ATTI ACCADEMIA NAZIONALE DEI LINCEI

CLASSE SCIENZE FISICHE MATEMATICHE NATURALI

RENDICONTI

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Chromosome constitution of newt interspecific hybrids of the genus Triturus (Urodela: Salamandridae)

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **61** (1976), n.6, p. 661–668.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1976_8_61_6_661_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1976.

Citogenetica. — *Chromosome constitution of newt interspecific hybrids of the genus* Triturus (*Urodela*: *Salamandridae*). Nota (*) di GIORGIO MANCINO, MATILDE RAGGHIANTI e STEFANIA BUCCI-INNOCENTI (**), presentata (***) dal Socio M. BENAZZI.

RIASSUNTO. — È stata studiata la costituzione cromosomica di larve ibride di tritone ricorrendo al C-*staining method*. La diploidia (in cui si verifica un egual contributo materno e paterno) è risultata la condizione più diffusa. Sono state anche rilevate larve aneuploidi (trisomiche) e poliploidi (triploidi e tetraploidi); la loro costituzione cromosomica ha permesso di interpretare l'origine di questi individui eteroploidi.

Esaminati nell'ambito della stessa cellula ibrida, i genomi delle diverse specie parentali appaiono differenziati, in maniera significativa, non solo per numero e morfologia delle C-bande, ma anche per il diverso contenuto di DNA nucleare e i diversi valori delle lunghezze cromosomiche.

INTRODUCTION

Hybridization is not considered to have played an important role in animal evolution because of the high degree of sterility normally caused by this condition (cf. Mayr, 1963; White, 1973). Nevertheless, the study of interspecific hybrids has provided useful information on several problems of evolutionary interest, such as those concerning the extent of reproductive isolating mechanisms among species, the high embryonic and larval hybrid mortality, the alteration of the sex ratio, the anomalies of meiotic events etc. (cf. Bataillon and Tchou, 1930 and 1932; White, 1946; Benazzi and Lepori, 1947; Lantz, 1947; Lantz and Callan, 1954; Benazzi, 1956; Spurway and Callan, 1960; Mancino and Scali, 1964; Kawamura and Nishioka, 1967; Scali and Mancino, 1968).

Recently, interspecific *Triturus* hybrids have been used also as a cytogenetic system which permits the comparison, within the same cell nucleus, of different specific genomes supposed to be under identical physiological conditions. Consequently, differences between parental species, based on data concerning chromosome size and shape, as well as amount and distribution of eu- and heterochromatin, gene arrangement and nuclear DNA C-values, have resulted highly significant for deductions referring to genetic and taxonomic relationships and to karyological evolution and speciation mechanisms (cf. Mancino *et al.*, in press).

In the present study we have carried on previous investigations (Ragghianti *et al.*, 1975) into the chromosome constitution of first generation newt species hybrids by means of the C-banding method: since this technique induces specific banding patterns on chromosomes, the identification within

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(*) This work was financially supported by C.N.R. Rome.

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(***) Nella seduta dell'11 dicembre 1976.

the hybrid complement of each parental set and of single elements is greatly facilitated. The results have confirmed the morphological and quantitative differences between specific genomes, and have revealed the occurrence of heteroploidy among first generation hybrids.

MATERIAL AND TECHNIQUES

Hybrids:

The interspecific hybrids here studied were obtained by heterospecific artificial insemination of the following *Triturus* species: *T. cristatus carnifex* (Laurenti, 1768), *T. vulgaris meridionalis* (Boulenger, 1882) and *T. alpestris apuanus* (Bonaparte, 1839).

The process of artificial insemination consists in the removal of sperm from the deferents and application of the sperm, concentrated or slightly diluted with a very small amount of NaCl 2 ‰, to oviductal eggs kept moist with the same saline. The females were previously injected with HCG (Human Chorionic Gonadotropin) which induces females to ovulate.

Controls:

Chromosomal observations were made in hatching larvae of *T. c. carnifex*, *T. v. meridionalis* and *T. a. apuanus* from eggs of freshly collected females not injected with HCG to verify the real occurrence of polyploidy in nature. Some observations refer also to larvae from eggs of injected females or from eggs inseminated with homospecific sperm, following the same manipulation of oviductal eggs and sperm which provides interspecific hybrids in the laboratory.

Cytological techniques:

First generation hybrid larvae were kept for 24 h in 3 ‰ colchicine (Sigma) as soon as they reached the hatching stage. In this phase, approximately corresponding to stage 34–35 of Gallien and Durocher (1957), the number of mitotic cells is sufficiently high to allow a broad karyological analysis. The larvae were subsequently fixed in 3 : 1 absolute ethanol plus glacial acetic acid. The preparations were made following the dry-ice method and then treated with the C-banding method according to Arrighi and Hsu (1971). The preparations showing no C-bands were used for numerical observations on ploidy and, when possible, for karyotypic reconstruction following only the specific morpho-metric data.

Criteria for karyotyping:

Each parental set was first recognized by comparing its banding appearance to the specific banding patterns of the parental species (cf. Mancino *et al.*, 1973 for *T. c. carnifex*; Nardi *et al.*, 1973 for *T. v. meridionalis* and Mancino *et al.*, in press, for *T. a. apuanus*), or, lacking C-bands, to the normal karyotypes. Then the haploid alignment was made according to the morphometric parameters (length and centromere index). The matching of the homologues in triploid and tetraploid complements showed the same difficulties and discrepancies which sometimes are encountered when karyotyping concerns pure species. The reason for such discrepancies (either differential effects of colchicine on coiling of single chromosomes or existence of « a polymorphism without known genetic consequences » according to Sybenga, 1972) still remains doubtful.

The chromosome morphology was defined using the terminology proposed by Levan, Fredga and Sandberg (1964).

It is useful to recall that all the European Salamandrid species have $n = 12$, $2n = 24$.

Terminology:

Winckler (1916), followed by a number of cytologists, used the term " heteroploidy " for indicating any deviation affecting the diploid number ($2n$) typical of the species or, more generically, its ploidy rate: then heteroploidy comprises the cases of eupolyploidy (eutriploidy, eutetraploidy, etc.) as well as aneuploidy which concerns non balanced deviations from the specific diploid complement (monosomy, nullisomy, polysomy, etc.) (see also Täckholm, 1922).

OBSERVATIONS AND DISCUSSION

The commonest chromosome constitution among first generation newt species hybrids is diploidy, where the haploid sets of both parent species are present (Table I). From a strictly numerical point of view, this constitution can be considered balanced and indicated as $2n = n♀ + n♂$ (Plate I). The calculation of the chromosome lengths of diploid F_1 hybrids has constantly indicated different values between the genomes of the 2 parental species (Table II). These differences can be related to the difference of nuclear DNA values characterizing the species used in our experiments (Olmo, 1973, 1974 and personal communication; Olmo and Morescalchi, 1975; Morescalchi, 1975; see also Mancino *et al.*, in press).

TABLE I

Chromosome constitution of interspecific hybrid newt larvae

Hybrids	No. Larvae	Ploidy			
		Diploidy ($2n, n♀+n♂$)	Aneuploidy ($2n, n♀+$ $+n♂+1C$)	Triploidy ($3n, 2n♀+n♂$)	Tetraploidy ($4n, 2n♀+2n♂$)
T. v. meridionalis ♀ × *T. c. carnifex* ♂	33	28	1	3	1
T. c. carnifex ♀ × *T. v. meridionalis* ♂ . . .	36	35	—	1	—
T. v. meridionalis ♀ × *T. a. apuanus* ♂	4	3	—	—	1

TABLE II

Statistical analysis on 8 metaphase cells of diploid hybrid larvae
T. c. carnifex ♀×T. v. meridionalis ♂

Hybrids	Total length of hybrid complement (μ)	Length of genome *T. c. carnifex* (μ)	%	Length of genome *T. v. meridionalis* (μ)	%
1	651	303	46.54	348	53.46
	590	270	45.76	320	54.24
2	561	262	46.70	299	53.30
3	560	260	46.43	300	53.57
	494	227	45.95	267	54.05
	456	210	46.05	246	53.95
4	750	350	46.67	400	53.33
	400	190	47.50	210	52.50

According to the method of paired comparisons calculated on actual lengths, $t = 4.13$ with $0.01 > P > 0.001$.

Aneuploid deviants from such constitution, for instance trisomic larvae, have been detected. Also triploid and tetraploid hybrids occur and their constitutions can be expressed as $3n = 2n♀ + n♂$ and $4n = 2n♀ + 2n♂$ respectively. In the first case, the karyological analysis has ascertained the presence of two sets from the maternal parent and one from the paternal parent: this can be interpreted as due to fertilization of an egg which was diploid because it retained the second polar body at meiotic anaphase II. Concerning tetraploidy, the presence of double sets of both parent species suggests that chromosome doubling occurred in the zygote or at very early cleavage, although such an event is considered rare in the normal ontogenetic development (Astaurov, 1969). As a rule, some specimens defined as triploid and tetraploid should be considered more properly aneutriploid and aneutetraploid mosaics: in fact each complement lacks one or more chromosomes because an elimination process can affect the single cells to a different extent during larval development. This elimination seems to preferentially concern the chromosomes belonging to the paternal set(s): if so, it can be interpreted as an attempt to restore the diploid complement of the maternal parent which provides also the egg cytoplasm.

A series of hybrids anomalous in the external appearance of the larvae (microcephaly, edema and ascites, undersized gills, lack of circulation, etc.)

turned out to possess a normally diploid chromosome complement. On the contrary, no relevant phenotypic anomaly was manifestly evident among the aneuploid and polyploid hybrid larvae studied here. These findings suggest fuller research on the frequency and the morphological consequences of possible karyopathies associated with the hybrid condition. Particularly noteworthy is the study of morphogenetic, anatomical and physiological alterations caused by the presence of 2 different specific genomes inside the same cell nucleus, as well as the analysis of the effects of the combination of both qualitative (hybridization) and quantitative (heteroploidy) chromosomal variations. This research can usefully run side by side with a biochemical and electrophoretic investigation which could allow the discovery of anomalies identifiable at the metabolic level related to an anomalous genetic constitution.

In detail, the results are the following:

a) *T. v. meridionalis* ♀×*T. c. carnifex* ♂.

The great majority of the larvae of this cross resulted to be normally diploid (Table I). However, one larva was trisomic, its diploid complement constantly including an extra chromosome belonging to the group of the shortest elements (group C): therefore this trisomy can be indicated as $2n = n♀ + n♂ + 1\,C$ (the species to which the extra chromosome belongs would presumably be the paternal parent). The phenotype of this trisomic larva was normal, meaning that this trisomy is compatible with apparently normal embryonic development and regular larval morphogenesis at least until the stage when the specimen was killed. On the other hand, we can recall that trisomy has been often detected among *Pleurodeles* adults obtained under experimental conditions (Gallien *et al.*, 1965; Gallien, 1969); one trisomic *Pleurodeles* female turned out to be fertile and have normally developed ovaries (Lacroix, 1967).

Among F_1 hybrids *T. v. meridionalis* ♀×*T. c. carnifex* ♂, there were also two eutriploid hybrid larvae, in which 2 complete sets of the maternal parent (*vulgaris*) and 1 of the paternal parent (*cristatus*) were present in all the mitotic metaphases ($3n = 2n♀ + n♂$) (Plate IV, fig. 1 and Plate II). Another triploid hybrid larva turned out to be a chromosomal mosaic constituted by eutriploid and aneutriploid cells (Plate III, *a* and *b*). Lastly, a hybrid specimen studied only on unstained preparations examined under phase-contrast was tetraploid, made up of eu- and aneutetraploid cells.

No heteroploid larvae of this cross appeared to be phenotypically anomalous.

b) *T. c. carnifex* ♀×*T. v. meridionalis* ♂.

Only one specimen was a triploid mosaic showing a number of chromosomes of about $3n$ (Table I). In spite of this anomalous constitution, the primitive presence of a double set of the maternal species can be proved by the existence of several, if not all, chromosome pairs of *T. c. carnifex*. Its constitution, therefore, can be indicated as $3n = 2n♀ + n♂$.

Four larvae were basically diploid and showed a number of rearrangements and anomalies, such as dicentric chromosomes (Plate IV, fig. 2 and Plate V), centric fragments which appear as telocentric chromosomes (Plate VI, fig. 1), acentric fragments and breaks, which can alter the normal mitotic feature.

In spite of these atypical constitutions, no larvae of this cross appeared to be phenotypically anomalous at the hatching stage.

c) *T. v. meridionalis* ♀ × *T. a. apuanus* ♂.

A case of tetraploidy was detected in this cross (Table I). This specimen appeared as a phenotypically normal larva. Its tetraploid constitution can be expressed by the formula $4n = 2n♀ + 2n♂$: in fact, part of the cells showed 48 (eutetraploid) chromosomes. Within their complements, 2 sets were recognized as belonging to *vulgaris* and 2 to *alpestris*. This suggests that a doubling of the diploid complement occurred in the zygote or in the first blastomeres at very early cleavage. Further attempts at doubling seem to account for the presence of rare cells with chromosome numbers of about 96 (octoploid) per nucleus.

However, a chromosome elimination seems to have concerned most cells. Their mitoses very often were affected by breaks resulting in the formation of dicentric chromosomes, and in the presence of centric and acentric fragments. Thus, besides eutetraploid cells, others have been found which show fewer chromosomes. Frequently, there were cells containing 46 chromosomes plus a dicentric. Plate VI, fig. 2 and Plate VII refer to one of these cells, where the entire diploid complement of the maternal parent (*vulgaris*) could be recognized.

Finally, it is noteworthy that polyploidy occurs in the anuran genera *Ceratophrys*, *Odontophrynus* and *Hyla* (Beçak *et al.*, 1966, 1967; Saez and Brum-Zorrilla, 1966; Bogart, 1967; Wasserman, 1972) as well as in the major frog families *Ranidae* and *Bufonidae* (Bogart and Tandy, 1976). These last authors, therefore, believe that polyploidy represents a widespread and important evolutionary phenomenon in anuran amphibians.

Differently, among Urodeles, spontaneous polyploidy is still considered very rare (Asher and Nace, 1971) and it is reported to be limited to the genera *Ambystoma*, *Eurycea* and *Notophthalmus* (Fankhauser, 1938, 1939, 1945; Fankhauser and Humphrey, 1942; Uzzell, 1963, 1964). In European Salamandrids, only one adult triploid specimen, a male of *T. taeniatus* (= *T. vulgaris*) in active although anomalous spermatogenesis, has been found in nature so far (Böök, 1940). By contrast, we have noticed a significant incidence of polyploidy (mainly triploidy) among the larvae used as a control in the present work (paper in preparation). These observations are in agreement with previous cytological findings concerning the presence of unreduced eggs (Humphries, 1956; Mancino and Poggi, 1963) and they are also in agreement with reports which show that triploidy is the commonest aberration among Salamandrid larvae (1–2 % according to Fankhauser, 1945).

Considering that the incidence of adult polyploids among F_1 hybrids is rather high (see Lantz and Callan, 1954; Mancino and Scali, 1964; Scali and Mancino, 1968), it can be assumed that the combination between polyploidy and hybridization could often represent an advantageous condition enabling allopolyploids to complete metamorphosis: a subject which needs further investigation.

REFERENCES

ARRIGHI F. E. and HSU T. C. (1971) – *Localization of heterochromatin in human chromosomes,* « Cytogenetics », *10*, 81–86.

ASHER J. H., jr. and NACE G. W. (1971) – *The genetic structure and evolutionary fate of parthenogenetic amphibian populations as determined by markovian analysis,* « Am. Zoologist », *11*, 381–398.

ASTAUROV B. L. (1969) – *Experimental polyploidy in animals,* « Annual review of genetics » (Roman H. L., ed.), pp. 99–126. Palo Alto, California.

BATAILLON E. and TCHOU S. (1930) – *Avortement de la gamétogenèse chez des hybrides d'Urodèles de deux ans,* « C. R. Acad. Sci. Paris », *191*, 690–692.

BATAILLON E. and TCHOU S. (1932) – *Croisements en retour (2^e génération) entre une femelle hybride d'Urodèle et les mâles des deux types parentaux,* « C. R. Acad. Sci. Paris », *195*, 449–452.

BEÇAK M. L., BEÇAK W. and RABELLO M. N. (1966) – *Cytological evidence of constant tetraploidy in the bisexual South American frog* Odontophrynus americanus, « Chromosoma », *19*, 188–193.

BEÇAK M. L., BEÇAK W. and RABELLO M. N. (1967) – *Further studies on polyploid amphibians* (Ceratophrydidae). I. *Mitotic and meiotic aspects,* « Chromosoma », *22*, 192–201.

BENAZZI M. (1956) – *Il sesso negli ibridi di tritoni e la questione della digametia sessuale negli urodeli,* « Ricerca Scient. », Suppl., *26*, 3–11.

BENAZZI M. and LEPORI N. G. (1947) – *Le gonadi nei maschi dell'ibrido* Triton cristatus ♀× Triton vulgaris ♂, « Sci. Genet. », *3*, 1–18.

BOGART J. P. (1967) – *Chromosomes of the South American amphibian family* Ceratophridae *with a reconsideration of the taxonomic status of* Odontophrynus americanus, « Can. J. Genet. Cytol. », *9*, 531–542.

BOGART J. P. and TANDY M., (1976) - *Polyploid amphibians: three more diploid–tetraploid cryptic species of frogs,* « Science », *193*, 334–335.

BÖÖK J. A. (1940) – *Triploidy in* Triton taeniatus *Laur.*, « Hereditas », *26*, 107–114.

FANKHAUSER G. (1938) – *Triploidy in the newt,* Triturus viridescens, « Proc. Am. Phil. Soc. », *79*, 715–739.

FANKHAUSER G. (1939) – *Polyploidy in the salamander* Eurycea bislineata, « J. Hered. », *30*, 379–388.

FANKHAUSER G. (1945) – *The effects of changes in chromosome number on amphibian development,* « Quart. Rev. Biol. », *20*, 20–78.

FANKHAUSER G. and HUMPHREY R. R. (1942) – *Induction of triploidy and haploidy in axolotl eggs by cold treatment,* « Biol. Bull. », *83*, 367–374.

GALLIEN L. (1969) – *Anomalies chromosomiques provoquées et mutations chez les Amphibiens,* « Ann. Biol. », *8*, 319–331.

GALLIEN L. and DUROCHER M. (1957) – *Table chronologique du développement chez* Pleurodeles waltlii *Michah.*, « Bull. Biol. France et Belgique », *91*, 97–114.

GALLIEN L., LABROUSSE M., PICHERAL B. and LACROIX J.-C. (1965) – *Modifications expérimentales du caryotype chez un Amphibien Urodèle Pleurodeles waltlii Michah. par irradiation de l'oef et la greffe nucléair,* « Rev. Suisse Zool. », *72*, 59–66.

HUMPHRIES A. A., jr (1956) – *A study of meiosis in coelomic and oviducal oocytes of* Triturus viridescens *with particular emphasis on the origin of spontaneous polyploidy and the effects of heat shock on the first meiotic division,* « J. Morph. », *99*, 97–135.

Kawamura T. and Nishioka M. (1967) – *On the sex and reproductive capacity of tetraploids in Amphibians*, « Gunma Symp. on Endocrinology », *4*, 23–39.

Lacroix J.-C. (1967) – *Obtention de femelles trisomiques fertiles chez l'Amphibien Urodèle* Pleurodeles waltlii *Michah.*, « C. R. Acad. Sc. Paris », *264*, 85–88.

Lantz L. A. (1947) – *Hybrids between* Triturus cristatus *Laur. and* Triturus marmoratus *Latr.*, « Proc. Zool. Soc. Lond. », *117*, 247–258.

Lantz L. A. and Callan H. G. (1954) – *Phenotypes and spermatogenesis of interspecific hybrids between* Triturus cristatus *and* Triturus marmoratus, « J. Genet », *52*, 165–185.

Mancino G., and Poggi M. (1963) – *Prime osservazioni sulla meiosi femminile in alcuni Anfibi Urodeli*, « Atti Acc. Naz. Lincei », *35*, 591–597.

Mancino G. Ragghianti M. and Bucci-Innocenti S. (1973) – *I cariotipi di* Triturus marmoratus *e* T. cristatus *studiati con il « C-staining method »*, « Rend. Acc. Naz. Lincei (Roma) », *55*, 559–564.

Mancino G., Ragghianti M. and Bucci-Innocenti S. – *Cytotaxonomy and cytogenetics in European newt species*, « The Reproductive Biology of Amphibians » (Taylor D. H. and Guttman S. I. eds.), New York: Plenum Press (in press).

Mancino G. and Scali V. (1964) – *Sviluppo embrionale, fenotipo e spermatogenesi di ibridi allopoliploidi da reincrocio*: Triturus helveticus/vulgaris ♀×T. vulgaris ♂, « Caryologia », *17*, 161–194.

Mayr E. (1963) – *Animal species and evolution*, Belknap Press, Harvard Univ., Cambridge, Mass.

Morescalchi A. (1975) – *Chromosome evolution in the Caudate amphibia*. In: « Evolutionary Biology » VIII (Dobzhansky, T., Hecht, M. K., Steere, W. C., eds.), pp. 339–387. New York and London, Plenum Press.

Nardi I., Ragghianti M. and Mancino G. (1973) – *Banding patterns in newt chromosomes by the Giemsa stain*, « Chromosoma », *40*, 321–331.

Olmo E. (1973) – *Quantitative variations in the nuclear DNA and phylogenesis of the Amphibia.* « Caryologia », *26*, 43–68.

Olmo E. (1974) – *Further data on the genome size in the Urodeles*, « Boll. Zool. », *41*, 29–33.

Olmo E., and Morescalchi A. (1975) – *Evolution of the genome and cell size in Salamanders*, « Experientia », *31*, 804–406.

Ragghianti M., Bucci-Innocenti S. and Mancino G. (1975) – *Indagine citogenetica su ibridi interspecifici di* Triturus (*Anfibi Urodeli*), « Boll. Zool. », *42*, 482–483.

Saez F. A. and Brum-Zorrilla N. (1966) – *Karyotype variation in some species of the genus* Odontophrynus (*Amphibia-Anura*), « Caryologia », *19*, 55–63.

Scali V. and Mancino G. (1968) – *Osservazioni sulla spermatogenensi di ibridi poliploidi* Triturus helveticus/vulgaris ♀ × T. vulgaris ♂, « Atti Soc. Tosc. Sc. Nat. », *75*, 8–18.

Spurway H. and Callan H. G. (1960) – *The vigour and male sterility of hybrids between the species* Triturus vulgaris *and* T. helveticus, « J. Genet », *57*, 84–117.

Sybenga J. – *General cytogenetics*, Amsterdam, London and New York: North-Holland/ American Elsevier.

Täckholm G. (1922) – *Zytologische Studien über die Gattung*, « Rosa. Proc. Okla. Acad. Sci. », *22*, 137–138.

Uzzell T. M., jr. (1963) – *Natural triploidy in salamanders related to* Ambystoma jeffersonianum, « Science », *139*, 113–115.

Uzzell T. M., jr. (1964) – *Relations of the diploid and triploid species of the* Ambystoma jeffersonianum *complex* (*Amphibia, Caudata*), « Copeia », *1964*, 258–300.

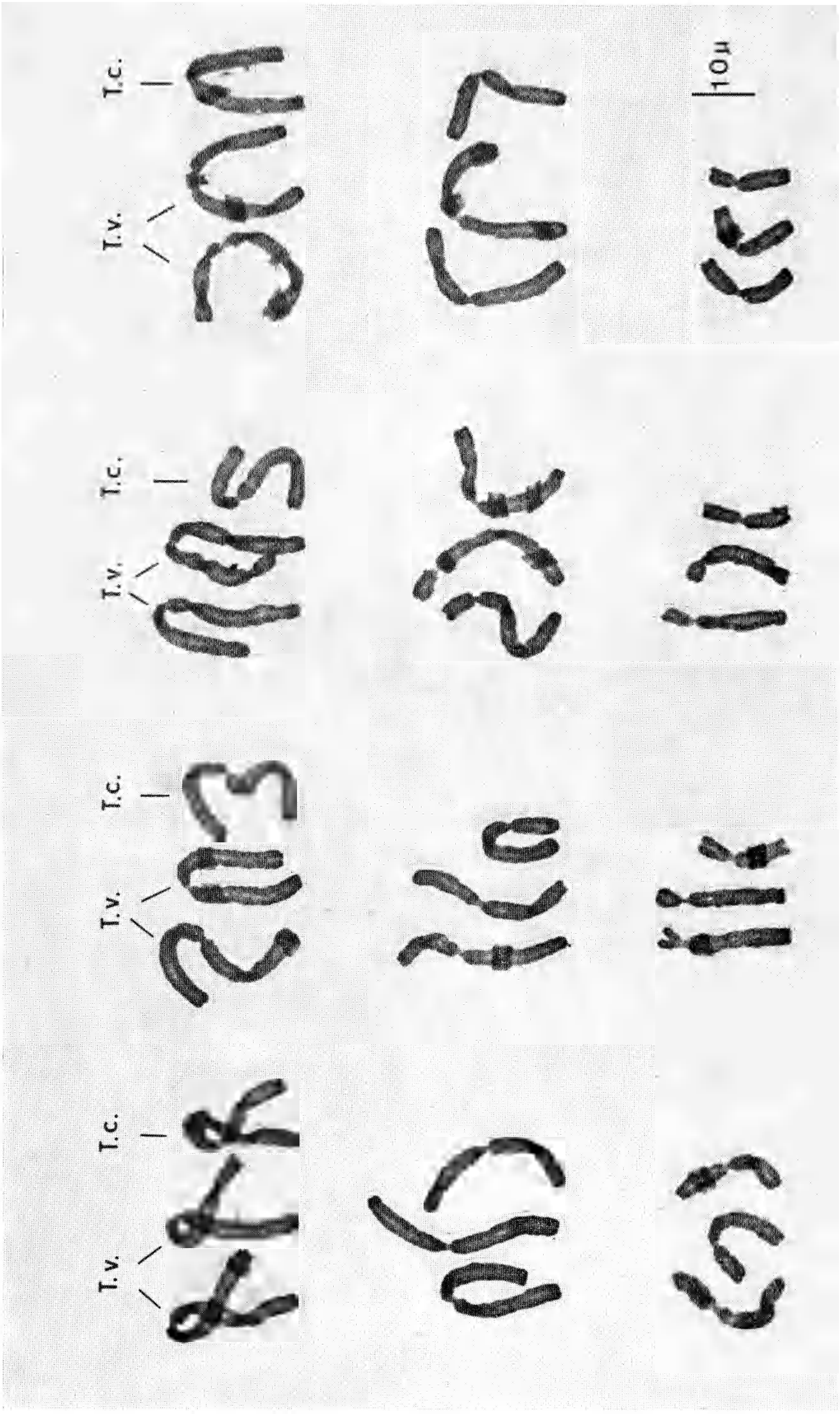
Wasserman A. (1970) – *Polyploidy in the common tree toad* Hyla versicolor *Le Conte*, « Science », *167*, 385–386.

White M. J. D. (1946) – *The spermatogenesis of hybrids between* Triturus cristatus *and* T. marmoratus (*Urodela*), « J. exp. Zool. », *102*, 179–207.

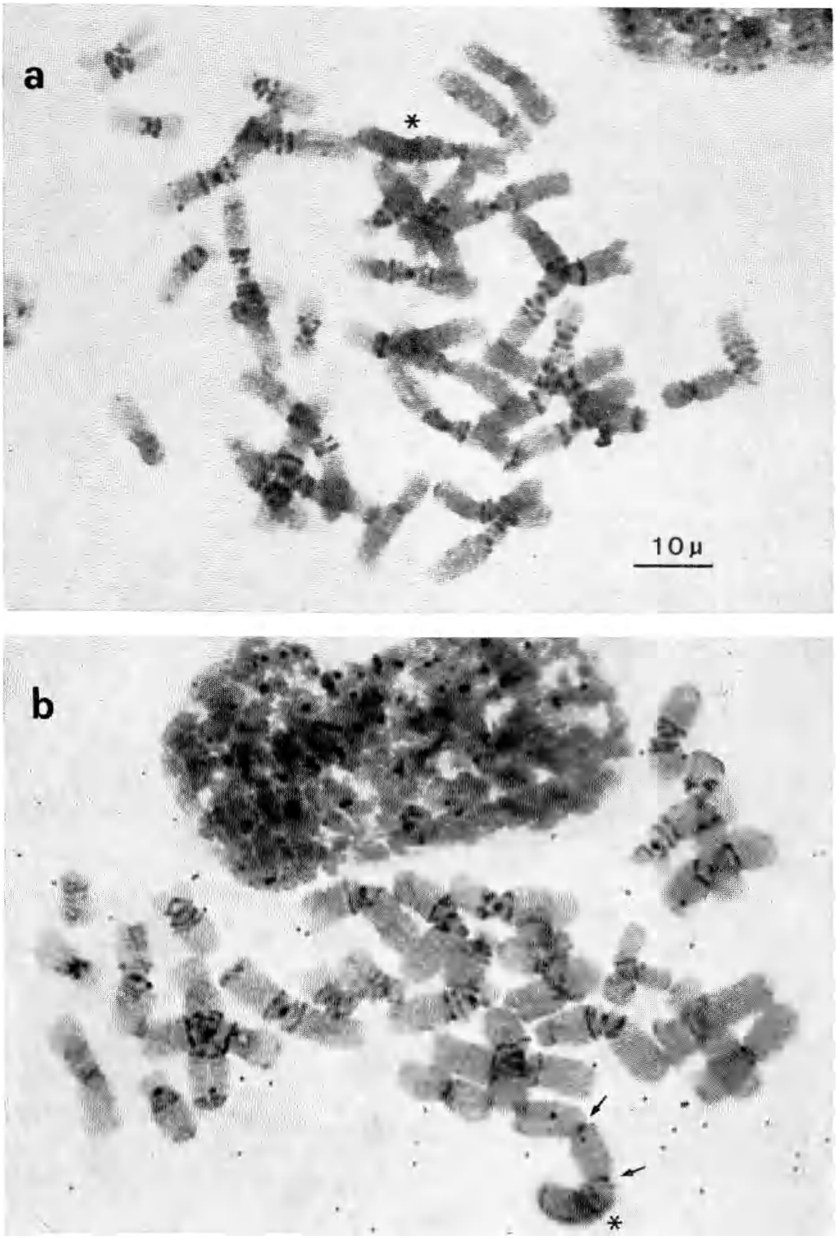
White M. J. D. (1973) – *Animal cyiology and evolution*, 3rd ed. London: Cambridge Univ. Press.

Winckler H. (1916) – *Über die experimentelle Erzeugung von Pflanzen mit Abweichen den Chromosomenzahlen*, « Z. Bot. », *8*, 471–531.

A diploid hybrid larva *T. c. carnifex* ♀ × *T. v. meridionalis* ♂: its karyotype comprises the 12 chromosomes of the haploid set of the maternal parent *cristatus* (upper alignment) and the 12 of the haploid set of the paternal parent *vulgaris* (lower alignment). Chromosome I of *T. c. carnifex* is represented by the shorter partner. C-banding method.



The metaphase chromosomes from Plate IV, fig. 1 into a karyotype.



A triploid hybrid larva *T. v. meridionalis* ♀ × *T. c. carnifex* ♂ characterized by a chromosomal mosaic. *a*) cell nucleus of 36 chromosomes; *b*) cell nucleus of 31 chromosomes + 1 dicentric. The dicentric is constituted by chromosomes of *cristatus*, one of which is undoubtedly chromosome I. Arrows indicate the 2 centromeres of the dicentric chromosome. * indicates the "heteromorphic" region of *cristatus* chromosome I. C-banding method.

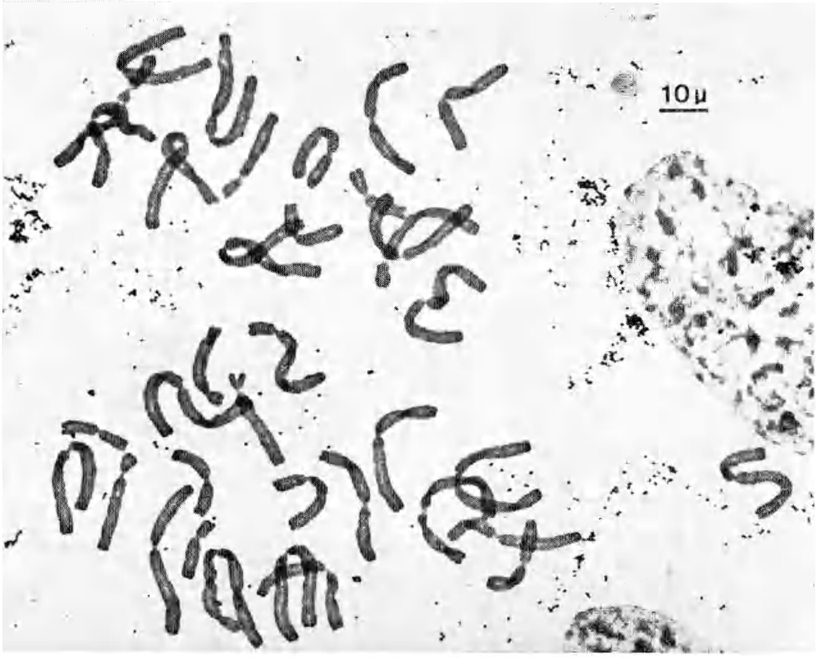
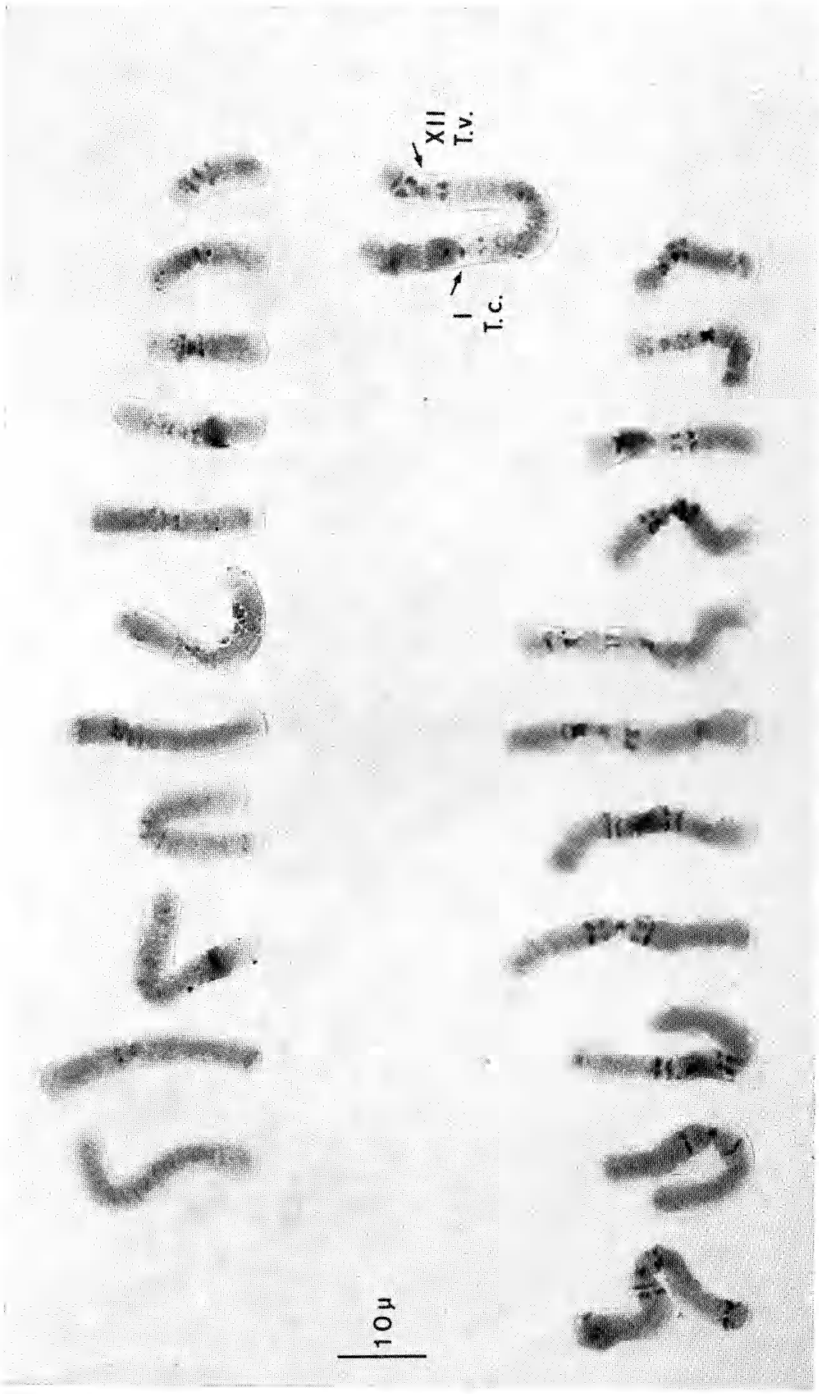


Fig. 1. – Metaphase cell nucleus of a triploid hybrid larva *T. v. meridionales* ♀ × *T. c. carnifex* ♂.

Fig. 2. – Metaphase cell nucleus of a hybrid larva *T. c. carnifex* ♀ × *T. v. meridionalis* ♂ (22 chromosomes + 1 dicentric). Arrows indicate the 2 centromeres of the dicentric chromosome (its constitution is shown in the next figure). C – banding method.



Karyotype of the cell nucleus of Plate IV, fig. 2. Upper alignment: chromosomes II–XII of *T. c. carnifex*. Lower alignment: chromosomes I–XI of *T. v. meridionalis*. Centre: the dicentric chromosome constituted by *cristatus* chromosome I and *vulgaris* chromosome XII. Centromeres are indicated by arrows. C-banding method.

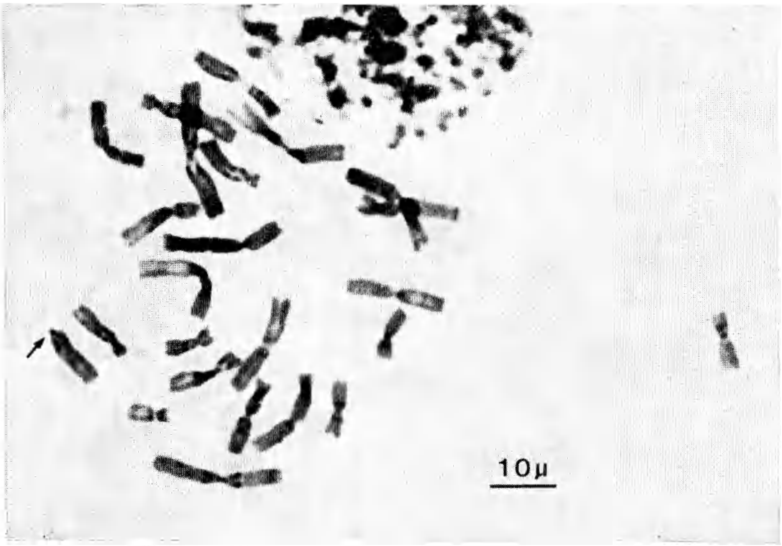


Fig. 1. – A diploid hybrid larva *T. c. carnifex* ♀ × *T. v. meridionalis* ♂. The centric fragment (telocentric chromosome) is indicated by an arrow. C – banding method.

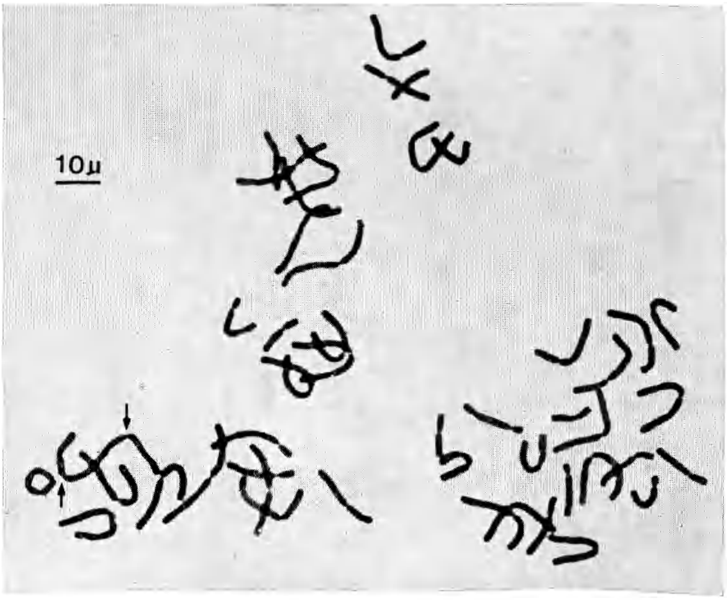
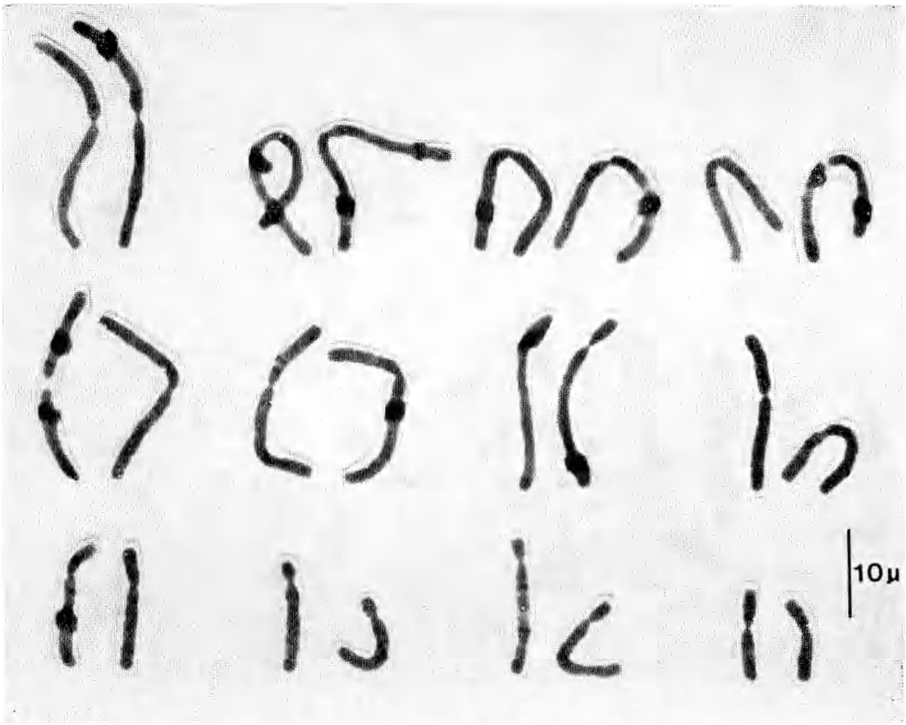


Fig. 2. – Mitotic chromosomes of a hybrid larva *T. v. meridionalis* ♀ × *T. a. apuanus* ♂: 46 chromosomes (24 of which belonging to *T. vulgaris*) + a dicentric, entirely constituted by *alpestris* chromosomes (arrows indicate the centromeres).



Same metaphase as Plate VI, fig. 2. The 24 chromosomes from the maternal parent have been arranged into a karyotype.