

Chromosome studies of Brazilian vespertilionids *Lasiurus cinereus* and *Lasiurus ega* (Mammalia, Chiroptera)

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ABSTRACT. Cytogenetical studies based on conventional coloration by Giemsa, C-banding and Ag-NOR were performed on 2 species of bats from the vespertilionid family: *Lasiurus cinereus* (Beauvois, 1796) and *Lasiurus ega* (Gervais, 1856). The 2n was 28 and FN was 48 in both species. The constitutive heterochromatin is located in centromeric regions in the two species and in the short arm of the subtelocentric X chromosome in *L. ega*. NORs were observed in the secondary constriction of the smaller autosome in both species.

KEYWORDS. Chiroptera, chromosomes, *Lasiurus*, C-banding, NORs.

INTRODUCTION

An important tool in the adaptive strategy of an organism is the karyotype. Chromosomal changes play an essential role in the evolution of the species. The number of linkage groups and the arrangement of genes and heterochromatin in chromosomes can affect the amount of variation among offspring, as well as phenotypic expression and gene regulation. Although the exact role that chromosomal change plays in the evolutionary process is not well understood, some authors have argued that a sudden chromosomal evolution is correlated with and facilitates morphological evolution and the rate of speciation (WILSON *et al.*, 1975; BUSH *et al.*, 1977; QUMSIYEH, 1994).

The family Vespertilionidae is among the largest taxa of mammals, representing 42 genera and 355 species (NOWAK, 1999). This family as well as other families of Chiroptera, has been studied from the cytogenetic point of view and the results have permitted to verify that the dynamics of chromosomal evolution varies in different chiropteran taxa. The vespertilionid bats show very little karyological diversity in both intrapopulational and intraspecific level, but when karyotypic diversity exists, it may have been brought about by Robertsonian or non-Robertsonian changes or by both (STOCK, 1983; VOLLETH & TIDEMANN, 1989; VOLLETH & HELLER, 1994 a, b).

Despite of the several cytogenetic studies in vespertilionid species, the karyological information is still insufficient to characterize or represent the karyotypic variation in this family. There are few chromosomal studies on Brazilian vespertilionid specimens (VARELLA-GARCIA *et al.*, 1989; FREITAS *et al.*, 1992).

The vespertilionid genus *Lasiurus* Gray, 1831 is endemic to North and South America. The genus is morphologically distinctive and comprises the tribe Lasiurini (BICKHAM, 1987; SIMMONS & GEISLER, 1998). Evolutionary relationships of *Lasiurus* to other vespertilionine genera are not established.

Morphological, karyological and biochemical data have permied some authors to hypothesizet that *Lasiurus* diverged early from the primitive vespertilionine stock (HALL & JONES, 1961; MORALES & BICKHAM, 1995).

The number of recognized species of *Lasiurus* fluctuates in the taxonomic literature and some studies have attempted to investigate the evolutionary relationships between species of *Lasiurus* and other vespertilionid genera by means of several approaches, such as cytogenetic and molecular studies (BICKHAM, 1987; MORALES & BICKHAM, 1995).

Karyological data have shown that the few chromosomes shared between *Lasiurus* and other vespertilionid genera are considered to be plesiomorphic (BICKHAM, 1979). This suggests that the *Lasiurus* species have an early divergence. In Brazilian specimens, only representatives of *L. borealis* (Muller, 1776) were analyzed (VARELLA-GARCIA *et al.*, 1989). Additional chromosomal investigations of these taxa should better resolve their phylogenetic relationships.

This paper describes the karyotypes and the localization of constitutive heterochromatin and nucleolar organizer regions (NORs) for *L. cinereus* and *L. ega*.

MATERIAL AND METHODS

The specimens of *L. cinereus* (DZSJRP16616, DZSJRP16645) and *L. ega* (DZSJRP16611, DZSJRP16644) analyzed were captured in São José do Rio Preto (20° 48' S, 49° 24' W), São Paulo state, Brazil and are deposited in the Coleção de Chiroptera do IBILCE/UNESP, São José do Rio Preto, SP, Brazil. Mitotic chromosome spreads were prepared after the customary colchicine arresting and hypotonic (1% sodium citrate) and fixation (methanol-acetic 3:1) treatments of fibroblast-like cells obtained from lung biopsies. Cultures were grown in Ham F-10 medium supplemented with 20% fetal calf serum, L-glutamine, penicilin, and streptomycin. CBG-banding and Ag-NOR staining were performed according to SUMNER (1972) and HOWELL & BLACK (1980) with the modifications referred to by VARELLA-GARCIA & TADDEI (1989).

RESULTS AND DISCUSSION

Lasiurus cinereus and *L. ega* presented a similar karyotype with a diploid and fundamental number of 28 and 48 respectively (fig. 1A, C).

The autosomes are composed of 10 pairs of chromosomes meta and submetacentric, ranging from large to medium (1-10); one pair of small subtelocentric

(12) and two pairs of small acrocentric (11 and 13). Despite the similar morphology of the autosomes, the X of *L. ega* is a medium submetacentric chromosome and the X of *L. cinereus* is a medium submetacentric chromosome. These results were similar to those observed in the literature for these two species (BAKER *et al.*, 1971; BICKHAM, 1979, 1987).

An interesting aspect is that one specimen of

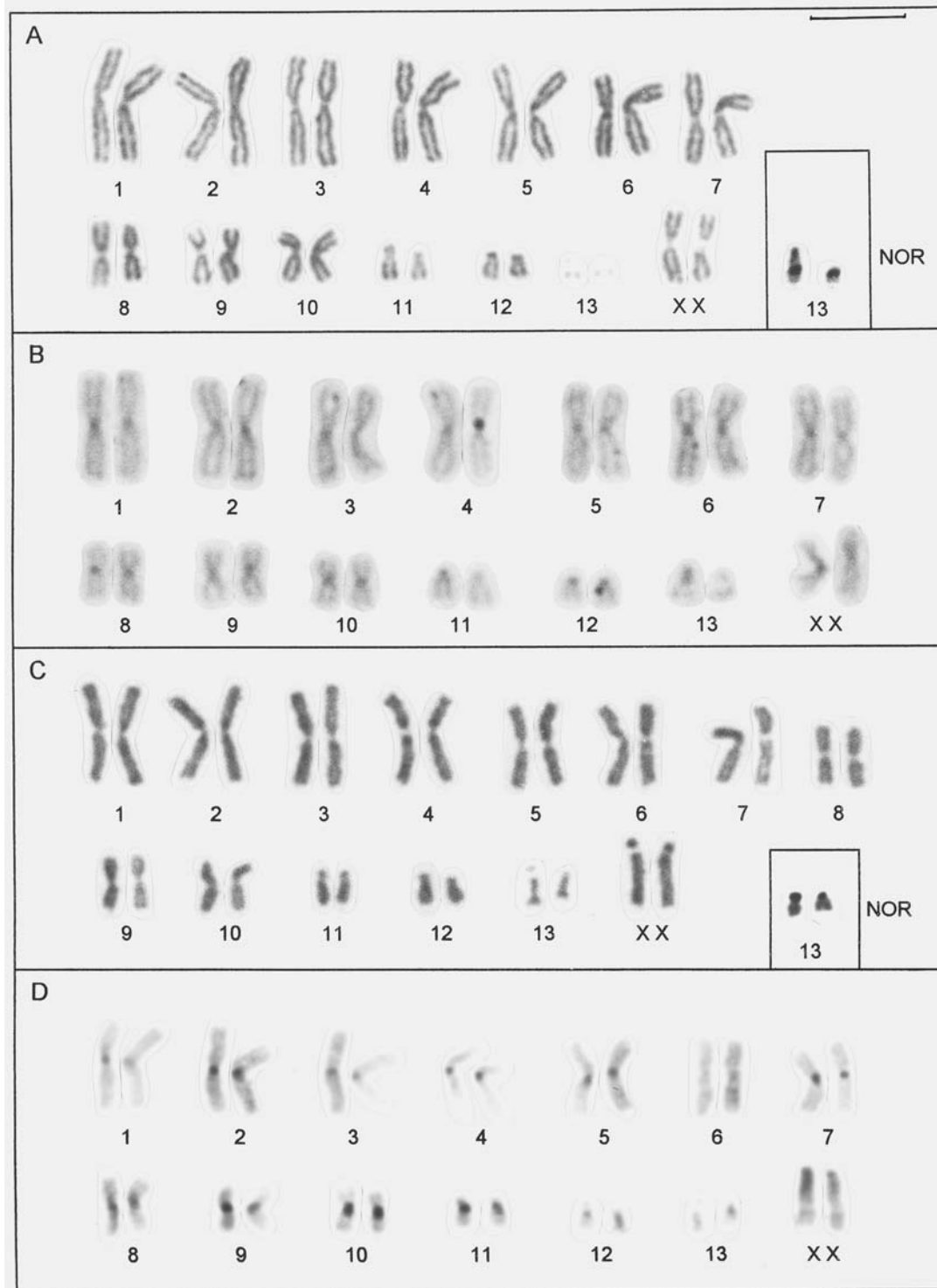


Fig. 1. Representative karyotypes with Giemsa staining, C-banding and nucleolar organizer regions of *Lasiurus cinereus* (A and B) and *L. ega* (C and D). The bar corresponds to 5 μm.

L. cinereus was chromosomally heteromorphic in size. In the chromosome pair with the secondary constriction (the smallest acrocentric) a conspicuous short arm was observed in one of the homologous (fig. 1A, inset). The secondary constrictions of the heteromorphic chromosomes were observed to carry the nucleolus organizer regions (NORs), which are described for the first time for this species (fig. 1A, inset). In *L. ega*, one secondary constriction was observed in the short arm of the lesser acrocentric autosome and these regions carried the NORs in *L. ega* (fig. 1C, inset).

The C-banding technique revealed the presence of heterochromatin only in the centromeric regions in the autosomes in both species, but with differences in the size of the heterochromatic blocks (fig. 1B, D). The heteromorphism in the size of small acrocentric autosomes in *L. cinereus*, uncommon in bats, was due to addition of heterochromatin in the small short arm. Unlike of the X chromosome in *L. cinereus*, which presents only centromeric heterochromatin, the short arm of the submetacentric X chromosome in *L. ega* is all heterochromatic. The heterochromatin present in the short arm of the acrocentric of *L. cinereus*, could have been resulted of the change occurred between autosome heterochromatic regions during the cell events causing the heteromorphism from that individual.

Studies of both banded and standard karyotypes reveal that chromosomal variation is widespread at the generic level in vespertilionid and most of the variation is due to Robertsonian fusions and fissions (BICKHAM, 1979). Despite this, variation at lower taxonomic level is uncommon in these bats. The only case of intraspecific chromosomal variation in vespertilionid was observed in the *Rhogeessa tumida-parvula* complex, which presents five cytotypes. A heteromorphic condition was not observed in the analyzed individuals, and the heterochromatin does not account for the observed chromosomal variation (BICKHAM & BAKER, 1977).

Generally the species of the family Vespertilionidae present conservative karyotypes, at least at the genus level. In species belonging to the same genus or a group of related genera the diploid and fundamental number are generally equal, or the fundamental number is the same, while there is a slight difference in the diploid number. Similarly to other genera of Vespertilionid, *Lasiurus* also presents a high level of conservation (BICKHAM, 1979, 1987). The karyotypes of *Lasiurus* have been interpreted as the most specialized among the Vespertilionidae, due to the reduction in their chromosomal number. Karyotypes similar to those observed in species of *Myotis* Kaup, 1829 (2n=44), *Eptesicus* Rafinesque, 1820 and *Histiotus* Gervais, 1856 (2n=50) have been interpreted as representing a primitive condition, and karyotypes with a lower diploid number, as observed in *Lasiurus*, could represent a derived state (BICKHAM, 1979). The condition observed in *Lasiurus* could have resulted from events such as pericentric inversions and Robertsonian translocations (fusions).

Despite that few differences have been observed

among the karyotypes of different species of *Lasiurus*, the results of the present study suggest that events such as small inversions and variation in the quantity and localization of heterochromatin have played an important role in the evolution of the *Lasiurus* species. Generally the heterochromatin in bat chromosomes is limited to the centromeric regions. When large variation in quantity and location has been observed, it is related to species differentiation. This was observed in chromosomes of Molossidae and Phyllostomidae species (MORIELLE-VERSUTE *et al.*, 1996; FINATO *et al.*, 2000; FARIA & MORIELLE-VERSUTE, 2002). More cytogenetic information is needed in order to understand the karyotype evolution within the *Lasiurus* genera and the Vespertilionidae family and to interpret the relationships with other Chiroptera families.

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