It includes the following studies:

The Genetic Complement

It comprises the genome (the DNA in the nucleus) and plasmon (the DNA in cytoplasmic organelles). Deoxyribonucleic acid is the essential material of heredity. It is believed that if the DNA composition of all species is known, their evolutionary course would become quite apparent. It is believed that the amount of deoxyribonucleic acid per chromosome set is constant for each species. But it is still not certain whether the ratios of the DNA

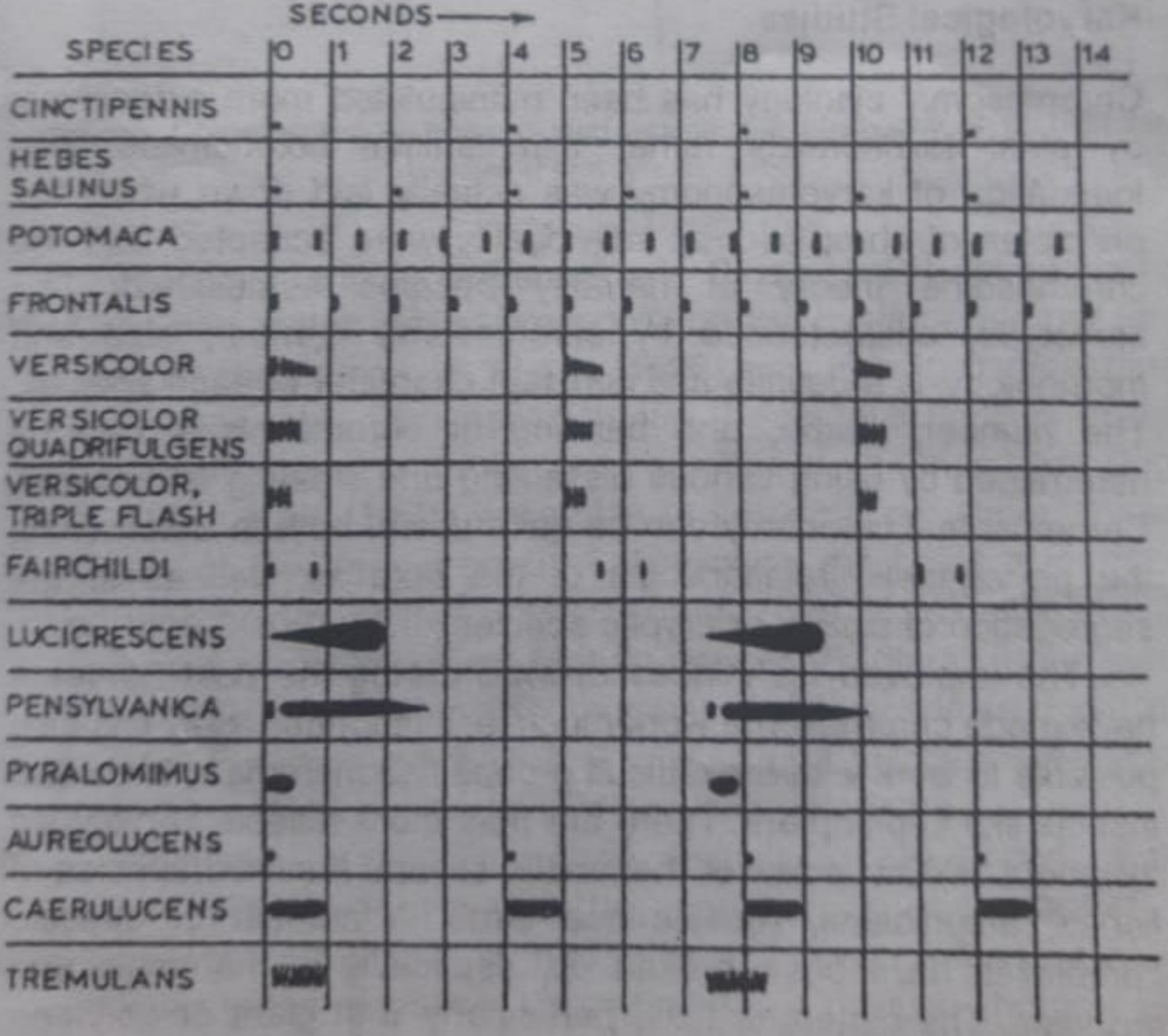

Fig. 29. Pattern of light flashes in some species of fireflies of the genus *Photuris*
(Modified from Barber, 1951)

content of the chromosomes are attributable to variation in the size of heterochromatic segments or they are associated with differences in the metaphase thickness. Even today it is not known that a given amount of DNA and proteins is stimulated at mitosis to become distributed into a particular number of chromosomes.

DNA Hybridisation

The discovery that "hybridisation" between single stranded DNA components from different origins can occur (Schildkrant *et al.*, 1961) provides a physico-chemical means for assessing genetic relatedness among species (Marmur *et al.*, 1963). In such studies the DNA is extracted from an organism and made to hybridise *in vitro* with the cell-lines of other organisms. These DNA matching techniques hold much promise in solving complex taxonomic problems. The taxonomic implications of these have been well reviewed by Hoyer *et al.* (1964). The incomplete fossil record in many animal groups may pose problems in solving the evolutionary or phylogenetic problems through these studies.

Karyological Studies

Chromosomal cytology has been manipulated more extensively by plant taxonomists rather than animal taxonomists. The foundation of karyotaxonomy was actually laid down when the principles of chromosomal individuality were accepted and the chromosome theory of heredity became established. The karyotype, characterised by chromosome number, size and morphology, is a definite and constant character of each species. The number, shape, and banding of chromosomes can be determined by using various dissecting and staining techniques. Chromosomal taxonomy can be quite useful both in determining the phylogenetic relationships of the taxa as well as in the segregation of sibling or cryptic species.

The improved techniques evolved during the past 30 years have made chromosome work much less laborious. Now it is also possible to work with the difficult groups like mammals, birds and insects like Lepidoptera. There are now more reliable karyotypes for about 1000 species of mammals, several hundred species of fishes, amphibians, reptiles and birds. A number of species complexes have been broken up, especially in mammals and urodeles. The dipterous flies, particularly with giant or polytene chromosomes and orthopterans, are the most suitable groups for

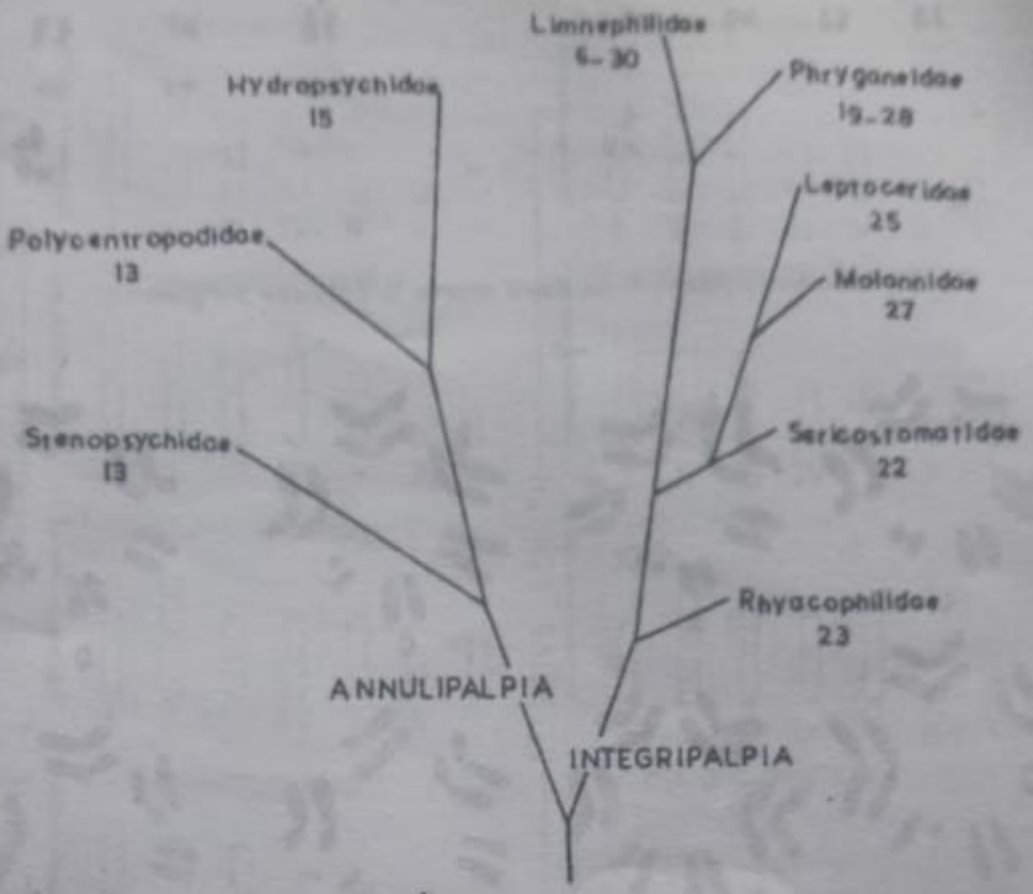

Fig. 30. Chromosome numbers plotted against the phylogeny of Trichopteran families (From Kiauta, 1968).

chromosomal studies. Patterson and Stone (1952) differentiated 16 species of the genus *Drosophila* on the basis of number and shape of chromosomes. Kiauta (1968) was able to demonstrate the phylogenetic relationship among the various families of the order Trichoptera on the basis of number of chromosomes (Fig. 30). Mittal *et al.* (1974) were able to separate two synonymised species of the earwig genus *Labidura* on the basis of number and morphology of their chromosomes (Fig. 31). Grewal (1982) separated some important fruit fly species (Diptera, Tephritidae) on the basis of the shape and number of chromosomes (Figs. 32, 33). He also discovered another population of *Bactrocera zonata* (Figs. 32, 33) on the basis of these characteristics.

Polyploidy is not common in animals except among wholly parthenogenetic forms. But the variation in the number of chromosomes occurs in many ways. One of the ways for reduction in the number of chromosomes is through chromosome fusion. In some groups the chromosome pattern has remained

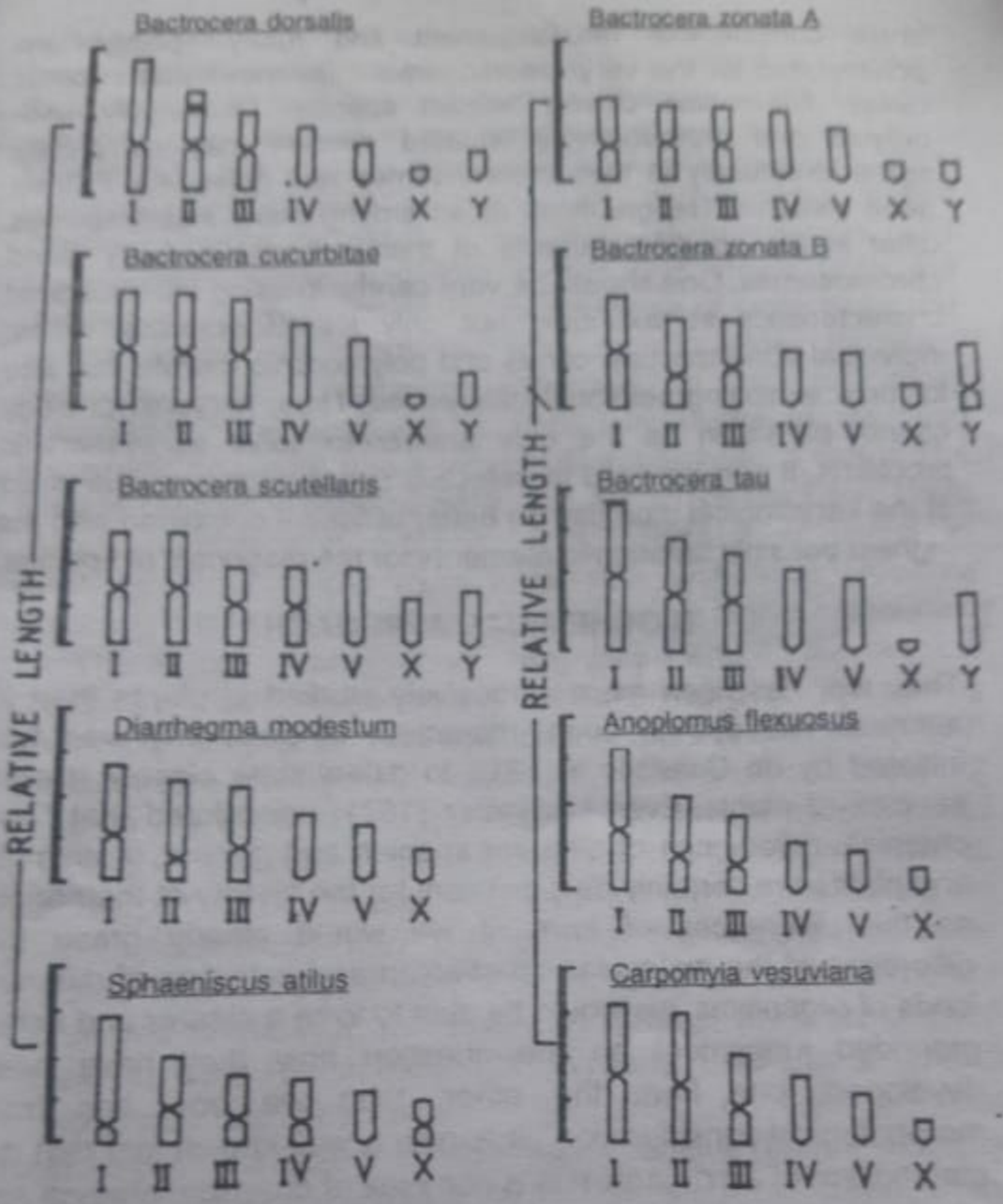

Fig. 33. Diagrammatic presentation of the idiograms of some fruit fly species of fig. 32.

acrocentrics) and Pyrgomorphidae (2n ♂ = 19 acrocentrics), while in others it has undergone distinct changes even in closely related species. For example among insects like Odonata, Diptera and Coleoptera, the chromosome number is fairly constant while in Lepidoptera, Trichoptera (insects), scorpions and fishes, it has shown marked variations. Moreover, it is also not true that the amount of chromosomal change reflects the amount of genetic change even though hereditary patrimony is carried by the chromosomes. The closely related species may

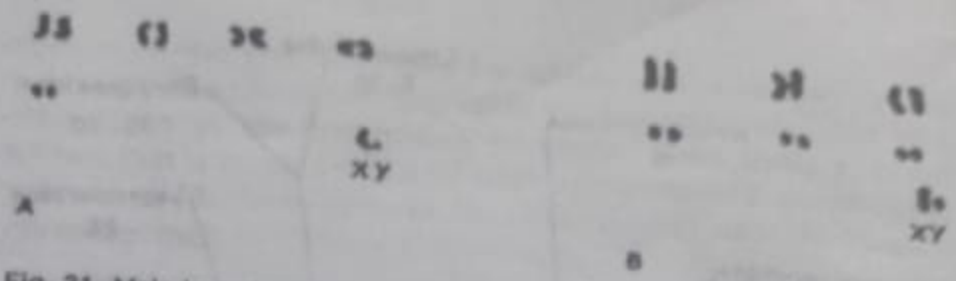

Fig. 31. Male karyotypes—A. *Labidura riparia*; B. *Labidura bengalensis*.

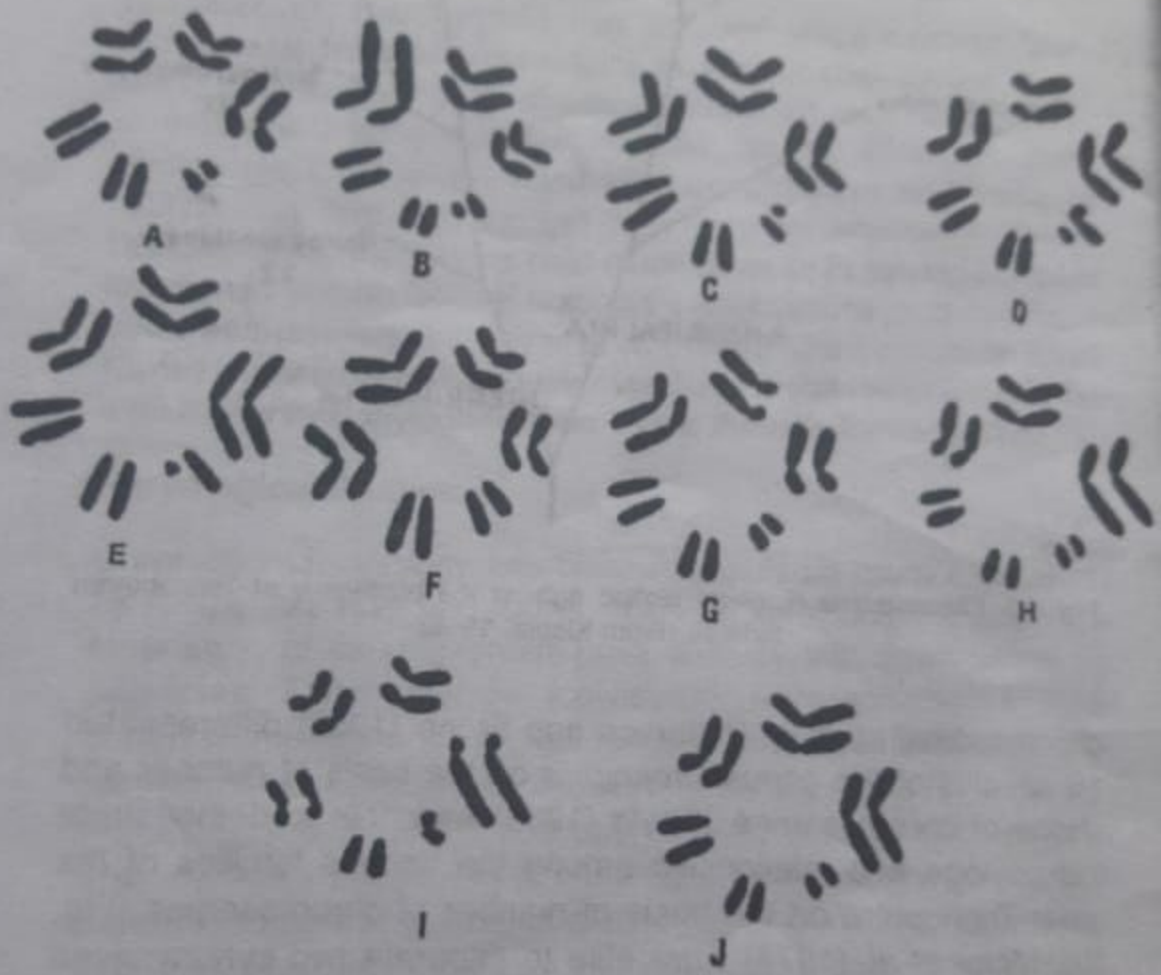

Fig. 32. Differences in the number and shape of chromosomes in some species of fruit flies (Diptera: Tephritidae), courtesy Dr. J.S. Grewal—A. Main *Bactrocera zonata*, B. another population of *zonata*, C. *B. dorsalis*, D. *B. cucurbitae*, E. *B. tau*, F. *B. scutellaris*, G. *Diarrhegma modesta*, H. *Anoplomus flexuosus*, I. *Sphaeniscus atilus*, and J. *Carpomyia vesuviana*.

substantially constant through long evolutionary stages (for example among grasshoppers, there is great uniformity in the Acrididae (2n ♂ = 23 acrocentrics); Pamphagidae (2n ♂ = 19

show considerable rearrangement and many species are polymorphic for the very chromosomal differences that in other cases differentiate closely related species. Conversely well-defined and reproductively isolated species may completely agree structurally in their chromosomes and differ only in their gene contents. Geographical races among many insect species differ in the banding patterns of their polytene salivary gland chromosomes. One should be very careful in using chromosomal characteristics in taxonomy not only for geographical races, individual abnormalities, clines and polymorphic morphs but also for taxa exhibiting seasonal differences. Thus, karyomorphology cannot be taken as the only answer to solve all systematic problems. It can be used in selective cases. However, the value of the karyological data can be better utilised if combined with the highest possible taxonomic elements for the diagnosis of species.

BIOCHEMICAL APPROACH

This, too, has been more extensively studied in plants than in animals. The use of such characters in taxonomy was first initiated by de Candolle in 1813 to differentiate closely related species of plants. Even Lankester (1871) speculated that "The chemical differences of different species and genera of animals and plants are certainly as significant for the history of their origin as the differences of form. If we would clearly grasp the difference of the molecular constitution and activities of different kinds of organisms, we would be able to form a clearer and better grounded judgement on the question how they have been developed, one from the other, than we now can from morphological consideration." It is now a well-known fact that the metabolism of an organism is a complex of chemical change and all morphology, behaviour and ecology of an organism must depend on its metabolism. The animal contains a large number of complex compounds like hormones, enzymes and other proteins with peptides, nucleic acids, amino acids and sugars. The biochemical taxonomic techniques are probably less subject to direct environmental influences and thus are more likely to reflect genetic divergence than many of the classic morphological analyses. The principal work of a biochemical taxonomist concerns the comparison and contrasting of compounds of the same class and performing the same function in different animal species, with regard to their properties as well as to their distribution in different organs of the body. Thus, the species can be differentiated on the basis of the amino acid sequences in the

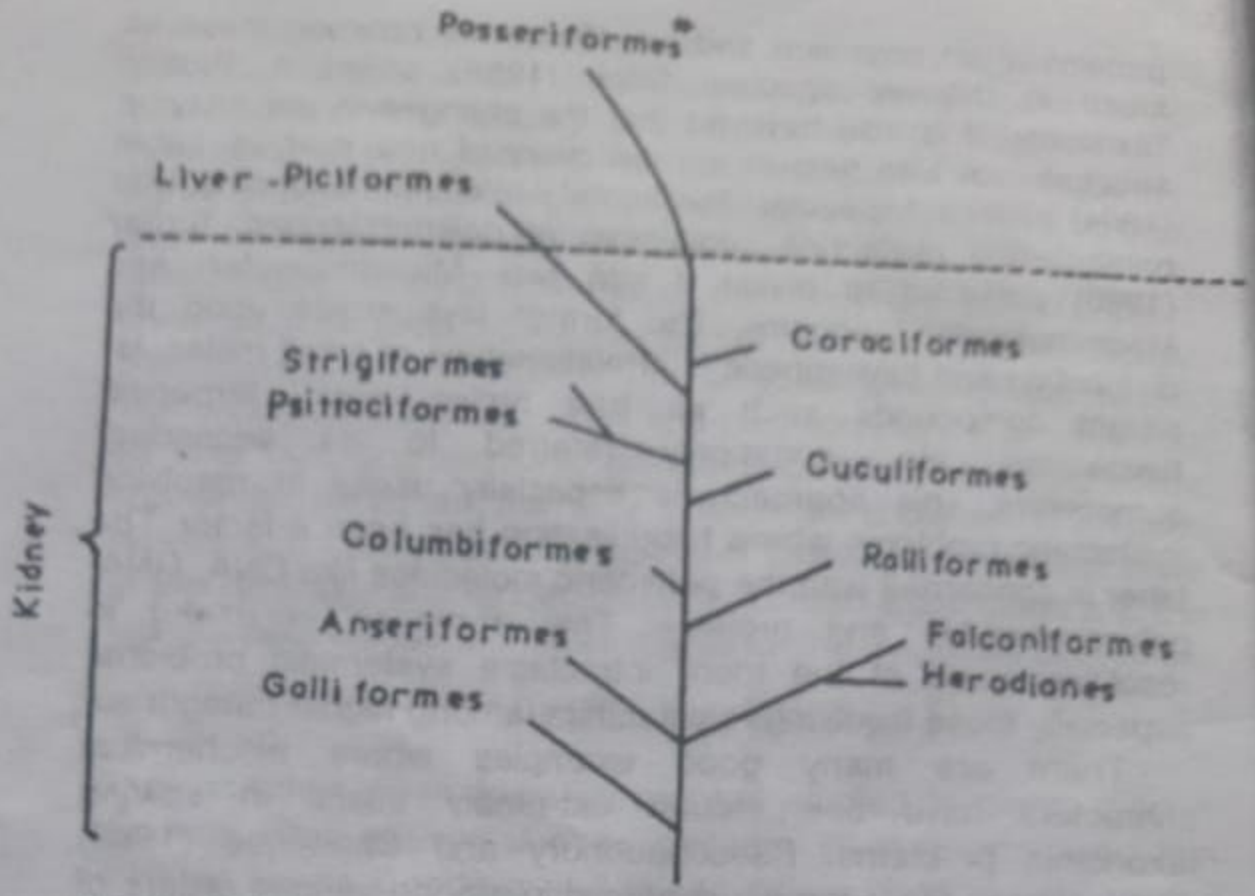

Fig. 34. Probable phylogenetic presentation in birds based on the sites of synthesis of L-ascorbic acid; in few both kidney and liver, in some only liver, and majority incapable.

Like morphological characters, chemical characters are also variable. A proper understanding of the taxonomic relationships of organisms requires comparison of a number of biochemical characters in combination with one another to reveal the diversity on biochemical patterns rather than on a single biochemical character. Since proteins and nucleic acids provide a reliable, though indirect, estimate of the degree of genetic homology among animals (Wilson and Kaplan, 1964), comparison of various characteristics of these chemical constituents as such are more suitable than other constituents for understanding their taxonomic relationships. The distribution of free amino acids in different organs of insects is of greater taxonomic value than their mere presence or absence or concentration in whole animals or in one of their tissues (Seshachar *et al.*, 1966). Brown (1967) did not find the distribution of metabolic amino acid 3-hydroxy-L-Kyneurenine, a wing pigment, as a useful taxonomic character in nymphalid butterflies. However, in mammals the classification of species, based on amino acid sequences of the peptides, agrees in general with the accepted one based on the morphological

proteins of an organism and on differences between these as found in different species. Crick (1958) called it 'Protein Taxonomy.' It is also believed that the changes in the enzyme structure can also help in the discovery of new species. Lahni (1964) calls it 'Molecular Taxonomy.' Molecular taxonomy was primarily the nucleotide sequences of polynucleotides. Turner (1966) preferred to divide it into two—Micromolecular- and Macromolecular-Taxonomy. The former lays stress upon the distribution and biosynthetic interrelationships of small molecular weight compounds such as free amino acids, terpenes, flavonoides, etc., commonly referred to as secondary compounds. This approach is especially useful in resolving systematic problems where hybridisation has been a factor. The latter is concerned with the polymeric molecules like DNA, RNA, polysaccharides and proteins. This approach is useful in resolving some of the more intractable systematic problems especially those involving relationships among higher categories.]

There are many good examples where biochemical characters have been found extremely useful in solving taxonomic problems. Basuchaudhury and Chatterjee (1969) demonstrated phylogenetic relationship among various orders of birds on the basis of the quantitative analysis of ascorbic acid (Fig. 34). In some birds it is produced in the kidney; in some, in the liver; in some, in both liver and kidney; and in others, in neither. Accordingly they clarified that the ancestral enzyme systems involved occurred first in kidney, were later somehow transferred to the liver, and finally, in some of the more evolved passerine birds, completely lost. Similarly, Brand *et al.* (1972) established the phylogeny of a group of fireants using biochemical characters of the highly unique fireant venoms. Walbank and Waterhouse (1970) corrected the phylogenetic affinities (based earlier on morphological data) of certain genera of Australian cockroaches after analysing their defence secretions.

Thus, the present biochemical approach is definitely helpful in solving many taxonomical problems. But this, too, is not useful in many cases. Moreover, such studies are possible only in the existing organisms and therefore it is difficult to trace the course of evolutionary history. It cannot lead to definite judgements with regard to the phylogeny of any organism whose fossil records are inadequate or lacking. Most of the biochemical taxonomic works are based on qualitative and quantitative differences in single chemical constituent of whole organisms or one of their tissues.

data (Blomback and Blomback, 1968).

Kinds of Chemical Approaches

These studies are taken up in five ways—immunological, chromatographic, electrophoresis, infrared spectrophotometry and histochemical. All these are concerned with elucidating the chemical composition of the tissues and the serum of the blood which carries the necessary chemicals to feed the cells both in the development and reproduction.

Immunological

This approach is based on the precipitin reaction preferred for the study of soluble antigens, such as those contained in animal sera or tissue extracts from plants or animals. It was first discovered by Rudolph Krauss (1897) in respect of micro-organisms. Nuttal (1901) was the first to extend its use in animal systematics. Boyden (1943, 1959) further elaborated its use in animal systematics with refined techniques. Its application is based on the fact that "the proteins of one organism will react more strongly with antibodies to the proteins of a closely related organism than to those of one more distantly related." An antigen (usually a protein), when injected into an animal, will stimulate that animal to generate compounds, and the *antibodies* will react with a high degree of specificity to the material that was injected. The animals with the antibodies are considered to be *immunised* and the process of immunisation is detected by the formation of a precipitate, the *precipitin*, when the soluble antigen is mixed with its immune serum in optimal proportions. It is also possible to determine whether an antigen is unique to a certain genus, species within the genus, or even to a particular strain within a species or it shows cross reactions.

Although this practice has been in use for over half a century, it has not yet benefited us as much as was expected. Irwin's work (1947) on the blood group genes for the specific classification of pigeons has been remarkable and has now been extensively applied to the study of primates. Some of the important achievements made possible through these studies in taxonomy have been discussed in the proceedings of the 'Kansas Symposium' (ed. Hawkes, 1968) and in 'Biochemical and Immunological Taxonomy of Animals' (ed. Wright, 1974).

Chromatography

It is a technique by which the constituents of a complex mixture can be separated and subsequently identified. It depends on the "different rates at which the compounds in a double mixture move along a porous medium, i.e., a piece of paper (paper chromatography) or a column of powdered chalk (column chromatography)." Paper chromatography has been widely used for comparing the chemical composition of closely related species, especially with regard to amino acids and peptides through ninhydrin treatment (spray), and purines, pyrimidines, or other compounds which either fluoresce or absorb ultraviolet light. The material to be analysed is prepared through two general approaches. Either pieces of tissues or small whole animals are squashed directly on to the filter paper or extracts are prepared from which soluble proteins precipitate so that the resultant solution contains only amino acids and small peptides. A minimum of 21 amino acids have been detected in homogenised adult mosquitoes. But one should be very cautious in using total homogenates, often hydrolysed as these show differences due to age, sex and physiological state.

Buzzati-Traverso and Rechnitzer (1953) were the first to apply this practice to animal systematics. They studied the amino acids of the muscle protein in different species of fish and found these characters extremely useful in segregating them. Kirk *et al.* (1954) distinguished seven species of land snails by their fluorescent patterns. Florkin and Jeuniaux (1964) discovered that the primitive hemimetabolous insects have low concentration of free amino acids in their haemolymph as compared to high concentration in holometabolous insects. Duchateau and Florkin (1958), Wyatt (1961), Chen (1962, 1966), Ball and Clark (1953), Micks (1954, 1956), Throckmorton (1962), Saxena *et al.* (1965), Micks *et al.* (1966), Seshachar *et al.* (1967), Harlow *et al.* (1969), and Stephen (1974) are other workers who have shown the importance of these studies in animal systematics.

Electrophoresis

This is another technique involving a similar movement of dissolved substances through a fixed medium, but here the movement is brought about by electrical potential differences. It is based on the fact that the "components of mixtures carry electric charges of varying amounts and so will move at different rates in salt solution through which a current is passed."

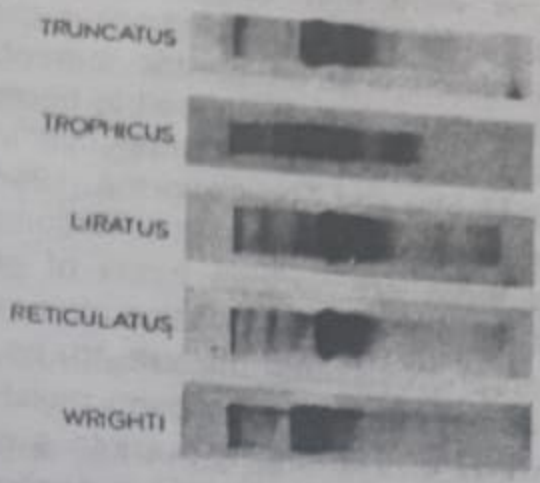

Fig. 35. Electrophoretic analysis of egg proteins of some species of the molluscan genus *Bulinus* (Modified from Wright, 1974).

complex molecule, for example haemoglobin, of one species is selected and its amino acid composition is compared with that of closely related or more distantly related species (Sande and Karcher, 1960). Manwell and Baker (1963) reviewed much of the systematic literature in this field and themselves discovered a sibling species of sea cucumber using these techniques. Manwell *et al.* (1967) also reviewed the utility of haemoglobin bands for the deduction of hybrids. Crenshaw (1965) also claims to have detected introgression in turtles through this approach. Other important works in this field are those of Handler (1964) and Bryson and Vogel (1965).

Infrared Spectrophotometry

It is based on the principle of absorption of infrared light by biological materials. The patterns thus formed depend upon their chemical composition and bring to light many features of taxonomic importance. So far this approach is mainly applied to micro-organisms (Randall *et al.*, 1951; Stevenson and Bolduan, 1952; Norris, 1959). Micks and Benedict (1953) for the first time applied this technique in the identification of mosquitoes. It is hoped that this technique, if applied extensively to other animal groups, can yield useful taxonomic information.

Histochemical Studies

When the same kind of tissues from different animal species may exhibit apparently the same functions, histochemical differences between them may be observed which could be of

Such techniques were first used by Tiselius (1937) to distinguish multiple fractions of serum proteins migrating through solution under the influence of an electric current. Since then these techniques have been greatly refined to permit even large numbers of different proteins in the same cyanins (Smithies, 1955; Hubby, 1963; Hubby and Throckmorton, 1964; Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Williams and Chase, 1967, 1968). Now there are various types of electrophoretic methods to study the molecular composition of complex proteins.

In paper electrophoresis the mixture to be analysed is poured on a strip of paper after it has been moistened with the salt solution. Each end of the strip is put into a container filled with solution. An electrode is submerged in each container and direct current is passed through the solution. The different components migrate at different rates according to their electric charges. When they are separated, their identification is made by various means. The paper, due to its high molecular absorptive qualities, variable pore size, and high electro-endosomatic buffer flow, was first replaced by agar gel, then starch and more recently by acrylamide gel. Each of these provided an increase in macromolecular differentiation. The media starch and acrylamide introduced a second dimension to protein separation. The use of polyacrylamide gels for the separation of proteins as a substitute for starch gel was first reported by Davis and Ornstein (1959) and Raymond and Weintraub (1959). Since then the technique has been extended and modified in various ways.

The electrophoretic investigations of insulin from oxen, horses, and sheep have shown that they are different; the ACTH of pigs is different from that of oxen; the vasopressin of oxen contains arginine but that of the pigs lysine. Such biochemical studies are of great help in solving the phylogenetic problems which otherwise do not receive enough support from taxonomy. Sibley (1960) analysed the egg-white protein of 359 species of non-passerine birds by paper electrophoresis. He was not only able to corroborate the standard classification of Mayr and Amadon (1951) and Wetmore (1960), but also to raise doubts concerning previous agreements and make suggestions for the relationship of taxa previously considered highly isolated. Wright (1974) made a breakthrough in molluscan taxonomy when he separated some species of the genus *Bulinus* through electrophoretic analysis of their egg protein (Fig. 35).

In another biochemical approach to taxonomy a single

taxonomic value. This can also help in the recognition of infraspecific groupings. The histochemical approaches involve distinctive microtechniques and specific staining reactions. The mode of fixation of material of such studies is of great importance as there should be no chemical alteration from what exists in life. These techniques have been employed in the qualitative and semi-quantitative analysis of proteins, free amino acids, enzymes, carbohydrates, lipids and nucleic acids including metal ions. Various dyes for staining are used for better perception of these characteristics. The use of cryostat microtomes, etc., and above all electron microscopes have made such studies more meaningful. This approach, when combined with other characteristics, can also be of great help in inferring taxonomic relationships amongst various animal groups.