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