CHROMOSOME NUMBER AND POLYPLOIDY IN AMPHIBIA

BY

B. R. SESHACHAR, D.Sc.

(*Department of Zoology, Central College, Bangalore*)

A VERY large amount of recent work has brought to light many important features in the chromosome cytology of Amphibia. Many of the early studies were confined to the chromosomes of the common Anura and Urodela and it is only recently that an extension of these studies has been made with reference to the other amphibians. Up till 1937 (Oguma and Makino) the chromosome numbers of one species of Apoda, 37 of Urodela and 30 of Anura were known. The chromosome number of one other species of Apoda has since been added (Seshachar, 1939).

From a study of the chromosome number in Amphibia it becomes clear that the variation in the chromosome number within the group obeys fixed laws. In Apoda, the number in only two species is known: $n = 21$ in *Ichthyophis glutinosus* and $n = 18$ in *Uræotyphlus narayani* (Seshachar, 1937; 1939). Among the Urodela the lowest number recorded is in *Proteus anguineus* (Stieve, 1920) where $n = 9$. In the majority of the Urodela belonging to the Amphiumidæ and Salamandridæ, the basal chromosome number shows an astonishing uniformity and might be taken to be the typical urodelan number. It is $n = 12$. In the Cryptobranchidæ and Hynobiidæ, however, there is a distinct departure. This variation is all the more striking because the difference between the basal urodelan number and that in the known examples of the above two families is very great. In fact, the latter is often more than twice the former. The numbers are as follows: $n = 20$ in *Hynobius retardatus* and varies from $n = 28$ in the majority of the species of *Hynobius* (*H. leechii*, *H. nigrescens*, *H. nebulosus*, *H. dunni*) to $n = 31$ in *Salamandrella keyserlingii*. In *Cryptobranchus allegheniensis* it is 31 and $n = 32$ in *Megalobatrachus japonicus*.

The variation is not so striking in Anura. *Bufo* appears to have $n = 11$ and in *Rana* n is generally 12. Many other species disclose this latter number (species of *Bombinator*, *Hyla*). The highest number observed in Anura is in *Alytes obstetricans* where $n = 16$ (Janssens & Willems, 1909).

From the foregoing account of the chromosome number in Amphibia certain conclusions can be drawn. The basal number in Amphibia appears to be $n = 12$. Wherever there are variations, in the majority of cases these variations may be traced to a fragmentation of the chromosomes resulting in a multiplication in the number. The work of the author has shown that the apparently diverse chromosome numbers in the two species of Apoda whose numbers are known, is deducible, according to Robertson's law, to the same basal number, which in this case is $n = 13$.

But the very large number of chromosomes found in examples of Cryptobranchidæ and Hynobiidæ cannot apparently be explained by Robertson's law and must have been brought about by a totally different kind of fragmentation from that which has resulted in the slight variations found in some species of Urodela, Anura and Apoda. The presence of a very large number of V-shaped chromosomes with atelomitic attachments precludes the application of this law. And apart from the fact that in these two families the basal number appears uniformly to be $n = 28$, nothing more can be said either about the origin or the significance of this change from the typical amphibian basal number of $n = 12$.

Vandel (1938) has, after a critical examination of the number of chromosomes in vertebrates, come to the conclusion that while in many vertebrates the basal number is $n = 12$, any increase (which is probably due to fragmentation) indicates a specialization and is correlated with the evolution of the group. He does not account for the enormous number of chromosomes found in Cryptobranchidæ and Hynobiidæ. While it is possible that these two families form exceptions to the rule, the origin of their chromosome numbers still remains to be determined. And while fragmentation in general might indicate an evolutionary specialization within the group, the fragmentation in the case of these two families (if it is fragmentation) must have a totally different significance.

In this respect Anura is a more stable group. None of the species whose chromosomes are known exhibits the huge variations seen in Urodela and the slight changes in number noticed here have probably been brought about by fragmentation and indicate specialization. As already observed, the chromosomes of only two species of Apoda have been known and it is desirable that our knowledge of this group is wider before any definite conclusions are drawn. But it is significant that these two species, when Robertson's law is applied, reveal a basal number, $n = 13$.

Polyploidy.—Generally in animals, polyploidy is rare and whenever it occurs, it has not the same significance as in plants. Among Amphibia, triploids have been reported in *Rana esculenta* (Hertwig & Hertwig, 1920) and in the urodeles, *Triton palmatus* (Fankhauser, 1934) and *T. viridescens* (Fankhauser & Kaylor, 1935). Parthenogenetic triploid larvæ have been reported in *Rana pipiens* (Parmenter, 1933) and in *Rana nigromaculata* (Kawamura, 1939). Triploid and tetraploid larvæ have been found in *Eurycea bislineata* (Fankhauser, 1939).

Experimentally it is possible to induce polyploidy and of all the methods the most productive so far as the Amphibia are concerned, is the temperature factor; and Fankhauser and his colleagues have thrown much light on this problem in the urodeles.

It is a well-known fact that low temperatures applied during meiosis in plants result in a non-reduction of the chromosomes in the gametes which therefore retain the diploid number (Belling, 1925). The fusion of such diploid gametes with normal haploid gametes produces triploid zygotes. While low temperatures produce gametic duplication of the chromosomes, high temperatures produce somatic doubling.

Rostand (1936) first applied these methods to frogs and toads and was at once successful. In his hybridization experiments between frogs and toads, he exposed eggs immediately after insemination (with sperms of a different genus) to refrigeration and produced normal diploid gynogenetic tadpoles from these eggs. But it was found on cytological examination that the male chromosomes had not fused with those of the female and therefore the diploid nature was due to quite a different cause. It was discovered that the haploid chromosomes of the female had become doubled due to refrigeration. It probably had happened this way: in many Amphibia the nucleus of the egg is in the metaphase (Frog) or anaphase (Urodele) of the second division at the time of insemination and so, refrigeration immediately after, prevented the completion of this division. The result was, that the chromosomes of mitosis which should have gone into the second polar body came to be retained in the egg, which therefore became diploid. On the same analogy it is found that in newts (*Triturus*), triploid larvæ can be obtained only by refrigerating the eggs immediately after they are laid. Even if refrigeration is delayed by half an hour, normal diploid larvæ result. The explanation is that when the egg is laid, the nucleus is in the second division of meiosis, which is completed in about an hour's time after laying. An inhibition of this division which is the only method of making the egg diploid, can take place by refrigeration if applied quite soon after the eggs are laid (Griffiths, 1941).

Polyploidy in animals always leads to abnormalities and to death. In the urodeles studied, the animals lived up to metamorphosis and in no instance could complete it.

It is a well-known fact that among plants and also in many animals polyploidy leads to gigantism. In animals, Vandel (1927) reports it in the isopod, *Trichoniscus*, Seiler (1927) in *Solenobia* and Artom (1928) in *Artemia*. But in Urodela, no gigantism, either in triploid or pentaploid *Triturus* or in tetraploid *Eurycea* is seen. The gigantism in polyploid plants and animals is generally due to the fact that while the cell number in organs remains the same, the cell size becomes very much larger. In polyploid newts on the other hand, the cell size is larger, but the cell number in each organ is reduced with the result that the size of the polyploid animal remains almost the same as that of the diploid one. Similar instances of polyploid plants, where in spite of larger size of the cells, the plant size on the whole remains normal is reported by Hagerup (1932) in *Euphorbia granulata*. Fankhauser (1941) recently found a single pentaploid *Triturus viridescens* whose body size was not different from that of the normal diploid individual of the same age, though the cell size was very much larger than the normal. This points to the conclusion that in newts there is some regulatory mechanism which comes into play in polyploid individuals and which reduces the cell number in the organs to offset the increase in cell size.

The cytology of the effect of abnormal temperatures in producing polyploidy can only be conjectured at this stage. It is clear that of the two structures of the cell in meiosis,—the chromosomes and the spindle,—temperature has its effect only on the spindle and not on the chromosomes, for the latter are seen to behave normally and to divide, but their separation into two distinct daughter nuclei is prevented. This is probably due to some disturbances in the spindle mechanism brought about by change of temperature.

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CANCER RESEARCH IN INDIA

THE TATA MEMORIAL HOSPITAL which His Excellency Sir Roger Lumley, Governor of Bombay, opened on 30th April 1941, is one of the benefactions which India owes to the illustrious Tata family. This splendidly equipped institution dedicated to the treatment of Cancer will serve not only the purpose of a hospital, but also that of an advanced centre of research for the study of this malignant disease.

In a special supplement to the *Times of India*, dated 1st March 1941, Dr. V. R. Khanolkar, Director of the Cancer Research Laboratory, writes: "The establishment of a hospital devoted to cancer research in Bombay on the lines of the Memorial Hospital in New York, is a departure which takes into account the shortcomings of purely experimental institutions in other parts of the world. Just now when a large part of the world is involved in a life and death struggle and the best energies of the human race are directed towards destructive activities, it is an important achievement to have started a humanitarian institution for the better care of people suffering from malignant diseases.

"The Tata Memorial Hospital is particularly fortunate, inasmuch as the Trustees have been farsighted enough to organise a place where besides study, treatment and laboratory research would be intimately co-ordinated and the clinician will be a research worker, and a laboratory investigator will have an opportunity of extending the experience gained from the laboratory to the hospital patient.

"The institution is unique in its conception inasmuch as most of the clinicians and the whole of the laboratory staff will be devoting their whole time to the work at the institution."