

## Phylogeny: Principles and Methods

Since evolution consists in genetic changes in populations of organisms in the course of time, the fundamental problem of phylogeny appears to be the analysis of these genetic changes which have occurred in the history of a species. At present the fossil record provides the best clues to the actual processes in evolution. Where the fossil record is either incomplete or lacking, varied comparison of living things is the only method available. To coordinate some of the results of current research on evolution, investigators from the fields of comparative morphology, paleontology, biochemistry, cytology, and genetics participated in a symposium, *Principles and Methods of Phylogeny*, which was sponsored by the American Society of Naturalists, and held on 27 December 1962 in Philadelphia.

In his introductory remarks, E. W. Caspari pointed out that phylogeny is a historical science which shares with other historical sciences, for example, history in the political sciences and historical geology and cosmology in the inorganic natural sciences, certain difficulties: the facts cannot be established by direct observation but have to be inferred from indirect evidence. Phylogenetic considerations are a necessary part of the full description of any biological system which complements the description of fundamental mechanisms that are assumed to be rather uniform in different organisms. Phylogenetic studies were regarded as the main problem area of biology in the late 19th century, and the phylogenetic tree worked out at that time is still taught in the schools, even though much doubt has been expressed about the validity of the methods employed at the time, particularly the "ontogenetic law."

In the last two decades, a number of living organisms have been found which have been assigned to a certain group, that is, a class or phylum, but

show a much more primitive organization than any previously known members. H. Sanders used one of these organisms, which he himself had first discovered, the Cephalocarida, to derive the phylogenetic relations in an old, well-differentiated group, the Crustacea. In the Cephalocarida, the serial homology of the appendages is particularly well expressed. The studies were extended to include the muscles of the appendages, the endoskeleton, the horizontal, dorsoventral, and longitudinal muscles of the thorax. In comparing these structures to those found in other Crustaceans, relations of the Crustacean groups to the Cephalocarida for each of these characters could be established. It turned out that for most of the characters studied, either the Malacostraca including the Leptostraca, or the Branchiopoda, or both, were most similar to the Cephalocarida. W. Bock considered a group of animals which is not very variable with respect to morphology, the birds. His discussion centered on the concept of homology, and the means of its establishment. In many shore birds, but not all, a secondary articulation of the lower jaw is found between the mandibular process and the lateral or medial edge of the basitemporal process. If all shore birds not possessing this process were extinct, the secondary articulation in this group would be regarded as monophyletic. Since this is not the case, we know that these processes are not homologous in the phylogenetic sense, but pseudohomologous. The survival of primitive forms is therefore essential for the establishment of phylogenetic homologies. In a uniform group, such as the birds, the genotype and consequently the developmental processes are rather similar, and restrict the possibilities of variation. Evolution may therefore proceed in different groups along parallel lines, because they have very similar genetic and developmental potentialities. Bock proposes therefore

to distinguish between homology which results from common descent and homology which results from evolutionary homodynamy.

R. F. Thorne discussed similar problems with respect to the flowering plants. In this group, the fossil record is very incomplete and not too useful, but many primitive forms are still surviving, and many different groups are connected by links still existing in the present flora. On the other hand, plants show many specializations as a result of adaptation to specific environmental conditions, and convergent evolution has been frequent in this group. It is possible, in most cases, to establish the phylogenetic relationships by comparison of a large number of characters, particularly to primitive members of the group.

Phylogenetic problems relating to microorganisms were discussed by A. W. Ravin. The difficulties in this group are similar to those found in other "uniform groups," such as birds and angiosperms. The fossil record is missing, the morphology is rather uniform, and convergent evolution has probably taken place. Phylogenetic relationships can, however, be established in this group by comparison of the genetic material itself, the DNA. Two methods, with different applications, lend themselves to this purpose. The ratio of the bases contained in the DNA is strikingly different in different groups of bacteria, and a specific range of base ratios is frequently characteristic for a group of microorganisms, such as the spore-forming bacilli, which also on morphological and physiological grounds are believed to be related. Inside one of the groups of closely related organisms, transformation by means of isolated DNA proves to be a sensitive indicator of phylogenetic relations.

DNA from pneumococcus, for example, can be used to transform related strains of streptococcus, using streptomycin resistance as a marker. The efficiency is always highest in homologous transformation and lower in more or less closely related species. Transformation between unrelated species is impossible. It can be shown that the failure of transformation between unrelated species is not due to lack of uptake of donor DNA, but to its inability to become incorporated into the host genome. Since the incorporation of donor DNA is assumed to proceed by pairing and crossing over with the

host genome, the transformation experiment supplies a sensitive test for the pairing ability of the two homologous chromosome segments, which is presumably dependent on similarity of the pattern of arrangement of the bases.

The genetic material can also be used for the establishment of phylogenetic relations in higher organisms. M. Wasserman showed that chromosome rearrangements in the genus *Drosophila* can be used for this purpose, and exemplified his conclusions by a discussion of the repleta group of this genus. Inversions are particularly useful, since they occur very rarely, and the independent reoccurrence of the same arrangement can be reasonably excluded. Since many arrangements found cannot have arisen from another known arrangement in one step, it is possible to arrange the occurring chromosomal patterns in series which correspond to phylogenetic series. It cannot be easily established in any one series in which direction the series is to be read; but consideration of all arrangements in a larger group can usually overcome this difficulty. Wasserman pointed out that in the repleta group the phylogenetic relations established by chromosome morphology agree well with the taxonomy based on morphological characters. But chromosomal divergence is not correlated with morphological divergence.

E. H. Colbert discussed the fundamental contributions that paleontological methods can make for our understanding of phylogeny, and illustrated his points by references to the evolution of the duckbill dinosaurs in the Upper Cretaceous of North America. Where a reasonably complete fossil record exists, there is usually no difficulty in establishing the true phylogenetic relations between species found in successive strata. Convergence and pseudohomology will arise in these cases too, but they are relatively easily detected and will not confuse the phylogenetic picture. Finally, the paleontological record can give us information on the time spans involved in particular evolutionary processes (about 24 million years for the history of the duckbill dinosaurs), and on the changes in the conditions on the earth which were occurring at the same time.

The biochemical implications of evolution were considered by S. S. Cohen. He pointed out that, contrary to the assumptions of earlier biochemists, differences in biochemical characters between different organisms are

not restricted to so-called superficial characters, such as pigments or cell-wall constituents, but that also very fundamental processes, such as the synthesis of DNA and RNA and their components and the pathways in the activation and synthesis of amino acids are by no means as homogeneous among different organisms as was originally supposed. A seeming contradiction consists in the fact that in different species of bacteria strong differences in DNA composition can be found, while the proteins depending on the DNA's appear to be much more similar in constitution and function. Since important basic steps in biochem-

ical syntheses are performed in different ways in different organisms, it may be concluded that biochemical processes change in the course of evolution, and that biochemical functions cannot only be lost, but also acquired. Differences and similarities in basic biochemical processes have not yet been investigated systematically; most of the material quoted is derived from bacteria and from vertebrates. But study of biochemical evolution promises to offer a very important tool for progress in the study of phylogeny.

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## Transfer of Genetic Information

The analysis of the genetic code and the clarification of mechanisms controlling gene expression are among the important contributions of molecular biology in recent years. At the AAAS meetings in Philadelphia, a symposium entitled *The Transfer of Genetic Information*, organized by Severo Ochoa and Philip Abelson, was concerned with recent progress in these areas. Three of the papers discussed the genetic code: "Characteristics of RNA code-words," Oliver W. Jones and Marshall W. Nirenberg (National Institutes of Health); "Synthetic polynucleotides and the amino acid code," Peter Lengyel, Joseph Speyer, Carlos Basilio, Albert Wahba, and Severo Ochoa (New York University); and "The doublet code and its implications," Richard B. Roberts (Carnegie Institution of Washington). The fourth speaker, Sol Spiegelman (University of Illinois) discussed the nature of genetic expression and its control in a talk entitled "Properties of the mechanism which reads the genetic book."

Since the announcement by Marshall Nirenberg in August 1961 that polyuridylic acid stimulates cell-free protein synthesizing systems to incorporate labeled phenylalanine into acid-insoluble peptide linkages, the characterization of the amino acid code has proceeded rapidly. It was soon found

by Ochoa and Nirenberg that copolymers containing uridine and varying amounts of one of the other three nucleotides found in naturally-occurring RNA stimulate the incorporation of other amino acids. The genetic work of Crick and coworkers had indicated that most probably a triplet of RNA nucleotides is the template for one amino acid. With this assumption a triplet code was constructed for the amino acids incorporated by the various copolymers.

In the earliest published data only copolymers which contained U could serve as templates for protein synthesis. No naturally-occurring RNA has been observed with such a large fraction of U. The data of Sueoka relating the acid composition of organisms to the GC content of their DNA can not be explained by a code containing a preponderance of U. To resolve this paradox Richard Roberts proposed a doublet code which (i) incorporated the results of Ochoa and Nirenberg; (ii) had a reasonable GC content; (iii) agreed with the Sueoka data; and (iv) was consistent with the idea that certain single amino acid changes produced in tobacco mosaic virus protein, as the result of treatment of the virus with nitrous acid, were the result of single nucleotide changes in the nucleic acid of the virus. Roberts reviewed this code in the symposium and pointed out