Experimental Hybridization Within the Genus Triturus
(Uroidea: Salamandridae)

III. Evidence for Crossing-over, True Chiasmata and Chromosomal Homologies
in the Spermatogenesis of F1 Species Hybrids,
T. cristatus carnifex ♀ × T. marmoratus ♂

Giorgio Mancino, Matilde Ragghianti and Stefania Bucci-Innocenti
Institute of Histology and Embryology, University of Pisa, 56100 Pisa, Italy

Abstract. Spermatogenesis in the F1 hybrid (2n = 24 = 12 ♀ + 12 ♂) between
the closely related newt species T. cristatus carnifex and T. marmoratus
was apparently normal up to pachytene. Many unpaired chromosomes were
present at diplotene and a typical diakinesis was lacking. Primary spermatocytes
at meta- and meta-anaphase contained up to 12 regular intergenomal
bivalents and a corresponding number of univalents when less then 12 H.
Most chiasmata were terminal or subterminal, some intercalary. Chiasmata
between corresponding heterospecific chromosomes can be reported as true:
real crossing over has taken place, proving the presence of primary chromo-
somal homologies between the 2 sets of the parental species. Evidence for
recombination is based on the segregation of particular markers (i.e., subter-
tinal C-bands and NORs) observed in certain chromosomes at metaphase
II. One chromatid of single chromosomes can show the T. cristatus “pheno-
type” and the other the T. marmoratus “phenotype”. A few primary sperma-
tocytes contain a certain number of irregular associations (intragenomal
or intrahaploid bivalents, irregular intergenomal bivalents, chromosome mul-
tivalents) joined by chiasmata which can be defined as anomalous. Other
abnormalities concern the occurrence of interlocked bivalents which occa-
sionally show an anomalous exchange between heterologous chromatids.
- Cytogenetic criteria useful to evaluate the taxonomic relationships between
different species have been discussed as well as some possible trends in
chromosome evolution and speciation within the genus Triturus.

Introduction

An accurate definition of the concept of species and of speciation mechanisms
is still fraught with problems, despite our increased knowledge and information
concerning both the animal and the plant kingdoms (cf. Dobzhansky, 1951;
Among the animals, the group of the urodele Amphibians provides useful mate-
rial for investigating chromosomal evolution and phylogenesis as it lends itself well to the application of up-to-date qualitative and quantitative karyological techniques, as well as to that of techniques for evaluating genetic distances by means of electrophoresis, immunoelectrophoresis and other biochemical methods (Barsacchi and Gall, 1972; Nardi et al., 1973; Olmo, 1973; Hedgecock and Ayala, 1974; Wilson et al., 1974; Pukkila, 1975; Macgregor and Mizuno, 1976; Old et al., 1977; Ragghianti et al., 1977; Sommerville et al., 1978; Varley and Morgan, 1978; Wake et al., 1978).

One of the most widely studied genera among Amphibians is Triturus, especially in respect to morphology, development and sexuality (cf. Spurway, 1945; Lantz, 1947; Benazzi, 1956) and meiotic anomalies of hybrids (White, 1946; Benazzi and Lepori, 1949; Spurway and Callan, 1950, 1960; Callan and Spurway, 1951; Lantz and Callan, 1954; Mancino, 1961; Mancino and Scali, 1964). By utilizing banding and molecular techniques, cytogenetics has demonstrated that a hybrid individual may be used as a highly suitable system for establishing the extent of any primary homology maintained by the sets of the two parent species after divergence from a common ancestor. A chromosomal analysis of the spermatogenesis and the oocyte lampbrush phase of F1 hybrids between unrelated species has already been carried out (Mancino et al., 1978 and in press).

The present paper deals with data obtained on the spermatogenesis of F1 hybrids between two closely related species, T. cristatus and T. marmoratus. On the basis of the results, cytogenetic criteria have been proposed for a better evaluation of the taxonomic distances between newt parent species, and certain chromosomal events connected with speciation have been hypothesized.

Material and Methods

T. cristatus (Laurenti, 1768) and T. marmoratus (Latreille, 1800) are 2 closely related species which occasionally give rise to natural hybrids where their ranges overlap (cf. Velle, 1959). These F1 hybrids have been reported as T. Blasii de l'Isle (1862) and T. Trouessarti Peracca (1886) depending on the direction of the cross (cf. Thorn, 1968). Accordingly, the two parental species have been included in the same species group or complex (Lantz, 1947).

Several females of T. c. carnifex were collected in ponds near Pisa, while the males of T. marmoratus were collected in the neighbourhood of Bordeaux and kindly supplied us by Dr. Silvio Bruno.

Ninety oviducal eggs of T. c. carnifex were artificially inseminated with sperm of T. marmoratus on 15 January 1976. Ninety percent eggs started cleavage. Two adult hybrids which displayed fully developed sexual characters of breeding males were first inspected on 29 December 1977: both of them had large fatty bodies and testes.

The hybrid T. c. carnifex ♀ x T. marmoratus ♂ (♀♂) (c/m♂1 in this paper) had a length of 10.5 cm; it was submitted to two testicular biopsies on 14 June 1978 and 21 September 1978. The hybrid male T. c. carnifex ♀ x T. marmoratus ♀ (♂♀) (c/m♀2 in this text) had a length of 10 cm and showed fully sized testes, entirely excised on 20 August 1978.

The hybrid material was not given any colchicine treatment. Testicular fragments of c/m♂1 were treated with distilled water for 10 min, then fixed in 1:3 glacial acetic acid-absolute ethanol for 20 min and finally dissociated and squashed in 45% acetic acid. The same technique was followed to make cytological preparations of testicular tissue of c/m♀2 apart from the hypotonic treatment. The preparations were made according to the dry ice method and then submitted to