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**Some considerations on the karyological evolution of
the Nearctic Salamandrids**

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Biologia. — *Some considerations on the karyological evolution of the Neoarctic Salamandrids* (*). Nota (**) di DONELLA LASCIALFARI, CARLA BOSCO, STEFANIA BUCCI-INNOCENTI, MATILDE RAGGHIANI e GIORGIO MANCINO, presentata dal Socio M. BENAZZI.

RIASSUNTO. — Sono stati studiati, ricorrendo al C-banding method, i cromosomi dei due generi di Salamandridi Neoartici, *Taricha* e *Notophthalmus*, i quali presentano numero cromosomico identico $2n = 22$, $n = 11$.

I cariotipi specifici e subspecifici appaiono largamente uniformi nell'ambito del genere *Taricha*; comunque essi non sono sovrapponibili e mostrano un diverso grado di reattività alla tecnica di bandeggio. Il cariotipo di *Notophthalmus* è solo apparentemente simile a quello di *Taricha*: esso presenta infatti cromosomi eterobrachiali di diverso tipo e con diversa distribuzione. Inoltre in *Notophthalmus*, il pattern delle C-bande è caratterizzato da una notevole quantità ed estensione dell'eterocromatina centromerica e da peculiari bande interstiziali e subterminali.

Nell'insieme, i dati cariologici avvalorano l'ipotesi che *Taricha* e *Notophthalmus* derivino da un unico progenitore europeo nel quale sia avvenuta la riduzione del numero cromosomico seguita da un particolare processo evolutivo che ha portato alle attuali divergenze morfologiche e strutturali dei cariotipi generici. Le minori differenze cromosomiche osservate nell'ambito del genere *Taricha* sembrano confermare le strette relazioni tassonomiche ipotizzate tra le singole specie e possono essere messe in relazione agli eventi cromosomici collegati ai processi di speciazione.

INTRODUCTION

The Neoafrican Salamandrids are represented by two genera: *Taricha* and *Notophthalmus*. The former comprises three species: *T. granulosa* (Skilton, 1849) with the subspecies *T. g. granulosa* (Skilton); *T. g. mazamae* (Mayers) and *T. g. twittyi* (Bishop); *T. rivularis* (Twitty, 1935); *T. torosa* (Rathke, 1833) with the subspecies *T. t. torosa* (Rathke) and *T. t. sierrae* (Twitty). The second genus comprises a single species *N. viridescens* (Rafinesque, 1820) with the subspecies *N. v. viridescens* (Rafinesque), *N. v. dorsalis* (Harlan) and *N. v. louisianensis* (Wolterstorff).

The chromosome number $2n = 22$, $n = 11$ shared by the Neoafrican Salamandrids differentiates them from the Palaearctic species which are characterised by $2n = 24$, $n = 12$ (Fankhauser and Kaylor, 1935; Henley and Costello, 1947; Makino, 1951; Seto and Pomerat, 1965). Significant morphological, ethological and physiological differences can be observed, however (Bishop, 1947; Coates and Metcalf, 1970; Arnold, 1977). Furthermore,

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the two Nearctic genera have quite different geographic distributions: *Taricha* is found throughout a wide coastal strip of Northwest America running from Alaska, to the North, to the South of California, while *N. viridescens* inhabits a vast area of Northeast America extending as far as Indiana and Michigan to the North, and Alabama to the South.

As we considered that any cytogenetic deductions made concerning the karyological evolution of the two genera are all the more valid the greater our knowledge of the morpho-structural characteristics of their karyotypes, it seemed useful to begin by preparing the hitherto unknown karyotype of *Taricha granulosa twittyi* and then to subject the chromosomes of as many *Taricha* and *Notophthalmus* species and subspecies as possible to banding techniques. The karyological data thus obtained were found to be useful in evaluating the degree of chromosomal differentiation reached by the two genera in the course of their evolution.

MATERIAL AND METHODS

The animals used in the present work consisted of 5 specimens of *T. granulosa twittyi* kindly made available by Dr Stevan Arnold (Chicago); 10 specimens of *T. rivularis* collected between April 30th and May 1st, 1977 near Camp Gualala on the Staggs Sprs. Rd (to Steward Pt.) in Sonoma County, California and 15 specimens of *T. torosa torosa* purchased from a specialised dealer at the end of Summer 1975 and whose collection sites are thus unknown. Also studied were 5 specimens of *N. viridescens viridescens* collected in the vicinity of Oxford (Ohio) and kindly sent to us by Dr Paul Daniel, Oxford.

All specimens received an intraperitoneal injection of 0.10 ml of colchicine 0.3% (Sigma, Che. Co.) before undergoing testicular biopsy. Whenever somatic tissues such as gut, spleen or liver were intended to be used, the specimens received a further two or three doses of colchicine at 48-hour intervals. The material thus removed was treated with hypotonic solution for 10 min and then fixed in 3:1 absolute ethyl alcohol—glacial acetic acid for 20–30 min and then dissociated in 45% acetic acid. Slides were then prepared by the normal dry-ice method. Some preparations were stained with dilute Giemsa in 0.01 M phosphate buffer (pH = 7); others were subjected to C-banding according to the method of Arrighi and Hsu (1971).

OBSERVATIONS

GENUS *Taricha*.

The chromosomes of *T. granulosa twittyi* were studied for the first time. The karyotype was divided into three groups (A, B, and C) of chromosome pairs of gradually decreasing length (Pl. I, Fig. 1). Group A comprises the four pairs of longer chromosomes: I, II and III were found to be meta-

centric (c.i. = 47.2; 44.7 and 42.2, respectively) while pair IV is submetacentric (c.i. = 35.8). Group B comprises the four pairs of medium-length chromosomes: pairs V and VI are metacentric (c.i. = 46.0 and 44.1) while pairs VII and VIII are submetacentric (c.i. = 36.7 and 36.5). Group C comprises the three smallest chromosome pairs (IX, X, XI) which are all metacentric (c.i. = 44.1, 46.7, 45.3). The *T. g. twittyi* karyotype thus consists of eight pairs of metacentric chromosomes and three pairs of submetacentrics, as our data shows to be true also in the other *Taricha* species and subspecies examined here. In this genus, therefore, no significant differences in general karyotype morphology have been observed, and the karyotypes may, on the whole, be considered as uniform even if not superimposable. These findings are in agreement also with the results of Seto and Pomerat (1965), Morescalchi (1975), Léon (1976), if allowance is made for the different methodologies and terminology used by these authors.

The application of the C-banding method to *T. g. twittyi* chromosomes has often revealed the presence of a slight centromeric granule. Giemsa-positive pericentric granules are present on one or both sides of the centromere, which are particularly evident on some of the B and C group chromosomes (Pl. I, Figs. 1-2). One of the longer metacentrics occasionally displays a band near the termination of the short arm (Pl. I, Fig. 2).

In the other two species examined the C-banding method gave the best results in *T. rivularis* as conspicuous simple pericentric bands are induced on all the chromosomes of the complement, particularly on those of groups B and C. A pair of longer metacentrics also shows clear subterminal tracts on the short arm. The centromeric regions are lightly marked only in a few preparations (Pl. II, Fig. 1). *T. t. torosa* is the species in which application of the C-banding method gave the least evident results (Pl. II, Fig. 2). The centromeric regions were lightly marked in a few chromosomes and light, dark-stained granules appear in one or both of the pericentric regions of part of the chromosome complement elements. The short arm of pair III has a Giemsa-positive tract in a terminal position, the long arm of chromosome IV in a subterminal position.

GENUS *Notophthalmus*.

Our work has confirmed the chromosome number indicated previously and has made it possible to define the morpho-structural characteristics of the karyotype of this genus which has already been analysed by Hutchison and Pardue (1975). However, these workers neglected to give the values of the centromeric indexes and, in order to define the morphology of the individual chromosomes and the C-band pattern, they used a different nomenclature to that normally used for the other Urodeles (Mancino *et al.*, 1977).

Also the chromosome complement of *N. v. viridescens* was divided into three groups (A, B and C) of chromosome pairs of gradually decreasing

length (Pl. III, Fig. 1). Group A consists of four pairs of the longest chromosomes (I, II, III and IV), all of which are metacentric (c.i. = 42.8; 45.7; 38.4 and 46.9, respectively). Pair III differs by being the most heterobrachial. Group B comprises the four medium-length chromosome pairs. Pairs V and VII were found to be submetacentric (c.i. = 31.4; 34.1), pair VI is metacentric (c.i. = 47.5) and pair VIII is subtelocentric (c.i. = 24.8), to use the terminology proposed by Levan *et al.*, (1964). Group C is composed of three pairs of shorter chromosomes (IX, X and XI), all of which are metacentric (c.i. = 42.9; 44.7 and 45.5, respectively).

After the application of the C-banding method all the chromosomes of the eleven pairs of the complement display a large, intensely stained centromere region (Pl. III, Figs. 1-2). Furthermore, on both sides of the centromere there are characteristic Giemsa-positive bands which, being situated at a more median position on the chromosome arms, perhaps because of the great amount of centromeric heterochromatin, may be more properly denoted as intercalary bands rather than pericentric ones as indicated in *Triturus* chromosomes (Mancino *et al.*, 1973; Nardi *et al.*, 1973). These bands are often resolved into one or more contiguous series of granules, especially when the chromosomes are somewhat despiralized. In agreement with Hutchison and Pardue (1975), one of the shorter pairs is characterized by a very thin Giemsa-positive band on the long arm. Also present are subterminal bands on the long arms of chromosomes I, III and V and on the short arm of chromosomes IV and VI (Pl. III, Figs. 1-2). These bands appear as simple tracts except in the subterminal region of the long arm of pair I. In fact, this region constantly displays two contiguous bands, the more distal of which is more conspicuous owing to its thickness and staining intensity (Pl. III, Fig. 1).

DISCUSSION

There are two hypotheses as to the origin of *Taricha* and *Notophthalmus*. The first of these, formulated by Noble (1931) and supported also by Steiner (1950), envisages an origin from two different stocks: *Notophthalmus* would be derived from an ancestral form from Western Europe; the genus *Taricha* would be of Asian origin. The second hypothesis was proposed by Wake and Özeti (1969) according to whom the two genera of Neartic Salamandrids derived from a common ancestral stock and their separation occurred before or during the Oligocene. At the moment, Wake and Özeti's hypothesis seems to be the most acceptable, not only in view of the more recent and up-to-date palaeontological and taxonomic data, but also because of the problems raised by chromosomal data regarding the acceptance of a dual origin for *Taricha* and *Notophthalmus*. Any such dual origin would, in fact, imply the existence of a karyological phenomenon of convergence, such as the loss of a chromosome pair (probably by the translocation of genetic material onto one or both of the complement pairs) occurring in a parallel fashion in the two ancestral stocks of different origin.

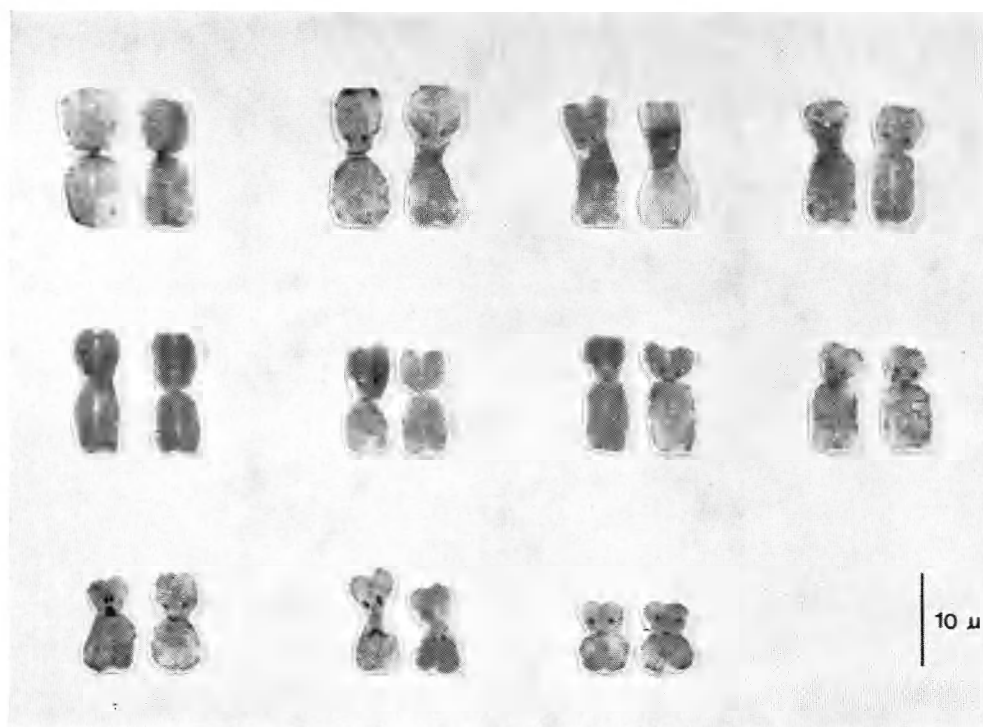


Fig. 1. - The karyotype of *T. granulosa twittyi* arranged from the same spermatogonium as in Fig. 2.



Fig. 2. - Spermatogonial metaphase of *T. g. twittyi* subjected to the C-banding method.



Fig. 1. - Mitotic metaphase of *T. rivularis* (gut; C-banding method).

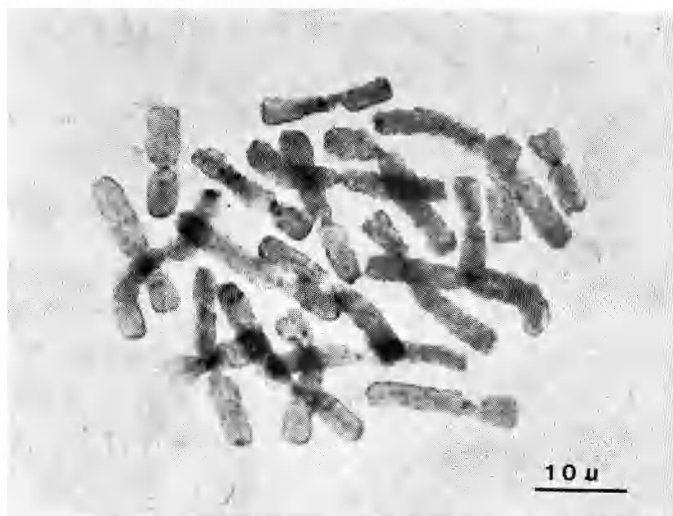


Fig. 2. - Mitotic metaphase of *T. torosa torosa* (testis; C-banding method).

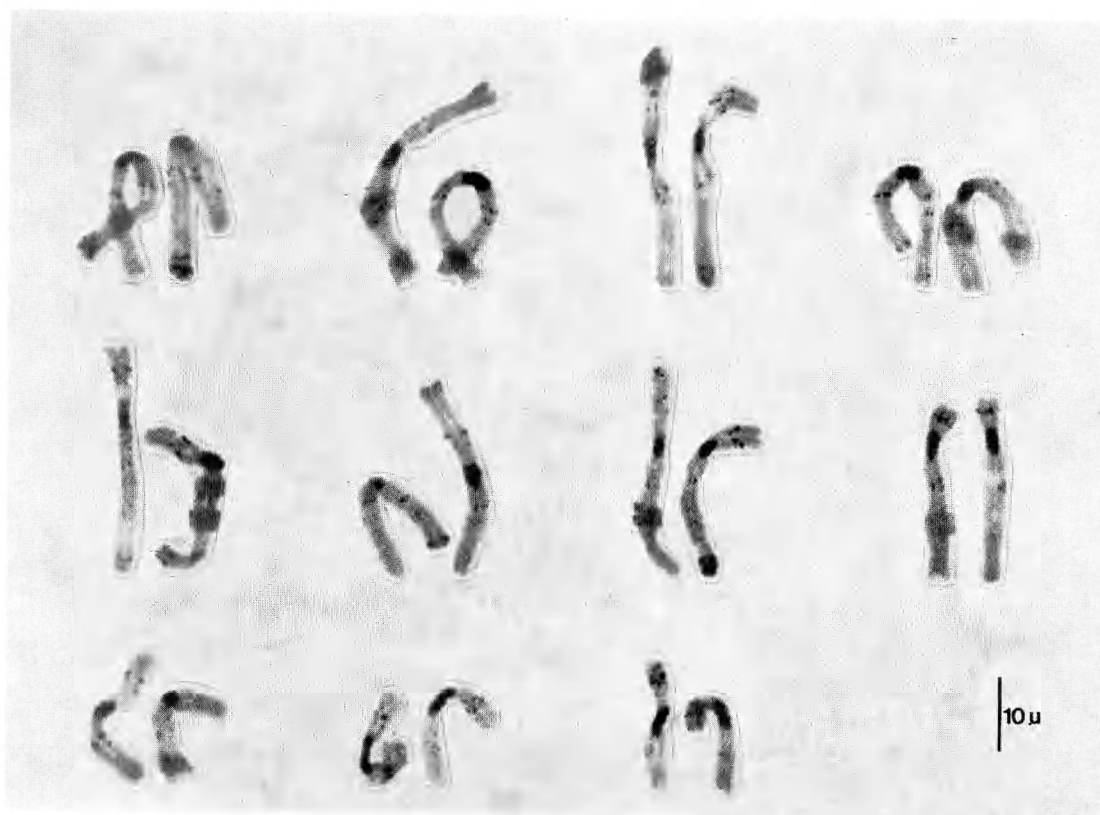


Fig. 1. - The karyotype of *N. viridescens viridescens* arranged from the same mitotic metaphase as in Fig. 2.

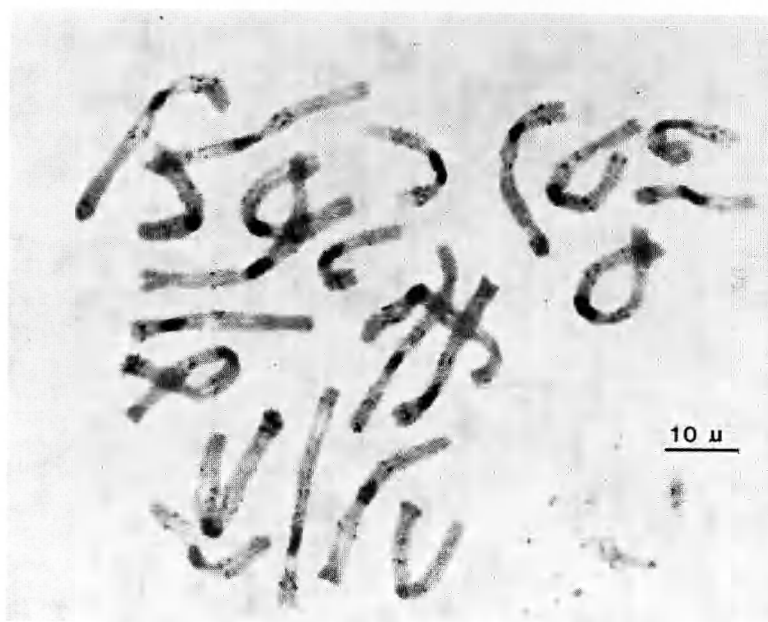


Fig. 2. - Mitotic metaphase of *N. v. viridescens* (gut; C-banding method).

In our present state of knowledge, such a convergence phenomenon would be hard to interpret. The hypothesis of a common origin would, on the other hand, imply the existence of a single, probably European, ancestral stock which, before the colonization of North America, underwent a series of rearrangements ending in the reduction of the chromosome number. Later geological phenomena, such as the glaciations mentioned by Steiner (1950), probably led to their separation and their different geographical distributions—*Taricha* to the West and *Notophthalmus* to the East—thus favouring the occurrence of further, although smaller rearrangements, resulting in the present different morpho-structural arrangement of the karyotypes. The hypothesis of a single ancestral stock would also be in line with Arnold's observations (1977) on the more general aspects of reproductive biology apparently shared by the European genus *Triturus* and the American ones *Taricha* and *Notophthalmus*. The smaller karyological differences we observed among *Taricha* species and subspecies may be related back to the successive speciation processes undergone by this genus according to mechanisms similar to those postulated for the genus *Triturus* (cf. Mancino *et al.*, 1977). The data obtained by the C-banding method, reported in the present paper, in conjunction with the nuclear DNA values known for the two genera (Bachmann, 1970; Olmo and Morescalchi, 1975), allow the *Taricha* species to be considered as strictly akin. This interpretation would be in agreement with the results of Twitty's interspecific hybridization experiments (1964) and the electrophoretic analysis data reported by Coates and Twitty (1967) and Hedgecock and Ayala (1974).

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