Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **65** (1978), n.5, p. 213–218.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1978_8_65_5_213_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1978.

Biologia. — *Contribution to the karyological knowledge of a few Hemidactyliini Plethodontids* (*). Nota di CARLA BOSCO, DONELLA LASCIALFARI e GIORGIO MANCINO, presentata (**) dal Socio M. BENAZZI.

RIASSUNTO. — Sono stati studiati i cromosomi di tre specie di Plethodontidi Hemidactyliini, due del genere *Pseudotriton* (*P. montanus* e *P. ruber*) e una del genere *Eurycea* (*E. longicauda*). Queste specie presentano numero comosomico $n = 14$, $2n = 28$. Anche se i tre cariotipi appaiono largamente simili da un punto di vista morfologico, non si possono considerare sovrapponibili. L'applicazione del C-banding method ha messo in evidenza notevoli differenze tra le due specie del genere *Pseudotriton*, in quanto in *P. montanus* si osserva una diversa distribuzione dell'eterocromatina rispetto a *P. ruber*. In *Eurycea* la risposta al C-banding method è abbastanza simile a quella data da *P. ruber*, essendo l'eterocromatina localizzata in un'ampia zona centromerica e in duplici bande pericentriche sulla coppia XIV. Questi dati cariologici non permettono quindi di confermare l'ipotesi di una netta separazione filogenetica dei due generi *Pseudotriton* e *Eurycea*; essi sembrano invece avvalorare l'idea suggerita da Bruce (1974) che le due specie di *Pseudotriton* qui esaminate si stiano separando filogeneticamente per una maggiore potenzialità evolutiva di *P. montanus*.

INTRODUCTION

In recent years increased interest by cytogeneticists and molecular biologists in the American Plethodontids has run parallel to the increasing amount of research into their taxonomy and geographical distribution, as well as their ecology, evolution and phylogenesis (Noble, 1931; Highton, 1962; Wake, 1966; Tilley, 1977). Among those contributing to the study of this family's chromosome complements, special mention must be made of J. Kezer. Besides ascertaining the chromosome numbers of many species, he was the first to report the existence of differentiated heterochromosomes and XY male digamety in the Central American genera *Oedipina*, *Thorius*, *Chiropterotriton* (provided it is not Mexican) (cf. Mancino and Barsacchi, 1966; Morescalchi, 1975; Mancino *et al.*, 1977). Many further contributions were made to karyological literature after the works of Kezer and Macgregor (1971, 1973), Macgregor and Kezer (1971, 1973), Macgregor *et al.* (1973) and Macgregor and Jones (1977) who pointed out the potential suitability of the Plethodontids as animals for cytological and cytomolecular studies. However, since according to Wake (1966) the family comprises 23 genera and

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(**) Nella seduta del 10 novembre 1978.

over 180 species, there are many large gaps in the available data, especially with regard to the Hemidactyliinae, which form one of the largest groups within the American Plethodontids. The present paper thus aims at contributing to our karyological knowledge of this tribe by illustrating the morphostructural characteristics of the karyotypes of two of the three species of the genus *Pseudotriton*: *P. montanus* (Baird, 1849) and *P. ruber* (Sonnini, 1802), and of *Eurycea longicauda* (Green, 1818), one of the eight species constituting the genus *Eurycea*.

Materials and Methods

Five individuals of *Pseudotriton montanus diastictus*, 7 individuals of *P. ruber schencki* and 5 of *Eurycea longicauda melanopleura* were used. Unfortunately the collection sites are not known as the specimens were purchased from specialised dealers. In this connection it is worth noting that *P. montanus diastictus* is found in the unfrozen plainlands of Southern Ohio, Central and Eastern Kentucky, Western and Southwestern Virginia and Eastern Tennessee, *P. ruber schencki* in the area south of Southern Blueridge, the Southwest of North Carolina and the Northeast of South Carolina, Eastern Tennessee and Northern Georgia, *E. longicauda melanopleura* in the mountainous areas of the U.S. interior, excluding the Southeast area, south of the Missouri, Northern and Southwestern Arkansas, Eastern Oklahoma, Southeastern Kansas (cf. Bishop, 1947).

Specimens were given three intracoelomic injections of 0.3 % colchicine at 48 h intervals. Gut, spleen and liver were then removed. In male individuals also the testes were excised. After removal, the material was placed in distilled water for 10 min., fixed for 20 min. in 3 : 1 absolute ethyl alcohol—glacial acetic acid, and then dissociated in 45 % acetic acid, squashed and prepared by the normal dry-ice method. A few slides were then stained with dilute Giemsa in phosphate buffer (pH 7); others were subjected to C–banding according to the method of Arrighi and Hsu (1971). Karyotype preparation was carried out on the basis of lengths and centromeric indexes, using the terminology proposed by Levan *et al.* (1964).

Results and Discussion

A) *Pseudotriton* karyology

The chromosome number was found to be $n = 14$, $2n = 28$ in both the species studied. This reflects the situation prevailing in the whole family, except for the Neotropical genera characterised by $n = 13$, $2n = 26$ (cf. Morescalchi, 1975). The fourteen chromosome pairs were conventionally arranged in three groups (A, B, and C) in order of decreasing length.

P. ruber schencki

Group A comprises the five longest chromosome pairs, four of which are metacentric (I, II, IV and V) with c.i. = 47.4; 46.0; 45.4; 48.2 respectively, and one sub-metacentric (c.i. = 34.5), statistically found to be III (Pl. I, Fig. 1). Chromosome IV displays a secondary constriction on the short arm. Group B comprises the five medium-length chromosomes, three of which are metacentric (VII, IX and X) with c.i. = 42.4; 46.7 and 48.8, respectively; pairs VI and VIII were found to be sub-metacentric, having c.i. = 36.7 and 34.8 (Pl. I, fig. 1). Group C comprises the four smallest chromosome pairs, of which XI and XIV are metacentric (c.i. = 39.9 and 42.6), while XII and XIII are sub-metacentric (c.i. = 35.7 and 31.7) (Pl. I, fig. 1).

P. montanus diastictus

Group A comprises the five longest chromosome pairs, four of which are metacentric (I, II, IV and V), with c.i. = 42.3; 47.3; 45.9 and 48.0, respectively; pair III is sub-metacentric with c.i. = 34.9 (Pl. I, fig. 2). Group B comprises the five medium-length chromosome pairs, one of which, VI, is a sub-metacentric with c.i. = 33.0, and four metacentric (VII, VIII, IX and X), with c.i. = 38.9; 45.4; 42.7 and 47.9, respectively (Pl. I, fig. 2). Group C comprises the four smallest chromosome pairs, two of which are metacentric (XI and XIV) with c.i. = 39.9 and 46.0 and two submetacentric (XII and XIII) with c.i. = 34.8 and 29.3 (Pl. I, Fig. 2).

The C-banding method has revealed a difference between the two *Pseudotriton* species studied. In *P. ruber schencki*, the technique has evidenced only the centromeric region and double pericentric tracts on one arm (probably the short one) of chromosome XIV, while in *P. montanus diastictus*, in addition to these areas, there are Giemsa-positive tracts in pericentric regions in most of the chromosome pairs (Pl. I, Fig. 3 and II, Fig. 2). Only one pair, XIII, displays a dark, subterminal tract on the long arm. The long arm of chromosome IV of *P. ruber schencki* displays a faintly stained area similar to those that in *Triturus* (Mancino *et al.*, 1973) and in *Pleurodeles* (Bailly, 1976) have been linked to the presence of nucleolus-organizing regions.

B) *Eurycea* karyology.

The chromosome number $n = 14$, $2n = 28$ reported by Morescalchi (1975) for *E. longicauda* has been confirmed also for the subspecies *E. longicauda melanopleura* and the fourteen chromosome pairs can be arranged in three groups (A, B and C) in order of decreasing length. Group A comprises the five longest chromosome pairs of which I, II, III and V are found to be metacentric, with c.i. = 46.4; 43.6; 40.4 and 47.2, respectively. Pair IV was found to be submetacentric, with c.i. = 37.1 (Pl. II, fig. 1). Group B comprises the five pairs of medium-length chromosomes. Pairs VII, IX and X are metacentric, with c.i. = 45.7; 47.2 and 48.0, respectively. Pairs VI and VIII are submetacentric with c.i. = 37.1 and 35.6 (Pl. II, Fig. 1). Group C comprises

the four smallest chromosome pairs; XI and XIV are metacentric, with c.i. = 42.3 and 46.8, while XII and XIII are submetacentric, with c.i. = 35.2 and 26.6 (Pl. II, Fig. 1).

The C-banding method extensively stains the centromeric regions (Pl. II, Fig. 3). Evidence has also been found of double tracts in an intercalary position on a single arm of both chromosomes XIV.

According to Wake (1966), *Pseudotriton* and *Eurycea* are two genera which became separated very early during phylogenesis of tribe *Hemidactyliini*. The karyological data discussed in the present paper are not sufficient to confirm that the two genera have attained different degrees of evolution. They merely show that the species examined, although having a common chromosome number, display karyotypes that are only apparently similar and in no case superimposable, not even within the genus *Pseudotriton*. Not even the structural differentiation, evaluated by means of a comparative examination of specific C-band patterns, appears to give more precise indications of the degree of evolution attained. Indeed, *P. ruber schencki* and *E. longicauda melanopleura* display karyotype bandings that are similar in their staining intensity and C-band centromere extension, as well as because of the presence of double bands on the only chromosome XIV. On the other hand, *P. montanus diastictus* displays variation in the heterochromatin distribution since, in addition to the C-band pattern already detected in the other two species, there are also pericentric bands on most of the chromosome pairs and subterminal Giemsa-positive bands on chromosome III alone, i.e. on the most heterobrachial element of the *Hemidactyliinae* complements studied here. Unfortunately, the nuclear DNA content values of these *Hemidactyliinae*, evaluated by Sexsmith (1968) and Olmo (1973) using different methods, do not seem to be comparable and are thus of no help in improving our evaluation of the genetic distances between the genera and species considered. It may be stated, however, that ecological observations seem to indicate that *P. montanus* is evolutionarily more active than *P. ruber*. *P. montanus* is not in fact restricted to its ancestral environment (muddy bottoms of mountain streams) but seems to have begun a process of moving away from such an environment towards plainland areas and a more land-based life (Bruce, 1974).

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EXPLANATION OF PLATES I–II

PLATE I.

Fig. 1. – The karyotype of *Pseudotriton ruber schencki* (mitotic metaphase; gut; sex: ♀; Giemsa).

Fig. 2. – The karyotype of *P. montanus diastictus* (mitotic metaphase; gut; sex: ♀; Giemsa).

Fig. 3. – Mitotic metaphase of *P. montanus diastictus*. Chromosomes XIII are indicated by →; chromosomes XIV are indicated by * (gut; sex: ♀; C–banding method).

PLATE II.

Fig. 1. – The karyotype of *Eurycea longicauda melanopleura* (mitotic metaphase; liver; sex: ♀; Giemsa).

Fig. 2. – Mitotic metaphase of *P. ruber schencki*. Chromosomes XIV are indicated by * (gut; sex: ♀; C–banding method).

Fig. 3. – Mitotic metaphase of *E. longicauda melanopleura*. Chromosomes XIV are indicated by * (gut; sex; ♀; C–banding method).

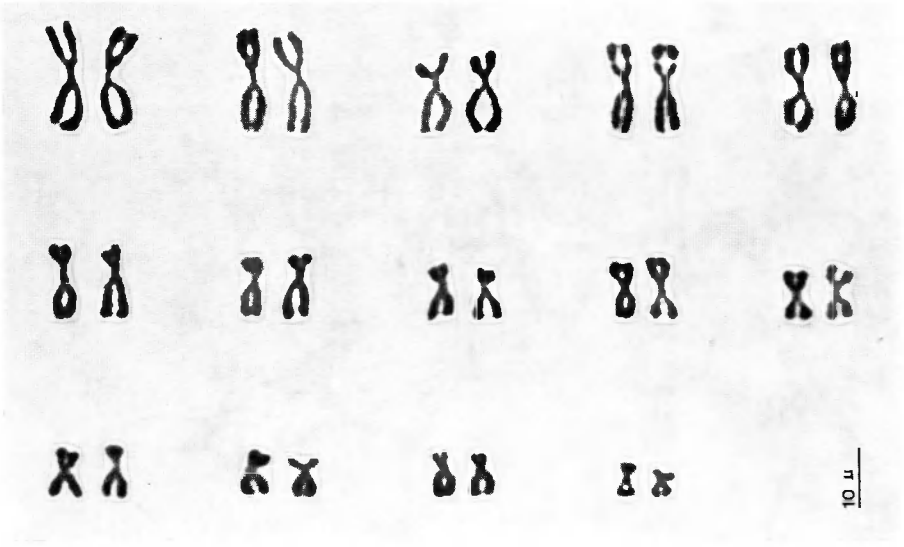

Fig. 1

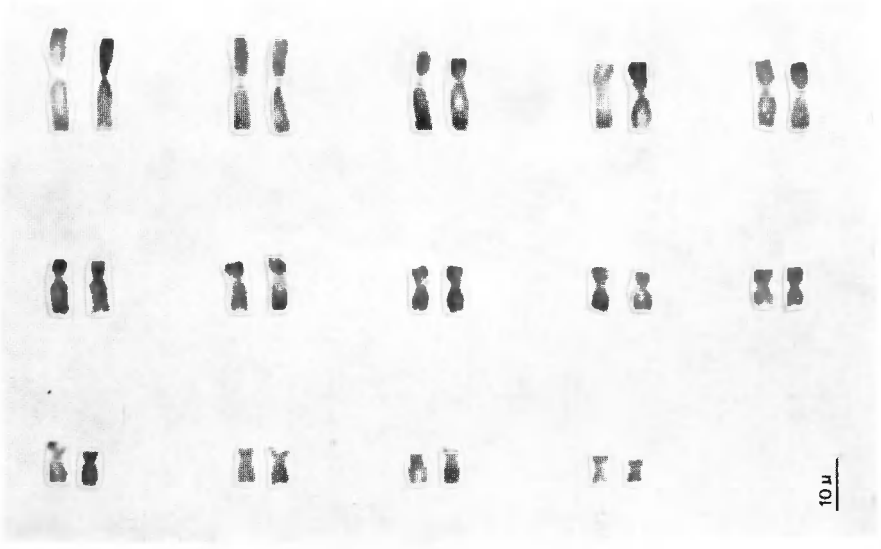

Fig. 2

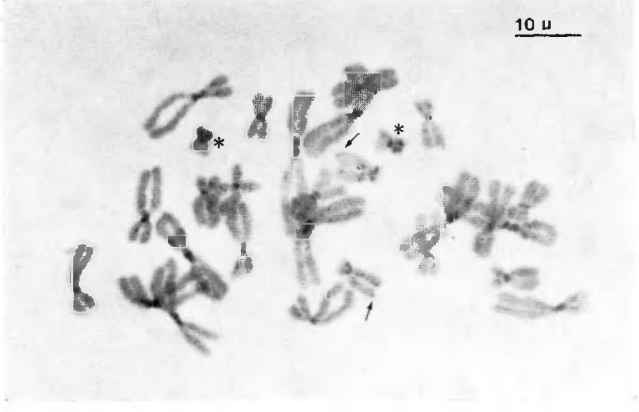

Fig. 3

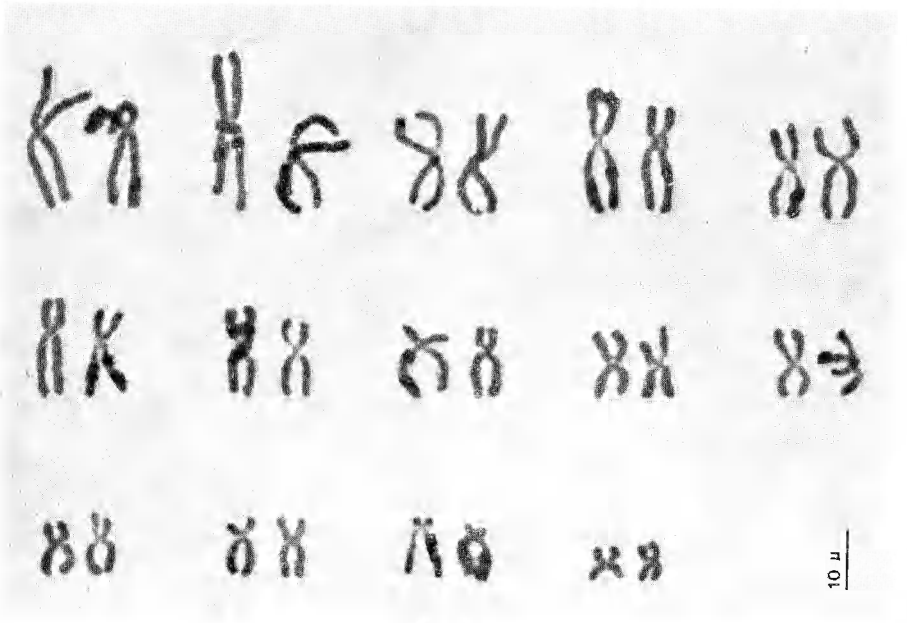

Fig. 1

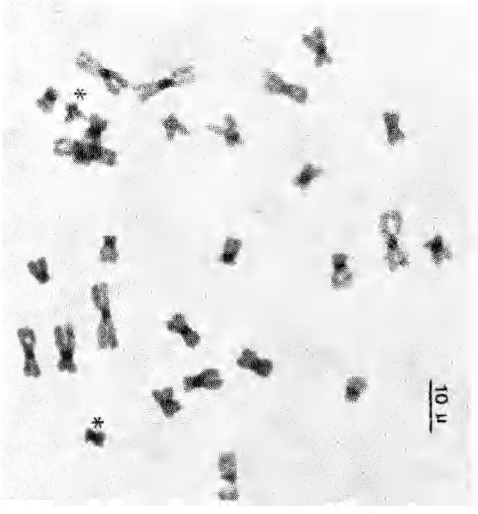

Fig. 2

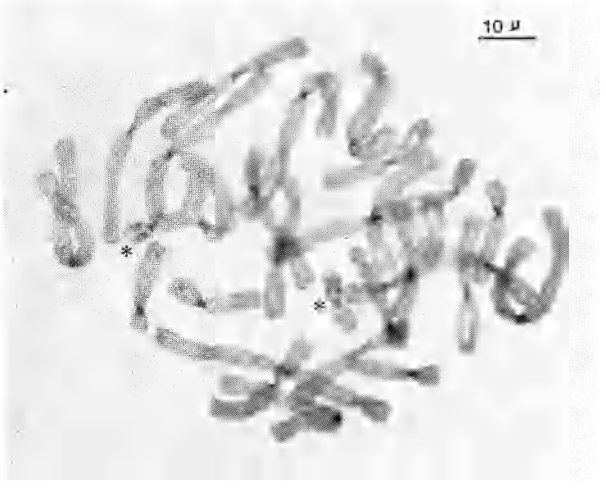

Fig. 3