20. EVOLUTION OF VERTEBRATE GENOMES

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The Darwinian concept of evolution has revealed that speciation has been dependent upon the process of natural selection. Natural selection, in turn, can be effective only if there is hereditary variability among individuals comprising the population. The identical genetic constitution offers no chance for natural selection to operate; thus, evolution is quite obviously the consequence of genetic changes that have accumulated within the genome. The genome can be defined as a set of genes contained within the haploid set of chromosomes of an organism. In the case of speciation from an immediate ancestor, genetic changes are no doubt due mainly to allelic mutations at the already existing gene loci. What used to be a rare mutant allele of the old species would become the wild-type allele in a new species. For instance, a major component of adult hemoglobin of man and cattle is $\alpha_2 \beta_2$. Alpha- and beta-chains are different polypetides produced by two independent gene loci. The alpha-chain in man, however, has an amino acid sequence different from that of the cattle alpha-chain. Apparently, a common ancestor to diverse species of placental mammals already had the gene loci for two component polypeptides of adult hemoglobin. A series of mutations at each of these two gene loci finally gave rise to the genes for alpha- and beta-chains of today's human. Another series of mutations at the same gene loci, on the other hand, produced the gene for alpha- and beta-chains of the cattle.

When the scope is broadened to consider the evolution of the sub-phylum *VERTEBRATA* as a whole, allelic mutations of already existing genes cannot possibly account for all the genetic changes that occurred during the past 300 million years. There apparently were creations of new gene loci. Invertebrates are incapable of producing antibodies as such. The gene loci for light- and heavy-chains which comprise γ-globulin molecules were obviously created *de novo* at the beginning of the vertebrate evolution.

The creation of new genes now emerges as the most important single factor of evolution within the phylum. In the biological system, however, nothing is created anew out of the blue sky. The new material is produced by modification of the old which already existed.

IMPORTANCE OF GENE DUPLICATION IN VERTEBRATE EVOLUTION — The extensive study carried out by Margoliash (1963) on molecular structures of Cytochrome C revealed the extremely conservative nature of the gene. Cytochrome C is the heme-containing protein which engages in the intracellular transportation

of oxygen. As such, it must have come to the existence soon after the cells made the first appearance on this earch as the unit of life. Yet, it was found that Cytochrome C of diverse organisms, from yeasts to man, not only have nearly the same molecular weight but also maintain similar amino acid sequences, in each instance about 104 amino acid residues making up a polypeptide chain. The clear implication is that the particular function assigned to the gene product imposes a severe limitation on that gene's freedom to mutate. If a change in the base sequence occurred at the wrong part of the DNA molecule, a new gene product would be unable to function as Cytochrome C. Such a mutation would quickly be eliminated.

Natural selection conserved only those mutations which were not deteriorative to the gene product's assigned function. The extremely conservative nature of the already existing genes indicate to us that the redundancy of genetic material was the prerequisite for the creation of new genes. By duplication, if the old gene had been represented twice within the genome, one of the duplicates was now free to mutate to an independent direction and acquire a new function.

In man and probably most other mammals, there are five independent gene loci for component polypeptides of hemoglobin. They are for α-, ε-, γ-, β-, and δ-chains.

It is the view of Ingram (1963) that the ancestry of all the five genes for five different components of hemoglobin can be traced back to a single ancestral gene. First, there was a duplication of this gene and by subsequent mutations to independent directions, one became a gene for myoglobin while the other became a gene for α-chain of hemoglobin. The ancient vertebrate when first emerged may have been able to produce only one type of a hemoglobin molecule which should be α_4. The genes for four other chains of hemoglobin are thought to have been derived from the multiplicates of the gene for α-chain.

Similarly, mammals and birds have three independent gene loci for component polypeptides of the enzyme, lactate dehydrogenase. They are known as A, B, and C (Markert, 1964; Blanco and Zinkham, 1962; Blanco et al., 1964). Originally there may have been only one gene locus for LDH, and the other two may have been produced by duplication of the orginal one.

The gene duplication can be accomplished in two different ways. The longitudinal duplication of a small segment of an individual chromosome would accomplish the purpose for a small number of genes closely linked together. In fact, regional duplication of small chromosomal segments appears to be occurring among mammals of today. For instance, γ-globulin molecule is made of two different kinds of polypeptide chains: the heavy-chain (H) with a molecular weight of about 60,000 and the light-chain (L) of about 20,000. In man, it is becoming increasingly clear that, instead of having one gene locus each for the H- and L-chains, the so-called H-chain locus is actually made of several very closely linked but slightly different genes; the same can be said of the so-called L-chain locus. There apparently were longitudinal multiplication of one ancestral gene for the H-chain and the other ancestral gene for the L-chain. In man, the genes for β- and δ-chains of hemoglobin are also very closely linked. The δ-chain gene must have been derived by a regional duplication of the β-chain gene.

While regional duplication of a small number of genes might have played an important role in speciation from an immediate ancestor, more drastic changes must have occurred to the genomes during the course of vertebrate evolution.

Simultaneous duplication of the entire set of genes can be accomplished by polyploidization. It can be assumed with reasonable certainty that a series of polyploidization of the ancestral genome have taken place sometime in the history of vertebrates which is, after all, 300 million years old.

INCOMPATIBILITY BETWEEN POLYPLOIDY AND THE WELL ESTABLISHED CHROMOSOMAL SEX-DETERMINING MECHANISM — Polyploidy, however, is incompatible with the well established chromosal sex-determining mechanism. When the diploid organisms with the XY/XX-scheme of sex-determining mechanism become tetraploid, the male has to maintain the 4AXXYY-constitution and the female, 4AXXXX. During meiosis of the 4AXXYY-male, the four sex elements may pair off as the XX-bivalent and the YY-bivalent. If such occurs, every gamete would be of the 2AXY-constitution. Consequently, all the offspring resulting from the mating between a tetraploid male and a tetraploid female would emerge as the intersex of the 4AXXXY-constitution. Even if two XY-bivalents are formed in individual spermatocytes of the tetraploid male, in 50 per cent of the cases the X and the Y would move to the same division pole at first meiotic anaphase, again resulting in the production of the intersex of the 4AXXXY-constitution. Polyploidy invariably disturbs the chromosal sex-determining mechanism.

Indeed, among vertebrates viable and fertile polyploid individuals have been found among amphibians where the Z and the W are still largely homologous to each other, but not in birds and mammals where the W and the Y became a highly specialized determiner of the heterogametic sex. Furthermore, it was found that even in amphibians, polyploid individuals are, as a rule, incapable of perpetuating themselves as polyploid by bisexual mating.

Polyploid individuals of amphibians were most thoroughly studied by Humphrey and his colleague (Humphrey and Fankhauser, 1956; Fankhauser and Humphrey, 1959) on *Ambystoma mexicanum* and *A. tigrinum*.

In the case of triploidy, males were uniformly of the 3AZZZ-constitution, while three kinds of sex chromosome constitutions, ZZW, ZWW, and WWW were found among females. Triploids of both sexes were of very poor fertility, and males were more sterile than females; therefore, it was not possible to perpetuate the triploid race by mating of triploid males and triploid females. When mated to diploid males, triploid females produced many tetraploids, revealing that these females ovulate triploid eggs. This may account for the presence of a gynogenic all-female triploid race found in *Ambystoma jeffersonianum* (Uzzell, 1963). Tetraploid, pentaploid, hexaploid, and heptaploid individuals of *A. mexicanum* and *A. tigrinum* also showed very poor fertility. In short, it appears that even in amphibians with the undifferentiated sex chromosomes, the serious obstacle which prevents the emergence of a bisexual polyploid race exists.

From the above, it may be deduced that various degrees of polyploidization of the ancestral genome must have occurred very early in evolution of vertebrates before the emergence of terrestial forms.

Our study on DNA-contents of various vertebrates confirms the above prediction and reveals the polyphyletic origin of the genomes of various vertebrates.

UNIFORMITY OF THE DNA CONTENT OF VARIOUS PLACENTAL MAMMALS — All placental mammals of today descended from a common stock of protoinsectivores which emerged at the dawn of the Cezonoic era. In terms of geological time, the history of placental mammals is brief indeed. Reflecting this recent origin

is the sameness of DNA content. Diverse speciation appeared to be accomplished
with little or no change in the total genetic content. Mandel and his colleagues
(1950) were among the first to show that each diploid nucleus of man as well
as cattle, sheep, pigs, and dogs contains about 7.0 mg $\times$ 10^{-9} of DNA, no more,
no less. Recently, we restudied this matter of DNA constancy by means of
microspectrophotometry. Six species representing four different orders were
chosen: man (*Homo sapiens*, 2n = 46) representing the order **PRIMATES**, the dog
(*Canis familiaris*, 2n = 78) representing the order **CARNIVORA**, the horse
(*Equus caballus*, 2n = 64) of the order **PERISSODACTYLA**, the mouse (*Mus
musculus*, 2n = 40), the golden hamster (*Mesocricetus auratus*, 2n = 44), and
the creeping vole (*Microtus oregoni*, 2n = 17/18) of the order **RODENTIA**. There
was no significant difference in DNA values between man, the horse, the dog,
the golden hamster, and the mouse. A single exception was the creeping vole
which had a DNA value 10% lower. This species (Ohno *et al.*, 1963) shares
with the other member of the rodent subfamily MICROTINAE, *Ellobius lutescens*,
2n = 17 (Matthey, 1953) the distinction of having the lowest diploid chromo-
some number known among placental mammals. Such a drastic reduction in the
number of chromosomes had to be accompanied by the loss of a number of cen-
tromeres with their adjacent heterochromatic chromosomal materials. This loss of
genetically unimportant heterochromatin would account for the 10% lower DNA
value found in the creeping vole (Atkin *et al.*, 1965).

It then follows that different species of mammals, by and large, share the
same kinds of gene loci, even if they belong to different orders. Allelic muta-
tions at each gene locus were mainly responsible for extensive diversification of
placental mammals.

The sameness of total genetic content, however, does not exclude the pos-
sibility that duplications of a small number of genes may have occurred during
speciation of mammals.

Regional gene duplications which occurred to a small number of genes of
different mammals, however, do not change the over-all picture of mammalian
evolution. Extensive speciation of placental mammals was accomplished without
substantial change in the total genetic content. Yet, placental mammals of today
display chromosome constitutions of infinite variety. The diploid number ranges
from a high of 80 in the primitive primate, *Tarsius bancanus* (Klinger, 1963)
to a low of 17 in two rodent species mentioned above. Nothing but acrocentric
chromosomes are found in the mouse (*Mus musculus*, 2n = 40), and only meta-
centrics in the chinchilla (*Chincilla laniger*, 2n = 64) (Galton *et al.*, 1965).
The enormous array of karyotypes reveals the extent to which the original auto-
somal linkage groups of a common ancestor has been shuffled around. An
autosomal equivalent to the human chromosome 21 may only be found among
his closest relatives, the chimpanzee (*Pan troglodytes*, 2n = 48) and the gorilla
(*Gorilla gorilla*, 2n = 48) (Hammerton *et al.*, 1963).

UNIFORMITY OF THE DNA CONTENT OF VARIOUS AVIAN SPECIES — It is be-
lieved that ancestral forms of modern birds were already in existence near the
end of the Jurassic period of the Mesozoic era. The fossil remains of the tooth-
ed bird, *Archeopteryx lithographia* found in slate deposits in Bavaria is said
to be 150 million years old. Thus, it is clear that the avian lineage branched
out from a reptilian lineage before the other reptilian lineage gave rise to a com-
mon ancestor to placental mammals.

Reflecting this independent evolution of the two classes of warm-blooded vertebrates is the fact that the male is the heterogametic sex in mammals, while in birds it is the female which is the heterogametic sex. The avian chromosome complements are also distinct from those of placental mammals in that they include numerous microchromosomes, each no larger than one micron in size.

In our experience, the diploid complements of present day birds belonging to the orders **PASSERIFORMES**, **COLUMBIFORMES**, **GALLIFORMES**, and **ANSERIFORMES** followed the common rule in that nine pairs of macrochromosomes or ordinary chromosomes and about 60 microchromosomes constituted each diploid complement. Members of the ordem **PSITTACIFORMES** were exceptional, having more macrochromosomes and fewer microchromosomes. For instance, twelve pairs of macrochromosomes and about 18 pairs of microchromosomes constituted the diploid complement of the Australian parakeet, *Melopsittacus undulatus* (Ohno et al., 1964).

Relative DNA values were measured on the canary representing the order **PASSERIFORMES**, the chicken (*Gallus gallus domesticus*) representing the order **GALLIFORMES**, the pigeon (*Columba livia domestica*) of the order **COLUMBIFORMES**, and the Australian parakeet of the order **PSITTACIFORMES**. As the extreme similarity in their diploid chromosome complements already indicated the uniformity in the total genetic content of various avian species, the above four species representing four diverse orders were deemed sufficient.

As expected, four representatives of the class **AVES** gave the uniform DNA value. The value, however, was 44-59% that of placental mammals.

The above finding on the total genetic content of avian species, on one hand, reveals the fact that polyploidy played no role in extensive speciation within the class **AVES** and, on the other hand, shows that the genome lineage which gave rise to the class **AVES** has long been in separation from that which eventually gave rise to placental mammals.

THE COEXISTENCE OF THE TWO GENOME LINEAGES IN THE CLASS **REPTILIA** — Reptiles of today can be compared with the twigs of a great tree which flourished during the early Mesozoic era. About 95% of all the different kinds of living reptiles belong to the order **SQUAMATA**, yet fossil remains indicate that this order never held greater importance than today. On the contrary, fossil beds in many parts of the world are strewn with shells of many kinds of turtles. The orders **CROCODYLIA** and **CHELONIA** had seen better days.

We have no way of directly assessing the genomes of ancient reptiles which constituted the huge limbs of a great tree and which produced the direct ancestor to the placental mammals on one hand and that to the birds on the other. It was fortunate that the studies on relative DNA content and chromosome constitutions of a limited number of living reptiles enabled us to discern the presence of two different genome lineages, one showing close affinity to that of the class **AVES** and the other to that of the class **MAMMALIA**.

As stated earlier, the abundant presence of microchromosomes characterize the avian chromosome complements. It has been known for some time that microchromosomes are also possessed by lizards and snakes which constitute the ordem **SQUAMATA**. While the exact number of microchromosomes in the diploid complement of each avian species is nearly impossible to determine, the number of microchromosomes in each lizard or snake species can be determined with ease.

The diploid complement of the alligator lizard (*Gerrhonotus multicarinatus*, 2n = = 46-48) belonging to the family ANGUIDAE of the suborder SAURIA, contains 12 pairs of microchromosomes, while a great majority of snakes constituting the suborder SERPENTES, possess 10 pairs of microchromosomes.

Aside from the possession of microchromosomes, there is yet another common characteristic which reveals that the reptilian order **SQUAMATA,** suborder SER-PENTES in particular, belong to the very same genome lineage which gave rise to the class **A V E S.** The female heterogamety of the ZZ/ZW-type also operates in snakes (Beçak *et al.*, 1962; Kobel, 1962). Furthermore, the avian Z-chromosome and the ophidian Z-chromosome may have been derived from the same ancestral chromosome, as both constitute about 10% of the genome or haploid set (Beçak *et al.*, 1964).

DNA content was measured on six representatives of the order **SQUAMATA** which were: the chameleon lizard (*Anolis carolinensis*, 2n = 36) of the family IGUANIDAE and the alligator lizard (*Gerrhonotus multicarinatus*, 2n = 46) of the family ANGUIDAE of the suborder SAURIA. The suborder SERPENTES was represented by the *Boa constrictor* (*Boa constrictor amarali*, 2n = 36) of the family BOIDAE, the gopher snake (*Drymarchon corais couperi*, 2n = 36), and the South American *X e n o d o n* (*Xenodon merremii*, 2n = 30) of the family COLUBRIDAE, and the South American jararaca (*Bothrops jararaca*, 2n = = 36) of the family CROTALIDAE. These six representatives of the order SQUAMATA demonstrated a DNA value of 60-67% that of placental mammals. The value obtained was only slightly more than that obtained for various avian species which was 44-59% that of placental mammals (Atkin *et al.*, 1965).

While the above finding should not be interpreted to mean that lizards and snakes of today were directly ancestral to birds, it reveals that an ancestral reptile which evolved to toothed birds belonged to the same genome lineage which independently gave rise to ancestral forms of modern members of the order **SQUAMATA**; there was no further polyploidization of this genome lineage. Among members of the order **SQUAMATA** the well differentiated heteromorphic Z- and W-chromosomes are seen only in the poisonous family CROTALIDAE and certain members of the family COLUBRIDAE of the suborder SERPENTES; others possess the primitive homomorphic sex elements. Yet, there apparently exist the effective barrier to prevent the evolution of a bisxual polyploid species. Triploid species of the Teiid lizard of the genus *C n e m i d o p h o r u s* were all females and apparently propagated by parthenogenesis (Pennock, 1965).

While present day members of the reptilian order **SQUAMATA** demonstrated the close kinship to the class **A V E S** members of the order **CROCODYLIA** and **CHELONIA** appeared to represent the pre-mammalian genome lineage.

The South American alligator (*Caiman sclerops*, 2n = 42) representing the order **CROCODYLIA** gave the DNA value as 84% that of placental mammals. The diploid chromosome complement of this species is totally different in character from those of snakes and lizards. In fact, there is a striking resemblance between the diploid complement of *C a i m a n* and that of one species of mammals, the rat (*Rattus norvegicus*, 2n = 42). To be sure, this extreme similarity is a pure coincidence. Nevertheless, there is little doubt that among present day members of reptiles, those belonging to the order **CROCODYLIA** demonstrate the closest kinship with placental mammals, not only in DNA value but in karyological characteristics as well. Although the lower diploid chromosome number of 32 has been reported on the North American alligator (*Alligator mississippien-*

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sis) and the African crocodile (*Crocodilus niloticus*), this reduction in chromosome number from 42 to 32 appears to be the result of simple Robertsonian translocations. The 10 largest pairs of acrocentrics of *C a i m a n* are represented as 5 pairs of metacentrics in *A l l i g a t o r* and *C r o c o d i l u s* (Hollingsworth, 1957; van Brink, 1959).

DNA value similar to that of placental mammals was also obtained on representatives of the order **CHELONIA**. The fresh-water soft-shell turtle (*Amyda ferox*, 2n = 66) and the desert tortoise (*Gopherus agassizi*, 2n = 52) gave DNA value 80 and 89% that of placental mammals, respectively. While observing their metaphase figures, however, it was noted that their karyological characteristics are not at all similar to those of placental mammals. Many small members can be regarded as microchromosomes. It appears that members of the order **CHELONIA** demonstrate the closest karyological affinity to the infraclass **P R O - T O T H E R I A**, rather than to either marsupials or placental mammals. A rather high diploid chromosome number of about 70 and 63 has been found in the duck-bill platypus (*Ornithorhynchus anatinus*) and the spiny anteater (*Tachyglossus aculeatus*) of the order **MONOTREMATA**. Many small members can be regarded as microchromosomes (Matthey, 1949; van Brink, 1959).

It would be of utmost interest to find out if the male heterogamety of the XY/XX-type operates in members of the orders **CROCODYLIA** and **CHELONIA** which represent the premammalian lineages. Unfortunately, the heteromorphic sex elements have not been found in these reptiles. No sex-linked gene is known, and the sex reversal experiments have not been performed on any of these species.

EXTREMELY HIGH DNA VALUES POSSESSED BY CERTAIN AMPHIBIANS WHICH SUGGEST THE POLYPHYLETIC ORIGIN OF TERRESTRIAL VERTEBRATES — It is known that birds, snakes, and lizards of today are the branches of one limb which originated from the ancestral reptile *O r n i t h o s u c h u s* mammals emerged from the other limb which was started from *L y c a e n o p s*. The fact that surviving members of the classe **REPTILIA** fell discretely into two groups (one group belonging to the preavian genome lineage and the other group belonging to the premammalian lineage) may be taken as an evidence that *O r - n i t h o s u c h u s* and *L y c a e n o p s* of ancient times already belonged to the two different genome lineages.

Reptiles, in turn, were derived from ancient amphibians grouped together as Labyrinthodonts. It appears that Labyrinthodonts were of many kinds representing diverse genome lineages. Most, if not all, of the amphibians of today belong to the genome lineages independent from both the preavian and premammalian lineages.

The most comprehensive survey on DNA values of various amphibians was carried out by Joseph Gall of Yale University; his results are quoted here with his kind permission. All the amphibian species surveyed by him demonstrated higher DNA values than that of placental mammals. DNA values demonstrated by tailless amphibians constituting the order **SALIENTIA** were still not as fantastically high as those demonstrated by members of the order **CAUDATA**.

Within the order **SALIENTIA**, the American toad (*Bufo americanus*, 2n = 22) representing the suborder PROCOELA gave the DNA value 137% that of placental mammals. The DNA value of the Leopard frog (*Rana pipiens*, 2n = 26) and the bull frog (*Rana catesbiana*, 2n = 26) of the suborder DIPLASIOCOELA

was 200% of the DNA value of placental mammals. In terms of the absolute content, the family RANIDAE contained 14.6 mg $\times 10^{-9}$ DNA in each diploid nucleus. While these values are high, they show close enough affinity to the pre-mammalian lineage. It is expected that if a truly extensive survey is done on tailless amphibians of today, the DNA value very similar to that of the premammalian lineage can be found in some of them.

On the contrary, members of the order **CAUDATA** showed absolutely no affinity to either the premammalian or the preavian lineages. Within this order, the lowest DNA value was found on the newt (*Triturus cristatus*, 2n = 24) of the suborder SALAMANDROIDEA. Yet, it was 830% that of placental mammals, and its close relative, *Triturus viridescens* (2n = 22) revealed even higher DNA value of 1300%. The Congo eel (*Amphiuma means*, 2n = 24) of the suborder PROTEIDEA, had the fantastically high DNA value of 2700% that of placental mammals.

Another interesting aspect of tailed amphibian genomes is that two closely related species belonging to the same family often demonstrated a remarkable difference in their DNA values. For instance, *Triturus cristatus* and *Triturus viridescens* belonged to the same family SALAMANDRIDAE, yet the DNA value of the latter was 50% greater than that of the former, despite the fact that both had the very similar diploid complements. Joseph Gall found that each lamp-brush bivalent of the latter was longer and had more loops than its counterpart of the former. On this basis, he believes that the increase in DNA value is due to regional duplication of chromosomal segments that occurred to *Triturus viridescens*.

It has been shown that the Z and the W or the X and the Y of amphibians are in such a primitive state of differentiation. The W or the Y is still a genetical equivalent of the Z or the X. This primitive state of sex chromosomes may permit polyploid evolution to exceptional membres of present day amphibians.

One species might represent a newly arisen tetraploid state of the old diploid species. The pioneering study by Saez (1964) has indicated that polyploid evolution may have occurred to South American frogs belonging to the family CERATOPHRYDAE. Indeed, the tetraploid nature of *Odontophrynus americanus* has been proven beyond any doubt by M. L. Beçak and her colleagues at this symposium. The 44 chromosomes can be arranged to 11 different kinds of homologues, and 11 quadrivalents rather than 22 bivalents were seen in meiosis. Thus, among amphibians of today, the increase of DNA content by both regional duplication and polyploidization might still be occurring to some extent. Nevertheless, so far as members of the order **CAUDATA** are concerned, it is clear that they belong to the genome lineage or lineages altogether different from both the preavian and premammalian lineages.

DIVERSE GENOME LINEAGES FOUND AMONG FISHES — The inevitable conclusion to be drawn from the above survey on DNA values of the four classes of terrestial vertebrates is that the evolution from Crossopterygian fishes to Labyrinthodont amphibians was polyphyletic. Today, the subclass **CROSSOPTERYGII** is represented only by the lung fish of the order **DIPNOI** and the coelocanth of the order **ACTINISTIA**. These surviving members of the lobe-finned fish must merely represent a fraction of the diverse genome lineages which were possessed by ancient Crossopterygian fishes ancestral to terrestrial vertebrates. As much as we have no way of obtaning the information on genomes from the fossils, we

must turn to members of the ray-finned fish constituting the subclass **NEOPTE-RYGII** as the source of indirect information on ancient genome lineages.

Our study, although limited to eight species of the class **PISCES**, appeared to confirm the polyphyletic origin of terrestrial vertebrate genomes (Ohno and Atkin, 1966).

It was found that surviving members of the order **DIPNOI**, the subclass **CROSSOPTERYGII** show close kinship only to tailed amphibians (the order **CAUDATA**). The DNA value, 3540% that of mammals, was obtained on the South American lung fish (*Lepidosiren paradoxa*, 2n = 38). According to Alfrey *et al.* (1955), the absolute DNA value for the African lung fish (*Protopterus*, 2n = 34) was 100 mg × 10^{-9} which is about 1400% that of mammals. The relatively low diploid chromosomes number, the absence of acrocentrics, the enormous size of individual chromosomes, and the very high DNA value found in the lung fish are all precise characteristics of the genomes maintained by present-day membres of the order **CAUDATA** of the class **AMPHIBIA**. Although the chronology of evolution suggests that the lung fish could not have been the direct ancestor of the tailed amphibians, it is apparent that both belong to the same particular genome lineage. This lineage is not directly related to the main genome lineages which gave rise to tailless amphibians, reptiles, birds, and mammals of today.

The DNA values which demonstrated the close kinship to the premammalian and preavian genome lineages were found among members of the subclass **NEOPTERYGII**.

The rainbow trout (*Salmo irideus*, 2n = 58-64) is the anadromous species belonging to the family SALMONIDAE of the order **ISOSPONDYLI**. The DNA value, 80% that of mammals, corresponded well with the values possessed by the orders **CROCODYLIA** and **CHELONIA** of the class **REPTILIA**; thus, this species and other members of the family SALMONIDAE may be regarded as belonging to the premammalian lineage. It is not my intention to imply that trouts and mammals constitute one direct line of descent. My view is that crocodiles, turtles, and mammals of today descended from a particular group of ancient Crossopterygian fish which already possessed the DNA value similar to that of trouts.

The DNA value similar to that possessed by the class **AVES** as a whole and also by the order **SQUAMATA** of the class **REPTILIA** was found on the goldfish (*Carrasius auratus*, 2n = 96-104) of the family CYPRINIDAE, the order **OSTARIOPHYSI**. The DNA value obtained on this species was 52% that of mammals. Thus, members of the family CYPRINIDAE may be regarded as belonging to the preavian genome lineage.

Our study on various members of the subclass **NEOPTERYGII** further revealed the presence of DNA values much smaller than any of the values possessed by terrestial vertebrates. Our notion that a series of polyploidization of an ancestral vertebrate genome occurred while vertebrates were still in aquatic forms appeared to be confirmed.

The DNA value of only 30% that of mammals was obtained on two members of the order **PERCIFORMES**. The green sunfish (*Lepomis cyanellus*, 2n = 46-48) of the family CENTRARCHIDAE and the discus fish (*Synphysodon aequifasciata*, 2n = 60) of the family CICHLIDAE.

The lowest DNA value, only 20% that of mammals, was found among two diverse groups of fishes. This value was obtained on the swordtail (*Xyphophorus hellerii*, 2n = 48), hornyhead turbot (*Pleuronichthys verticalis*, 2n = 48), and fantail sole (*Xystreurys liolepis*, 2n = 48).

From the taxonomical point of view as well as from their natural habitats, the swordtail and the flatfish are as remotely related as they can be among members of the subclass **NEOPTERYGII**. The swordtail, a Central American fresh-water fish long bred in the aquarium, belongs to the order **MICROCYPRINI**, while two species of the flatfish belong to different families of the order **HETEROSOMATA**: the hornyhead turbot to the right-eyed flounder family, PLEURONECTIDAE, and the fantail sole to the left-eyed flounder family, BATHIDAE. Their natural habitat is the ocean bottom. The swordtail and the flatfish apparently had identical diploid complements made of 48 acrocentrics gradually declining in size and the lowest DNA value.

We propose to regard these ray-finned fishes as the retainers of the original diploid lineage of ancestral vertebrates. The original diploid lineage then had the DNA value, 20% that of mammals. In terms of the absolute value, this lineage contained $1.4 \text{ mg} \times 10^{-9}$ DNA in each nucleus.

It then follows that the green sunfish and the discus fish belong to the ancient triploid lineage, while the ancient pentaploid lineage is represented by the goldfish and among terrestial vertebrates, by lizards, snakes, and birds.

The rainbow trout, crocodiles, and turtles may be regarded as representing the octa- and nonaploid lineages, and placental mammals, the decaploid lineage.

All three constituent polypeptides A, B, and C of the mammalian lactate dehydrogenase have been found to exist in avian species as well as in many of the ray-finned fishes (Blanco *et al.*, 1964; Markert and Faulhaber, 1965). These findings on lactate dehydrogenase are in conformity with the view that in vertebrates, any DNA values above 20% that of placental mammals indicate polyploid lineages; therefore, sufficient gene duplication has occurred to these genomes. Flatfish of the order **HETEROSOMATA**, on the other hand, revealed the presence of the A-polypeptide only (Markert and Faulhaber, 1965).

SUMMARY

It appears that gene duplication played a most important role in the evolution of vertebrates. A new gene with a new function arose from a duplicate of the old gene. When the same gene was represented twice within the genome, one redundant gene was allowed to mutate to an independent direction and acquire a new function, while the original function was maintained by the other.

Admittedly, regional duplication of a small number of genes might still be occurring to individual species of higher vertebrates. A series of polyploidization of the ancestral diploid lineage, however, appeared to have occurred while vertebrates were still in aquatic forms nearly 300 million years ago. Among fishes of today, some appear to retain the ancient diploid lineage which contain $1.4 \text{ mg} \times 10^{-9}$ DNA per diploid nucleus. Placental mammals as a whole appear to belong to the ancient decaploid lineage, while birds represent the ancient pentaploid lineage. Once the chromosomal sex-determining mechanism is well established, no further polyploidization is possible.

As a result, diverse species of placental mammals contain the identical amount of DNA in the diploid complement, 7.0 mg $\times$ 10^{-9}. Speciation within the infra-class **EUTHERIA** is accomplished almost exclusively by allelic mutations with little change in the total number of gene loci. The same can be said of various avian species. Among reptiles of today, snakes and lizards belong to the pre-avian pentaploid lineage. Crocodiles and turtles, on the other hand, show close kinship to the decaploid mammalian lineage.

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