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Short Communication

New insights into telomeric DNA sequence $(TTAGGG)_n$ location in bat chromosomes

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ABSTRACT. Molossidae species, *Cynomops abrasus* (2n = 34, fundamental number, FN = 64), *Eumops auripendulus* (2n = 42, FN = 62), *Molossus rufus* (2n = 48, FN = 64), *Molossops temminckii* (2n = 48, FN = 64), and *Nyctinomops laticaudatus* (2n = 48, FN = 64), and Phyllostomidae species, *Phyllostomus discolor* (2n = 32, FN = 60), have karyotypes with different chromosome and fundamental numbers, different localization of constitutive heterochromatin, and different numbers and location of nucleolar organizer regions (NORs). Fluorescence *in situ* hybridization with a human probe of the telomeric sequence $(TTAGGG)_n$ produced fluorescent signals in telomeric regions of the six bat species' chromosomes; in *E. auripendulus*, pericentromeric signals were also observed in the acrocentric and subtelocentric chromosomes. A relationship between telomeric sequences and NORs, and

between telomeric sequences and constitutive heterochromatin was detected in chromosomes bearing NORs in *C. abrasus*, *M. temminckii*, *N. laticaudatus*, and *P. discolor*. No interstitial signal was observed in the meta- or submetacentric chromosomes of these species.

Key words: Molossid; Phyllostomid; Chiroptera; Telomeric sequence; Fluorescence *in situ* hybridization

Cytogenetic studies using conventional staining and banding techniques have shown that karyotype evolution in the order Chiroptera, in most families, is characterized by variability in morphology, chromosome and fundamental numbers (Baker and Bass, 1979; Baker et al., 1979; Bickham, 1979; Baker and Bickham, 1980; Varella-Garcia et al., 1989; Souza and Araújo, 1990; Volleth et al., 2001; Marchesin and Morielle-Versute, 2004; de Faria and Morielle-Versute, 2006). Comparisons of chromosome banding patterns support the hypothesis that Robertsonian and tandem chromosomal fusions were the main events in the karyotype evolution of Chiroptera, and the reduced chromosomal number found in some species of phyllostomid and molossid bats may have resulted from such events (Morielle-Versute et al., 1996; de Faria and Morielle-Versute, 2006; Ao et al., 2006, 2007; Mao et al., 2008).

Robertsonian rearrangements and tandem fusions involve especially telomeres at chromosome termini. They are constituted by specialized nucleoprotein complexes associated with a specific DNA, telomeric DNA, which maintains chromosome stability and integrity (Meyne et al., 1989; Blackburn, 1991; Zakian, 1995). Despite their characteristic location in telomeres, telomeric repeat sequences $(TTAGGG)_n$ have been detected not only in telomeres but also in centromeric and interstitial chromosomal regions in a wide variety of vertebrate species (Meyne et al., 1989, 1990; Vermeesch et al., 1996; Garagna et al., 1997; Fontana et al., 1998; Andrades-Miranda et al., 2002; Pagnozzi et al., 2002; Castiglia et al., 2006; Ventura et al., 2006). Although the origin of these interstitial sequences has not yet been investigated in detail, they may be remnants of chromosome rearrangements that occurred during karyotype evolution (Ruiz-Herrera et al., 2008).

The karyotypes of the species *Molossus rufus*, *Molossops temminckii* and *Nyctinomops laticaudatus* show 2n = 48, and their fundamental number (FN) is 64. *Cynomops abrasus* and *Eumops auripendulus* exhibit a smaller number of chromosomes, with 2n = 34 (FN = 64) and 2n = 42 (FN = 62), respectively (Warner et al., 1974; Morielle-Versute et al., 1996). Warner et al. (1974) suggested that 48 could be the ancestral diploid number for this family, since 23 species and eight genera show 2n = 48.

In Phyllostomidae, the species *Phyllostomus discolor* has a diploid number of 2n = 32 (FN = 60). The results so far obtained for this family suggest that the condition observed in genus *Macrotus* or *Phyllostomus* is the closest to the primitive condition (Patton and Baker, 1978; Varella-Garcia et al., 1989; Baker et al., 1989).

In order to better characterize Chiroptera karyotype evolution and the possible events that occurred in the species differentiation, we analyzed the distribution of TTAGGG telomeric sequences conserved in vertebrates, in the chromosomes of six bat species: *C. abrasus*, *E. auripendulus*, *M. rufus*, *M. temminckii*, and *N. laticaudatus* (Molossidae), and *P. discolor* (Phyllostomidae).

Mitotic chromosome spreads were prepared from a bone marrow cell suspension using standard techniques. To find the location of telomeric DNA sequences, fluorescence

in situ hybridization was performed using the digoxigenin-labeled deoxynucleotide oligomer $(TTAGGG)_n$ as a probe (P5097-DG, Oncor - all telomeres), according to the method described previously (Finato et al., 2000).

Hybridization signals were observed at the termini of almost every chromosome in all individuals of the species *C. abrasus*, *M. rufus*, *M. temminckii*, *N. laticaudatus*, and *P. discolor* (Figure 1). In *E. auripendulus*, most telomeres did not show any hybridization signal with the telomeric probe; however, intense signals were observed in centromeric and pericentromeric regions of several subtelocentric and acrocentric chromosomes (Figure 1A). Despite the variation in the intensity of the signals among chromosomes and among species, no interstitial signal was observed in any of the chromosomes. The absence of telomeric signals was observed in another bat species, *Carollia perpicillata*, in which the hybridization signals with telomeric probe were very faint or absent in most chromosomes (Faria and Morielle-Versute, 2002). This finding was interpreted by the authors as being due to a reduced copy number of the telomeric repeat, resulting from extensive telomeric association and/or rearrangements undergone by the chromosomes of *Carollia*. According to Meyne et al. (1990), the distribution of telomeric sequences could be related to the evolutionary status of the species, where primitive species will have telomere-only patterns, evolving species will have several non-telomeric sites, and highly evolved species will have either non-telomeric sites or a telomere-only pattern. The results of our study support this idea, because most of the species analyzed (*M. rufus*, *M. temminckii*, *N. laticaudatus*, and *P. discolor*) displayed probable ancestral karyotypic conditions and exhibited hydridization signals only at telomeric sites.

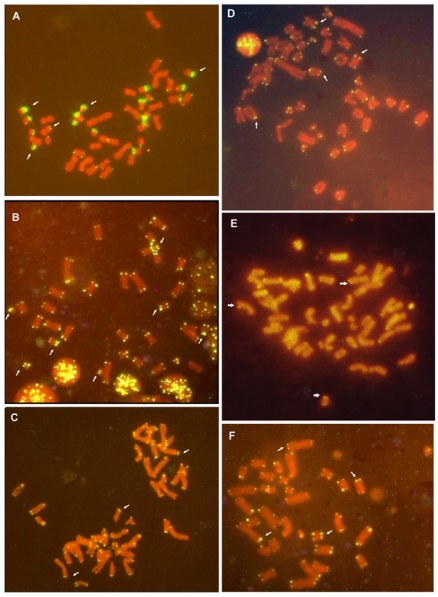

Figure 1. Localization of the telomeric repeat $(TTAGGG)_n$ in *Eumops auripendulus* (A); *Cynomops abrasus* (B); *Nyctinomops laticaudatus* (C); *Molossus rufus* (D); *Molossops temminckii* (E), and *Phyllostomus discolor* (F). Arrows in *C*, *D*, *E*, and *F* indicate signals at the chromosome termini, the arrows in *A* point to centromeric and pericentromeric signals in subtelocentric and acrocentric chromosomes, and the arrows in *B* indicate a more intense signal at telomeric sites of the chromosomes bearing nucleolar organizer regions.

Also remarkable is the fact that the hybridization signals observed in the telomeric regions of five pairs of subtelocentric autosomes in *C. abrasus* (Figure 1B), in three pairs in *M. temminckii*, and in one pair in *N. laticaudatus* and *P. discolor* (not indicated) are coincident with the nucleolar organizer regions (NORs). Subtelocentric chromosome pairs 11, 12, 13, 15, and 16 of *C. abrasus*, 4, 5, and 8 of *M. temminckii*, 5 of *N. laticaudatus*, and 15 of *P. discolor* are NOR-bearing chromosomes in these species, and also coincide with the presence of telomeric C-banding in pair 15 of *C. abrasus* (Morielle and Varella-Garcia, 1988; Varella-Garcia et al., 1989; Morielle-Versute et al., 1996) (Figure 2).

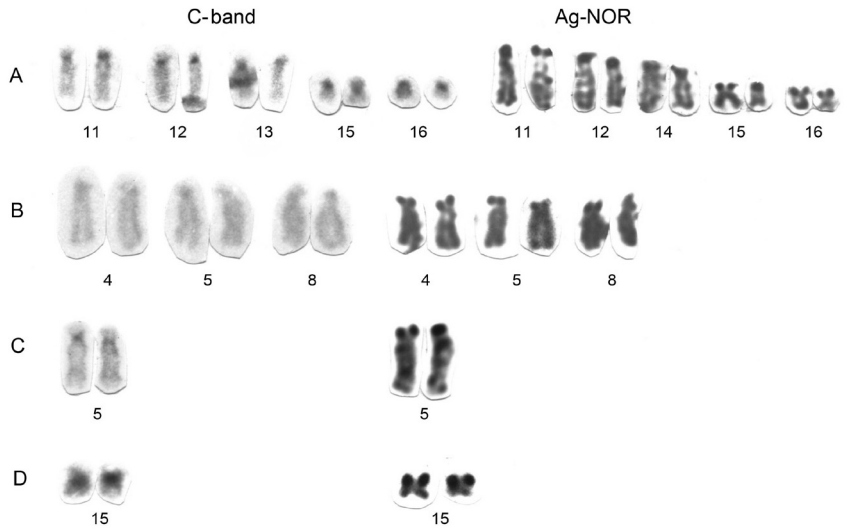

Figure 2. Chromosomes bearing nucleolar organizer regions (NORs) with C-banding and Ag-NOR staining in *Cynomops abrasus* (A); *Molossops temminckii* (B); *Nyctinomops laticaudatus* (C), and *Phyllostomus discolor* (D).

These results provide evidence of the complexity of repetitive DNA composition in bats. They suggest that their telomeric sequences, which are associated with heterochromatin in centromeric and telomeric regions and with the NOR in *C. abrasus*, *M. temminckii*, *N. laticaudatus*, and *P. discolor*, differ from those of other species in the composition of repetitive DNA sequences of heterochromatic and/or ribosomal DNA, since other species do not exhibit hybridization signals in regions bearing heterochromatin and NORs. Furthermore, this fact reinforces the heterogeneity of satellite DNA and indicates that, in some bat species, the telomeric sequences are a component of heterochromatin and ribosomal DNA.

It is not clear yet how telomeric sequences became part of C-heterochromatin and NORs, although the presence of telomeric sequences as a component of C-heterochromatin or satellite DNA has already been demonstrated in several mammals, including bats (Garagna et al., 1995, 1997; Ono and Yosida, 1997; Liu and Fredga, 1999; Pagnozzi et al., 2000; Finato et al., 2000; Faria and Morielle-Versute, 2002). Even if no interstitial signal in biarmed chromosomes was observed that could be evidence of remnants of telomere-telomere fusion events between two ancestral acrocentric chromosomes, the occurrence of interstitial telomeric se-

quences in centromeric regions of biarmed chromosomes in species of bats has already been identified, including two species of the genus *Eumops*: *E. perotis* (2n = 48) and *E. glaucinus* (2n = 42) (Ono and Yoshida, 1997; Finato et al., 2000; Faria and Morielle-Versute, 2002).

Similar to what was observed in the species analyzed in the present study, in some vertebrates, interstitial sequences $(TTAGGG)_n$ were not retained when Robertsonian fusions occurred (Garagna et al., 1995; Nanda et al., 1995; Vermeesch et al., 1996; Castiglia et al., 2006). According to Fontana et al. (1998), such findings may be explained either by the complete loss of telomeric segments during the fusion process or by the fact that chromosome rearrangements by fusion took place earlier in the evolution process, and residual telomeric sequences were then probably lost as a consequence of successive molecular processes.

Therefore, unlike what has been previously observed for other species of *Eumops*, the results obtained in the present study indicate the occurrence of Robertsonian fusion during the chromosomal evolution of *E. auripendulus*, *C. abrasus*, and *P. discolor* (Morielle-Versute et al., 1996; de Faria and Morielle-Versute, 2006), with loss of telomeric DNA sequences, and suggest different situations involving telomeric sequences in chromosomal rearrangements in bat species.

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