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Cryptic polyploidy in sharks and rays as revealed by DNA renaturation kinetics

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Zoologia. — *Cryptic polyploidy in sharks and rays as revealed by* DNA *renaturation kinetics* (*). Nota di ETTORE OLMO, VINCENZO STINGO, GAETANO ODIERNA e TERESA CAPRIGLIONE, presentata (**) dal Corrisp. G. CHIEFFI.

RIASSUNTO. — I selaci presentano delle dimensioni genomiche che sono fra le più alte tra i vertebrati e non sembrano correlate con la filogenesi della classe.

È stato effettuato uno studio sulla cinetica di rinaturazione del DNA di due Batoidei: *Raja asterias e Torpedo marmorata*, che differiscono nel contenuto di DNA nuclare, e di un Galeomorfo: *Scyliorhinus stellaris* che possiede una dimensione genomica simile a quella di *Torpedo*.

I risultati ottenuti mostrano che torpedine e sciliorino hanno un genoma poliploide rispetto alla razza, anche se questo non trova riscontro nel numero diploide dei cromosomi.

L'esistenza di casi di poliploidizzazione in due superordini di condroitti abbastanza lontani fa pensare che questo meccanismo di evoluzione genomica abbia giocato un ruolo importante nella filogenesi di questa classe.

Cartilaginous fishes have genome sizes which are among the biggest so far found in vertebrates with even very wide interspecific variations which do not seem to be correlated with the phylogenesis of this group (Stingo, 1979; Stingo *et al.*, 1980).

It has been suggested that, as in various other vertebrates, such differences in DNA content may not depend on a different number of structural genes, but on variations in repetitive DNA amount.

Accordingly, we have carried out a study through renaturation kinetics on the presence and amount of repetitive DNA fractions in the genome of three species of cartilaginous fishes: *Raja asterias* and *Torpedo marmorata* belonging to the same superorder as the Batoidea but with very different DNA amounts (7 and 14 pg/N), and *Scyliorhinus stellaris* belonging to another superorder (Galeomorphii) with a 12.3 pg/N DNA content rather similar to that of *Torpedo*.

MATERIALS AND METHODS

DNA renaturation kinetics was studied in *Raja asterias*, *Torpedo marmorata* (Batoidea) and *Scyliorhinus stellaris* (Galeomorphii). The samples investigated were supplied by the Zoological Station of Naples.

(*) Lavoro eseguito presso l'Istituto di Istologia ed Embriologia dell'Università di Napoli.

(**) Nella seduta del 26 giugno 1980.

DNA was extracted from blood, liver and testis cells of one or two samples per species using Marmur's technique (1963) partially modified by the addition of several enzymatic digestions and phenol deproteinization.

DNA was then fragmented into pieces of about 0.3 Kbases through sonication with a Branson sonifier cell disruptor. Each sample was subjected to three 30 sec. impulses with a tip 12.7 mm. in diameter, at 120 watts. The fragment lengths were controlled by agarose gel electrophoresis using as standard lambda DNA digested with ECO R1 and Hind 3.

Reassociation kinetics was performed by means of the optical method for the COT-values from 0.5×10^{-1} to 3×10^{-1} and HAP chromatography for the COT-values from 3×10^{-1} to 1×10^{4}. Reassociation by the optical method was performed with a Gilford 250 thermostatic spectrophotometer according to the method of Britten *et al.* (1974). Reassociation by HAP chromatography was performed according to the batch method (Paetken and Langman, 1975). The values obtained with the optical method were made uniform with those obtained with HAP chromatography by dividing the relative COT-values by 2 (Britten and Kohne, 1966; Tobler *et al.* 1972).

Results

Fig. 1 depicts the DNA renaturation curves of the 3 species assayed. The results obtained are listed in Tables 1 and 2.

The analysis of reassociation kinetics shows that in all the three selachian species DNA is constituted by 4 kinetic components nearly always present in the same percentages: a fraction which renatures faster than COT 0.5×10^{-3} and is probably constituted by palindromic DNA, a highly repeated fraction, an intermediate and a slowly reassociating one.

In *Raja asterias* the $COT\frac{1}{2}$ value of the slow fraction is equal to the $COT\frac{1}{2}$ value expected for single-copy DNA calculated on the basis of the haploid value according to the following formula: $\frac{G}{Gcoli} \times COT\frac{1}{2}$ coli, where G is the amount of haploid DNA of the species assayed, Gcoli and $COT\frac{1}{2}$ coli are respectively the *E. coli* haploid genome (0.0047 pg/N according to Cairns, 1963) and the $COT\frac{1}{2}$ value of *E. coli* DNA, that, in our stardard conditions is 5.75 Msec.

Conversely, in *T. marmorata* and *S. stellaris* the $COT\frac{1}{2}$ value of the slow fraction is found to be about half the expected $COT\frac{1}{2}$ value and is very similar to that of the ray, though these species have a genome size which is twice or almost twice that of *Raja*.

Such a divergence between the $COT\frac{1}{2}$ value of the slow fraction and that expected for single-copy DNA shows that this fraction contains—in part at least—sequences present more than once in the genome. In this connection it is to be noted that the relationship between the expected and actual $COT\frac{1}{2}$ values of the slow fraction is very close to 2. Moreover, if we compare the

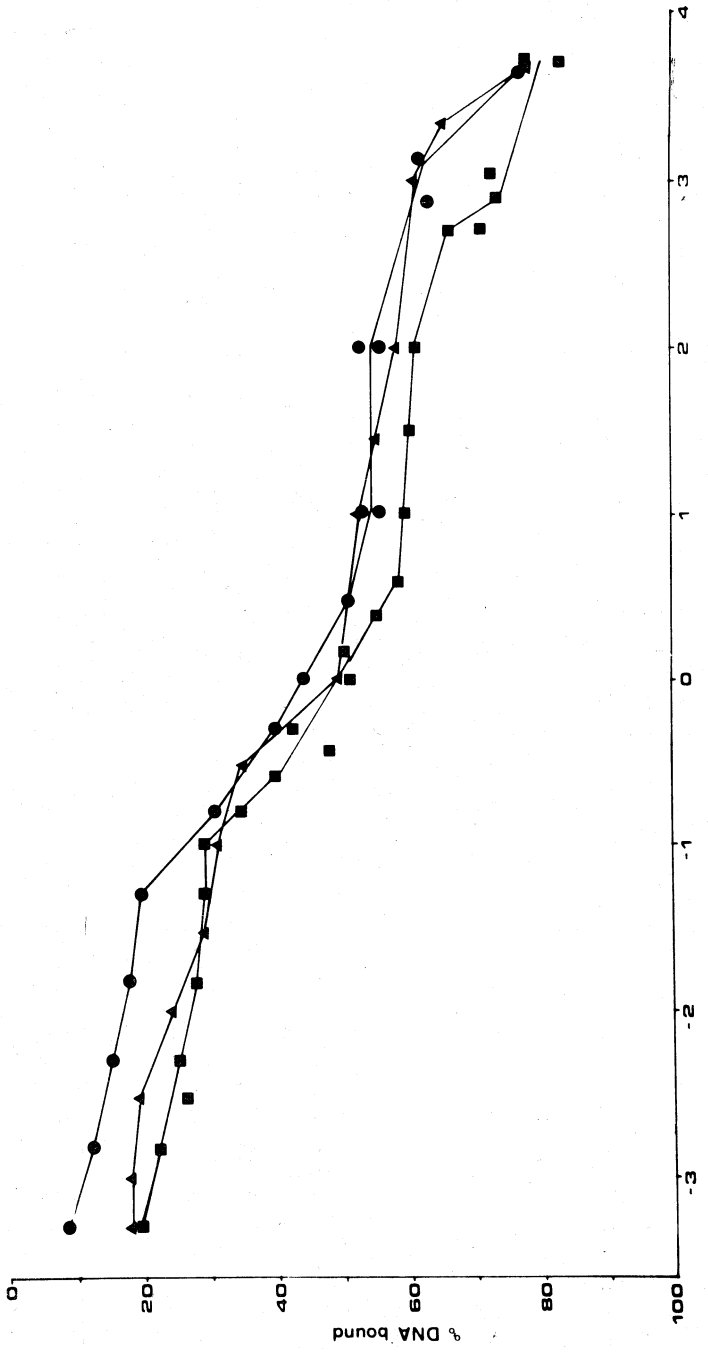

Fig. 1. – DNA renaturation curves of the three selachian species assayed. ●: *Raja asterias*; ■: *Scyliorhinus stellaris*; ▲: *Torpedo marmorata*. The kinetic parameters are shown in Table I.

TABLE 1.

Kinetic analysis of Selachian DNA.

Species	Foldback %	Highly repetitive				Middle repetitive				Slow component			
		%	Rate M^{-1} sec^{-1}	Cot $\frac{1}{2}$ Msec	Repet. freq. $\cdot 10^6$	%	Rate M^{-1} sec^{-1}	Cot $\frac{1}{2}$ Msec	Repet. freq. $\cdot 10^4$	%	Rate $\cdot 10^{-4}$ M^{-1} sec^{-1}	Cot $\frac{1}{2}$ Msec	Cot $\frac{1}{2}$ exp. Msec
R. asterias	8.5	11.5	250	.004	1.1	34.5	2.86	.35	1.0	45.5	2.4	4281	4282
T. marmorata	18.5	13.0	100	.010	0.9	27.5	1.41	.71	1.2	41.5	1.9	5129	8563
S. stellaris	19.5	10.0	294	.003	2.2	32.0	2.38	.42	1.8	38.5	2.3	4365	7523

TABLE 2.

Amounts in absolute values of various DNA *fractions in the three selachian species assayed.*

	R. asterias			T. marmorata			S. stellaris		
Foldback	0.6	1.4		2.5	4.3		2.4	3.6	
Highly rep.	0.8		3.8	1.8		8.1	1.2		7.5
Middle rep.	2.4			3.8			3.9		
Slow comp.	3.2			5.9			4.8		

amounts in absolute value (pg/N) of slowly reassociating and repeated DNA (Tab. 2), we can observe that in *Torpedo* and *Scyliorhinus* both these types of DNA are present in an amount twice that of the analogous fractions of ray.

DISCUSSIONS AND CONCLUSIONS

The results on reassociation kinetics suggest that the genomes of *T. marmorata* and *S. stellaris* are polyploid if compared with the *R. asterias* genome.

The genome of *Torpedo* might derive by polyplodisation from a genome very similar to that of the ray. In fact, these two species belong to the same superorder and Rajiformes are considered the most primitive forms of the superorder, while the Torpediniformes would be the most highly evolved group (Compagno, 1973).

The hypothesis of evolution by polyploidy seems to be in contrast with the chromosome number in the two species, *Raja asterias* having $2n = 98$ and *T. marmorata* a lower diploid number ($2n = 86$) (Stingo, 1979). In any case this may be explained by assuming that *Torpedo* derives from a species with the same genome size as the ray but with a lower diploid number, or that in *Torpedo* polyploidisation was followed by a progressive and wide chromosomic rearrangement as has been often observed in various organisms (Nagl, 1978).

As in *T. marmorata*, also in *Scyliorhinus* the genome is polyploid and is hypothesized to derive from an ancestral genome similar to that observed in many *Carcharhinidae*, species closely related to the *Scyliorhinidae* but with DNA contents which are often half that found in this family (see Stingo *et. al.*, 1980).

The presence of polyploidisation in two superorders of Selachians, not closely related, leads us to think that this genomic evolutionary mechanism is very common in this class and has played a leading role in its phylogenesis.

Cases of polyploidy have been frequently described also in bony fishes (Ohno, 1970). Therefore it may be suggested that genomic evolution occurred with substantially similar mechanisms in all the so-called " fishes ".

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