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Short Communication

Chromosomal localization of the 18S-28S and 5S rRNA genes and (TTAGGG)*n* sequences of butterfly lizards (*Leiolepis belliana belliana* and *Leiolepis boehmei*, Agamidae, Squamata)

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Abstract

Chromosomal mapping of the butterfly lizards *Leiolepis belliana belliana* and *L. boehmei* was done using the 18S-28S and 5S rRNA genes and telomeric (TTAGGG)*n* sequences. The karyotype of *L. b. belliana* was 2n = 36, whereas that of *L. boehmei* was 2n = 34. The 18S-28S rRNA genes were located at the secondary constriction of the long arm of chromosome 1, while the 5S rRNA genes were found in the pericentromeric region of chromosome 6 in both species. Hybridization signals for the (TTAGGG)*n* sequence were observed at the telomeric ends of all chromosomes, as well as interstitially at the same position as the 18S-28S rRNA genes in *L. boehmei*. This finding suggests that in *L. boehmei* telomere-to-telomere fusion probably occurred between chromosome 1 and a microchromosome where the 18S-28S rRNA genes were located or, alternatively, at the secondary constriction of chromosome 1. The absence of telomeric sequence signals in chromosome 1 of *L. b. belliana* suggested that its chromosomes may have only a few copies of the (TTAGGG)*n* sequence or that there may have been a gradual loss of the repeat sequences during chromosomal evolution.

Key words: chromosomal mapping, FISH, interstitial telomeric site, Leiolepidinae, ribosomal RNA gene, telomeric sequence.

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Squamate reptiles, the most diverse reptilian order, have traditionally been classified into three suborders: Amphisbaenia (worm lizards), Serpentes (snakes) and Lacertilia (lizards). Lizards can be further classified into six infraorders (Iguania, Gekkota, Scincomorpha, Diploglossa, Dibamia and Platynota) (Uetz, 2011). The butterfly lizards (*Leiolepis*, Agamidae, Iguania) are burrowers inhabiting Southeast Asia. These lizards show a wide variety of karyotypes and sexual systems. In Thailand, there are three species of *Leiolepis* (*L. belliana*, *L. reevesii rubritaeniata* and *L. boehmei*) that are barely distinguished from other congeneric species by their typical scale and skin coloration (Peters, 1971). *Leiolepis belliana* occurs as two subspecies, *Leiolepis belliana belliana* and *L. belliana ocellata. Leiolepis b. belliana* occurs throughout the Thailand, while *L. b. ocellata* is found in the upper northern region and *L. reevesii rubritaeniata* occurs only in the northeast. All of these species are bisexual. Putative unisexuality has been reported for the diploid *L. boehme*i, for which only females have been detected in the provinces of Songkhla and Nakhon Si Thammarat in southern Thailand (Darevsky and Kupriyanoya, 1993; V. Aranyavlai, 2003, PhD thesis, Chu-

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lalongkorn University, Bangkok). Our previous phylogenetic analysis of nuclear genes revealed that *L. reevesii rubritaeniata* (2n = 36) and *L. b. belliana* (2n = 36) are more closely related to each other than to *L. boehmei* (2n = 34) (Srikulnath *et al.*, 2010).

Ribosomal RNA genes are tandemly arrayed repeats that are believed to have evolved in a concerted manner. Since all copies of ribosomal RNA genes are homogenous within individuals and species, they represent an important source of information for karyological characterization and evolutionary relationships (Srikulnath, 2010). Telomeric (TTAGGG)*n* sequences are commonly found at telomeres (Meyne *et al.*, 1990) but are also observed at non-telomeric sites known as interstitial sites (ITSs) (Ventura *et al.*, 2006; Srikulnath *et al.*, 2009a). ITSs can form large blocks of telomeric sequences known as heterochromatic ITSs (het-ITSs) that are located mainly in centromeric or pericentromeric regions (Ruiz-Herrera *et al.*, 2008). ITSs may be the remnants of chromosomal rearrangements that provided the information involved in karyotypic evolution (Meyne *et al.*, 1990). The 18S-28S rRNA genes are generally located on a pair of microchromosomes or chromosome 2q in Iguania (Porter *et al.*, 1991).

In our previous study, we mapped the 18S-28S and 5S rRNA genes and telomeric (TTAGGG)*n* sequences of *L. reevesii rubritaeniata* chromosomes (Srikulnath *et al.*, 2009a). The 18S-28S gene was located at the secondary constriction of the long arm of chromosome 1, and the 5S rRNA gene at the pericentromeric region of chromosome 6. Hybridization signals for (TTAGGG)*n* sequences were observed at the telomeric ends of all chromosomes and interstitially at the same position as the 18S-28S rRNA genes, suggesting that in *L. reevesii rubritaeniata* telomere-to-telomere fusion probably occurred between chromosome 1 and a microchromosome where the 18S-28S rRNA genes were located (Srikulnath *et al.*, 2009a).

In the Leiolepidinae, a cytogenetic map has been reported for only one species (*L. reevesii rubritaeniata*) (Srikulnath *et al.*, 2009a,b). In the present study, we used fluorescent *in situ* hydridization (FISH) to map the 18S-28S and 5S rRNA genes and telomeric (TTAGGG)*n* sequences in *L. b. belliana* and *L. boehmei* as representative species of *Leiolepis* in Thailand.

Live specimens of *L. b. belliana* and *L. boehmei* were collected from Chonburi (13°4’0” N and 101°0’0” E) and Songkla (7°12’36” N and 100°33’36” E) provinces, respectively. Their sexes were determined morphologically and confirmed by internal genital anatomy. All experimental procedures with the lizards conformed to the guidelines established by the Animal Care Committee at Hokkaido University (Japan). Although *L. b. ocellata* used to be found in Thailand it was not available for this study. Mitotic chromosomal preparations of *L. boehmei* were obtained from fibroblast cultures of heart, lung and mesentery, as described by Srikulnath *et al.* (2009a), whereas mitotic and meiotic chromosomes of *L. b. belliana* were prepared from testes, according to Imai *et al.* (1981).

Chromosomal locations of the 18S-28S rRNA genes, 5S rRNA genes and telomeric (TTAGGG)*n* sequences were determined by FISH, as previously described (Matsuda and Chapman, 1995; Srikulnath *et al.*, 2009a). Dual-color FISH was done to compare the chromosomal locations of telomeric (TTAGGG)*n* sequences with those of the 18S-28S rRNA genes, whereas single FISH was use to investigate the chromosomal location of the 5S rRNA genes.

The chromosomal numbers of *L. b. belliana* and *L. boehmei* were 2n = 36 (12 macrochromosomes and 24 microchromosomes) and 2n = 34 (12 macrochromosomes and 22 microchromosomes), respectively (data not shown). These results were identical to those previously reported (V. Aranyavlai, 2003, PhD thesis, Chulalongkorn University, Bangkok).

Chromosomal mapping of the 18S-28S and 5S rRNA genes is important for karyological characterization of a species and for establishing phylogenetic relationships since these genes occur in multiple copies that facilitate detection (Pendás *et al.*, 1994; Srikulnath, 2010). In Iguania, the 18S-28S rRNA genes are generally located on a pair of microchromosomes or chromosome 2q (Porter *et al.*, 1991), whereas the nucleolar organizer region is located on chromosome 6 of *Tropidurus* species, the karyotypes of which are similar to *Leiolepis* (Kasahara *et al.*, 1987). In contrast, FISH signals for the 18S-28S rRNA genes in *L. b. belliana* and *L. boehmei* were restricted to the secondary constriction in the subtelomeric region of the long arm of chromosome 1 (Figure 1A,C,D,F,G,I). These features were comparable to those of *L. reevesii rubritaeniata* (Srikulnath *et al.*, 2009a).

In previous work, we showed that the 5S rRNA genes in *L. reevesii rubritaeniata* were located in the pericentromeric region of the long arm of chromosome 6 (Srikulnath *et al.*, 2009a). The same localization was also observed in *L. b. belliana* and *L. boehmei* (Figure 2). These findings indicate that the position of major and minor ribosomal RNA genes may be the same among species of *Leiolepis*. Cytogenetic studies of more leiolepidine species, especially *Uromastyx* sp. which is classified in the same subfamily, are required to confirm this suggestion. Such studies would shed light on the chromosomal locations of the 18S-28S and 5S rRNA genes in the conserved karyotypes of Leiolepidinae and Iguania.

The distribution of telomeric sequences provides preliminary information on the processes involved in karyotypic evolution (Meyne *et al.*, 1990; Srikulnath, 2010). In the present study, the (TTAGGG)*n* sequences were localized to the telomeric ends of all chromosomes of *L. b. belliana* and *L. boehmei* (Figure 1B,C,E,F,H,J). The hybridization signals were weak on macrochromosomes, whereas high intensity signals were observed on almost all

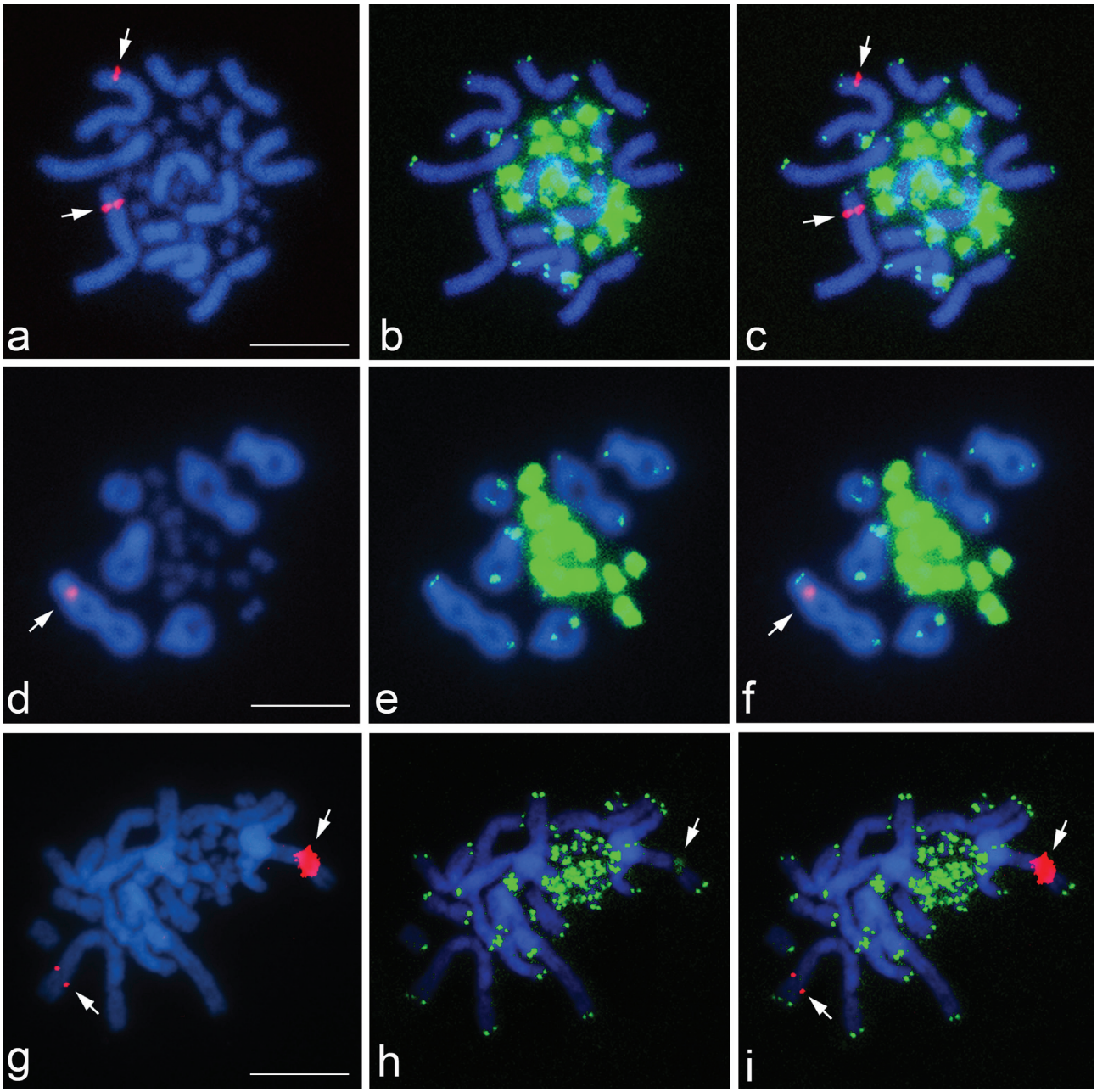

Figure 1 - Chromosomal localization of the 18S-28S rRNA genes and (TTAGGG)*n* sequences in *L. b. belliana* mitotic metaphase (A-C) and meiotic (D-F) chromosome, and *L. boehmei* mitotic metaphase chromosome (G-I). Hybridization patterns of the 18S-28S rRNA genes (red) (A,D,G) and (TTAGGG)*n* sequences (green) (B,E,H) in DAPI-stained chromosomes and their co-hybridization pattern (C,F,I). Arrows indicate FISH signals of the 18S-28S rRNA genes (A,C,D,F,G,I), and interstitial telomeric sites (ITSs) (H,I). Scale bars = 10 μm.

microchromosomes, which suggests site-specific amplification of the (TTAGGG)*n* sequences on these chromosomes. These findings were similar to those of *L. reevesii rubritaeniata* in our previous study (Srikulnath *et al.*, 2009a). However, microchromosome-specific amplification of telomeric repeats has not been reported in other squamate reptiles (Pellegrino *et al.*, 1999). Comparable hybridization patterns have also been observed in birds, including several species of Galliformes, Anseriformes and Passeriformes (Nanda *et al.*, 2002).

Interstitial telomeric sites (ITSs) appear to be relics of chromosomal rearrangements such as fusions or inversions (Go *et al.*, 2000). Hybridization signals for interstitial telomeric sites were found at the secondary constriction in the subtelomeric region of the long arm of chromosome 1 in *L. reevesii rubritaeniata*, where the (TTAGGG)*n* sequences co-localized with the 18S-28S rRNA genes (Srikulnath *et al.*, 2009a). This same arrangement was also found in *L. boehmei*. In contrast, 18S-28S rRNA genes are generally located on a pair of microchromosomes or chromosome 2q

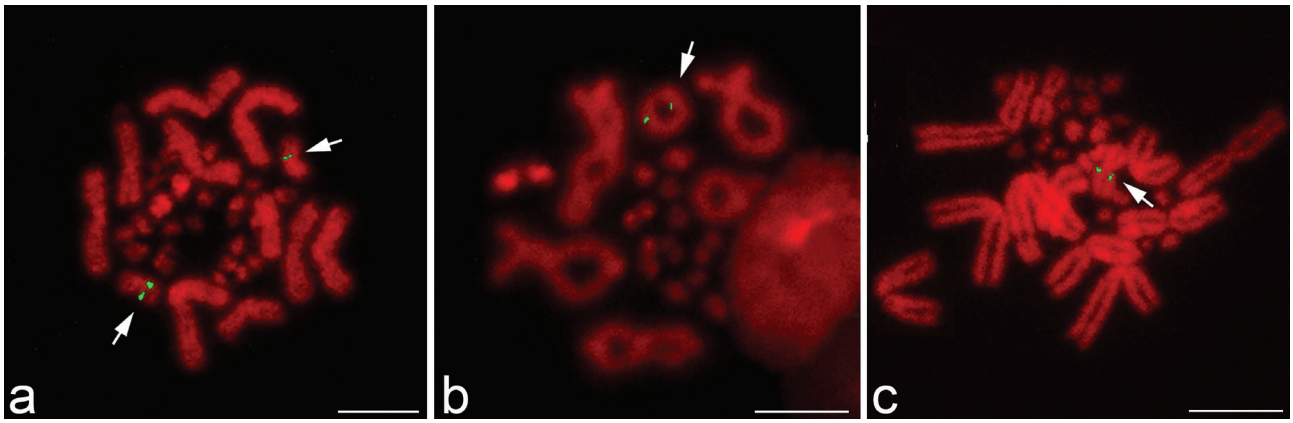

Figure 2 - Chromosomal localization of the 5S rRNA genes in *L. b. belliana* mitotic metaphase (A) and meiotic (B) chromosomes, and *L. boehmei* mitotic metaphase chromosomes (C). Hybridization patterns of the 5S rRNA genes (green) in PI-stained chromosomes. Arrows indicate FISH signals of the 5S rRNA genes. Scale bars = 10 μm.

in Iguania (Porter *et al.*, 1991). These results suggest the possible occurrence of telomere-to-telomere fusion between a microchromosome with the 18S-28S rRNA genes and the distal end of chromosome 1 in the lineage of *L. reevesii rubritaeniata* and *L. boehmei*.

Comparison of the chromosomal maps for the butterfly lizard (*L. reevesii rubritaeniata*) and Japanese four-striped rat snake (*Elaphe quadrivirgata*) also indicated that co-localization of the 18S-28S rRNA genes and ITSs in the subtelomeric region of LRE1q may be the result of a small paracentric inversion (Srikulnath *et al.*, 2009b). This inversion may have occurred between the proximal region of the *DYNC1H1* (dynein, cytoplasmic 1, heavy chain 1) gene and the distal region on LRE1q after the telomere-to-telomere fusion of the ancestral LRE1q and a microchromosome with the 18S-28S rRNA genes, which generally persist in Iguania (Srikulnath *et al.*, 2009b).

An alternative explanation could be that since telomeres cap the end of chromosomes to protect them from deteriorating and fusing with neighboring chromosomes then chromosomal reorganization would lead to telomere exclusion and an absence of ITS at the fusion site. In contrast, the blockade of ITS may produce a fragile site leading to chromosomal breakage (Bolzán and Bianchi, 2006). Hence, ITSs may represent possible fission points at which new telomeres can be formed by pre-existing telomeric repeats (Ruiz-Herrera *et al.*, 2008). This conclusion suggests that telomere formation may have occurred at the secondary constriction of chromosome 1 in the lineage of *L. reevesii rubritaeniata* and *L. boehmei*. Comparison of the karyotypes of *L. reevesii rubritaeniata* and *L. boehmei* indicated that the macrochromosomal features were identical and conserved throughout the suborder Iguania (Olmo and Signorino, 2005).

Thus, the evidence of comparative mapping and the location of 18S-28S rRNA genes and ITS might also verify the process of their transposition to different chromosomes in *Leiolepis* and Iguania. However, no ITS was found in chromosome 1 of *L. b. belliana*. In equids, ITS signals have been observed on chromosome 1 of *Equus quagga burchelli*, but are absent from the chromosomes of other equids. The absence of ITSs on chromosome 1 of *L. b. belliana* could be the result of a low number of copies of (TTAGGG)*n*, making it impossible to detect the sequence, or may represent a gradual loss of the repeat sequences during chromosomal evolution (Santini *et al.*, 2002). Further molecular cytogenetic characterizations of other *Leiolepis* and *Uromastyx* species are required to clarify the possible location of (TTAGGG)*n* sequences in Leiolepidinae and Iguania.

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