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CHROMOSOMA

Chromosome and C-heterochromatin polymorphisms in the Italian newt, *Triturus italicus*

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Abstract. A combined chromosome and C-heterochromatin polymorphism in pair 12 in the complement of the newt species, *T. italicus* is described. The C-heterochromatin polymorphism is presumably due to a loss in the proximal C-band, whereas the chromosomal polymorphism has its origin in two different independent pericentric inversions both including the centromere and the proximal C-band of chromosome 12. The double-inversion polymorphism has a wide distribution over the range and follows a clear bipolarity between a northern area where the karyotype is homomorphic for the standard type of pair 12 (ST/ST) and an opposite area where the ST type is completely replaced by variant M_1 and M_2 metacentric chromosomes 12. Various karyophylogenies are possible, but the simplest and the most probable presumes an ancestral karyotype of ST/ST and a mechanism of gradual replacement of the heterobrachial chromosome ST by two independent pericentric inversions. The present data are discussed in relation to existing theories on karyological evolution of Urodeles and the functional significance of telocentric chromosomes suggested by Sessions et al. (1982).

Introduction

Conclusions on karyological differentiation between taxa and on chromosomal events underlying speciation in salamandrids are soundly based on several reliable reports (Mancino et al. 1977; Schmid 1980; Birnstein 1982). The cytogenetics of the salamandrids has been advanced by recent molecular and banding techniques that allow a more detailed analysis of the structure of mitotic and meiotic chromosomes.

Ragghianti et al. (1980) detected a polymorphism in shape and C-banding pattern of chromosome 12 of the newt, *Triturus italicus,* collected in a restricted part of its home range. The present report extends the study of this complex polymorphism to several populations scattered over most of the range. The results suggest that newt chromosomes are more susceptible to rearrangement than previously believed (Morescalchi 1975; Macgregor 1982) and that heterobrachial chromosomes tend to become metacentric. This may also account for a chromosomal variation recently detected in the karyotype of *T. vittatus* (Bucci-Innocenti et al. 1983). Investigation of more cases of polymorphism in *Triturus*, could contribute to understanding the mechanisms of karyotype evolution and speciation in the genus.

Material and Methods

The range of *T. italicus* (Peracca) includes all of central and southern Italy (except the major islands). It is delimited to the north by a hypothetical line joining the Marches and the Gulf of Gaeta. The material was collected from 21 sites scattered over eight Italian regions covering nearly the entire specific range. Populations are indicated by letters that refer to the collection sites (Fig. 2):

Ge = Genga (Ancona, the Marches). Ponds at about 320 m above sea level, 25 specimens (4 males, 3 females, and 18 larvae) collected May, 1982.

CSM = Colle San Marco (Ascoli-Piceno, the Marches). Four small, shallow ponds, interconnected and receiving water from a small spring at about 800 m above sea level, 20 specimens (10 males and 10 females) collected April, 1981.

Ci = Civitella (Teramo, the Abruzzi). Slow-moving, shallow stream, full of vegetation near shore, about 530 m above sea level, 20 specimens (11 males and 9 females) collected April, 1981.

Bu = Bucchianico (Chieti, the Abruzzi). Reservoir full of vegetation at about 300 m above sea level, 17 specimens (3 males, 4 females, and 10 larvae) collected April, 1981.

MCe = Monti del Cerro (Campobasso, Molise). Large swamp with scant vegetation about 800 m above sea level, 84 specimens (10 males, 9 females, and 65 larvae) collected April, 1980.

Vi = Vinchiaturo (Campobasso, Molise). Pond with borders full of vegetation about 625 m above sea level, 255 specimens (19 males, 25 females, and 211 larvae) collected May, 1979 and April, 1981.

MA = Maranola (Latina, Lazio). Eleven artificial pools at about 750 m above sea level, 17 specimens (2 males, 5 females, and 10 larvae) collected October, 1982.

CS = Cerreto Sannita (Benevento, Campania). Mountain brook at about 400 m above sea level; 24 specimens (4 males, 5 females, and 15 larvae) collected October, 1979 and April, 1980.

CP = Cerreto Piscone (Avellino, Campania). Reservoir receiving water from a small spring and a nearby pond with borders full of vegetation at about

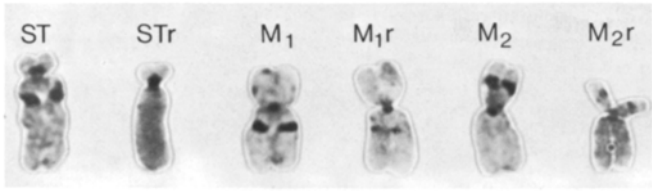

Fig. 1. Variants of chromosome 12. *ST* subtelocentric (c.i. = 0.195) with thick proximal C-band on long arm; ST_r subtelocentric (centromere index = 0.221) with reduction of proximal C-band; M_1 metacentric (c.i. = 0.387) with thick proximal C-band on long arm; M_{1r} metacentric (c.i. = 0.410) with reduction of proximal C-band; M_2 metacentric (c.i. = 0.415) with thick proximal C-band on short arm; M_{2r} metacentric (c.i. = 0.379) with reduction of proximal C-band

620 m above sea level, 96 specimens (10 males, 10 females, and 76 larvae) collected April, 1980.

CV = Casal Velino (Salerno, Campania). Interconnected reservoirs about 150 m above sea level, 50 specimens (15 males, 16 females, and 19 larvae) collected April, 1980 and April, 1981.

FU = Foresta Umbra (Foggia, Apulia). Shallow, narrow canal connecting an artificial lake (Cutino d'Umbra) and a large pool (Lago di Otri) at about 800 m above sea level, 40 specimens (22 males and 18 females) collected April, 1981.

CM = Cassano delle Murge (Bari, Apulia). Pond at about 340 m above sea level, 38 specimens (6 males, 6 females, and 26 larvae) collected April, 1981.

CO = Conversano (Bari, Apulia). Artificial tanks built inside the so-called Laghi di Conversano at about 250 m above sea level, 10 specimens (4 males, 5 females, and 1 larva) collected October, 1982.

Cis = Cisternino (Brindisi, Apulia). Pond with scarce vegetation at 394 m above sea level, 28 specimens (5 males and 23 females) collected October, 1979.

Ce = Ceglie Messapico (Brindisi, Apulia). Large and very deep pond at about 300 m above sea level, 43 specimens (8 males, 10 females, and 25 larvae) collected April, 1980.

Cig = Masseria Cigliano (Taranto, Apulia). Small artificial lake and slow-moving stream at about 178 m above sea level, 67 specimens (15 males, 21 females, and 31 larvae) collected April, 1980.

Ma = Matera (Matera, Basilicata). Permanent swamp (called Pantani) at about 350 m above sea level, 70 specimens (9 males, 10 females, and 51 larvae) collected April, 1980.

CaV = Campomaggiore Vecchio (Potenza, Basilicata). Reservoir and small pond at about 525 m above sea level, 45 specimens (27 males and 18 females) collected May, 1980 and April, 1981.

Ca = Camastra (Potenza, Basilicata). Pond full of vegetation at 800 m above sea level, 61 specimens (10 males, 3 females, and 48 larvae) collected June and October, 1979 and April, 1980.

Cas = Castrovillari (Cosenza, Calabria). Reservoir at about 350 m above sea level, 39 specimens (7 males, 3 females, and 29 larvae) collected May, 1982.

SSe = Santa Severina (Catanzaro, Calabria). Reservoir at about 325 m above sea level, 10 specimens (2 males, 1 female, and 7 larvae) collected April, 1980.

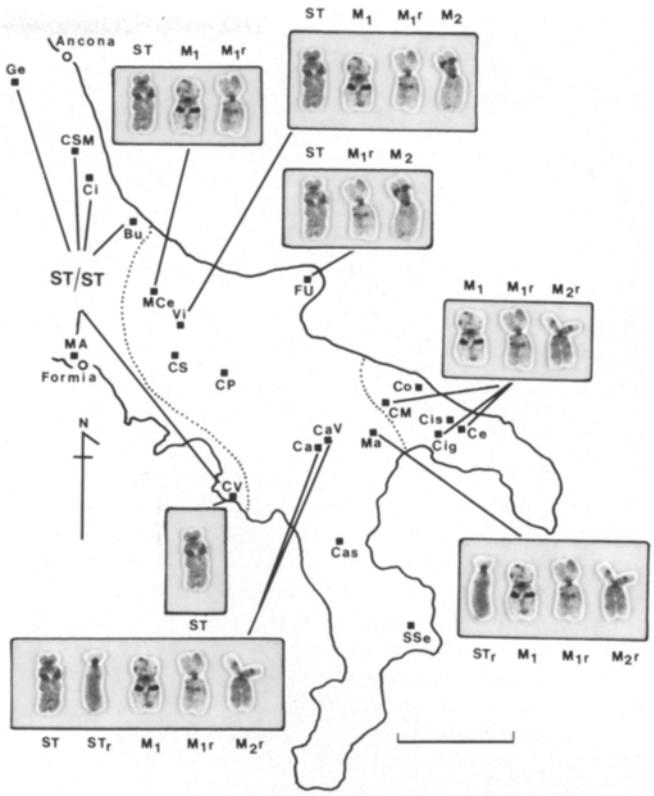

Fig. 2. Location of the 21 collection sites of *T. italicus*. The northern boundary of the range approximately connects the two towns, Ancona and Formia. Schematic distribution of the various types of chromosome 12 (only some populations have been indicated). Further explanation in text. Bar represents 100 Kms

At each site we collected as many specimens as possible. Of the 1059 that were cytologically studied, 203 were adult males; 214, adult females; and 642, larvae of undetermined sex.

Larvae were treated with 3‰ colchicine (Sigma) for 24 h. The adults were intracoelomically injected with 3‰ colchicine 24 h before testicular material was excised. A second injection preceded excision of somatic tissues, such as gut and spleen. All material was treated with distilled water for 10 min as a hypotonic treatment. Larvae and tissues excised from adults were fixed in a freshly prepared fixative (3 parts absolute ethanol: 1 part glacial acetic acid), then squashed in 45% acetic acid. Cytological preparations were made according to the dry-ice method and chromosomes were banded by the Giemsa technique according to Arrighi and Hsu (1971). Nomenclature and abbreviations indicating shape of individual chromosomes are those proposed by Levan et al. (1964). Oogenesis was investigated on lampbrush chromosomes from oocytes according to the technique described by Gall (1966).

Results

Inter- and intrapopulation variation

The various chromosome 12 types found so far are shown in Figure 1. Metacentric chromosomes 12 are assumed to have been derived from subtelocentric (ST) ones by either of two pericentric inversions (pi 1 being the larger inversion and pi 2, the shorter). All types of chromosome 12 can

Table 1. Karyomorphs of *T. italicus* in populations where chromosomes 12 can be involved in rearrangements

Site	No. of specimens		Karyomorphic distribution																			
			ST/ST	ST/ST$_r$	ST$_r$/ST$_r$	ST/M$_1$	ST/M$_{1r}$	ST/M$_2$	ST/M$_{2r}$	ST$_r$/M$_1$	ST$_r$/M$_{1r}$	ST$_r$/M$_{2r}$	M$_1$/M$_1$	M$_1$/M$_{1r}$	M$_{1r}$/M$_{1r}$	M$_1$/M$_2$	M$_1$/M$_{2r}$	M$_{1r}$/M$_2$	M$_{1r}$/M$_{2r}$	M$_2$/M$_2$	M$_2$/M$_{2r}$	M$_{2r}$/M$_{2r}$
MCe	adults:	19	15			4																
	larvae:	65	47			15	3															
CS	adults:	9	7				2															
	larvae:	15	9				6															
CP	adults:	20	19			1																
	larvae:	76	67			9																
Vi	adults:	44	24			8	6	4							1	1						
	larvae:	210	128			29	19	22						2	3	3		2		2		
FU	adults:	40	18				3	16												3		
Ca	adults:	13			5					1		2		1	1		2					1
	larvae:	48		2	8		2		2	2	6	7	2	1			4		5			7
CaV	adults:	45			2		1		1	7	8	2	3	6	1		4		6			4
Ma	adults:	19									1	2		4			6		2			4
	larvae:	51								1		2	5	4	2		20		11			6
CM	adults:	12													2				9			1
	larvae:	26													10				13			3
Co	adults:	9												3	2				1		1	2
	larvae:	1												1								
Cis	adults:	28											2	1	2	1	6		9			7
Ce	adults:	18											2	1	1		6		5			3
	larvae:	25											1	6	3				11			4
Cig	adults:	36											3	9	4		5		7			8
	larvae:	31											3	6	5		4		11			2
Cas	adults:	10							1										5			4
	larvae:	29					1		3						1				8			16
SSe	adults:	3																	2			1
	larvae:	7													4				2			1

Further explanation in text

be affected by a reduction (indicated by r) of the proximal C-band.

Some populations (Ge, CSM, Ci, Bu, MA, and CV) were characterized by the exclusive presence of the ST/ST karyomorph (Fig. 2). In the remaining part of the range, there were rearrangements (Tables 1 and 2; Fig. 2). The ST/ST karyomorph was still the most frequent at MCe, CS, and CP, where types M_1 and M_{1r} occured in heterozygous combinations (ST/M_1 and ST/M_{1r}).

The ST/ST karyomorph was frequent at Vi and FU, where both pi 1 and 2 and r are encountered. Consequently pair 12 showed various combinations of different chromosome types, although M_1 and M_{1r} chromosomes were prevalent at Vi, and M_2 chromosome, at FU (Table 2).

One larva from Vi not included in the tables, had a karyological peculiarity. Its pair 12 was ST/M_1 in some cells, whereas in others ST was combined with a perfectly metacentric chromosome, different from both M_1 and M_2 types.

The ST/ST karyomorph did not occur in Basilicata (Ca and CaV), but ST was found combined with ST_r (ST/ST_r karyomorph) or with the various metacentric chromosomes. Here different metacentric chromosomes (M_1, M_{1r}, and M_{2r}) occured in various combinations (Table 1; Fig. 2). In a third locality of Basilicata, indicated as Ma, the ST chromosome type was completely replaced by ST_r which was found only in heterozygous condition with metacentrics. The most frequent karyomorphs were combinations of the two metacentric chromosomes (Tables 1 and 2; Fig. 2).

In southern Apulia (CM, Co, Cis, Ce and Cig) only metacentric chromosomes 12 were found (Tables 1 and 2; Fig. 2). As in Basilicata, M_2 was almost completely replaced by M_{2r}, and M_1 and M_{1r} had nearly identical frequencies (Table 2).

Only two localities in Calabria, Cas and SSe, are represented, and a wider sampling in this area is necessary. The ST type was still found at Cas, although in heterozygous condition with metacentric types. Again the most frequent karyomorphs were those with metacentric chromosomes (Table 1). All karyomorphs found at SSe contained only metacentric chromosomes (Table 1).

From these results it appears that there is a bipolarity between localities (i.e., Ge, CSM, Ci, Bu, or MA, CV) where the karyotype was homomorphic for pair 12 (ST/ST) and localities (such as those of southern Apulia) where the karyotype was polymorphic for pair 12 (metacentrics of the various types). The ST chromosome 12 coexisted with all variants in the intermediate region (Fig. 2). This bipolarity is most evident between CV and southern Apulia. Along

Table 2. Frequencies of the various types of chromosome 12 in ten populations of *T. italicus*

Site	Number of specimens	Frequency					
		ST	ST_r	M_1	M_{1r}	M_2	M_{2r}
CV	adults: 31	1.0	0	0	0	0	0
	larvae: 19	1.0	0	0	0	0	0
	50	1.0	0	0	0	0	0
MCe	adults: 19	0.89	0	0.11	0	0	0
	larvae: 65	0.86	0	0.12	0.02	0	0
	84	0.87	0	0.11	0.02	0	0
CP	adults: 20	0.97	0	0.03	0	0	0
	larvae: 76	0.94	0	0.06	0	0	0
	96	0.95	0	0.05	0	0	0
Vi	adults: 44	0.75	0	0.10	0.09	0.06	0
	larvae: 210	0.78	0	0.08	0.07	0.07	0
	254	0.77	0	0.09	0.07	0.07	0
FU	adults: 40	0.69	0	0	0.04	0.27	0
Ca	adults: 13	0	0.50	0.15	0.12	0	0.23
	larvae: 48	0.06	0.34	0.12	0.15	0	0.33
	61	0.05	0.38	0.12	0.14	0	0.31
CaV	adults: 45	0.02	0.23	0.26	0.26	0	0.23
Ma	adults: 19	0	0.08	0.26	0.19	0	0.47
	larvae: 51	0	0.03	0.34	0.19	0	0.44
	70	0	0.04	0.32	0.19	0	0.45
Cis	adults: 28	0	0	0.21	0.25	0.02	0.52
Ce	adults: 18	0	0	0.31	0.22	0	0.47
	larvae: 25	0	0	0.16	0.46	0	0.38
	43	0	0	0.22	0.36	0	0.42
Cig	adults: 36	0	0	0.28	0.33	0	0.39
	larvae: 31	0	0	0.26	0.43	0	0.31
	67	0	0	0.27	0.38	0	0.35

an imaginary line joining these localities there is a gradual replacement of ST by ST_r and of ST_r by the metacentric chromosomes (Table 2; Fig. 2).

A statistical analysis of the double-inversion polymorphism, performed on populations from which enough specimens had been collected, has shown that they are in a Hardy-Weinberg equilibrium (Table 3). In this and the following analyses, we considered only the three characters: subtelocentric, metacentric M_1, and metacentric M_2 (types ST and ST_r were pooled, as were M_1 and M_{1r} and M_2 and M_{2r}). No significant difference between adults and larvae in a population was found when frequencies of chromosome types were compared by a contingency test; this allowed us to combine adults and larvae from a single population when comparing different populations. A comparison between the frequencies of chromosome types of two populations of Apulia, Ce and Cig, showed that there were no significant differences ($\chi^2 = 1.027$, $0.50 > P > 0.30$). Therefore, we can regard the distribution of the chromosome types in southern Apulia as homogeneous. In contrast, comparisons made between populations from southern Apulia and from localities in which rearrangements either occur (e.g., CaV, CP, Vi and FU) or do not occur (e.g., CV and CSM) showed significant differences in the distribution of chromosome types. For example, between Apulia (Cig) and Basilicata (CaV) there were very significant differences ($\chi^2 = 38.420$, $P < 0.001$).

Cytogenetics of spermatogenesis

As an extension of previous studies on spermatogenesis of ST/M_1 and other karyomorphs (Ragghianti et al. 1980), meiotic configurations and frequency and location of chiasmata were investigated in several more karyomorphs.

Pairing and chiasma formation were not affected when the two partners 12 were largely homologous or differed

Table 3. Distribution of expected karyomorphs in nine populations of *T. italicus*. Frequencies of ST, M_1 and M_2 are calculated from Table 2

Site	Number of specimens	Expected karyomorphs						χ^2	Probability
		ST/ST	ST/M_1	ST/M_2	M_1/M_1	M_2/M_2	M_1/M_2		
MCe	adults: 19	15.05	3.72	–	0.23	–	–	0.251	$0.70 > P > 0.50$
	larvae: 65	48.07	15.65	–	1.28	–	–	1.657	$0.20 > P > 0.10$
CP	adults: 20	18.82	1.16	–	0.02	–	–	0.044	$0.90 > P > 0.80$
	larvae: 76	67.16	8.57	–	0.27	–	–	0.292	$0.70 > P > 0.50$
Vi	adults: 44	24.75	12.54	3.96	1.59	0.16	1.00	0.572	$0.95 > P > 0.90$
	larvae: 210	127.76	49.14	22.93	4.73	1.03	4.41	1.072	$0.80 > P > 0.70$
FU	adults: 40	19.04	2.21	14.90	0.06	2.92	0.87	1.353	$0.80 > P > 0.70$
Ca	adults: 13	3.25	3.51	2.99	0.95	0.69	1.61	4.459	$0.30 > P > 0.20$
	larvae: 48	7.68	10.37	12.67	3.50	5.23	8.55	2.471	$0.50 > P > 0.30$
CaV	adults: 45	2.81	11.70	5.18	12.17	2.38	10.76	4.275	$0.30 > P > 0.20$
Ma	adults: 19	0.12	1.37	1.43	3.85	4.20	8.03	0.463	$0.95 > P > 0.90$
	larvae: 51	0.05	1.62	1.35	14.32	9.87	23.79	5.073	$0.20 > P > 0.10$
Ce	adults: 18	–	–	–	5.05	3.98	8.97	0.919	$0.50 > P > 0.30$
	larvae: 25	–	–	–	9.61	3.61	11.78	0.110	$0.80 > P > 0.70$
Cig	adults: 36	–	–	–	13.39	5.48	17.13	3.204	$0.10 > P > 0.05$
	larvae: 31	–	–	–	14.76	2.98	13.26	0.590	$0.50 > P > 0.30$

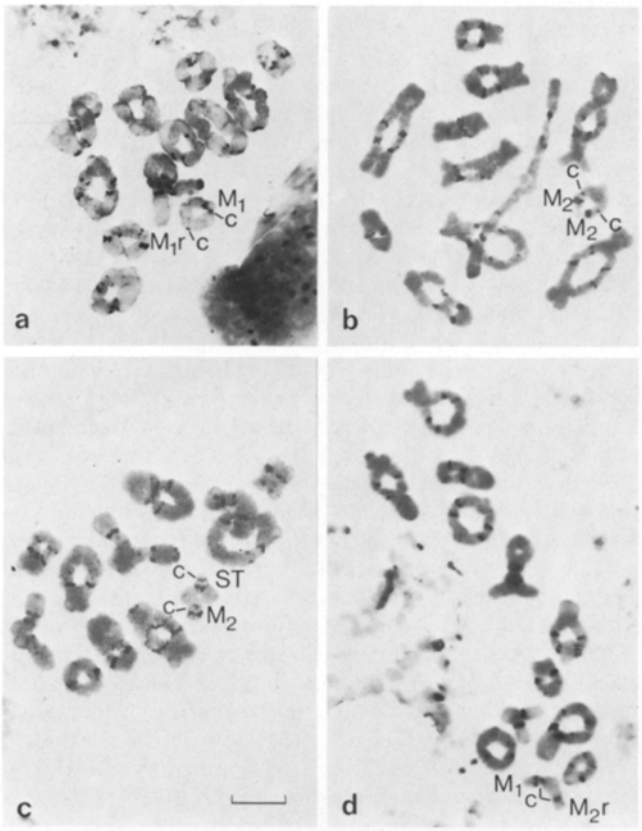

Fig. 3a–d. Configurations of C-banded bivalents 12 in metaphase I spermatocytes. **a** M_1/M_{1r}, **b** M_2/M_2, **c** ST/M_2, **d** M_1/M_{2r}. *c* centromere. Further explanation in text. Bar represents 10 μm

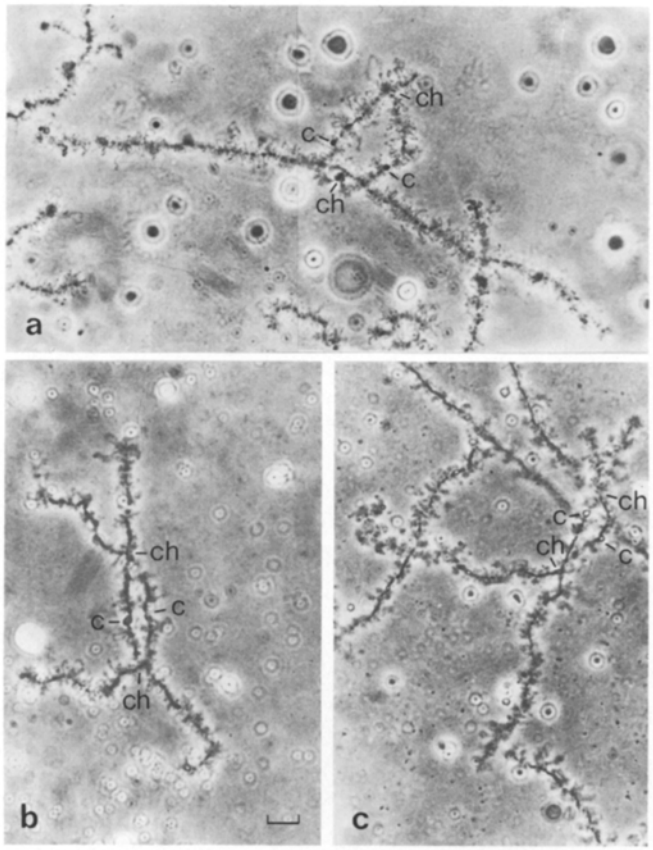

Fig. 4a–c. Shape of lampbrush bivalent 12 and location of chiasmata in ovarian primary oocytes, as seen in phase contrast. **a** ST/ST, **b** M_{1r}/M_{1r}; **c** M_{2r}/M_{2r}. *c* centromere; *ch* chiasma. Bar represents 10 μm

Table 4. Shape of the bivalents 12 and number and location of chiasmata in metaphase I spermatocytes of karyomorphs containing different types of chromosomes 12

Site	Specimen no.	Karyomorphs	No. of cells	No. of cells with		No. of chiasmata		
				Univalents	Rod bivalents	Intercalary	Subterminal	Terminal
FU	4	ST/M_2	24	2 (8.33%)	22 (91.67%)[a]	7 (30.43%)	6 (26.09%)	10 (43.48%)
FU	5	ST/M_2	24	3 (12.50%)	21 (87.50%)	15 (71.43%)	2 (9.52%)	4 (19.05%)
FU	9	ST/M_2	19	0	19 (100%)	12 (63.16%)	6 (31.58%)	1 (5.26%)
Cis	4	M_1/M_{2r}	20	0	20 (100%)	2 (10.00%)	12 (60.00%)	6 (30.00%)
CaV	12	M_{1r}/M_{2r}	11	0	11 (100%)	0	4 (36.36%)	7 (63.64%)

[a] One spermatocyte had two chiasmata

only in respect to the C-band. The bivalent 12 was usually ring-shaped in metaphase I spermatocytes of both M_1/M_{1r} and M_2/M_2 karyomorphs (91.3% and 78.1%, respectively). There were generally two, terminally located chiasmata (Fig. 3a and b).

The bivalent 12 was always rod-like and unichiasmate in spermatocytes I from karyomorphs with differently structured partners: ST/M_2, M_1/M_{2r}, and M_{1r}/M_{2r} (Table 4). Figure 3c shows the most frequent shape of the bivalent 12 in ST/M_2 where the single chiasma was intercalary, between the long arm of ST and the long arm of M_2. This location confirmed our previous assumption on the size of pi 2. The subterminal or terminal location of the single chiasma in ST/M_1 bivalents confirmed the size of pi 1. Chromosomes 12 occasionally appeared as univalents in both ST/M_1 and ST/M_2 karyomorphs. Their frequencies (4.63% and 7.46%, respectively) did not differ significantly ($t=0.784$, $0.50>P>0.40$). In the gametes, ST/M_2 was evenly distributed. We analyzed 65 metaphase II spermatocytes finding chromosomes 12 of ST type in 33 cells, of M_2 type in 31 cells, and an anomalous chromosome 12, probably derived from a bivalent 12 with a single chiasma located in the inversion region, in 1 cell.

The bivalent 12 is also rod-like and unichiasmate in karyomorphs consisting of metacentric but differently rearranged partners, M_1/M_{2r} and M_{1r}/M_{2r}. The single chiasma is located either subterminally or terminally between the short arm of M_1 or M_{1r} and the long arm of M_{2r} (Fig. 3d).

Table 5. Lampbrush bivalents 12 in different karyomorphs

Karyomorphs	No. of specimens	No. of oocytes studied	No. of oocytes mapped	No. of oocytes with		
				1 chiasma	2 chiasmata	3 chiasmata
ST/ST	14	192	33	5	20	8
ST/M_1	1	20	5	5	–	–
ST/M_{1r}	1	20	4	4	–	–
ST_r/M_{1r}	2	34	8	8	–	–
ST/M_2	1	15	4	4	–	–
M_{1r}/M_{1r}	2	46	6	–	6	–
M_{2r}/M_{2r}	5	81	12	–	7	5
M_1/M_2	1	34	7	7	–	–
M_1/M_{2r}	1	36	2	2	–	–
M_{1r}/M_{2r}	8	163	35	35	–	–

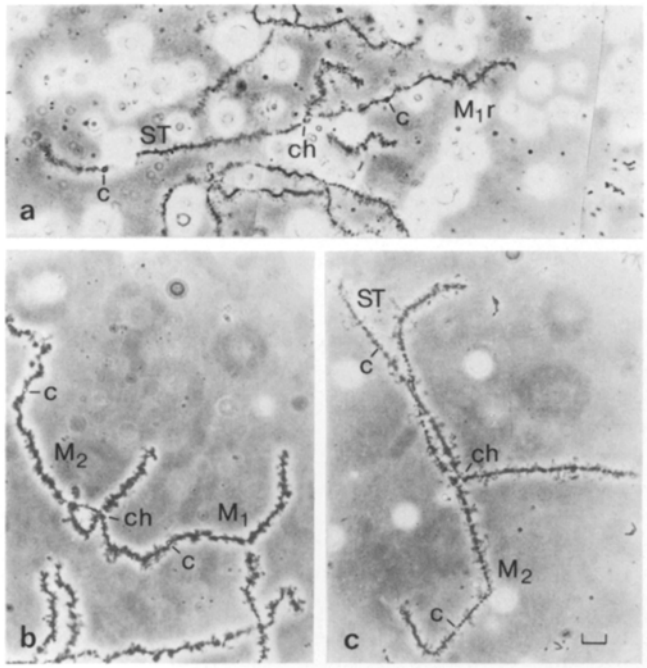

Fig. 5a–c. Shape of lampbrush bivalent 12 and location of chiasmata in ovarian primary oocytes, as seen in phase contrast. **a** ST/M_{1r}, **b** M_1/M_2, **c** ST/M_2. Bar represents 10 μm

A second terminal chiasma between the long arm of M_1 and the short arm of M_{2r} was found in a ring bivalent 12 in only one metaphase I spermatocyte (not included in Table 4) of male no. 4 from Cis.

Cytogenetics of oogenesis (lampbrush phase)

The lampbrush bivalent 12 of the ST/ST karyomorph had generally two procentric chiasmata (Mancino and Barsacchi 1969; Fig. 4a), although a third chiasma could be formed between the long arms, usually in an intercalary region. As expected, lampbrush bivalent 12 was also ring-shaped and has two procentric chiasmata in M_{1r}/M_{1r} and M_{2r}/M_{2r}, where pair 12 was made of two entirely homologous partners (Fig. 4b, c; Table 5). In M_{1r}/M_{1r} a bivalent 12 in a single oocyte was found to have two chiasmata, both between the short arms (procentric and intercalary respectively). In M_{2r}/M_{2r} a third intercalary chiasma between the long arms was frequently present. Lampbrush bivalent 12 of ST/M_1, ST/M_{1r}, and ST_r/M_{1r} karyomorphs, however, showed only a single subterminal chiasma between the long arm of the subtelocentrics and the short arm of the metacentrics, as expected from the supposed extent of both pericentric inversions (Fig. 5a; Table 5). Bivalent 12 of ST/M_2 had a single chiasma in the intercalary region between the long arms of ST and M_2 (Fig. 5c; Table 5). Analogously, in M_1/M_2, M_1/M_{2r} and M_{1r}/M_{2r} lampbrush bivalent 12 showed a single subterminal chiasma between the short arm of M_1 or M_{1r} and the long arm of M_2 or M_{2r} (Fig. 5b; Table 5); such a chiasma was terminally located only in a single oocyte from a specimen of M_{1r}/M_{2r}.

Discussion

Various hypothetical karyophylogenies can account for the chromosome and C-heterochromatin variations in *T. italicus* on the basis of the results of the present paper and the overall knowledge of karyological evolution of amphibian Urodeles (Morescalchi 1975; Mancino et al. 1979; Macgregor 1982). The two simplest karyophylogenies assume that the ancestral karyotype was homomorphic for pair 12, either ST/ST or M/M. The first seems to us the more probable as it agrees with the widely accepted view of karyological evolution in salamanders that symmetrical karyotypes originate from asymmetrical karyotypes by either Robertsonian centric fusion or pericentric inversion (Morescalchi 1975; Sessions et al. 1982). Telocentric chromosomes are considered to have been derived from bi-armed chromosomes only in various genera of advanced groups such as the plethodontids (Léon and Kezer 1978; Kezer and Sessions 1979). However, these same authors do not seem to discard the general view of Urodela karyological evolution which includes the concept of replacement of telocentrics by fully bi-armed chromosomes (Sessions et al. 1982).

The presumed ancestral karyotype (ST/ST) is still present and represents the only karyomorph of some localities at the periphery of the range (Ge, CSM, Bu, Ci to the north, MA and CV to the west). These populations can be considered as relicts. The gradient between these localities and southern Apulia shows that the mechanism of replacement of the subtelocentric ST by metacentric chromosomes is still in progress. The present polymorphism can be accounted for by the occurrence of two independent pericentric inversions: ST → M_1 and ST → M_2 (Fig. 6). Inversion pi 1 presumably occurred in southern Apulia and changed the centromere position of an ST chromosome 12. The resulting M_1 type gradually spread out, first locally and afterwards over continental Italy. Successful diffusion was ensured by the maintenance of a high degree of fertility in the heterozygotes, as we can judge from the meiotic configurations of ST/M_1 karyomorphs. The lack of reproductive barriers due to rearrangements is a prerequisite for the diffusion of a new chromosome type and for the formation of new populations that are not separated reproductively from the ancestral population (Hedrick 1981). While the M_1 type was still spreading out, the second pericentric inversion, pi 2, occurred, presumably independently, in southern Apulia or in a region close to it. The cytogenetic results presented here (shape of bivalents and location and mean frequency of chiasmata in those karyomorphs with M_2, such as ST/M_2 and M_1/M_2) seem to exclude the formation of M_2 from M_1 by an overlapping pericentric inversion. Presumably pi 2 spread because of the same factors

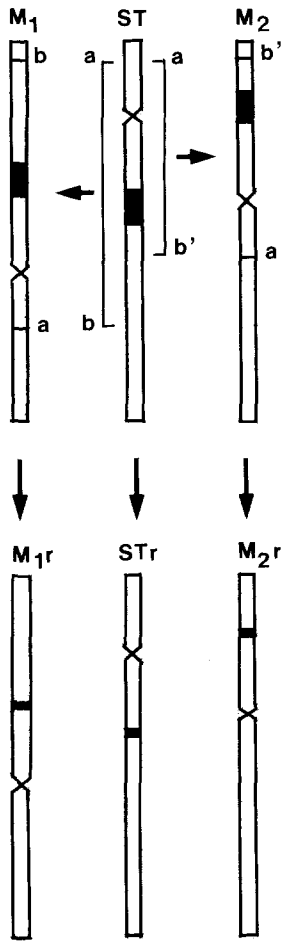

Fig. 6. Scheme of the most probable derivation of the various chromosome types from ST. The break points for the inversion pi 1 are indicated by the *bracket ab*; and for the inversion pi 2 by the *bracket ab'*

that favored the successful diffusion of pi 1. Subsequent fixation of both pericentric inversions is thought to have occurred by elimination of the ST type in and around southern Apulia.

According to this karyophylogeny, the most advantageous cytological condition now is a complement made entirely of pairs of metacentrics. The double inversion polymorphism of pair 12 of *T. italicus* may represent an intermediate step in the conversion of the last pair of subtelocentrics into a pair of metacentrics. It will be followed by a subsequent selection of the most suitable metacentric, since the present metacentrics still maintain a certain degree of susceptibility to rearrangement, as the cytological mosaic discovered in the Vi population shows. Considering the significance of inversions for the maintenance of gene blocks free of recombination (Sybenga 1972), the possible role of heterotic effects of inversion polymorphisms cannot be excluded.

The karyophylogeny that supposes an ancestral karyotype with an M/M pair 12, seems to us less likely. In fact we would assume that both M_1 and M_2 chromosome types have derived from a hypothetical isobrachial metacentric chromosome (M); however, the presumed ancestral karyotype homomorphic for pair 12 (M/M) or at least the M type was never found among the present day populations in any part of the range. Moreover, it seems too complicated to explain the last steps of this sequence, namely the conversion of either M_1 or M_2 or both metacentrics into ST.

The large proximal C-band of chromosome 12 of *T. italicus* can be involved in a C-heterochromatin polymorphism, as shown by M_1 and M_{1r}, (which usually coexist over most of the range), M_2 and M_{2r} (M_{2r} replaces M_2 nearly completely in southern Apulia and Basilicata) and ST and ST_r (ST_r predominates or completely replaces ST in Ca, CaV, and Ma). The differences in size in the proximal C-band can be attributed to a loss of heterochromatin because ST, believed to be the most primitive, shows a large C-band on its long arm (Fig. 6). Evidence for real loss of C-heterochromatin is given by preliminary histochemical data (Pellicciari et al., in preparation). This interpretation differs from ones that postulate a gain in heterochromatic segments (John and King 1977). In contrast to Keyl (1965), we can also exclude any close relationship between the reduction in size (or deficiency) of C-heterochromatin and the shift of the centromere by pericentric inversion because the break points are outside the heterochromatic segment. We can assume that a factor, inducing the reduction of C-heterochromatin size, expressed itself in southern Apulia and possibly in Basilicata after pi 2 arose, but before M_2 had spread through continental Italy. In fact, the reduction affected a small part of the remaining ST chromosome distribution, part of the M_1 chromosomes which were already numerous at that time, and many of the newly formed M_2 chromosomes. The frequency of chromosomes 12 with a large proximal C-band is still high. Therefore we can assume that the C-band is related to some gene function or the preservation of heterotic gene blocks in chromosome 12.

The proposed karyophylogeny (ST/ST homomorphy for pair 12, fixation of metacentrics, and elimination of subtelocentrics) may open a discussion on the relative functional significance of asymmetric or symmetric chromosomes, including the possibility that ribosomal genes, located in or close to the proximal C-band of chromosome 12 of *T. italicus*, are involved in the double inversion polymorphism and C-heterochromatin reduction. Sessions et al. (1982) claim that centromere position may not be neutral for gene function and that telocentrics could be regarded as the combined product of random changes in centromere position and the effects of such changes on chromosome function. Kezer and Sessions (1979) explain the polymorphism of *Aneides ferreus* by accepting, as the most probable and simplest theory, a karyophylogeny from an ancestral T/T towards a derived ST/ST karyotype.

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