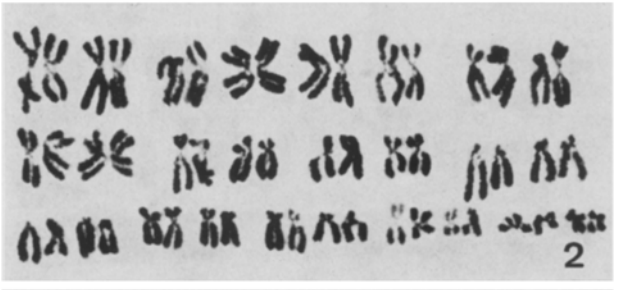
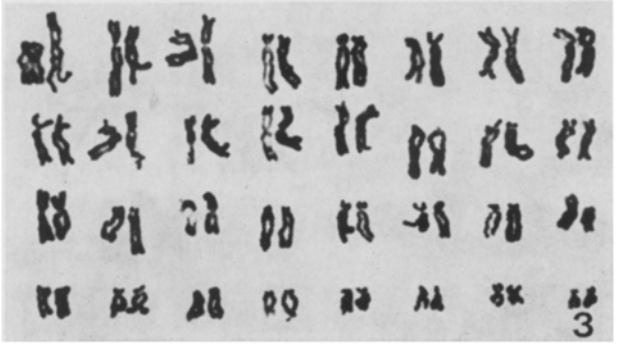
Sirenids: a Family of Polyploid Urodeles?

The Sirenids (Amphibia, Caudata) are a small group of permanently larval Urodeles whose phyletic relationships are still debated[1]; the family includes the monotypic *Pseudobranchus* (*P. striatus*) and *Siren*, with 2 species (*intermedia* and *lacertina*). Only little karyological information is available on Sirenids, and it concerns only *Siren*[2].





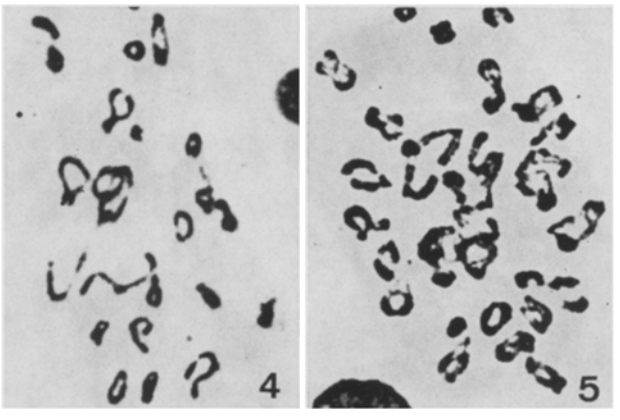


Fig. 1. The karyotype of *Siren intermedia*.
Fig. 2. The karyotype of *Siren lacertina*.
Fig. 3. The karyotype of *Pseudobranchus striatus*.
Fig. 4. Spermatocyte bivalents of *S. lacertina*.
Fig. 5. Spermatocyte bivalents of *P. striatus*.

We have made new observations on the chromosomes of these problematic Urodeles using the current squash techniques; moreover, we have calculated by histophotometrical methods the amount of the nuclear DNA content of the 3 species (for technical details, cf.[3]).

The dwarf siren (*S. intermedia*) has 46 chromosomes in the somatic set; the karyotype includes all bi-armed, meta- (*m*) or submeta-centric (*sm*) chromosomes which may be arranged in 23 pairs of homologues ($2n = 46$, $n = 23$; NF– or number of chromosome arms– = 92) (Figure 1). Even if many chromosomes might be grouped in quartets, this karyotype is the most 'diploid' of the 3 species of Sirenids (cf. below in the text); we have not found meiotic chromosomes in this species.

The great siren (*S. lacertina*) has 52 chromosomes in the somatic tissues; many elements are *m*, *sm* or *st* (subtelocentric) and 4 chromosomes are telocentric (*t*) (NF = 100). These chromosomes may be grouped in 26 pairs ($2n = 52$, $n = 26$) or also in 13 quartets of morphologically very similar elements, which suggests a possible tetraploid condition in this species ($4n = 52$, $n = 13$) (Figure 2). In the male meiotic line, only 26 bivalents (and no quadrivalents) can be found (Figure 4): thus, in the case of tetraploidy, it might have been achieved by allopolyploidy[4].

The mud siren (*P. striatus*) has 64 chromosomes in the somatic set and 32 bivalents in the spermatocytes (Figures 3 and 5); this species has more *st* and *t* elements than *S. lacertina* (NF = about 120). Most chromosomes of *P. striatus* could be grouped in quartets, but this is not possible for some of them (i.e., the 2 largest chromosomes of the set, which are *m*, do not have equal partners).

The nuclear DNA amount of *S. intermedia* corresponds to about 108 picograms per nucleus (pg/N), that of *S. lacertina* to 114 pg/N, and that of *P. striatus* to 91 pg/N: hence, the species with the highest chromosome number (*P. striatus*) has less nuclear DNA than both species of *Siren*, while *S. lacertina*, the most 'tetraploid' of the three, has the highest DNA amount. In their high DNA content the Sirenids are similar to other paedogenetic families of *Caudata* (Proteids, Cryptobranchids and Amphiumids)[2,3].

Since the species of Sirenids with the higher chromosome numbers have more *t* and *st* elements, many interspecific karyological differences might depend on Robertsonian mechanisms of centric fusion/fission often complicated by further pericentric inversions.

The karyotype of Sirenids seems peculiar in the *Caudata*: the advanced families of this order (Ambystomatids, Plethodontids, Amphiumids and Salamandrids) have lower chromosome numbers ($2n$ between 28 and 22), the intermediate Proteids have $2n = 38$, while the primitive families (Hynobiids, Cryptobranchids) have chromosome numbers ($2n$ from 64 to 40) which sometimes are similar to those of the Sirenids, but in this case show many microchromosomes (very small, dot-like elements) which are absent in the karyotype of the latter family.

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The Sirenids seem to show (in various degrees) traces of a tetraploid condition in their chromosome set: indeed, the peculiar karyotype found in the family becomes comparable to that of other families of *Caudata* only on the hypothesis of its derivation from a numerically lower chromosome set by polyploidy (and further chromosome changes, typical of each species and tending to 'diploidize' the newly arisen tetraploid karyotype). Such mechanisms could have been of importance for achieving gene duplication during the evolution of Vertebrate genomes[5]; however, Sirenids might represent the first example of a whole amphibian family karyologically differentiated by polyploidy[2,6].

Riassunto. I tre Sirenidi viventi hanno 46, 52 e 64 cromosomi; il corredo di *S. lacertina* sembra tetraploide ($4n = 52$). Viene fatta l'ipotesi che anche i corredi delle altre due specie derivino per (allo-)poliploidia da corredi a minor numero cromosomico, come si ritrovano nelle famiglie «superiori» dell'Ordine.

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5 October 1973.

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[7] Research carried out through a contribution from the C.N.R.

Hemoglobin J β Baltimore in a Fourth French Canadian Family

Fourteen families with hemoglobin J β Baltimore have been described, 7 of whom are caucasians of British or Dutch extraction, 3 are caucasians of French-Canadian stock, 3 are negroes and 1 is of mixed (caucasian-negro) parentage[1,2]. We report the discovery of the hemoglobin type in a fourth French Canadian family, thereby adding evidence to the concept that the gene for this rare variant occurs only in certain population groups.

Materials and methods. The hemoglobin type of a healthy, 16-year-old white boy came to our attention during hemoglobin electrophoresis while his basic health status was being evaluated in a health maintenance program for low-income families.

The proband was adolescent, of asthenic build, with no skeletal abnormalities. He was 165 cm tall and weighed 67 kg. Heart, lungs and abdominal findings were normal; the spleen and other lymph glands were not palpable. Physical and mental developments were normal. Previous illnesses were unremarkable; he and other members of the family had been treated at the center for frequent bouts of streptococcal pharyngitis.

Hematologic values were normal. White cell and differential counts were within normal limits; platelets and red cells were of normal morphology. The urinalysis was normal. Electrophoresis of blood was a routine procedure in the evaluation of health status of participants in the health maintenance program.

He was a fraternal twin, the 3rd of 6 children (Figure 1). The paternal line was French Canadian, whose antecedents had emigrated from the Montreal area to northern Maine and eventually to New York State. No relationship was known with the 3 other French Canadian families carrying Hb J β Baltimore, all of whom had also moved to northern New England from the Province of Quebec.

A fast-moving band of hemoglobin was found in the proband's hemolysate during electrophoresis on Titan III cellulose acetate plates in a Zip-Zone electrophoresis chamber, with Supre Heme Buffer of pH 8.4 at a constant voltage of 400 V for 15 min at room temperature (Helena Laboratories, Beaumont, Texas). The variant's mobility was similar to that of other Hb J β Baltimore variants identified in this laboratory. Densitometry indicated that the variant comprised approximately half (52%) of the entire hemolysate volume, a proportion similar to that in hemolysates from patients with hemoglobin J Baltimore[2].

Hemolysates from the father and 2 brothers also contained the fast-moving band in the same proportion (Figure 1).

Structural studies were carried out with 15 ml of the proband's toluene-extracted hemolysate[3], as described previously[1]. The variant hemoglobin was separated and eluted from the other hemoglobins by vertical polyacrylamide gel electrophoresis[4,5], and the eluate concentrated to approximately 10 g per 100 ml by vacuum dialysis[6]. Recombinant products obtained by hybridization were observed on cellulose acetate[7,8].

The mutation site was also localized by a method in which the hemoglobin chains in the dialyzed eluate were cleaved with sodium *p*-hydroxy mercury benzoate (HMB)[9] and separated in horizontal starch gel electro-

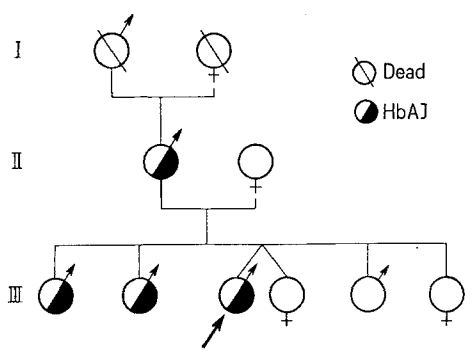

Fig. 1. Pedigree of proband with Hb J β Baltimore.

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