Trends of karyological evolution in Pelobatoid frogs[1]

A. Morescalchi, E. Olmo and V. Stingo

Institute of Histology and Embryology, The University, Faculty of Sciences, via Mezzocannone 8, I-80134 Naples (Italy), 23 May 1977

Summary. The Pelobatids have a basal karyotype of 26 chromosomes, with 6 pairs of large and 7 of small chromosomes (6 + 7). From this karyotype, shown by *Leptobrachium*, *Pelobates* and *Scaphiopus*, those of *Megophrys* and *Spea* (5 + 8) may be derived, as well as that of *L. pelodytoides*, with only 24 chromosomes. *Pelodytes* has 24 chromosomes and a karyotype 7 + 5 differing from that of typical Pelobatids under several respects.

Amphibians belonging to the family Pelobatidae are generally considered transitional between the primitive (Archaeobatrachia) and the advanced (Neobatrachia) anurans. In some worker's opinion, they are a phyletically sterile branch, nevertheless by their morphology, notably as concerns the Oriental ones (Megophryinae), Pelobatids are the most generalized when compared to the various radiations of Neobatrachia[2].

Pelobatid history is well-known since the Early Tertiary[3]. The eocenic (maybe also cretaceous) *Eopelobates* is close to extant Megophryines from Asiatic South East, but it also possesses several features of spadefoots (Pelobatinae), of holarctic diffusion. Both Megophryines and Pelobatines might therefore descend from forms similar to *Eopelobates*, then diverging during climatic crises in the Early Coenozoic[4]. On the other hand, less certain are the relations of these anurans with *Pelodytes*, known in North America since the Miocene[5] and of relict diffusion in Euroasiatic lands today. Some workers allocate it to a pelobatid subfamily (Pelodytinae), while others held it different enough from 'true' Pelobatids to classify it in a family per se (Pelodytidae), though in the same suborder (Pelobatoidea[2]).

Information is available in the literature on the karyology of *Pelodytes punctatus* and several Pelobatines; more rarely on Megophryines[6]. Fresh data have been gathered in our laboratory on the chromosome morphology of some species from the 3 pelobatoid groups, and in some cases the nuclear DNA-content was histophotometrically evaluated[7]. It is felt that in the light of present knowledge on this subject the overall results obtained by us allow a

1 Research supported by a contribution from the Italian C. N. R.

2 O. A. Reig, Physis *21*, 109 (1958). I. Griffiths, Biol. Rev. *38*, 241 (1963). M. K. Hecht, Syst. Zool. *12*, 20 (1963). J. A. Tihen, Am. Zool. *5*, 308 (1965). J. D. Lynch, Univ. Kans. Publ. Mus. nat. Hist. *53* (1971). W. R. Heyer, Smithson. Contr. Zool. *199* (1975).

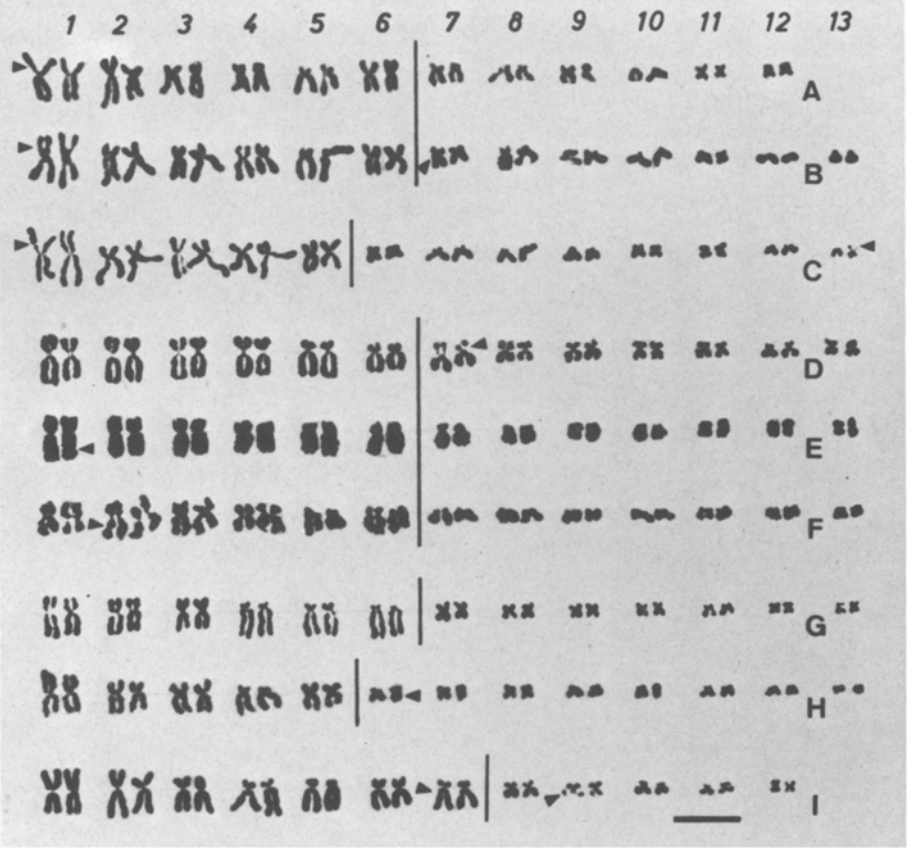
3 Z. V. Špinar, Sb. ústřed. Ust. geol. *19*, 457 (1952); R. G. Zweifel, Am. Mus. Novit. *1762* (1956); A. G. Kluge, Los Ang. County Mus. Contr. Sci. *113*, 1 (1966).

4 R. Estes, Bull. Mus. comp. Zool. *139*, 293 (1970).

5 E. H. Taylor, Kans. Univ. Sci. Bull. *27*, 61 (1941).

6 A. Morescalchi, in: Cytotaxonomy and Vertebrate Evolution, p. 233. Ed. A. B. Chiarelli and E. Capanna. Academic Press, London 1973.

7 E. Olmo, Caryologia *26*, 43 (1973).



Karyotypes of Pelobatids belonging to the subfamilies Megophryines (A–C); Pelobatines (D–H) and Pelodytines (I). A: *Leptobrachium pelodytoides;* B: *L. hasselti;* C: *Megophrys nasuta;* D: *Pelobates cultripes;* E: *P. fuscus;* F: *P. syriacus;* G: *Scaphiopus* (*Scaphiopus*) *holbrooki;* H: *Scaphiopus* (*Spea*) *hammondi;* I: *Pelodytes punctatus.*
Vertical lines separate large from small chromosomes; arrows point to the more constantly visible heterochromatic (nucleolar) segments. The line (bottom right) is 10 μm.

first cytotaxonomic approach to the evolutionary history of these interesting anurans.

The species studied here, with their chromosome numbers and sometimes their nuclear DNA-amounts (in pg per nucleus), are listed in the table. The figure shows the diploid chromosome sets of the species in question, along with that of *Scaphiopus holbrooki*, investigated by Wassermann[8], which was used by us (with some changes in the arrangement of the homologue pairs) in view of a thorough discussion of karyological relations within the Pelobatids.

Except *Leptobrachium pelodytoides* (Megophryines) and *Pelodytes punctatus*, both with 2 n = 24, all the other species studied by us have 2 n = 26. The chromosome numbers of 4 species other than those considered here, are known from the literature, e.g. *Leptobrachium nigrops* and *Megophrys monticola* (Megophryines)[6], *Scaphiopus couchii* and *Spea bombifrons* (Pelobatines)[8,9]. All these species have 2 n = 26, and their karyotypes are similar to those of the respective genera (or subgenera) of the species inspected here.

The figure shows that in all Pelobatoids under study, chromosomes are biarmed (meta-, submeta-, or subtelocentric). The chromosome morphology, as also seen in the figure, differs little between different species, major divergencies consisting in the localization of the 'step' between larger and smaller chromosomes in each karyotype (see below) and in the chromosomal localization of the heterochromatic zones (may be the nucleolus organizers).

As regards the nuclear DNA-content, the species in which it was estimated exhibit medium or medium-high values among Anura[6,7]; in other Pelobatines undergoing a rapid larval development (*Scaphiopus, Spea*), lower values (from 3.6 to 1.6 pg/N) were reported by other workers[10]. These divergencies may plausibly be related to adaptive factors, characteristic of the life of each species (namely, the rate of DNA-replication, cell division, etc.[6,7,10]).

The 26 chromosome set, being typical of the most generalized Megophryines (e.g. *L. hasselti*)[11] and of all Pelobatines, is plausibly the basic complement of the family from which the 24 chromosome set of *L. pelodytoides*, and perhaps also that of *Pelodytes*, are derived. A 26 chromosome set is also typical of most basal groups from the various families of the Neobatrachia, while it is practically absent in the extant Archaeobatrachia[6]: from a karyological standpoint, Pelobatids appear closer to the former than the latter anuran groupment. In particular, the karyotype of Pelobatids is strongly reminiscent of that of some Leptodactylids[12]: unless it is a question of precise parallelisms in the karyological differentiation of the 2 groups, the above findings might be of systematic interest.

Many workers believe that Pelobatids are derived from a discoglossid stock[2]. Among extant Discoglossidae, *Alytes* has 2 n = 38, *Discoglossus* 2 n = 28 and *Bombina* 2 n = 24. If the karyological differentiation on the last family took place, as seems likely, through progressive reduction of the chromosome number[6], forms with 26 chromosomes, karyologically akin to extant Pelobatids, may have been present within the Discoglossids.

As concerns the karyologic differentiation within Pelobatids, the figure shows that 2 karyologic 'formulae' occur in the species with 13 homologue pairs. The 1st consist of 6 pairs of larger and 7 of smaller chromosomes (6 + 7), the 2nd of 5 larger and 8 smaller pairs (5 + 8). The 6 + 7 karyotype is found in *L. hasselti*, in the 3 *Pelobates* species and in the *Scaphiopus* species of the *Scaphiopus* subgenus: these are the most generalized forms within Megophryines and Pelobatines[3,4,11]. Should the conservativity in the anatomical features of those species find its counterpart at the karyological level as well, the hypothesis might be advanced that the 6 + 7 karyotype is the primitive one within extant Pelobatids. From this karyotype, through a simple deletion in a large element, the 5 + 8 karyotype, which characterizes *Megophrys* among Megophryines and the species of *Scaphiopus* of the subgenus *Spea* among Pelobatines, may have arisen, perhaps independently in the 2 subfamilies[6,8].

L. pelodytoides (6 + 6 karyotype) has retained the 6 pairs of larger homologues of the 'primitive' karyotype, but seems to have lost a pair of smaller elements. Apparently, this is a fairly common pattern of karyological evolution among Amphibians[6].

Pelodytes has 12 pairs of homologues like *L. pelodytoides*, but it displays a divergent 7 + 5 karyotype. None of the typical Pelobatids studied seems to possess 7 pairs of larger homologues, which however are present in *Discoglossus* (7 + 7) and in several Leptodactylids (among which *Heleophryne*, with a 7 + 6 karyotype)[6].

Owing to its smaller chromosome number, *Pelodytes* appears karyologically more differentiated than typical Pelobatids. Its karyotype might have arisen from a 6 + 7 one through unequal translocation of 2 small chromosomes, fused into a single larger element (as reported in *Rana, Litoria* and other anurans)[6,13], or else it might have originated by starting from karyological formulae capable of evolving both in a 6 + 7 and in a 7 + 5 karyotype. In other words, karyological findings, as anatomical ones, further emphasize the peculiar condition of *Pelodytes* among Pelobatoids, namely, the fact that this genus though being karyologically more differentiated than several typical Pelobatids, does not bear any particular resemblance with any of them.

Species	2 n	Karyologic formula	DNA (pg/N)
Megophryinae			
Leptobrachium hasselti	26	6 + 7	
L. pelodytoides	24	6 + 6	9.1
Megophrys nasuta	26	5 + 8	5.6
Pelobatinae			
Pelobates cultripes	26	6 + 7	
P. fuscus	26	6 + 7	8.2
P. syriacus	26	6 + 7	9.0
Scaphiopus (*Spea*) *hammondi*	26	5 + 8	
Pelodytidae			
Pelodytes punctatus	24	7 + 5	

8 A. O. Wasserman, SWest. Nat. *15*, 235 (1970).
9 A. O. Wasserman and J. P. Bogart, Copeia *1968*, 303.
10 O. B. Goin, C. J. Goin and K. Bachmann, Copeia *1968*, 532. E. Sexsmiths, Thesis, Univ. Toronto 1968. K. Bachmann, Q. J. Fla Acad. Sci. *35*, 225 (1972).
11 R. F. Inger, Fieldiana, Zool. *52* (1966).
12 A. Morescalchi, Experientia *23*, 1071 (1967). J. P. Bogart, Acta zool. lilloana *28*, 19 (1971).
13 A. Morescalchi, Caryologia *18*, 193 (1965). J. I. Menzies and J. Tippett, J. Herpet. *10*, 167 (1976).