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Structural Characteristics of Genome Organization in Amphibians: Differential Staining of Chromosomes and DNA Structure

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Summary. Differential staining patterns on amphibian chromosomes are in some respects distinct from those on mammalian chromosomes; C-bands are best obtained, whereas G- and Q-bands are either unobtainable (on anuran chromosomes) or coincide with C-bands (chromosomes of urodeles). In amphibians, rRNA genes are located at secondary constrictions, but in urodeles they are also found at other chromosome sites, the positions of these sites being strictly heritable. DNA content in amphibian cells is tens and hundreds times higher than in mammals. DNA contents in anurans and urodeles differ within certain limits: from 2 to 25 pg/N and from 30 to over 160 pg/N respectively. Species characterized by slow morphogenesis have larger genomes. Genome growth is normally due to an increase in the amount of repetitive DNA (mostly intermediate repetitive sequences), the amount of unique sequences being almost constant (11 pg/genome in urodeles, and 1.5 pg/genome in anurans). In anurans in general no satellite DNA was found, whereas such fractions were found in many *Urodela* species. Nucleosome chromatin structure in amphibians is identical to that of other eukariotes. It is postulated that differences in chromosome banding between amphibians and mammals are due to differences in chromatin packing which in turn is related to the distinct organization of DNA repetitive sequences. It is likely that fish chromosomes have a similiar structure. A comparison of such properties as the chromosome banding patterns, variations in nuclear DNA content and some genome characteristics enable us to group fishes and amphibians together as regards chromosome structure, as distinct from amniotes – reptiles, birds and mammals. It is probable that in the ancient amphibians – ancestors of reptiles – chromatin packing underwent a radical transformation, following changes in the organization of DNA repetitive sequences.

At present, differential staining techniques are widely used for studying chromosomes of various classes of animals. In particular, they are employed in the study of chromosome homology in mammals, localization of the satellite DNA and nucleolar organizer regions (NORs) (Evans et al. 1973; Nadler et al. 1974; Dutrillaux et al. 1975; Wurster-Hill and Gray 1976; Zakharov 1977 and others). Those investigations seem to be especially interesting where banding techniques are used along with classical methods of molecular biology to determine evolutionary relationships between organisms (see, for example, Dev et al. 1975; Hatch et al. 1976; Mitchell et al. 1977). Studies of chromosome banding patterns and molecular characteristics of some *Drosophila* species allow us to establish phylogenetic and evolutional relationships between sibling species with similar morphology and karyotypes (Holmquist 1975). In other words, those studies enable us to connect the molecular level of genome evolutionary changes with the chromosome level.

The following staining techniques appear to be the most popular (Dutrillaux and Lejeune 1975; Zakharov 1977): G- and C-banding techniques (various Giemsa staining methods) and the Q-banding technique using fluorescent dyes. The G-banding technique involves a pretreatment of chromosome preparations with proteolytic enzymes (usually with trypsin), urea, chelates, and saline solutions (SSC, in particular), with a subsequent application of Giemsa stain (whose components are azure dyes and eosin). Such treatment reveals stained bands on the chromosomes of many mammals, the result being a specific pattern for each chromosome pair.

C-banding involves a pretreatment of chromosome preparations with an alcali which is followed by incubation in the SSC solution and staining with Giemsa. The C-technique reveals regions of structural heterochroma-

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tin enriched with satellite DNA. With Q-staining using fluorescent dyes (quinacrine and its derivatives) mammalian chromosomes acquire characteristic fluorescent banding patterns.

There are other banding techniques – R-banding for example, which results in the reversed staining pattern of the G- and Q-bands. Finally, N- and Ag-AS-techniques have been recently used for the localization of NORs (sites of ribosomal DNA) on chromosomes (Funaki et al. 1975; Goodpasture and Bloom 1975).

There is a great amount of data in the literature on the staining patterns of amphibian chromosomes; these animals are the traditional objects of evolutionary and molecular biological research. In this review these data are analysed and discussed in connection with the characteristics of amphibian DNA and chromosome structure.

1. Cytological Data: Differential Chromosome Staining in Urodele and Anuran Amphibians

Since most experiments have been carried out on urodele chromosomes (with the result that differences between them and anuran chromosomes have been established) data on urodele and anuran chromosomes will be discussed separately. With regard to the karyotypes of amphibians in general, it should be noted that there is an evolutionary tendency towards the acquisition of karyotypes composed of a low number of metacentric chromosomes, regularly decreasing in size (called "symmetrical" karyotypes) (Morescalchi 1973; 1977). With the exception of some members of the *Cryptobranchoidea* suborder (animals with "asymmetrical" karyotypes (consisting of a large nuber of meta- and acrocentric chromosomes and dot-like microchromosomes) practically all the species in question have "symmetrical" karyotypes.

a. Chromosomes of Urodeles

In primitive urodeles C-banding was obtained on the mitotic chromosomes of two species of the Cryptobranchoids, viz. *Andrias japonicus*, 2n=60 (the family *Cryptobranchidae*), and *Hynobius keyserlingii*, 2n=62 (the family *Hynobiidae*). In the former case the centromeres of almost all chromosomes were stained, with the exception of two pairs of middle-sized chromosomes and four pairs of microchromosomes (Morescalchi et al. 1977). Using N-banding (pretreatment of chromosomes with an acid) a NOR was localized in the third chromosome pair of the salamander. C-banding patterns in *Hynobius keyserlingii* were unusual: on all the chromosomes, except microchromosomes a specific staining pattern was obtained and the chromosomes looked like G-banded mammalian chromosomes (Graphodatsky et al. 1978). By using N-technique it was also possible to localize the NORs at the secondary constrictions of two chromosome pairs in *Hynobius retardatus*, 2n=40 (Funaki et al. 1975).

The most detailed data have been obtained on members of the following genera: *Pleurodeles, Triturus, Notophthalmus, Euproctus, Cynops, Aneides* of the families *Salamandridae* and *Plethodonthidae*. Of those, the differential staining in *Pleurodeles waltii* Michah, 2n=24, seems to have been studied most extensively. After staining mitotic chromosomes with quinacrine mustard or Hoechst 33258 intensively fluorescing Q-bands at the secondary constrictions of almost all chromosomes could be seen (Bailly 1972; 1976; 1977; 1979; Bailly et al. 1973). Weaker fluorescence was observed in the centrometric regions of most chromosomes, and it disappeared altogether if the animals previously had been kept in cold water. The satellites of the third and eleventh chromosome fluoresced considerably less than other regions – this may be due to a high content of non-histone proteins (Bailly 1972; 1976). The locations of the majority of the bands appearing during G-staining at pH 9.0 (Labrousse et al. 1972; Bailly et al. 1973) coincided with the locations of Q-bands (Bailly 1976; Bailly et al. 1973). Centromeres of all chromosomes and telomeres of the second pair were also revealed during C-staining (Bailly 1977). It is noteworthy that the number of bands on the chromosomes of urodeles is much less than in the case of mammals – only 2 (at the secondary constrictions of each arm) or 3 (including the centromere) in the case of Q-banding, and 1 or 2 in the case of C-banding.

Of all members of the genus *Triturus, T. cristatus carnifex*, 2n=24, has been studied most thoroughly (Mancino et al. 1973; 1977; Ragghianti et al. 1974; Schmid and Krone 1976; Rudak and Callan 1976). The exposure of embryos to cold led to the formation of secondary constrictions near the centromeres in both arms of most chromosomes. The third, fourth and fifth chromosomes were an exception, showing no such constrictions; the first chromosome had two constrictions in its long arm, also two constrictions were observed in the short arms of the sixth and twelfth chromosomes. Besides, the sixth and ninth chromosomes had the normally observed secondary constrictions carrying NORs. Having been subjected to Giemsa the cold-induced secondary constrictions stained intensively. In the third, fourth and fifth chromosomes (showing no constrictions) interstitial bands were also seen on the long and short arms. The NORs of the sixth and ninth chromosomes were not stained. The resulting bands coincided with C-bands on mitotic chromosomes from liver cells of adults that had not been subjected to cold treatment, and also with C-bands on the spermatogonial chromosomes of animals that had been preliminary kept in water maintained at a temperature of 2°C (in the later case, besides the constrictions, the centromeres were also stained). It

seems that the stained regions are characterised by an especially high degree of chromatin packing and are potentially capable of forming secondary constrictions (Rudak and Callan 1976).

It should be noted that the long arms of the first chromosome in *T. cristatus carnifex* were stained differently from other chromosome regions: after C-banding or after the animals had been exposed to cold heteromorphic segments showed a spotty pattern resembling "mitotic spiralization" (Mancino et al. 1973; Rudak and Callan 1976); sometimes even 7 C-bands occured (Schmid and Krone 1976). The heteromorphic segments was also stained intensively during C-banding of *T.c. carnifex* lampbrush chromosomes (Morgan 1978).

Similar results have been obtained on the chromosomes of other *Triturus* species (2n=24). In *T. cristatus cristatus* and in *T.c. dobrogicus* C-banding revealed a pattern analogous to that obtained during the staining of *T.c. carnifex* chromosomes (Rudak and Callan 1976; Mancino et al. 1977). G- or C-bands, usually identical to Q-bands, have been shown in *T. marmoratus marmoratus, T. montandoni, T. alpestris apuanus, T.a. alpestris, T. italicus, T. vulgaris meridionalis, T.v. vulgaris, T. helveticus,* and *Notophthalmus (= Triturus) viridescens* (Mancini et al. 1973; 1977; Nardi et al. 1973; Ragghianti et al. 1974; 1978; 1980; Hutchinson and Pardue 1975; Schmid and Krone 1976; Schmid et al. 1979). C-staining of heteromorphic segments of one of the chromosome pairs of *T.a. alpestris* and *T.h. helveticus* males, similar to the banding of a *T.c. carnifex* heteromorphic segment was obtained (Schmid et al. 1979). In *N. viridescens,* as well as in *T. cristatus* and *P. waltlii,* there proved to be a clear-cut correlation between the locations of bands on chromosome arms and the regions of cold-induced secondary constrictions (Hutchinson and Pardue 1975). Thus, the ability of the regions of cold-induced secondary constrictions to be Giemsa-stained is a characteristic feature of chromosomes of some urodeles.

On the whole, the C-banding pattern of *Triturus* lampbrush chromosomes seems to be similar to the C-banding pattern of mitotic chromosomes (Mancino et al. 1978; Ragghianti et al. 1978; Schmid et al 1979). Thus, all centromers of *T. marmoratus* lampbrush chromosomes, as well as most telomers, nucleolar organizer region and some inserted structures were stained (Batistoni et al. 1974); the same regions did not include ^{3}H-uridine (Nardi et al. 1972). An elaborate comparison of the C-banded katyotypes of *T. alpestris, T. vulgaris* and *T. helveticus* and the maps of their lampbrush chromosomes showed that some of the C-banding regions seem to correspond to the structures inserted on the lampbrush chromosomes (Schmid et al. 1979).

On C-staining of mitotic chromosomes of two species of the genus *Euproctus,* 2n=24, centromeres and pericentric regions in all but one of the chromosome pairs and also the long arms of one pair were stained (Bucci-Innocenti et al. 1978). In the case of *Cynops pyrrhogaster,* 2n=24, centromeres were not stained, only a band was obtained on each arm (Schmid and Krone 1976), while in *Aneides ferreus,* 2n=28, mainly centromeres were C-stained (Kezer and Sessions 1979).

The above results can be *summarized* as follows. C-bands are the easiest to obtain on the chromosomes of urodeles; locations of G-, C-, and Q-bands are often identical. Few banding patterns, usually only centromeres, the regions of the cold-induced secondary constrictions and sometimes also telomeres and some interstitial regions are stained.

b. C-banding Patterns of Urodele Chromosomes and rDNA Localization

Some attempts to discover the relationship between the banding ability of chromosome regions with the function of rDNA loci were made. A partial coincidence of C-banded regions and rDNA loci location was shown in *T. vulgaris meridionalis* chromosomes (Nardi et al. 1977; 1978). In situ hybridization experiments revealed sites of 18S+28S rRNA genes not only in NORs (the secondary constriction of the 11th chromosome), but in additional sites also (at the telomeric, centromeric and C-band regions of some chromosomes). Hybridization with the NORs has been observed in all specimens, while the hybridization with additional sites varied from one specimen to another. The data concerning the location of rRNA genes was confirmed by the results of $AgNO_3$-staining: in males the secondary constriction of one homologue of the eleventh chromosome was stained, as well as two intercalary sites in the long arm of the same chromosome and one site in one homologue of the eighth chromosome (Ragghianti et al. 1977). The distribution of rDNA sites is strictly heritable (Batistoni et al. 1978).

Coinciding banding patterns and rDNA loci have also been found in two *Euproctus* species: there were C-bands at secondary constrictions, while additional rDNA sites could be detected only by the Ag-AS-technique (Bucci-Innocenti et al. 1978). However, one should be cautious about the results of staining with silver: experiments on the chromosomes of *T.v. meridionalis* have shown that Ag-positive regions exist that do not contain rDNA (Nardi et al. 1978).

In other *Triturus* species, and also in *N. viridescens,* no correlation has been found between the location of rDNA in chromosomes and C-staining (Barsacchi-Pilone et al. 1974; Hutchinson and Pardue 1975). It should be noted, however, that in those species some rDNA sites are located not only at secondary constrictions, but also in other chromosome regions (Hutchinson and Pardue 1975; Ragghianti et al. 1977).

It can be assumed that in other newt and salamander species rDNA is also partly localized in C-banding regions. Thus the in situ hybridization in *Taricha granu-*

losa, 2n=22 (which is closely related to *Triturus*), has shown that 18S and 28S RNA genes are located near the centromere in the short arm of the ninth chromosome and in the telomeres in the eleventh chromosome pair, while in *Batrachoseps wrighti*, 2n=26 (the family *Plethodontidae* – the most specialized lungless salamanders) they were localized in the pericentric region in the long arm of the large chromosome (León 1976).

The genes for 5S RNA are also sometimes located in C-staining regions. In *N. viridescens*, the greater part of 5S DNA is localized in the pericentric regions of all acrocentric chromosomes (Hutchinson and Pardue 1975; Pukkila 1975). In the same regions a highly repetitive satellite DNA is localised (Barsacchi and Gall 1972). The 5S RNA genes are either embedded into this DNA or are a part of the highly repeated sequences (Hutchinson and Pardue 1975). Besides, a small part of 5S DNA in *N. viridescens* is localized in the middle of the long arm of one of the small submetacentrics. In *Triturus marmoratus* the sites of 5S DNA are localized only in one region in the middle of the long arm in the tenth chromosome (Barsacchi-Pilone et al. 1974), and in *T. vulgaris meridionalis* in a single locus in an intercalary position of the long arm of chromosome XI (Barsacchi-Pilone et al. 1977).

In various species of the family *Plethodontidae* three types of localization of the 5S RNA genes sites have been found: in most cases in the short arms of long chromosomes, sometimes in the long arms on long chromosomes, but in some cases they were localized in centromeric and telomeric regions (Macgregor and Kezer 1973; Macgregor and Mizuno 1976; León 1976; León and Kezer 1978). Besides, in some *Plethodon* species, as in *N. viridescens*, the satellite DNA was localized in pericentric regions (Macgregor and Kezer 1971; Macgregor et al. 1973). It can be assumed, therefore, that in these animals, too, the 5S RNA genes are partly located in C-banding regions of the chromosome.

In summary it can be stated that in most cases C-bands in urodeles do not correlate to rRNA loci; sometimes, however, some of the rDNA sites are found in the banded regions. A specific feature of the location of the 18S and 28S RNA genes in urodeles is that they are found both in NORs and in other chromosome sites in the form of small rDNA blocks, the position of these sites being strictly heritable. This fact is not quite in accordance with the theory that the 18S and 28S RNA genes have a fixed position in eukaryotic chromosomes (Lima-de-Faria 1976). The 5S RNA genes also may be interspersed. Sometimes their location coincides with the location of the satellite DNA.

c. *Chromosomes of Anuran Amphibians*

The available data on differential staning of anuran chromosomes is rather limited. G-banding experiments have been carried out on the chromosomes of primitive species of *Anura-Xenopus laevis*, 2n=36 (Matsui 1974), and *X. mulleri*, 2n=36 (Stock and Mengden 1975); however, the published data does not allow us to discover the banding patterns of all the chromosomes. The banding pattern revealed after trypsin treatment of *X. laevis* chromosomes resembled "mitotic spiralization" of chromosomes (Matsui 1974). In *X. mulleri*, where the bands were obtained after trypsin and urea treatments, the pattern was somewhat similar to that in mammals, but the number of bands per chromosome unit seems to have been greater, and the pattern was considerably different from bandings in birds, turtles and snakes (Stock and Mengden 1975). After a weak treatment of the mitotic chromosomes of *X. laevis* with an acid the telomeres of almost all the chromosomes and the secondary constriction of the twelfth chromosome were stained; strong treatment (N-banding to localize the NORs) produced bands only at the secondary constriction of the twelfth chromosome (Matsui 1974; Funaki et al. 1975). This is the site of 18S+28S rDNA (Pardue et al. 1973). 5S RNA genes in both *Xenopus* species were localized in other regions, namely in the subtelomeric regions of the long arms of the majority of chromosomes (Pardue et al. 1973; Pardue 1974).

C-bands have been obtained on the chromosomes of 4 *Hylidae* species and 18 *Bufonidae* species (Schmid 1978a; Obara et al. 1975; Birstein, 1981a). Usually centromeres, and in many cases also telomeres (often more weakly) and the regions of secondary constrictions were stained. In all 3–5 bands per chromosome were revealed. In the experiments of other authors centromeres and sometimes pericentric regions of *Bufo* chromosomes were stained (Graphodatsky et al. 1978; Birstein, 1981a).

C-bands were also obtained in the related *Bufonidae Leptodactilus ocellatus* and *Odontorphynus*. In *L. ocellatus*, 2n=22, centromeres of all chromosomes were stained, and also 1–2 or 5 bands were observed in different regions of various chromosomes (Bianchi et al. 1973). In many instances the position of bands coincided with the localization of late-replicating DNA and secondary constrictions (they are present in all chromosomes of this species), but DNA of the staining centromeres in six chromosomes was not late-replicating. This has led the authors to the assumption that the centromeric heterochromatin is heterogenous.

In the diploid *Odontophrinus americanus*, 2n=22, there are secondary constrictions on the fourth and eleventh chromosomes, while in the teraploid sibling species *O. americanus*, 4n=44, and in *O. cultripes*, 2n=22, the constrictions are present only on the eleventh chromosome. In *O. americanus*, 2n, C-bands appeared only at secondary constrictions of the fourth chromosomes, while in *O. americanus*, 4n, and in *O. cultripes* they appeared at secondary constrictions of

the eleventh chromosomes (Ruiz and Beçak 1976). Besides, in fresh chromosomal preparations of the latter specimen intercalary bands were obtained on the second, third and sixth chromosomes, while in old ones centromeres of all chromosomes were also stained.

N-staining confirmed the results of C-staining; in the diploid *O. americanus* in the regions of the secondary constrictions of the eleventh chromosomes there were no bands, consequently, it did not contain rDNA (active rDNA, at any rate) (Ruiz and Beçak 1976). The secondary constrictions of the fourth chromosomes in *O. americanus*, 2n and of the eleventh chromosomes in *O. americanus*, 4n were positively stained. On the whole, the number of C-bands in the diploid and tetraploid *O. americanus* is proportional to the DNA content and the number of rDNA cistrons.

C-bands have also been obtained on the chromosomes of some species of the genus *Rana* (Raicu and Georgmăneanu 1977; Schmid 1978b; Heppich 1978; Graphodatsky et al. 1978; Birstein, 1981a). After relatively strong treatments (trypsin, NaOH) only centromeres become stained, while using $Ba(OH)_2$ both centromeres and other regions are stained.

Thus C-bands appear on chromosomes of a variety of anuran species. As regards G-staining, all authors are unanimous: all attempts to obtain G-bands in anurans, that would be similar to G-bands in mammals, have failed (Raicu and Georgmăneanu 1977; Schmid 1978b; Graphodatsky et al. 1978; Birstein, 1981a). During G-staining chromomere-like structures resembling mitotic spiralization appear first, which disappear as the treatment proceeds, and the chromosomes become "swollen" and deformed (Birstein, 1981a). We know of only one successfull attempt where clear G-bands were obtained after staining the chromosomes of the haploid *Rana pipiens* cell line ICR 2A, n=13 (Freed 1975–1976). The number of G-bands per chromosome unit length was considerably greater than that of mammalian chromosomes. It is difficult to tell whether those bands were due to a different chromatin state in the haploid cells cultured in vitro or to a modified staining technique.

Like G-bands, Q-bands on anuran chromosomes appear rarely. Usually after quinacrine staining the majority of chromosome regions fluorescence with a uniform and weak intensity, however fluorescing regions are mostly exceptions (Pardue 1974; Badaev et al. 1974; Ruiz and Beçak 1976; Schmid 1978a, b; 1980; Birstein, 1981a). In the case of *Xenopus mulleri* chromosomes (but not of *X. laevis* chromosomes) the localization of intensive fluorescing Q-bands correspond to the regions of the localization of AT-rich satellite DNA (Pardue 1974). In Schmid's experiments (Schmid 1978a, b; 1979; 1980) C-band positive regions of chromosomes of many *Bufo* species and some other *Anura* fluoresced brighter (intensive Q-bands, 3-6 per haploid set) or weaker (regions of secondary constrictions, some telomers) than the most parts of chromosomes (euchromatin). The mithramycin and chromomycin A_3-stainings (fluorescent GC-specific antibiotics) generally resulted in a pattern with the same regions but opposite to those obtained with quinacrine stain: the weaker a region fluoresced with quinacrine, the stronger was the intensity of the fluorescence with antibiotics (Schmid 1980).

As has been shown using N- or Ag-techniques, in most anurans studied (species of the genera *Xenopus, Odontophrynus, Hyla, Bufo, Rana*) rDNA is located only at secondary constrictions of one of the chromosome pairs (Funaki et al. 1975; Ward 1977; Ruiz and Beçak 1976; Schmid 1978a, b; Beck and Mahan 1979; Aleksandrovskaya et al. 1979). Secondary constrictions are often C-band positive heterochromatic regions (Ruiz and Beçak 1976; Freed 1977; Schmid 1978a, b). By contrast the sites of 5S RNA genes in *X. laevis* and *X. mulleri* are located in subtelomeric regions of the long arms of most of or all chromosomes (Pardue et al. 1973; Pardue 1974).

In summary, compairing banding patterns on chromosomes of anurans and urodeles, it should be noted that in the former G- and often Q-bands do not appear, whereas in the latter, if they do, their positions coincide with C-bands. As in urodeles, C-bands in anurans usually occur in centromeric, but sometimes in telomeric and other regions; on the whole, C-banding patterns may vary more than in urodeles. In anuran chromosomes cold-induced secondary constrictions do not appear. The genes for 18S+28S rRNA are located at secondary constrictions, and, unlike urodeles, anurans have no additional rDNA sites.

In general, the whole picture in the case of amphibians is quite different from that of mammals with their characteristic G- and Q-banding patterns. This difference may be due to the specific organization of DNA in amphibians, or to chromatin packing in chromosomes, or both.

2. DNA Structure in Amphibians

a. The Genome Size

One of the principal molecular features of amphibian cells is their high nuclear DNA content which can be tens, hundreds or even thousands times as greater than that in mammals (Morescalchi 1973; Fredga 1977). The DNA content shows a very high degree of interspecific variability: from 30 to over 160 pg/N in urodeles and from 2 to 25 pg/N in anurans (Morescalchi 1973; Olmo 1973; Olmo 1973; Olmo and Morescalchi 1978; Bachmann and Nishioka 1978). Among different species with the same chromosome number and belonging to the same genus the DNA content may differ by a factor of about 1.5-2.0 (Morescalchi 1973; Bachmann and Nishioka 1978).

In southern anurans genome size is smaller than in northern species within the same genus (Bachmann and Blommers-Schlösser 1975; Bachmann and Nishioka 1978). Apparently, this is a general rule for all genera (Morescalchi 1973; 1977). There is an inverse correlation between the nuclear DNA content in anurans and the rate of embryonic development: in animals with larger genomes metamorphosis is completed later than in species with smaller genomes (Goin et al. 1968; Bachmann and Nishioka 1978). A similar correlation can be observed among urodele species: species with paedogenetic forms (i.e. forms which can reproduce themselfs in the larval stage) have larger genomes (Morescalchi 1973).

Nuclear volume, cell volume and cell surface area show a linear and direct correlation to the nuclear DNA content (experiments on erythrocytes of amphibians) (Olmo and Morescalchi 1978). As DNA content increases, cell volume and, to a lesser extent, cell surface area both increase, and the cell surface area/cell volume ratio tends to decrease. These correlations are characteristic of both anurans and urodeles with a DNA content of less than 70 pg/N; among urodeles with a higher nuclear DNA content there is a tendency to the stabilization of the cell surface area/cell volume ratio. Probably, these correlations are due to the fact that Amphibia, lacking physiological mechanisms capable of controlling the rate of oxidative metabolism in the organism as a whole, exercise this control at least at the cellular level (Morescalchi 1977). In any case, variations in the genome and cells sizes seem to determine the adaptive opportunities of amphibians, which are greater than in other vertebrates (Olmo and Morescalchi 1978).

Thus a high nuclear DNA content and a correlation between its great variability with the length of morphogenesis is a characteristic feature of amphibians. Therefore their DNA must have an organization that would allow an easy enough change in the number of genome fractions responsible for the morphogenesis rate.

According to cytological data, variations in DNA content do not radically change chromosome morphology, which may point to a distinctive characteristic of chromatin packing in amphibian chromosomes. It should be remembered that both the structure of the DNA and the means of chromatin packing provide the possibility for the packing of the "excess" DNA in the chromosomes of amphibians. It seems that all these characteristics are directly connected with the banding patterns of anuran chromosomes.

b. Genome Organization in Amphibians

The pioneer works on the genome structure in amphibian had pointed out that genome size increases as the amount of repetitive DNA increases (Straus 1971; Morescalchi and Serra 1974; Rosbash et al. 1974). Experimental studies of the kinetics of DNA reassociation revealed that in *Andreas japonicus,* 2n=60, 2C=93 pg (the most primitive of the urodeles studied), and in *Necturus maculosus,* a species with a very high DNA content (2C=165 pg), DNA reassociates very quickly, due to a great number of repetitive DNA fractions in their genomes (Morescalchi and Serra 1979). DNA of more advanced species, both with a high *(Amphiuma means,* 2n=28, 2C=150 pg, family *Amphiumidae*) and a low (species of *Plethodontidae* and *Salamandridae,* 2C=30-60 pg) DNA content ressociated more slowly and the reassociation curves were more complex. It is interesting to note that, judging by the kinetic data, the genome of *Taricha torosa,* 2n=22, 2C=56 pg, is more complex than the genome of *Triturus cristatus carnifex,* 2n=24, 2C=44 pg, although the genus *Taricha* is considered to derive from the genus *Triturus.* On the whole, it seems that species of evolutionarily advanced families have more complex genomes than species of more primitive groups (Morescalchi and Serra 1974).

Further experimental studies of the reassociation kinetics confirmed the hypothesis that both in urodele and anuran amphibians the difference in the genome size is due to the different repetitive DNA content. DNA of two anuran species – *Xenopus laevis,* 2n=36, 2C=6 pg, and *Bufo bufo,* 2n=22, 2C=14 pg – and two urodele amphibians – *Triturus cristatis,* 2n=22, 2C=46 pg, and *Necturus maculosus,* 2n=38, 2C=104 pg – have been investigated (Baldari and Amaldi 1976). Analysis of the reassociation curves for DNA fragments of a length from 200 to 250 nucleotides has led to the conclusion that in DNA of *X. laevis* and *T. cristatus* there are sequences of three classes: highly repetitive and foldback, intermediate repetitive and unique sequences, whereas in DNA of *B. bufo* and *N. maculosus* there are four sequence classes, since in those species intermediate seguences consist of two components, I and II. The whole picture of the genome structure of these four species can be seen from Table I. It is worth noting that in DNA of *X. laevis* the number of palindromes per genome is very great and equals 100,000 (in mice it is 40,000 and only 4000 in *Drosophila*) (Perlman et al. 1976). Probably, this number is also great in other amphibians.

Thus it seems that the difference in DNA content between *X. laevis* and *B. bufo* (3 and 7 pg per haploid genome respectively) almost wholly depends on the difference in the content of repetitive fractions, including particularly highly repetitive and intermediate sequences, while the amount of unique sequences is approximately the same in the two species (about 1.5 pg per haploid genome). Similarly, the difference in DNA content between *T. cristatus* and *N. maculosus* (23 and 52 pg per genome respectively) is due to the different content of highly repetitive and intermediate sequences, the amount of unique sequences being equal (about 11 pg per genome).

Table 1. Genome structure of four amphibian species*

Species	2n	C, pg	Highly repetitive and foldback sequences			Intermediate repetitive sequences					Unique sequences		
			Amount		Average length, nucleotides	Amount				Average length, nucleotides	Amount		Average length, nucleotides
			pg/genome			pg/genome		%			pg/genome		
				%		I	II	I	II			%	
Xenopus laevis	36	3	0.2	7	480	0.9		30		380	1.9	63	1600
Bufo bufo	22	7	1.4	20	560	3.5	0.7	50	10	400	1.4	20	760
Triturus cristatus	24	23	2.3	10	420	9.9		43		350	10.8	47	1340
Necturus maculosus	38	52	10.4	20	480	24.4	5.2	47	10	370	12.0	23	880

*Data from Baldari, Amaldi 1976; 1977.

The interspersion period of repetitive and unique sequences in renaturated DNA, both pretreated with nuclease S_1 and non-treated, was analyzed by electron microscopy and agarose gel electrophoresis (Baldari and Amaldi 1977). The length of the interspersed repetitive sequences in DNA of the species of both orders turned out to be the same – 300 to 400 base pairs (380 nucleotides on an average). The length of unique sequences in DNA of *B. bufo* (760 nucleotides) was shorter than in DNA of *X. laevis* (1600 nucleotides) and the length of unique sequences in DNA of *N. maculosus* (880 base pairs) was shorter than in DNA of *T. cristatus* (1340 nucleotides). Consequently, species with a higher DNA content have shorter unique sequences than species with a lower DNA content (see Table I). Besides, in the course of the evolution of *Anura* and *Urodela* there were apparently no changes in the length of repetitive sequences despite their different number in genomes.

More detailed studies of the intermediate repetive sequences have revealed that those which are accumulated in long clusters increase in number roughly proportionally to the increase of genome size (Bozzoni and Beccari 1978). The number of short repetitive sequences (about 300 nucleotides) grown unproportionally rapidly; however, it should be remembered that in species with a high DNA content the percentage of highly repetitive sequences also increases and an additional new fraction, component II, appears (Baldari and Amaldi 1976).

Although the means of change in genome size in the two *Amphibia* orders discussed seem to be quite similar, and the quantity of intermediate repetitive sequences seem to be mostly responsible for the changes in DNA content, the primary structure of repetitions is very different in each case. Thus the percentage of hybridization of different anuran DNAs is high: 60 to 80% in the case of hybridization between DNA of *Rana* species, and 8 to 25% for species of different families (the percentages of hybridizations of *R. ridibunda* DNA with *Bufo bufo* and *Hyla japonica* DNA were 25 and 20% respectively, and *X. laeves* and *Bombina variegata* DNA – 8-16%) (Mednikov et al. 1976). A similar picture is observed among urodeles: the percentage of hybridization of DNAs of closely related species of the same group of the large genus *Plethodon* is 40-60%, while for distant species it is less than 10% (Mizuno and Macgregor 1974). Hybridization between an anuran *(R. ridibunda)* and an urodele (*Salamandra salamandra* and *N. maculosus*) DNA was very low – 2 to 6% (Mednikov et al. 1976) – which points to considerable structural differences between repetitive DNA sequences in the species of these two orders.

As regards intermediate sequence fractions in urodeles, it has been shown that, unlike distant species, related species have identical, evolutionarily stable sequences (Mizuno et al. 1976; Macgregor et al. 1977: Macgregor and Jones 1977). Closely related species have much in common (over 90% of hybridization between DNA of *Plethodon cinereus cinereus,* C=20.0 pg, and *P. hoffmani*, C=21.4 pg, or *P. nettingi*, C=18.2 pg), but distantly related species have only about 10% of intermediate repetitive sequences in common. Thus, in the case of *P.c. cinereus* and *P. dunni,* C=38.8 pg, the percentage of DNA hybridization was 12.1%, that is about 6.000 "common" repetitive sequenses per genomes (Mizuno et al. 1976; Macgregor et al. 1977), and the percentage of all intermediate sequences is about 50 and 80% per genome respectively (Mizuno and Macgregor 1974). In situ hybridization of middle repetitive fractions has shown that in *P. cinereus* these sequences are non-uniformly distributed along the mitotic chromosomes, apparently in blocks (Macgregor Andrews 1977; Macgregor 1978). Similar experiments with *Triturus cristatus carnifex* chromosomes have revealed that the heteromorphic segments of the first chromosome pair are enriched

with middle and high repetitive sequences (Macgregor 1978; 1979).

Structural differences between DNA from urodeles and anurans can also be seen during the analytic centrifugation of DNA in CsCl. In the course of the experiment (Thiery et al. 1976) DNA of *X. laevis* was divided into two components, p=1.699 and 1.704 g/cm^3 (92% and 8% of genome, respectively), while in the case of *Pleurodeles waltlii* there were three components: major, p=1.7036 g/cm^3 (85%), minor, p=1.706 g/cm^3 (7%), and a satellite, p=1.709 g/cm^3 (9%). Similar experiments revealed satellites in DNA of other urodeles: some *Plethodon* species (up to 4 to 20%; in some of the species no such fractions were found (Macgregor and Kezer 1971; Macgregor et al. 1973) and *Triturus viridescens* (Barsacchi and Gall 1972). In all those cases, as was noted earlier, *in situ* hybridization showed the satellite DNA to be located in the centromeric heterochromatic regions.

In DNA from *X. laevis* a satellite (p=1.712 g/cm^3, 0,3% of genome) was found only on combined Cs_2SO_4–Ag^+ and CsCl analysis (Thiery et al. 1976). In a similar study of *P. waltlii* DNA two satellites were found, p=1.686 and 1.716 g/cm^3 (0,4 and 0,2% of the genome , respectively). Structural differencies between DNA from *X. laevis* and *P. waltlii* were also confirmed by the combined centrifugation of DNA pretreated with a mixture of restrictases (Macaya et al. 1976).

Satellite DNA (two AT-rich satellites making up about 4% of the total DNA) has been found on centrifugation in CsCl of *X. mulleri* DNA (Stern 1972; Pardue 1974). On of those satellites (p=1.683) was localized in the short arms of almost all (except two) chromosome pairs, that is in those regions which, as was pointed out above, fluorescence after being stained with quinacrine mustard (Pardue 1974). Since chromosomes of most anurans show no Q-bands and in two *Anura* species (*X. laevis* and *R. pipiens*) no satellite DNA has been found (Thiery et al. 1976; Sueoka 1961), satellites in *X. mulleri* seem to be rather an exception from the general rule.

The facts presented above can be *summarized* as follows. DNA from urodeles and anurans differ in a number of characteristics: the amount of unique sequences differ by one order (1.5 pg in anurans and 11 pg in urodeles; such a great number of unique sequences is surprising in itself); in anurans practically no satellite DNA was found; the percentage of hybridization of repetitive DNA of animals belonging to the two orders is low. However, the genome growth (and, probably, decrease) seems to be governed by the same mechanism in both orders, the relative length of unique sequences decreases by 1.5-2 times, while the proportion of repetitive fractions increases – proportionally to the genome growth in the case of clusters, and unproportionally in the case of short interspersed repetitions. How this is connected with genome activity, particularly with slow larval development of the organism, is not quite clear.

The differences in DNA characteristics between *Anura* and *Urodela* species are not great as compared with the differences between amphibians and higher vertebrates, particularly mammals. For instance, by the centrifugation in CsCl of the DNA of 11 mammals and two bird species three major components have been found (p=1.697, 1.704 and 1.709 pg/cm^3) (Thiery et al. 1976). In mammals their relative contents vary insignificantly: 50-60%, about 25% and about 5-10% respectively. In the same work, as was pointed out above, in CsCl amphibian DNA was divided into three components: major, minor and – in the case of urodeles – a satellite. Another feature of amphibian DNA (of anurans, at any rate) is a high content of palindromes (Perlman et al. 1976) and a high level of methylation, 4% for *X. laevis* cell culture DNA to compare with 1.0-2.9% for the total mammalian DNA (Browne et al. 1976).

It used to be an accepted theory that most vertebrates have an identical general DNA structure resembling that found in *X. laevis* (Davidson et al. 1975) where about 50% of the genome consist of closely interspersed repetitive and unique DNA sequences whose average length is 300±100 and 800±200 nucleotides respectively. In the rest of the DNA repetitions are rare and spaced at about 4000 nucleotides (Davidson et al. 1973). Now it has become evident that no such clear-cut universalization exists. For example, genome organization in many birds is apparently quite different (Arthur and Straus 1978; Epplen et al. 1978; Ginatulin et al. 1979), for more on which see below. Moreover, recent data on the repetitive sequence arrangement in amphibians does not quite agree with the results of the pioneer works. It is probably the way in which repetitive and unique sequences are interspersed that constitutes the difference between details of genome structure in amphibians and mammals.

Distinctive features of amphibians can sometimes also be seen on the protein level (that is in some structural genes).Thus no immunological relatedness has been found between serum albumins from frogs of the genus *Rana* and from some species belonging to other vertebrate classes (Wallace and Wilson, 1972). Similar results have been obtained for transferrin from *Pleurodeles waltlii* (Foucrier et al. 1976). In both cases cited antiserums precipitated only with proteins from members of that family of *Amphibia* to which the studied species belonged (Walace et al. 1971; Foucrier et al. 1976). This is not a hard-and-fast rule: the antiserum to *R. pipiens* lactate dehydrogenase M_4 reacted with the same enzyme from species of other vertebrate classes (Salthe and Kaplan 1966).

c. rDNA Structure and Transcription in Amphibians

At present, the structure of ribosomal DNA in amphibians has been studied rather comprehensively; its details have been found different from those of mammalian rDNA. It has also been shown that the process of rDNA transcription is different in animals of these two classes.

As was noted earlier, in most amphibians rDNA (18S+28S rRNA genes) is located in NORs occuring in one chromosome pair (see above). rDNA content of various *Urodella* is higher compared to the amount of rDNA in *Anura*. Thus, the ratio of rDNA content in *X. laevis* (C=3 pg) to those in *Plethodon* species (C=20.0-38.8 pg) or in *Ambystoma opacum* (C=30.1 pg) is 1:3, to those in *Oedipina uniformis* (C=62 pg) is 1:5 and to those in *Triturus carnifex* (C=33.5 pg) is 1:7 (Vlad 1977). Therefore rDNA content increases with increasing of genome size, but the relative proportion of rDNA decreases with the increase of DNA content.

rDNA of *X. laevis* can be displaced from the bulk of nuclear DNA as a distinct satellite which bands on CsCl gradients at a buoyant density of 1.723 g/cm^3 and comprises about 0.2% of the genome (Birnstiel et al. 1968). It consists of repeating units with a molecular weight of 7.3-8.7x10^6 daltons; about 500 such units per haploid genome are arranged in tandem (Wellauer et al. 1976).

Only parts of the repeating units are transcribed into 40S rRNA precursor and non-transcribed spacers occupy about one-third of the total rDNA length (Wellauer et al. 1976; Buongiorno-Nardelly et al. 1977; Fedoroff 1979). The sequence coding for the precursor of rRNA is constant in length and exactly the same in sequence in different repeating units. The transcription unit is flanked by short nonrepetitive spacer sequences. The remainder of the nontranscribed spacer varies in length from about 2.7 kb to about 9 kb in different repeating units. On whole spacer DNA consists of 7 regions, three of which are internally repeated (Boseley at al. 1979; Moss et al. 1979). These nontranscribed regions differ in sequence because in the first one clusters of about 100 nucleotide pairs are repeated, and in the other two repeating units of 81 and 60 nucleotide pairs are interspersed.

The transcribed rDNA of *X. laevis* is highly methylated (up to 99%), whereas in spacers non-methylated regions are concentrated (Bird and Southern 1978). This is a peculiar feature of rDNA of somatic tissues only, because amplified oocyte rDNA is less methylated. It has been proposed that a complex organization of spacers may be connected with their function: spacers may include centers of RNA-polymerase binding and promotor zones (Moss et al. 1979). In experiments with rDNA of *X. laevis* and *X. mulleri* it was shown that the spacer rDNA evolves considerably faster than the transcribed regions (Brown et al. 1972).

In mammals rDNA can be located in only one site of the haploid set (for example, in the gibbon – Wartburton et al. 1975) or in several sites (for example, there are three such sites in the rat *Rattus norvegicus* (Szabo et al. 1978) and five in man (Henderson et al. 1972; Evans et al. 1974). The number of rRNA genes also varies: in mice it is about 100 (Gaubatz et al. 1975), in *Rattus norvegicus* – about 100-360 (Melli et al. 1971), and in the cat – about 1000 (Szabo et al. 1978). Although many details of rRNA gene organization in mammals remain unknown, it has been found that the size of repetitive units in them is about twice as great as it is in the case of amphibians (16.2-21.3x10^6 and 8x10^6 daltons respectively) (Southern 1975; Blin et al. 1976; Arnheim and Southern 1977; Stambroock 1978; Meunier-Rotival et al. 1979). As in amphibians, the absolute size of rDNA repeating units in mammals can also vary, due to variations in spacer length (Arnheim and Southern 1977; Stambroock 1978; Krystal and Arnheim 1978). Thus in human rDNA repetitive units of the length of 43.7, 28.6 and 53.9 kb have been discovered (Wellauer 1979).

The difference in rDNA gene size between amphibians and mammals is accompanied by the difference in transcript size: in all poikilotherms – fishes, amphibians and reptiles – the size of pre-rRNA is less (38-40S, or 2.6-3.0x10^6 daltons; the same values have been determined for many plants and insects) than in birds and mammals – 45S (Perry et al. 1979: Wellauer and David 1974; Rungger and Crippa 1977). In mammals it is greater than the sum of 18S and 28S by almost 80%, while in polikilotherms the difference is only 25-50%. The size of 28S RNA in amphibians is much smaller (1.39-1.55x10^6 daltons) than in birds and mammals (1.58x10^6 and 1.71x10^6 daltons respectively), while the size of 18S is approximately the same (0.7x10^6 daltons) (Loening 1968; Rogers and Klein 1972). It should be noted that the molecular weight of 28S RNA varies among species of the two *Amphibia* orders: 28S RNA in *X. laevis* is larger than in *Triturus cristatus* and *Plethodon cinereus* (1.55x10^6, 1.39x10^6 and 1.45x10^6 daltons respectively) (Rogers and Klein 1972).

Both rDNA and rRNA are evolutionarily conservative. Thus rDNA of *X. laevis* hybridizes with DNA of various eukaryots (Sinclair and Brown 1971) and is frequently used for the localization of rDNA sites in chromosomes of different animals (e.g. Evans et al. 1973; Hsu et al. 1975). On the other hand, two thirds of the conserved similar regions are present in 18S rRNA of various animals (Gerbi 1976) and the 5'-end nucleotide sequences in 18S RNA are identical in all the vertebrates studied (Sakuma et al. 1976). One third of the conserved regions is present in 28S rRNA and the 5'-end sequence in 28S RNA in *X. laevis* is different from that in other vertebrates (Gerbi 1976; Sakuma

et al. 1976). This is in accord with the above-mentioned difference in size between 28S RNA molecules in different animals. Positions of methyl groups in 18S and 28S rRNA molecules are not the same in *X. laevis* and mammals (Khan et al. 1978).

5.8S rRNA transcribed from the region between 18S and 28S-genes and remaining bound to 28S rRNA in ribosomes is evolutionarily very stable; 5.8S RNA sequence in the hen and the turtle *Terrapene carolina* is 99% homologous with that in human cells (Nazar and Roy 1976; Khan and Maden 1977), while 5.8S rRNA sequences in *X. laevis* and *X. borealis (mulleri)* are 97% homologous, being especially distinct at the 3'-end, where in amphibians there is C and not U, as in other animals (Khan and Maden 1977; Ford and Mathieson 1978).

All these features of rDNA show that it is very conservative and probably relatively autonomous. The latter is also evidenced by the presence of RNA-polymerase I in the nucleoli of animal cells (Reeder and Roeder 1972), which transcribes rDNA and remains bound to the NORs in mitotic chromosomes (Matsui et al. 1978). In amphibians the autonomy of rDNA is also demonstrated in its ability to amplify in ovary cells.

In spite of the noted differences in size between rRNA genes and their transcripts in cold- and warm-blooded animals, there seems to be no reason to believe that there are radical differences in chromatin and chromosome structure of these regions between different animals, taking into account the evolutionary conservatism of rDNA. The difference in gene size rather is due to different rates of processing in warm- and cold-blooded animals. Anyway, rDNA organization in amphibians supports the suggestion that rDNA are not randomly localized: these sites take a fixed position with respect to centromeres and telomeres (Lima-de-Faria 1976). The fact that in mammals rDNA is often located in several chromosomes does not fully comply with this rule.

d. 5S RNA Genes in Amphibians

A few words about 5S DNA seem to be essential. As was noted above, in urodeles 5S RNA genes can be located both in one site of a specific chromosome (e.g. in the *Triturus* species – Barsacchi-Pilone et al. 1974) and in several sites, in different chromosomes (e.g. in *Notophthalmus viridescence* Hutchinson and Pardue 1975; Pukkila 1975). In anurans, 5S RNA genes have been localized only in *Xenopus laevis* and *X. mulleri* – in both cases in the telomeric regions of long arms of (probably) all chromosomes (Pardue et al. 1973; Pardue 1974).

In mammals, 5S RNA genes are located at one or two sites: in the long arm of the first chromosome in man (Steffensen et al. 1975), and in homologous regions in chimpanzee, gorilla and orang-outang (Henderson et al. 1976); at one site in the cat and, probably, mouse, but at two sites in the Norwegian rat – in the NOR in one chromosome, and the telomeric region in another (Szabo et al. 1978).

Some detailed data on 5S RNA structure has been obtained for *X. laevis* and *X. mulleri* (Brown et al. 1971; Brown and Sugimoto 1973; Fedoroff 1979). There have been found 24,000 and 9,000 5S RNA genes per genome, respectively. The average size of repetitive units in *X. laevis* is $0.5\text{-}0.6 \times 10^6$ daltons and $1.2\text{-}1.5 \times 10^6$ daltons in *X. mulleri*, the 5S RNA gene size being equal in both species – about 0.08×10^6 daltons. Thus a greater portion of the repetitive sequence is presented by a non-transcribed spacer, the average spacer length in *X. mulleri* being twice as great as in *X. laevis*. It is interesting that in these two species the spacer DNA is practically non-homologous (Brown and Sugimoto 1973), while 5S RNAs in them differ from one another only by one nucleotide substitution (Ford and Brown 1976).

In mammals, the number of 5S RNA genes is much smaller than in *Xenopus*: about 2000 copies per genome in HeLa cells (Hartlin and Attardi 1971), in the case of the DNA of diploid human cells the number appears to be 2-3 fold lower (Szabo et al. 1978).

The homology of the 5S RNA sequences in vertebrates is very great (Lind et al. 1978; Denis and Wegnez 1978). The 5S RNA nucleotide sequence is identical in all the mammals studied (man, mouse, hamster, ox, dolphin, and kangaroo rat). There is one inconsistency with the usual phylogenetic views, namely, the 5S RNA sequence in reptiles is closer to that in modern mammals than in birds (Denis and Wegnez 1978). On the whole, the rate of the 5S RNA gene evolution is quite low: 90 of 120 residues in 5S RNA of all the vertebrates studied are identical. Consequently, 5S RNA genes, like 18 and 28S RNA genes, are quite conservative regions of vertebrate genomes.

It should be noted, however, that only the sequences of 5S, 18S and 28S RNA genes are rather conserved in evolution, the rate of the spacer region evolution being quite high. Divergence time for *X. laevis* and *X. mulleri* is about 10 million years (Bisbee et al. 1977); in the case of rDNA homology of spacer regions has decreased by 20% during this period (Brown et al. 1972), and in the case of 5S DNA spacer sequences became almost nonhomologous (Brown and Sugimoto 1973). The lability of the spacer DNA is also confirmed by the polymorphism of its size and the nucleotide sequence (Buongiorno-Nardelli et al. 1977).

It is quite probable that the position of 5S DNA in the chromosomes of vertebrates is non-randon, exactly as the location of rDNA in eukaryotes (Lima-de Faria 1976). Thus in amphibians, these genes are located mainly near telomers or centromers (see above). It seems possible that in the interphase nuclei of eukaryotes 5S RNA genes take a precise position; for instance,

it was found that in *Drosophila melanogaster* interphase nuclei 5S RNA genes are situated between the nucleolus and the nuclear membrane (Steffensen 1977).

In contrast to phylogenetically higher vertebrates, in anurans *(Xenopus)* a dual 5S RNA gene system has been found to exist: the genes of somatic cell (mentioned above) and 5S genes which are active in oocytes (Wegnez et al. 1972; Ford and Brown 1976). The oocyte-type 5S DNA in *X. laevis* consists of reiterated repetitive units, approximately 700 nucleotides long; about half of this length is occupied by a rather complex spacer, the other half includes a 5S RNA gene, the so-called "pseudogene" (which is probably a result of the 5S RNA gene duplication), and a region between those two (Jacq et al. 1977; Fedoroff and Brown 1978; Miller et al. 1978; Fedoroff 1979). The oocyte-type 5S RNA genes in *X. laevis* and *X. mulleri* have envolved much faster than the genes of somatic cells and independently of them (Ford and Brown 1976; Denis and Wegnez 1978). The oocyte 5S DNA is probably transcribed by the specific RNA-polymerase III (Parker and Roeder 1978).

A similar dual encoding 5S RNA system has been found in Teleost fish, namely, in *Tinca tinca* (Denis and Wegnez 1977), but here the oocyte genes have evolved more slowly than 5S RNA genes of somatic cells (Denis and Wegnez 1978). It is not yet known how frequently these two 5S RNA gene types occur in different amphibians and fishes. It would be equally interesting to find out whether other vertebrate species have dual systems for 5S RNA coding, or this is a feature of amphibians and fishes, determined by their development.-

3. Chromatin Structure in Amphibians

Switching from DNA content and organization in amphibians to DNA packing in chromatin, it should be stated, regretfully, that very little is known about the details of chromatin structure in amphibians. An electron microscopic study of metaphase chromosomes of somatic cells and chromosomes of the first meiotic division of *Odontophrynus americanus*, 4n, revealed chromatin fibers consisting of globules (nucleosomes) about 80 Å in diameter and loops with 160 Å globules (Beçak et al. 1977). In the condensed oocyte or spermatocyte chromatin of this animal the size of globules of supernucleosomes consisting of 8-10 nucleosomes 80 Å in diameter may be as great as 300 Å (Beçak and Fukuda 1979). Chromatin of the snake *Xenodon neuwiedii* has the same structure (Beçak et al. 1977). Similar nucleosome characteristics are typical for bird and mammalian chromatin (Georgiev and Bakaev 1978).

The nucleosome repeating length of 5S DNA is 175±5 base pairs (bp) in both red blood cells and liver of *X. laevis*, suggesting that these specific genes may have a constant chromatin structure, independent of the organization of the bulk chromatin in the two tissues (in blood cells and liver the nucleosome repeating length of the majority of the chromatin is 189±5 bp correspondingly) (Humphries et al. 1979). In addition, 5S DNA is less susceptible to staphylococcal nuclease than is bulk DNA in nuclei from both cell types.

The association of nucleosomes with DNA in the formation of amphibian chromatin subunits (experiments on *X. laevis* rDNA) is probably not base-sequence specific (Reeves 1977). The results of electron microscopic study of oocyte and lamprush chromosome chromatin of *X. laevis, Triturus alpestris, T. cristatus* showed that nucleosome frequency is a function of transcription: nucleosomes are not seen in the heavily transcribed regions and are formed only after the inactivation of the regions (Scheer 1978). Thus, in lampbrush chromosomes beaded nucleosome structure of chromatin fibrils is present only in the transcriptionally inactive regions.

The existing data on chromatin proteins in amphibians is also very scarce. The electrophoretic mobility and distribution of nonhistone chromatin proteins in liver and kidney tissue are different in amphibians *(R. pipiens)* and other vertebrates (the turtle *Chelydra serpentina* and rat (Wu et al. 1975). In some amphibians (kidney chromatin of *X. laevis*) a fraction of histone HI has been found which is characteristic of these species (Risley and Eckhardt 1974). The electrophoretic mobility of histones H2A and H2B in amphibians (liver chromatin of *R. pipiens*) is different from that in mammals (Donna and Gorovsky 1975). The distribution and mobility of histones in amphibian embryos *(Cynops pyrrhogaster, X. laevis, X. borealis)* are dissimilar to those in adults (Imoh 1975; Blackler and Cassidy 1978).

It is noteworthy that in erythrocytes of birds, reptiles and fishes there is histone H5, which does not occur in mammals (Edwards and Hnilica 1968; Seligy et al. 1976). Similarly the amino acid compositions of histone H2B of erythrocytes are different in various vertebrate classes (the available data includes *X. laevis, Crocodilus niloticus*, the hen and the calf), the rate of the evolution of these histones being 7-8 times as high as that of histones H3 and H4 (Van Holden et al. 1978).

Perhaps the most interesting results have been obtained studying the testis-specific histones (Kasinsky et al. 1978). During spermatogenesis histone H1 is replaced by another histone (histones), specific for spermatosoids. While in reptiles (snakes and lizards) and mammals there are histones of uniform type (in reptiles there are two histones the electrophoretic mobility of which is close to that of protamins), quite a variety of testicular histones has been found in amphibians. Some genera (e.g. *Bufo*) are characterised by uniform histones, while in others *(Xenopus, Scaphiopus)* there is great variation in the amount and mobility of histone fractions in closely related species. Similar variations have been observed in fish testicles (Bols and Kasinsky 1976).

Immunochemical experiments (Ashmarin et al. 1975; 1976) have revealed a great difference between chromatin proteins in amphibians and other vertebrates. During the reaction of liver chromatin of *R. temporaria* with the homologous antiserum 9 precipitation zones were seen; with the antiserum to liver chromatin of the turtle *Testudo horsfieldi,* there was only one weak zone, and no precipitation occured during the reaction with the antiserum to the rat and hen chromatin. Turtle chromatin reacted with the antiserums to the frog and hen chromatins, but not that of rat, while the rat chromatin precipitated with all heterologous serums. It can be stated, therefore, that there is little immunological similarity between chromatin proteins of the frog and those of other vertebrates.

These data enable us to conclude that the first level of chromatin packing in amphibians, the nucleosome structure, does not differ from the chromatin structure in other animals. This is not surprising, since chromatin structures in all eukaryotes are likely to be basically the same (Frado et al. 1977; Lohr et al. 1977; Georgiev and Bakaev 1978). An analysis of a higher level of chromatin packing in amphibians *(Triturus viridenscens,* lampbrush chromosomes) and mammals (meiotic chromosomes of the Chinese hamster) has also failed to reveal any substantial difference between these two types of chromatin packing (Basu 1977). These results have shown that chromomeres in chromosomes are formed by randomly oriented fibres of coiled DNP, and in interchromomeric regions fibres run parrallel several time in longitudinal arrays. However, a great specificity of chromatin proteins in amphibians (of course, it should be remembered that very scarce data is available at present) does not contradict our assumption of a possibly specific chromosome structure in these animals.

4. Mechanisms of Chromosome banding and Chromosome Structure in Amphibians

Although the mechanism of G-, C- and Q-banding has not been determined so far, it seems essential, in order to reveal a possible difference between chromosome organizations in mammals and amphibians, to observe briefly some basic details of banding of mammalian chromosomes.

a. The Mechanism of Chromosome Banding in Mammals

By using light and electron microscopy it was established that the pretreatment of mammalian chromosomes with proteolytic enzymes or DNase I causes changes in the chromosome surface: on G-bands the collapsed chromosomal morphology was seen; these are the regions where chromatin seems to be densely packed (Burkholder 1975; Gormley and Ross 1976; Burkholder and Weaver, 1977). Distances between identical bands may vary depending on treatment conditions (Takayama 1979).

It is assumed that, in the case of the metaphase chromosomes, the number and arrangement of G-bands are roughly the same as of the chromomeres of pachytene chromosomes (Okada and Comings 1974; Comings and Okada 1975). Pachytene chromosomes and banding of prometapahse chromosomes show that chromosome bands are composed of multiple sub-bands, there are 2-5 sub-bands in each major G-band (Luciani et al. 1975; Comings 1977; Yunis et al. 1978). It seems possible that there are sub-divisions of chromomers (and bands) – so-called "rosetts" composed of loops of DNA clustered around centers of nonhistone proteins (Okada and Comings 1979).

G-band DNA is late replicating and AT-rich (Comings 1977). In interphase these DNA regions are condensed and attached to the inner surface of the nuclear membrane or connected to the nuclear matrix and are almost inactive (Comings 1977; Yunis et al. 1977). After enzyme pretreatments these condenced blocks may be revealed as G-banding regions of chromatin (Bianchi et al. 1977).

Usually many authors connect the mechanism of G-banding (as of C-banding) with the difference in histone and nonhistone proteins content in different chromosome regions (Comings et al. 1973; Holmquist and Comings 1976). Although histones do not play a significant role in G-banding, there is some data on removing histone H1 from non-staining regions (Holmquist and Comings 1976). It seems possible that some nonhistone proteins take part in the high compaction of G-banded regions (Burkholder and Weaver 1977). Apparently, this group of proteins includes the proteins of the nuclear matrix (Comings 1977). One can not exclude the opportunity that proteins of the recently found by some authors (Adolph et al. 1977; Paulson and Laemmli 1977; Jeppesen et al. 1978) chromosome scaffolding structure also take part in G-banding.

In addition to the already mentioned components, specific chromosomal RNA was found in G-banded regions (Pierpont and Yunis 1977; Byarugaba 1978). It seems possible that in G-banding some polysaccharides or glycoproteins take part since hyaluronidase or lysozyme pretreatments change the chromosome staining pattern (Khachaturov et al. 1975; Birstein, 1981b).

The Q-banding mechanism in mammalian chromosomes is not quite clear either. As was mentioned above, it is known that flourescence is enhanced when quinacrine and 33258 Hoechst are bound to AT-rich DNA regions and quenched when they are bound to GC-rich sequences (Comings et al. 1975; Comings and Drets 1976; Jorgensen et al. 1978). A positive correlation between fluorescence brightness and base ratio was established in some regions of quinacrine-stained human chromosomes (Kornberg and Engels 1978). It seems probable that nonhistone proteins affect the interaction between fluorochromes and chromosomes (Comings et al. 1975; Comings and Drets 1976).

On the basis of the data described one can *summarize* that G-, C- and Q-bands reflect the structural differentiation of chromatin (different state of compaction) which is also present in metaphase chromosomes. The relative positions of G- and C-banding regions in interphase nuclei, possibly, determine a certain spatial arrangement of active chromatin regions which is necessary to successful transcription in cells of each tissue (Steffensen 1977; Jones 1978).

b. Hypothesis of Amphibian Chromosome Organization

Coming back to amphibians, it will be recalled that many authors have failed to obtain G- and Q-bands on anuran chromosomes, while on urodele chromosomes the locations of few G-, C- and Q-bands often coincide. Taking into account the banding pattern, genome size and DNA and chromatin organization in amphibians and mammals one can suppose that chromosome structure in these animals (in particular, chromatin compaction) is different.

Recently it was proposed that the absence of G- and Q-bands on amphibian (and plant) chromosomes is due to the very high degree of spiralization of amphibian chromosomes in comparison with the mammalian chromosomes (Greilhuber 1977; Schmid 1978a; 1980). Thus, the comparison of human chromosomes with those of anurans (some *Bufo* species) shows that the mean DNA content per nm of chromosome length in metaphase is 1.5 to 3 times greater in the *Anura* (Schmid 1978a). If one assumes, that G-bands in prometaphase correspond to chromomers in pachytene, and taking into account DNA content per chromosome and the length of mitotoc and meiotic chromosomes, one can see that the G-bands on amphibian (and plant) chromosomes would be placed so close that they could not be resolvable in a light microscope (Greilhuber 1977).

Such an explanation seems to be unacceptible in the cases when the amphibian genome size is comparable with the mammalian genome size, but G-bands are not revealed on chromosomes of these animals (for example, *X. laevis*, 2C=6.3 pg – see above, or *Pelodytes caucasicus*, 2C=4.0 pg – Aleksandrovskaya et al. 1979). It seems possible to propose that the structure of amphibian mitotic chromosomes let DNA content per chromosome (which means the change of the amount of repetitive DNA) change without any serious change in chromosome size and morphology.

Possibly, there are fusions of chromomeres (subbands) in amphibian metaphase chromosomes (at least of anurans) as in mammalian chromosomes. Such a conclusion can be made on the basis of the following data. At first, in the case of chromosomes of *Rana pipiens* cells (haploid cell line ICR 2A) G-bands were distributed rather regularly along the chromosomes (Freed 1975-1976). Secondly, in our own experiments on G-banding of anuran chromosomes we pointed out that after trypsin or saline pretreatment "mitotic spiralization" appeared, and chromomere-like structures were observed (Birstein, 1981a). After a prolonged treatment anuran chromosomes became deformed and swollen. On the whole, it seems that the removed substances are distributed rather uniformly along the chromosomes and the chromomeric structures are more susceptible to the attack of denaturating agents than in the case with mammalian chromosomes.

C-banded structures of the heteromorphic arm of *Triturus cristatus carnifex* chromosome I (Mancino et al. 1973; Rudak and Callan 1976) may be the extreme manifestation of such chromomers in mitotic chromosomes. Thus, 20-30 active chromomers of lampbrush heteromorphic segments correspond to only a few C-bands of mitotic chromosomes (Macgregor 1978; 1979). It must be emphasized that these chromomers and corresponding sites of mitotic chromosomes are highly enriched with high and intermediate repetitive DNA (Macgregor and Andrews 1977; Macgregor 1978; 1979).

The increase in chromomere number in lampbrush chromosomes rises along with the increasing of the genome size, as was shown on some Plethodons (Vlad and Macgregor 1975; Macgregor et al. 1976). Since the increase in DNA content is due mostly to the repetitive DNA, then the lampbrush chromomers of Plethodons seem to be enriched with repetitive sequences (Macgregor et al. 1976). On the other hand, repetitive DNA is distributed non-randomly along the mitotic chromosomes of urodeles (Macgregor and Andrews 1975; Macgregor et al. 1976). It seems possible that these sites may be the sites of fusion of lampbrush chromomers; moreover, data on heteromorphic segment of *T. cristatus* points out that such fusing chromomeres may be enriched with the same family of repeated sequences. Possibly the orientation of chromatin fibers in lampbrush chromosomes promotes such fusion; DNA in chromomeres is oriented randomly, in iterchromomeric regions – mostly longitudinally (Basu 1977).

In conclusion one can *summarize* that the lack of G- and Q-bands on mitotic amphibian chromosomes, analogous to the mammalian ones, may reflect the difference in the structural differentiation of chromosomes of animals of different classes. It seems possible that the difference is not only in the actual chromosome spiralization, but also in the system of the distribution of clusters of repeated sequences along the chromosomes and the fiber compaction in the regions enriched with repetitive DNA. One can suppose that the chromatin enriched in repeated sequences in amphibian chromosomes composes chromomere-like structures, which are distributed quite uniformly along the chromosomes. Such chromosome organization might give us the opportunity to change the content of the repeated sequences during the change of genome size without principal changes in the chromosome morphology. The opinion that the

changes in genome size result in local changes of DNA in amphibian chromosomes was advanced earlier (Ullerich 1970).

Some data concerning spermatosoid histones present indirect evidence of a dissimilarity between chromosome organization in amphibians and mammals: while in amphibians histone H1 is replaced by different histones, in all mammals investigated the substitution histones have similar characteristics (Kasinsky et al. 1978).

The organization of C-banded regions in amphibian and mammalian chromosomes, especially in centrometric regions, seems to be similar. In particular, these regions are enriched with repetitive DNA (see above). The intercalar C-banding regions of *Triturus* chromosomes, and probably of other urodeles, seem to have a specific organization since dense structures correspond to them in lampbrush chromosomes (Schmid et al. 1979). Multiple C-bands, revealed on *Hynobius keyserlingii* chromosomes (Graphodatsky et al. 1978), may correspond to the described chromomeres of mitotic chromosomes. Since intercalar bands on anuran chromosomes are obtained by different authors differently, these regions do not have a highly condensed structure.

The characteristic features of amphibian chromosome structure might be determined by DNA sequence organization, which, in turn, is connected with genome expression. It is not known in what way the growth of the repetitive DNA fraction leads to the elongation of amphibian development – whether this is due to structural complications of regulatory regions of the genomes (Zuckerkandl 1976), or to variations in nuclear volume (Cavalier-Smith 1978). It is possible to suppose that repetitive DNA in amphibians, as in *Drosophila* (Korochkin 1977; Steffensen 1977) is significant for determining the spatial organization of chromatin in interphase nuclei and for time regulation of gene action. In any case, in amphibian oocytes a part of repeated sequences is transcribed (Davidson and Hough 1971; Sommerville et al. 1976) and this process is stage specific, at one stage of oogenesis certain intermediate repetitive DNA sequences are exposed for transcription but not at other stages (Macgregor 1978;1979). This fact points to the possible participation of repetitive DNA in the regulation of transcription. Nevertheless, the major part of transcribed DNA in amphibian oocytes, of course, consists of unique sequences (Rosbash et al. 1974). It is interesting to add, that heterogeneous nuclear RNA size increases during development (gastrula and tailbud RNAs of *Rana pipiens* were compared) and this is correlated to an increasing degree of turnover of this RNA (Shepherd and Flikinger 1979).

It should be emphasized again that the increase in the repetitive DNA fraction leads to prolonged morphogenesis and paedogenesis. The change in genome size probably results in a change of transcription: as was mentioned above, the size of 40S-pre rRNA in urodeles is greater than in anurans. In any case, the specific organization of repeated sequences (regulatory systems?) in amphibians determines a slower evolutionary rate of karyotypes and organisms as compared with mammals (Wilson et al. 1974; Wilson 1976; Cherry et al. 1978). It should be added that so far the difference between unique DNA contents in the two amphibian orders is not clear, as well as the high rate of evolution of the majority of repeated sequences.

5. Chromosome Structure in Amphibians – a Stage of Evolution?

One may well ask: are all the described features of chromosome and chromatin structure characteristic only of modern amphibians or do they represent a stage in eukaryotic genome structure evolution? The existing data on chromosome banding patterns and genome structure in fishes, reptiles and birds seems to point to the conclusion that the chromosome organization in amphibians constitutes a stage in the evolution of the chromosomes of vertebrates. Although we have no direct proofs of this view, it seems to us that much of data described below does not contradict it.

a. Chromosome Banding Patterns and DNA in Fish

Although the number of chromosomes in fishes is normally relatively great (Kirpichnikov 1974), their banding patterns are similar to those in amphibians: G- and Q-bands practically are not revealed on chromosomes (Abe and Muramoto, 1974; Thorgaard 1976; Kilgerman and Bloom, 1977). In some fishes, Q-bands are seen only on heteromorphic F-bodies of chromosomes of males (Howell and Bloom 1973; Kligerman and Bloom 1973). Using C-technique, centromeres and pericentric regions are stained (Zenzes and Voiculescu 1975; Thorgaard 1976; Kligerman and Bloom 1977), sometimes also secondary constrictions (Thorgaard 1976; Barshene 1978) or other regions are too (Zenzes and Voiculescu 1975). On the whole, the banding patterns on fish chromosomes are more similar to those on anuran chromosomes than on urodelean ones.

The presently known characteristics of fish genomes are in many respects similar to the characteristics of amphibian genomes. Although the amount of nuclear DNA in the majority of fish species is lower than in man (on average, 2C=2 pg/N – Cimino 1974), the range of variation within the class may run as high as a hundred times (Hinegardner 1976). In some dipnoan species the DNA content is very high, approximating that in urodeles: in *Protopterus aethiopiens* 2C=241 pg/N (Pedersen 1971). Probably, the increase in DNA content in urodeles on the one hand, and in dipnoans on the other, are convergent processes (Morescalchi 1973) with similar mechanisms. On the whole, the changes in DNA content of fishes and amphibians are possibly convergent: in north-

ern forms of amphibians the DNA content is greater than in southern ones; similarly, in deep-sea fishes, it is higher than in the related shallow-marine species (Ebeling et al. 1971). Among fishes polyploidy occurs even more frequently than among amphibians (Fredga 1977).

On centrifugation of fish DNA *(Salmo salar, Opsanus lan)* is CsCl only the major component has been found (Thiery et al. 1976). This peak is more symmetrical than DNA peaks of vertebrates of other classes. It has been proposed that the single DNA component in fishes with small genomes could be the ancestor of the main DNA component of evolutionarily more recent vertrebrates (Thiery et al. 1976).

In some cases, apart from the main component, the satellite fraction also was found (Helleiner et al. 1974; Thiery et al. 1976). According to reassociation kinetics this DNA consists of repeating units about 4400 base pairs long (Helleiner et al. 1974). On the whole, the genome organization in fishes (the presence of several repetitive fractions and unique sequences) is similar to the DNA organization in other vertebrates (Vladychenskaya et al. 1975; 1976; Kupriyanova 1976). Probably, a greater specialization of fish is attended by a decrease in DNA content (Hinegardner and Rosen 1972) and, possibly, by the growing repetitive fractions/unique fractions ratio (Vladychenskaya et al. 1975). The changes in genome size in diploid species are due mainly to differences in the amount of intermediate repetitive DNA (Schmidtke et al. 1979).

All these specific features of genome structure in fishes do not contradict the possibility that chromatin and chromosome organizations in fish are analogous to those in amphibians.

b. Chromosome Banding Patterns and DNA in Reptiles and Birds

Chromosomes of reptiles and birds stain as well as mammalian chromosomes. G-banding patterns have been obtained on chromosomes of various reptiles: snakes, turtles, lizards (Stock and Mengden 1975; Bickham and Baker 1976; King and Rofe 1976; Bull 1978; Graphodatsky et al. 1979); in some cases banding patterns in turtles were found to be similar to those in birds (Stock and Mengden 1975). In all the specimen studied centromeres are C-staining; intercalary regions or telomeres are also often stained as well as the sex W-chromosome (Bull et al. 1974; Singh and Ray-Choudhuri 1975; Bickham and Baker 1976; Bull 1978; Graphodatsky et al. 1979). On chromosomes of birds G-bands are easily revealed (Wang and Shoffner 1974; Stock et al. 1974; Stock and Mengden 1975; Bulatova 1977); in addition centromeres and the sex W-chromosome are C-positive (Steffos and Arrighi 1971; Wang and Shoffner 1974; Stock et al. 1974).

DNA content in reptiles and birds approximates those in mammals: 3.0-7.0, 3.4-4.6 and 6.0-11.6 pg/N respectively (Hinegardner 1976). On centrifugation in CsCl, however, DNA of reptiles behaves differently to DNA of mammals and birds (experiments on the hen and the gull *Larus argentatus*) in which three major components have been found in the same experiments (Thiery et al. 1976). DNA of reptiles studied is closer to DNA of amphibians: in DNA of the lizard *Iguana iguana* only two major components have been found, p=1.702 and 1.706 G/cm^3 (85 and 15% of genome), and in DNA of the turtle *Testudo graeca* – two major components, p=1.702 and 1.714 G/cm^3 (64 and 27%), the minor and satellite components (5% and 4% respectively). According to these data the general organization of reptilian DNA seems to be intermediate between the DNA structure of amphibians and birds, but the results of reassociation experiments show that the genome structure of reptiles differs from the genome structure of birds and is similar to those of amphibians and mammals (Epplen et al. 1979).

Specific satellite DNA has been localized in the W-chromosome of snakes, which is the functional homologue of the Y-chromosome of mammals (Singh et al. 1976). Reptiles are the first vertrebrates in the phylogenetic row that have morphologically different sex chromosomes. It is interesting that there is a considerable homology between the satellite DNA of the W-chromosome of reptiles and birds (Singh et al. 1976). In birds, however, satellite DNA is present, apart from the W-chromosome, in microchromosomes (Stefos and Arrighi 1974). The nucleosome chromatin structure in reptiles is identical to that in other vertebrates (Beçak et al. 1977).

A characteristic feature of DNA of many birds is that the relative content of unique fraction is higher than in DNA of other vertebrates. In the chicken unique DNA composes 80% of the genome (Stefos and Arrighi 1974; Jimenes et al. 1974; Arthur and Straus 1978), but in the genomes of other birds unique DNA content is lower, approximating that in mammals (60-70%) (Shields and Straus 1975; Epplen et al. 1978). In the chicken, 7-8% of the genome consist of fast-reassociating foldback sequences, and 11% – of sequences repeating about ten times per genome (Shields and Straus 1978); probably, these fractions were revealed on centrifuation in CsCl in the form of the satellite DNA. It is interesting that many of the repetitive sequences of the chicken genome are conserved as they were found in the genome of such a distant species as the ostrich, although their amount is considerably lower (Eden et al. 1978). On the whole, in chicken DNA unique sequences are interspersed with long repetitive sequences and the genome organization is closer to that of *Drosophila* and some other insects than to the *Xenopus*-type genome organization of most vertebrates (Shields and Straus 1978; Epplen et al. 1978; Ginatulin et al. 1979).

The specificity of bird evolution has also been noted at the structural gene level: the rate of evolution of some

proteins studied is, probably, 2-4 times lower than in other vertebrates (Prager et al. 1974). Besides, in birds no correlation between genome and cell size has been detected: large cells have been found in bird species of large size (Szarski 1976).

The data cited makes it possible to conclude that as regards genome size and structure, as well as chromosome structure, reptiles, birds and mammals are quite close to each other.

The above presented properties common to fish and amphibian genomes (viz. a great range of variety in nuclear DNA content and polyploidization and the similarity between chromosome banding patterns) seem to provide a good argument for grouping them together, as distinct from more developed vertebrates – amniotes. Amniotes, in their turn, are quite similar to each other as regards DNA and chromosomal characteristics. An additional argument for distinguishing fishes and amphibians from amniotes with respect to DNA organization (and, possibly, packing) is the difference in properties between testis-specific histones.

If we accept that amphibian chromosomes have a distinctive structure (in particular, high spiralization) similar to that of fish chromosomes, then we may assume that in some amphibians, which were the ancestors of reptiles, radical changes in the chromatin packing occurred, following changes in the organization of repetitive DNA sequences and a decrease in total DNA content. Such changes are probably characteristic only of amniotes, and they occured only in the phylogenetic row of vertebrates. In plants and many insects, on the other hand, as well as in amphibians and fishes, G-bands have not been obtained so far, although C-banding patterns in them may be rather complex (Greilhuber 1977), which point to different chromatin packing, as compared with that in amnioteis. Further investigations of details of DNA structure and chromatin organization will give insight into the mechanism of those changes.

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References

Abe S, Muramoto J (1974) Proc Jpn Acad 50:507–511
Adolph K W, Cheng S M, Laemmli U K (1977) Cell 12:805–816
Aleksandrovskaya T O, Birstein V J, Mazin A L (1979) Zool Zh 58:1365–1373
Arnheim N, Southern E M (1977) Cell 11:363–370
Arthur R R, Straus N A (1978) Can J Biochem 56:257–263
Ashmarin I P, Kalyshentseva Yu E, Konarev V G, Sidorova V V, Fedorova N A (1975) Zh Evol Biokhim Fiziol 11:515–519
Ashmarin I P, Fedorova N A, Kalyshentseva Yu E (1976) Zh Evol Biokhim Fiziol 12:367–369
Bachmann K, Blommers-Schlösser R (1975) Zool Anz 194:13–31
Bachmann K, Nishioka M (1978) Copeia N 2:225–229
Badaev N S, Borisov Yu M, Zelenin A V, Iordansky A B, Pankova N A (1974) Tsitologia 16:1030–1032
Bailly S (1972) C R Acad Sci D275:1267–1269
Bailly S (1976) Chromosoma 54:61–68
Bailly S (1977) Biol Cell 30:3a
Bailly S (1979) C R Acad Sci D288:105–108
Bailly S, Guillemin C, Labrousse M (1973) C R Acad Sci 276: 1867–1869
Baldari C T, Amaldi F (1976) Chromosoma 59:13–22
Baldari C T, Amaldi F (1977) Chromosoma 61:359–368
Barsacchi G, Gall J G (1972) J Cell Biol 54:580–591
Barsacchi-Pilone G, Nardi I, Andronico F, Batistoni R, Durante M (1977) Chromosoma 63:127–134
Barsacchi-Pilone G, Nardi I, Batistoni R, Andronico F, Beccari E (1974) Chromosoma 49:135–153
Barshene Ya V (1978) Genetica 14:2029–2036
Basu S (1977) Biopolymers 16:2315–2328
Batistoni R, Andronico F, Nardi I, Barsacchi-Pilone G (1978) Chromosoma 65:231–240
Batistoni R, Nardi I, Pilone G B (1974) Chromosoma 49:121–134
Beçak M L, Fukuda K (1979) Experimentia 35:24–26
Beçak M L, Fukuda K, Carneiro S M (1977) Experientia 33: 1314–1316
Beck M L, Mahan J T (1979) Copeia 341
Bianchi N O, Bianchi M A, Matayoshi T (1977) Cytologia 42: 425–432
Bianchi N O, Bianchi M S, Vidal-Rioja L (1973) Caryologia 26, 39:397–403
Bickham J W, Baker R J (1976) Chromosoma 54:201–219
Bird A P (1978) J Mol Biol 118:49–60
Bird A P, Southern E M (1978) J Mol Biol 118:27–47
Birnstiel M L, Spiers J, Purdom T F, Jones R, Loening U E (1968) Nature 219:454–463
Birstein V J (1981a) Zool Zh 60:246–256
Birstein V J (1981b) Tsitologia 23:396–403
Bisbee C A, Baker M A, Wilson A C, Hadji-Azimi I, Fishberg M (1977) Science 195:785–787
Blacker A W, Cassidy D M (1978) Dev Biol 63:224–232
Blin N, Stephenson E C, Stafford D W, (1976) Chromosoma 58: 41–50
Bols N C, Kasinsky H E, (1976) J Exp Zool 198:109–114
Boseley P, Moss T, Mächler M, Portmann R, Birnstiel M (1979) Cell 17:19–32
Bozzoni T, Beccari E (1978) Biochem Biophys Acta 520:245–252
Brown D D, Wensink P C, Jordan E (1971) Proc Natl Acad Sci USA 68:3175–3179
Brown D D, Wensink P C, Jordan E (1972) J Mol Biol 63:57–73
Brown D D, Sugimoto K (1973) J Mol Biol 78:397–415
Browne M J, Turnbull J F, Burdon R H (1976) Biochem Soc Trans 4:1130–1131
Bucci-Innocenti S, Lascialfari D, Ragghianti M, Mancino G (1978) Experientia 34:1002–1004
Bulatova N Sh (1977) In: Hybrid and mutation cytogenetics and karyotype evolution. Novosibirsk "Nauka":248–259
Bull J (1978) Can J Genet Cytol 20:205–209
Bull J J, Moon R G, Legler J M (1974) Cytogenet Cell Genet 13:419–425
Buongiorno-Nardelli M, Amaldi F, Beccari E, Junakovic N (1977) J Mol Biol 110:105–117
Burkholder G D (1975) Exp Cell Res 90:269–278
Burkholder G D, Weaver M G (1977) Exp Cell Res 110:251–262
Byarnagaba W (1978) Chromosoma 67:275–283
Cavalier-Smith T (1978) J Cell Sci 34:247–278
Cherry L M, Case S M, Wilson A C (1978) Science 200:209–211
Cimino M C (1974) Chromosoma 47:297–307
Comings D E (1977) Chromosomes Today 6:19–26

Comings D E, Avelino E, Okada T A, Wyandt H E (1973) Exp Cell Res 77:469–493
Comings D E, Drets M E (1976) Chromosoma 56:199–211
Comings D E, Kovacs B W, Avelino F, Harris D C (1975) Chromosoma 50:111–145
Comings D E, Okada T A (1975) Exp Cell Res 93:267–274
Davidson E H, Calan G A, Angerer R C, Britten R J (1975) Chromosoma 51:253–259
Davidson E H, Hough B R (1971) J Mol Biol 56:491–506
Davidson E H, Hough B R, Amenson C S, Britten R J (1973) J Mol Biol 77:1–23
Denis H, Wegnez M (1977) Dev Biol 59:228–236
Dennis H, Wegnez M (1978) J Mol Evol 12:11–15
Dev V G, Miller D A, Tantravahi R, Schreck K R, Roderick T H, Erlanger B F, Miller O J (1975) Chromosoma 53:335–344
Donna A, Gorovsky M A (1975) J Cell Biol 64:389–397
Dutrillaux B, Lejeune J (1975) Adv Hum Genet 5:119–156
Dutrillaux B, Rethore M O, Lejeune J (1975) Ann Génét 18: 153–161
Ebeling A W, Atkin N B, Setzer P Y (1971) Am Nat 105:549 –561
Eden F C, Hendrick J P, Gottlieb S S (1978) Biochemistry 17: 5113–5121
Edwards L J, Hnilica L S (1968) Experientia 24:228–229
Epplen J T, Diedrich U, Wagenmann M, Schmidtke J, Engel W (1979) Chromosoma 75:199–214
Epplen J T, Leipoldt M, Engel W, Schmidtke J (1978) Chromosoma 69:307–321
Evans H J, Buckland R A, Sumner A T (1973) Chromosoma 42: 383–402
Fedoroff N (1979) Cell 16:697–710
Fedoroff N V, Brown D D (1978) Cell 13:701–716
Ford P J, Brown R E (1976) Cell 8:485–493
Ford P J, Mathieson T (1978) Eur J Biochem 87:199–214
Foucrier J, Chalmneau M Th, Boffa G A (1976) Comp Biochem Physiol 53B:555–559
Frado L L Y, Annunziato A T, Woodcock C L F (1977) Biochim Biophys Acta 475:514–520
Fredga K (1977) Proc R Soc Lond B 199:377–3971
Freed J J (1975–1976) Inst Cancer Res 21st Sci Rep 136–137
Freed J J (1977) In Vitro 13:200
Funaki K, Matsui S, Sasaki M (1975) Chromosoma 49:357–370
Gaubatz J, Prashas N, Cutler R G (1976) Biochem Biophys Acta 418:358–375
Georgiev G P, Bakaev V V (1978) Mol Biol 12:1205–1230
Gerbi S (1976) J Mol Biol 106:791–816
Ginatulin A A, Ginatulina L K, Vorontsov N N, Timofeeva M Ya (1979) Dokl AN SSSR 244:1018–1021
Goin O B, Goin C J, Bachmann K (1968) Copeia N 3:533–540
Goodpasture C, Bloom S E (1975) Chromosoma 53:37–50
Gormley I P, Ross A (1976) Exp Cell Res 98:152–158
Graphodatsky A S, Grigoriev O V, Isaenko A A (1978) Zool Zh 57:1279–1280
Graphodatsky A S, Grigoriev O V, Isaenko A A (1979) Zool Zh 58:1074–1076
Greilhuber J (1977) Theor Appl Genet 50:121–124
Hatch F T, Bodner A J, Mazrimas J A, Moore D H (1976) Chromosoma 58:155–168
Hatlin L E, Attardi G (1971) J Mol Biol 56:535–553
Helleiner C W, Huh T Y, Sangater S M (1974) Comp Biochem Physiol 48B:11–24
Henderson A S, Atwood K C, Yu M T, Wartburton D (1976) Chromosoma 56:29–32
Henderson A S, Wartburton D, Atwood K C (1975) Proc Natl Acad Sci USA 71:3394–3398
Hennen S, Mizuno S, Macgregor H C (1975) Chromosoma 50: 349–36
Heppich S (1978) Z Zool Syst Evolutionsforsch 16:27–39
Hinegardner R (1976) In: Molecular Evolution Ayala F J (ed), Sinauer Assoc Inc Mass, pp 179–199
Hinegardner R, Rosen D (1972) Am Nat 106:621
Holmquist G (1975) Nature 257:503–506
Holmquist G (1979) Chromosoma 72:203–224
Holmquist G P, Comings D E (1976) Science:599–602
Howell W M (1977) Chromosoma 62:361–367
Howell W M, Bloom S E (1973) Nature 245:261–263
Hsu T C, Spirito S S, Pardue M L (1975) Chromosoma 53:25 –36
Humphries S E, Young D, Carroll D (1979) Biochemistry 18: 3223–3231
Hutchinson N, Pardue M L (1975) Chromosoma 53:51–69
Imoh H (1977) Exp Cell Res 108:57–62
Jacq C, Miller J R, Brownlee G G (1977) Cell 12:109–120
Jeppesen P G N, Banhier A T, Saunders L (1978) Exp Cell Res 115:293–302
Jiménez de E S, Gonzalez J L, Domingez J L, Saloma E S (1974) Eur J Biochem 45:25–26
Jones K W (1978) Z Morph Anthrop 69:143–171
Jorgensen K F, Saude van de J H, Lin C C (1978) Chromosoma 68:287–302
Kasinsky H E, Huang S Y, Kwank S, Mann M, Sweeney M A J, Yee B (1978) J Exp Zool 203:109–126
Kezer J, Sessions S (1979) Chromosoma 71:65–80
Khachaturov E N, Barsky V E, Galkina I G, Stonova N S, Maksimaldo Yu B (1975) Dokl Akad Nauk SSSR Ser Biol N 6:873–876
Khan M S N, Maden B E H (1976) Nucleic Acids Res 4:2490 –2506
Khan M S N, Salim M, Maden B E H (1978) Biochem J 169: 531–542
King M, Rofe R (1976) Chromosoma 54:75–87
Kirpichnikov V S (1974) Usp Sovrem Biol 78:404–422
Kligerman A D, Bloom S E (1977) Cytogenet Cell Genet 18: 182–196
Kornberg J R, Engels W R (1978) Proc Natl Acad Sci USA 75: 3382–3386
Korochkin L I (1977) Interaction of genes in development. Moscow. "Nauka", 248–255
Krystal M, Arnheim N (1978) J Mol Biol 126:94–104
Kupriyanova N S (1976) Repetitive nucleotide sequences in the genome of loach (Midgurnes fossilis L.) Cand Th
Labrousse M, Guillemin C, Guillemin L (1972) C R Acad Sci 174:1063–1065
León P E (1976) J Cell Biol 69:287–300
León P E, Kezer J (1978) Chromosoma 65:213–230
Lima-de-Faria A (1976) Hereditas 83:1–22
Lind A Ya, Villems R L, Uuskula L S (1978) Usp Sovrem Biol 86:3–18
Loening H E (1968) J Mol Biol 38:355–365
Lohr D, Corden J, Tatchell K, Kovacic R T, Van Holde K E (1977) Proc Natl Acad Sci USA 74:79–83
Luciani J M, Devictor M, Morazzani M R, Stahl A (1976) Chromosoma 57:155–163
Macaya J, Thiery J P, Bernardi G (1976) J Mol Biol 108:237 –254
Macgregor H C (1978) Philos Trans R Soc Lond Ser B 283: 309–318
Macgregor H C (1979) Chromosoma 71:57–64
Macgregor H C, Andrews C (1977) Chromosoma 63:109–126
Macgregor H C, Horner H, Owen C A, Parker I (1973) Chromosoma 43:328–348
Macgregor H C, Jones C (1977) Chromosoma 63:1–9
Macgregor H C, Kezer J (1971) Chromosoma 33:167–182
Macgregor H C, Kezer J (1973) Chromosoma 42:415–426
Macgregor H C, Mizuno S (1976) Chromosoma 54:15–20
Macgregor H C, Mizuno S, Vlad M (1976) Chromosomes Today 5:332–339

Mancino G, Ragghianti M, Bucci-Innocenti S (1977) In: "The reproductive Biology of Amphibians", Taylor D H, Cuttman S I (eds), Plenum Press, New York London, pp 411–447
Mancino G, Ragghianti M, Bucci-Innocenti S (1978) Chromosoma 69:27–47
Mancino C, Ragghianti M, Innocenti S B (1973) Atti Accad Naz Lincei Rc Ser 8 55:559–564
Matsui S (1974) Exp Cell Res 88:88–94
Matsui S, Weinfeld H, Sandberg A A (1978) J Cell Biol 79:116a
Mednikov B M, Shubina E A, Phillipovitch S Yu (1976) Biol Nauki N 9:21–26
Melli M, Whitfield C, Rao K V, Richardson M, Bishop J O (1971) Nature (London) New Biol 231:8–12
Meunier-Rotival M, Cortadas J, Macaya G, Bernardi G (1979) Nucleic Acid Res 6:2106–2123
Miller J P, Cartwright E M, Brownlee G G, Fedoroff N V, Brown D D (1978) Cell 13:717–725
Mitchell A R, Seuanez H N, Laurie S S, Martin D E, Gosden J R (1977) Chromosoma 61:345–358
Mizuno S, Andrews Ch, Macgregor H C (1976) Chromosoma 58: 1–31
Mizuno S, Macgregor H C (1974) Chromosoma 48:239–296
Morescalchi A (1973) In: Cytotaxonomy and vertebrate evolution. Chiarelli A B, Capanna E (eds), Academic Press, London, New York, pp 233–348
Morescalchi A (1977) Boll Zool 44:287–294
Morescalchi A, Odierna G, Olmo E (1977) Experientia 33: 1579–1581
Morescalchi A, Serra V (1974) Experientia 30:487–489
Morgan G T (1978) Chromosoma 66:269–280
Moss T, Boseley P G, Portmann R, Birnstiel M L (1979) Experientia 35:972
Nadler C F, Hoffmann R S, Woolf A (1974) Experientia 30: 744–746
Nardi I, Barsacchi-Pilone G, Batistoni R, Andronico F (1977) Chromosoma 64:67–84
Nardi I, Lucchini S de, Barsacchi-Pilone G, Andronico F (1978) Chromosoma 70:91–99
Nardi I, Ragghianti M, Mancino G (1972) Chromosoma 37:1–22
Nardi I, Ragghianti M, Mancino G (1973) Chromosoma 40:321 –331
Nazar R N, Roy R K, (1976) FEBS Lett 72:111–116
Obara Y, Kogame K, Saito K (1975) CIS N 18:15–17
Okada T A, Comings D E (1974) Chromosoma 48:65–71
Okada T A, Comings D E (1979) Chromosoma 72:1–14
Olmo E (1973) Caryologia 26:43–68
Olmo E, Morescalchi A (1978) Experientia 34:44–46
Pardue M L (1974) Cold Spring Harb Symp Quant Biol 38: 475–482
Pardue M L, Brown D D, Birnstiel M L (1973) Chromosoma 42: 191–193
Parker C S, Roeder R G (1977) Proc Natl Acad Sci USA 74:44 –48
Paulson J R, Laemmli U K (1977) Cell 12:817–828
Pedersen R A (1971) J Exp Zool 177:65–78
Perlman S, Phillips C, Bishop J O (1976) Cell 8:33–42
Perry R P, Cheng T Y, Freed J J, Greenberg J R, Kelley D E, Tartof K D (1970) Proc Natl Acad Sci USA 65:609–616
Pierpoint M E, Yunis J J (1977) Exp Cell Res 106:303–308
Prager E M, Brush A H, Nolan R A, Nakanishi M, Wilson A C (1974) J Mol Evol 3:243–262
Pukkila P J (1975) Chromosoma 53:71–89
Ragghianti M, Bucci-Innocenti S, Mancino G (1977) Experientia 33:1319–1321
Ragghianti M, Bucci-Innocenti S, Mancino G (1978) Caryologia 31:243–256
Ragghianti M, Bucci-Innocenti S, Mancino G (1980) Chromosoma 77:333–345
Ragghianti M, Innocenti S, Mancino G (1974) Atti Accad Naz Lincei Rc Ser 8 55:764–770
Raicu P, Georm̆neanu C (1977) J Hered 68:343–344
Reeder R H, Roeder R G (1972) J Mol Biol 67:433–441
Reeves R (1977) Eur J Biochem 75:545–560
Resley M S, Eckhardt R A (1974) J Cell Biol 63:286
Rogers M E, Klein G (1972) Biochem J 130:281–288
Rosbash H, Ford P J, Bishop J O (1974) Proc Natl Acad Sci USA 71:3746–3750
Rudak E, Callan H G (1976) Chromosoma 56:349–362
Rungger D, Crippa M (1977) Prog Biophys Mol Biol 31:247– 269
Ruiz I R C, Beçak W (1976) Chromosoma 54:69–74
Sakima K, Kominami R, Muramatsu M (1976) Eur J Biochem 63:339–350
Salthe S N, Kaplan N O (1966) Evolution 20:603
Schmid M (1978a) Chromosoma 66:361-368
Schmid M (1978b) Chromosoma 68:131–148
Schmid M (1979) Chromosoma 75:215–234
Schmid M (1980) Chromosoma 77:83–103
Schmid M, Krone W (1976) Chromosoma 56:327–347
Schmid M, Olert J, Klett C (1979) Chromosoma 71:29–55
Schmidtke J, Schmitt F, Leipoldt M, Engel W (1979) Comp Biochem Physiol 64B:117–120
Seligy V, Roy C, Dove M, Yaguchi M (1976) Biochim Biophys Res Commun 71:196–202
Sheer U (1978) Cell 13:535–549
Shepherd G W, Flickinger R (1979) Exp Cell Res 120:73–77
Shields G F, Straus N A (1975) Evolution 29:159–166
Sinclair J H, Brown D D (1971) Biochemistry 10:2761–2769
Singh L, Purdom I F, Jones K W (1976) Chromosoma 59:43–62
Singh L, Ray-Choudhuri S P (1975) Nucleus 18:163–166
Sommerville J, Macolm D B, Callan H G (1976) Phil Trans R Soc Lond B 283:359–366
Southern E M (1975) J Mol Biol 90:503–517
Stambrook P J (1978) Chromosoma 65:153:159
Steffensen D M (1977) Chromosomes Today 6:247–253
Steffensen D M, Prensky W, Mutton D, Hamerton J L (1975) Cytogenet Cell Genet 14:434–438
Stefos K, Arrighi F E (1971) Exp Cell Res 68:228–232
Stefos K, Arrighi F E (1974) Exp Cell Res 83:9–13
Stern R (1972) Carnegie Inst. of Washington, Yearbook 71, 22
Stock A D, Arrighi F S, Stefos K (1974) Cytogenet Cell Genet 13:410–418
Stock A D, Mengden G A (1975) Chromosoma 50:69–77
Straus N A (1971) Proc Natl Acad Sci USA 68:799–802
Sueoka N (1961) J Mol Biol 3:31–40
Szabo P, Lee M R, Elder F B, Prensky W (1978) Chromosoma 65:161–172
Szarski H (1976) Int Rev Cytol 44:93–111
Takayama S (1976) Chromosoma 56:47–54
Thiery J P, Macaya G, Bernardi G (1976) J Mol Biol 108: 219–235
Thorgaard G H (1976) Cytogenet Cell Genet 17:174–184
Ullerich F H (1970) Chromosoma 30:1–37
Van Holden P, Strickland W N, Brandt W F, Holt C van (1978) Biochim Biophys Acta 533:278–281
Vlad M (1977) J Cell Sci 24:108–118
Vlad M, Macgregor H C (1975) Chromosoma 50:327–347
Vladychenskaya N S, Kedrova O S, Antonov A S (1975) Biol Nauki N 7:105–110
Vladychenskaya N S, Kedrova O S, Antonov A S (1976) Biol Nauki N 10:15–20
Wallace D G, Wilson A C (1972) J Mol Evol 2:72–86
Wang N, Shoffner R N (1974) Chromosoma 47:61–69
Ward O G (1977) Can J Genet Cytol 19:51–57
Wartburton D, Henderson A S, Atwood K C (1975) Chromosoma 51:35–40

Wegnez M, Monier T, Denis H (1972) FEBS Letters 25:13–20
Weisblum B, Haenssler E (1974) Chromosoma 46:255–260
Wellauer P K (1979) J Mol Biol 128:289–303
Wellauer P K, David I B, Brown D D, Reeder R H (1976) J Mol Biol 105:461–486
Wellauer P K, David I B (1974) J Mol Biol 89:379–395
Wilson A C (1976) In: Molecular Evolution. Ayala F J (ed), Sinauer Assoc Inc Mass, pp 225–234
Wilson A C, Sarich V M, Maxson L R (1974) Proc Natl Acad Sci USA 71:3028–3030
Wu F C, Elgin S C R, Hood L E (1975) J Mol Evol 5:87–101
Wurster-Hill D H, Gray C W (1976) Cytogenet Cell Genet 15: 306–331
Yunis J J, Kuo M T, Saunders G F (1977) Chromosoma 61: 335–344
Yunis J J, Sawyer J R, Ball D W (1978) Chromosoma 67: 293-307
Zakharov A F (1977) Human Chromosomes: Problems of the Linear Organization. Moscow, Meditzina 128–174
Zenzes M T, Voiculescu I (1973) Genetica 45:531–536
Zuckerkandl E (1976) J Mol Evol 9:73–104

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Note added in proof. After the review has been completed new data on the problems discussed above appeared in the literature. The following facts are most important: C-bands on the chromosomes of one of the most primitive anurans, *Ascaphus truei*, 2n = 46, were obtained (Green et al. 1980). After the $Ba(OH)_2$-pretreatment of chromosomes, the centromers of all chromosomes and the telomers of many of them were stained. Besides, interstitial bands on homologous of three pairs (one band per arm) were seen. The C- and Ag-AS-banding of *Odontophrynus* chromosomes was made more precisely (Ruiz et al. 1981). In *O. americanus*, 2n = 22 and 4n = 44, the C-bands appeared in the same positions in the regions of the secondary constrictions in the 4th and 11th chromosomes, Ag-AS-bands in the same positions of the 4th chromosomes (*O. americanus*, 2n = 22) or of the 11th chromosomes (*O. americanus*, 4n = 44). In *O. cultripes*, 2n = 22, C-bands appeared at the secondary constrictions of the homologues of the 11th pair and in *O. carvalhoi*, 2n = 22, at the secondary constriction in one arm of the 8th chromosomes (in the latter species there are constrictions in both arms of the 8th chromosomes). Ag-AS-bands coincided with these C-bands. In *O. americanus*, 2n and 4n, and in *O. cultripes*, intercalar C-bands were also seen on the 2nd, 3rd and 6th chromosomes; centromers and telomers of almost all chromosomes were stained. A slight inter- and intrapopulation polymorphism in the number and position of C- and Ag-AS-bands was found. A conclusion similar to ours concerning the particular character of G-banding process in the case of anuran chromosomes was made after experiments on G-staining of *Rana pipiens* chromosomes: almost all the conventional methods of G-banding resulted in "uniformly spaced light and dark regions of a beadlike character" (Manago et al. 1980). As for the anuran DNA structure, it was shown that in clusters of repetitive DNA of *R. berlandieri* one class of repetitive sequences is interspersed with others (Graham and Schanke 1980). It was found that the satellite DNA of *Triturus cristatus carnifex*, which is localized in C-band positive regions of mitotic and meiotic chromosomes of the first pair, is transcribed in lampbrush chromosomes during oogenesis (Varley et al. 1980). A stage-specific transcription from both strands of a satellite DNA interspersed with histone genes was found in *Notophthalmus viridescens* lampbrush chromosome loops (Dias et al. 1981). It remains unknown if these data are indicative of the specificity of transcription in lampbrush chromosomes or of a certain functional role of these DNA sequences. Many new experiments on the density gradient centrifugation of fish (33 species belonging to 12 Teleostei and one Chondrichthyes orders were investigated) and mammalian (mouse, human) DNAs in CsCl supported the conclusion that the DNA sequence organization of cold- and warm-blooded (or, according to our assumption, of anamniotes and amniotes) is different (Hudson et al. 1980; Cuny et al. 1981). The first group is characterized by DNAs exhibiting a low degree of compositional heterogeneity whereas the DNAs of the second one are highly heterogeneous; they contain dG-dC-rich components which are either absent or very scarce in the DNA of cold-blooded vertebrates. There are new examples of very good and informative G- and C-banding patterns on the reptilian and bird chromosomes in the literature (in contrast to the amphibian and fish ones), which let the authors make conclusions on chromosomal evolution in serpents (Mengden and Stock 1980) and birds (Ryttman and Tegelström 1981).

Cuny G, Soriano P, Macaya G, Bernardi G (1981) Eur J Biochem 115:227–233
Diaz M O, Barsacchi-Pilone G, Mahon K A, Gall J G (1981) Cell 24:649–659
Graham D E, Schanke K (1980) Nucleic Acids Res 8:3875–3894
Green D M, Daugherty C H, Bogart J P (1980) Herpetologica 36:346–352
Hudson A, Cuny G, Cortadas J, Haschemeyer A E V, Bernardi G (1980) Eur J Biochem 112:203–210
Manago J N, Spina F L, Beckert W H (1980) Cytologia 45:561–569
Mengden G A, Stock A D (1980) Chromosoma 79:53–64
Ruiz I R G, Soma M, Becak W (1981) Cytogenet Cell Genet 29:84–98
Ryttman H, Tegelström H (1981) Hereditas 94:165–170
Varley J M, Macgregor H C, Nardi I, Andrews C, Erba H P (1980) Chromosoma 80:289–307