

Studies in Biology no. 143

Animal Taxonomy

H. E. Goto



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General Preface to the Series

Because it is no longer possible for one text book to cover the whole field of biology while remaining sufficiently up to date, the Institute of Biology proposed this series so that teachers and students can learn about significant developments. The enthusiastic acceptance of 'Studies in Biology' shows that the books are providing authoritative views of biological topics.

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Preface

In recent years there has been a radical change in the study of taxonomy. It no longer deserves the reputation it still has in certain quarters, even among some professional biologists, of being a rather dry and static subject dealing with specimens preserved in museums. It is now an exciting field of research in which rapid advances are being made both in practice and theory. It employs techniques developed in the chemical, physical and mathematical sciences and takes regard of theoretical studies in evolution and classification. It is, in addition, of very great importance to biologists working in other fields for it is now recognized that precise identification may be vital in their work.

The subject is now so extensive that the coverage here has to be very selective. Some of the topics omitted are dealt with excellently in the works listed under Further Reading.

My very great thanks are due to Valerie Forge without whose assistance, typing and proof-reading this book would never have been completed. I should like to thank Professor R. G. Davies for his useful comments on Chapter 8; Dr C. Loumont (née Vigny) of Geneva for the loan of her original photographs of the sound spectrograms shown in Fig. 5-1, and to Drs C. A. Wright and D. R. Ragge of the British Museum (Natural History) for the special preparation of Figs. 3-5 and 5-2 respectively.

London, 1982

H.E.G.

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1 Introduction

Modern taxonomy is a very broad subject encroaching on the theory and practice of many scientific disciplines. The rise of experimentation at the turn of the century gave rise to the temporary eclipse of studies in systematic biology and classification. However, during this period conflicting data arose from experiments on closely-related species in, for example, physiological, embryological and biochemical investigations. As a result the need for accurate identification became obvious. There was also a growing need to record, store, retrieve and pass on accurate information about the increasingly large number of species. This was undoubtedly one of the causes that led to a revival of interest in taxonomic studies.

The *New Systematics*, as it came to be known after the publication in 1940 of J. S. Huxley's book of that name, is quite different from the discipline as previously understood. It is now based to a large extent on the experimental sciences that once caused its eclipse. The new philosophy derived from the *Synthetic Theory* of evolution and the modern biological concept of the species also had a very significant effect on the subject. In the last 10–15 years enormous developments, both procedural and conceptual, have been made, especially in the areas of chemo-, immuno-, and numerical taxonomy.

Some indication of the size of the problem confronting a taxonomist may be seen from the following data. More than one million species of animals have been named; over 13 000 new species and nearly 1000 new subspecies are described in most years. In the same period about 2000 new genera and subgenera are erected, and some 24 000 nomenclatorial changes made at levels above the species. Of the new species over half are usually insects.

Ernst Mayr has estimated that about 99% of all birds are now known and that about 90% of mammals and reptiles have already been discovered. In many groups, however, the number is very much smaller: less than 10% of marine invertebrates, for example, have probably been described. Most of the 13 000 new species that are described each year belong to relatively unknown categories like marine invertebrates, but occasionally even new mammals are found. For instance, in 1973 a new marsupial mouse belonging to the family Dasyuridae was discovered in South Australia. Perhaps some 20 to 30 years may elapse before another new mammal will be found.

In general the number of different kinds of animals tends to increase, but in very well studied groups such as the birds there is actually a decrease. This is because named species when originally described may not represent true biological species. Instead they may simply be variants of one sort or another within a single species. It has also frequently occurred that different names have been given to the same species of animal. As these are discovered so the number of true species decreases. C. A. Wright (1971) has described how in 1758 one

2 INTRODUCTION

species of the African snail *Bulinus* was known, this was *B. senegalensis*. There remained only a single species in this genus until about 1830 when the number began to increase. By 1910 there were approximately 100 known species. Since then there has been an intensive study of the genus because some of its members were found to be important vectors of the parasitic worm *Schistosoma* that causes a great deal of human suffering in many tropical countries. As a result we now know that there are approximately only 30 true species.

Correct identification is vital in many biological fields, especially in ecology and various areas of applied zoology such as parasitology, medical and agricultural entomology, and in conservation. Two examples will suffice to demonstrate the importance of correct identification.

There is a complex of species of moths in the genus *Scirpophaga* whose larvae cause severe economic loss in some tropical and subtropical crops, particularly rice and sugar-cane, by boring into their stems. There are very few external characters that can be used to separate the species of *Scirpophaga* reliably. These moths are mostly white with few or no markings on the body or wings. When markings do occur they are very often unreliable for identification purposes. This situation has led to frequent misidentification and a great deal of confusion. Inappropriate control measures have resulted from incorrect identification. Studies by A. Lewvanich (1972) have revealed new characters particularly in the genitalia. These now enable identifications to be made with a very high degree of certainty. For example, it is now possible to separate *S. nivella*, a very important pest of rice from *S. excerptalis* that damages sugar-cane. The identification and biology, and therefore control measures, of these two species had hitherto been confused.

The second example is now a classic one. It was realized early this century that the distribution of malaria did not always coincide with that of the mosquito *Anopheles maculipennis*, its supposed vector in Europe. Even when the parasite (*Plasmodium*) was introduced into non-malarial areas where *A. maculipennis* was present, the disease was not transmitted. Such a situation occurred after the First World War when no cure was known for the disease and when troops returned home carrying the malaria parasite. This remained a mystery for some years until it was noticed in parts of Holland where malaria was endemic that the breeding sites of *A. maculipennis* differed in the malarial and non-malarial areas.

Table 1 Some differences between the species and subspecies of the *Anopheles maculipennis* complex.

Species/Subspecies	Oviposition Site	Hibernation	Carrier
<i>A. maculipennis</i> s. str.	cool running freshwater	yes	no
<i>A. labranchiae</i>	warm brackish water	no	serious
<i>A. l. atroparvus</i>	cool brackish water	no	slight
<i>A. messeae</i>	cool standing freshwater	yes	rarely
<i>A. melanoon</i> (= <i>subalpinus</i>)	standing freshwater (especially rice fields)	no	no
<i>A. sacharovi</i> (= <i>elutus</i>)	shallow standing freshwater or brackish water	no	serious

Where malaria occurred, the mosquito was found to breed in warm brackish water; where it was absent, *A. maculipennis* bred in cool, fresh water. The vector was believed to be a particular variety of the species and was named *labranchiae*. The non-carrier was called *messeae*.

An intensive study of the ecology of *A. maculipennis* (in its original broad sense) led to the discovery of five sibling (very closely-related and similar) species, and of one subspecies. The true *A. maculipennis* was found in fact to be a non-carrier. Each of these species, and the subspecies, were found to differ in their ecology, biology and in the degree to which they acted as a carrier of the malaria parasite (Table 1).

A. labranchiae is more southerly in its distribution than its subspecies, *atroparvus*, but where they overlap in central Italy they hybridize. In the F_1 generation the females are fertile but the males are usually sterile. It is for this reason that *atroparvus* is given subspecific rather than specific status.

In addition to the differences indicated in Table 1, we now know that each of these species and the subspecies differ in geographical distribution, behaviour, reproductive compatibility and fertility of hybrids. Marked differences are also found in the colour patterns on the egg and the fine structure of the egg floats. The patterns have been shown by scanning electron microscopy to result from different degrees of development of surface microtuberculation obscuring more or less of the darkly coloured inner layer of the egg shell.

Chromosome studies have revealed interspecific differences, mainly translocations and paracentric inversions (p. 48). These are especially noticeable in the banding patterns of the giant (polytene) chromosomes.

Similar species complexes are now well known in other groups of mosquitoes as well as in some quite unrelated organisms.

1.1 Definition of terms

It is necessary at the outset to define the basic terms used in taxonomic studies, i.e. *systematics*, *taxonomy*, *classification* and *nomenclature*. In the past, and still to a considerable extent today, the first two of these terms have been used rather loosely and synonymously to imply, in a broad sense, the science of classification. There is now a strong tendency to restrict the scope of these terms and to define them more precisely.

In 1952, R. F. Blackwelder and A. A. Boyden contributed a paper entitled 'The Nature of Systematics' to the first number of the journal *Systematic Zoology* in which they defined systematics as 'the entire field dealing with the kinds of animals, their distinction, classification and evolution'. SIMPSON, in his *Principles of Animal Taxonomy* (1961) preferred to extend the scope of the definition to include not only 'distinction and evolution' but 'any and all relationships' among living organisms. This broad and all-embracing attitude is the hallmark of modern systematics. He defined taxonomy as 'the theoretical study of classification, including its bases, principles, procedures and rules'. Classification he defined as 'the ordering of animals into groups or sets on the basis of their relationships, that is of association by contiguity, similarity or both'. Two types of classification are implied here: contiguity implies genetic or

phyletic (phylogenetic) relationship. Similarity, on the other hand, implies only what is called a phenetic relationship.

Construction of classifications based on contiguity is attempted by those who try to attain a 'natural' or 'biological' system reflecting genetic affinity and, as far as possible, phylogeny. A truly phylogenetic classification is never really possible without extensive fossil evidence.

Classifications based on similarity alone probably approach very near to a phylogenetic one if a sufficiently large number of characters is taken into consideration. This is the method employed in numerical taxonomy.

Classification results in the construction of a *hierarchy* such as that shown in Table 2. The ranks or categories shown on the left indicate levels in the hierarchy. Organisms or groups of organisms at a particular level (i.e. in a particular category) are known as *taxa*. Some intermediate categories, such as superfamily and subfamily, and their corresponding taxa exist but these are not all shown in the figure.

Table 2 Classification of the St. Kilda Wren, *Troglodytes troglodytes hirtensis*, as an example of a taxonomic hierarchy.

Category	Taxon	Vernacular name
Kingdom	Animalia	animals
Subkingdom	Metazoa	multicellular animals
Phylum	Chordata	chordates
Subphylum	Vertebrata	backboned animals
Class	Aves	birds
Subclass	Neornithes	—
Superorder	Neognathae	—
Order	Passeriformes	perching birds
Family	Troglodytidae	wrens
Genus	<i>Troglodytes</i>	wrens
Species	<i>troglodytes</i>	common wren
Subspecies	<i>hirtensis</i>	St. Kilda wren

The terms category and taxon are frequently confused. Mayr defines a taxon as 'a taxonomic group of any rank sufficiently distinct to be worthy of being assigned to a definite category', i.e. the 'groups' or 'sets' of Simpson mentioned above. The limits defining taxa are entirely subjective in nature, except perhaps in the case of those in the categories of biological species and subspecies. The concept of a biological species is one of populations that are interbreeding or potentially interbreeding in the wild, and normally reproductively isolated from other similar groups by one or more of a number of types of barrier, see MAYR (1970).

The term taxon is employed in a somewhat different way by numerical taxonomists who use it to refer to a group defined by similarity only, i.e. the species is a *morphospecies* rather than a biological species.

1.2 Taxonomic procedure

The tasks of a practising taxonomist are usually divided into three phases:

1. *The analytical or alpha phase* This includes the basic procedures of the systematic zoologist. These are: (a) the arrangement of animals into easily recognizable groups that are phenotypically more-or-less homogeneous. Such groups are known as *phena*. When first recognized at the specific level they may not even be morphospecies. They may conceal a number of species within a complex. Frequently different forms of a polymorphic species, races (or subspecies) of a polytypic species, males and females in a sexually dimorphic species, and even sometimes juveniles and adults of the same species fall into different *phena*. The taxonomist must unite such *phena* correctly into a single taxon. The term *phenon* is used differently by numerical taxonomists (see p. 55). (b) The description of new taxa. At this stage these are usually limited to a few criteria just sufficient to characterize the taxon and to distinguish it from other similar taxa. These primary descriptions must be adequate to enable subsequent identification. (c) The provision of suitable names in accordance with the rules of the *International Code of Zoological Nomenclature*. At this stage species are known as nominal species. The provision of universally accepted names is essential for accurate future reference.

2. *The synthetic or beta phase* This stage, also referred to as macrotaxonomy, comprises mainly the ordering of species into categories in the hierarchy, i.e. the provision of a classification. At first this is constructed from the maximum number of available characters, i.e. it is a purely phenetic classification.

3. *The biological or gamma phase* This phase is concerned mainly with the analysis of infraspecific variation leading to the study of the origin, evolution and determination of the biological species and its subspecies.

In the majority of groups of animals taxonomic studies have not yet progressed beyond the alpha and beta stages. It is only in some vertebrates, especially the birds, and a few insect orders such as Lepidoptera (butterflies and moths) that any real progress has been made in gamma taxonomy.

No consideration is given in this book to the basic procedures of taxonomic discrimination, description, key construction and nomenclature. These are excellently covered in MAYR (1969), PANKHURST (1978), JEFFREY (1973) and the *International Code of Zoological Nomenclature*.

Similarly, although the philosophy underlying the construction of classifications is currently a major one for discussion among taxonomists, no detailed consideration will be given here. The main point of dispute in this controversy is whether 'recency of common descent' should be the only criterion in classification. Acceptance of this *cladistic* view, as pointed out by Mayr would relate the crocodiles more closely to the birds than to other reptiles even though they are closer to the latter 'as far as the total gene composition is concerned'. No account is taken of the degree of divergence and associated radical genetic change. The other main theory is that of *Evolutionary Classification* championed by Mayr. According to this view one should consider not only cladistic relationships, but also phenetic and chronistic ones. The last two relate to degree and rate of divergence. For fuller discussions, the reader is referred to HENNIG (1966), MAYR (1969) and SNEATH and SOKAL (1973).

2 Taxonomic Characters

The basic procedure in any taxonomic study from the analytical through the synthetic to the biological phase always involves the consideration of a number of taxonomic characters. MAYR (1969) defined a taxonomic character as 'any attribute of a member of a taxon by which it differs from a member of a different taxon'. Interpreted in this way characters may be of any morphological kind, external, internal, macroscopic, microscopic or ultrastructural; they may be chemical, physiological, behavioural, developmental, ecological, geographical or any other kind of attribute of the organisms.

2.1 Selection of characters

In selecting taxonomic characters, certain precautions must always be borne in mind. They must not be too variable within a particular taxon whether this be due to phenotypic or genotypic cause. Size, colour and patterning are examples of attributes often falling into this category. For instance variation in size may be related to nutrition; colour to food difference, insolation or humidity and patterning may vary with age. This is not to say that these characters are always unsatisfactory. In the primitive insect genus *Entomobrya* (order Collembola), for example, colour patterning, although often very variable between individuals and at different stages in the life history, is sometimes the only character by which some species of the genus can at present be separated. Such *unreliable characters* are usually only revealed after a considerable knowledge of a particular taxon has been amassed. Inevitably, therefore, in the alpha phase of taxonomy many characters of this nature are employed and have later to be abandoned. Frequently a character may be reliable or 'good' in one group, but unreliable in another to which it may even be closely related. In other genera related to *Entomobrya*, for example, colour and patterning are relatively constant within a species and therefore constitute valuable taxonomic characters.

The different conditions exhibited by a single character such as brown or grey pelage in a mammal are known as the *character states*. Characters with states showing reduction of one sort or another as a result of evolutionary change are also considered to be rather poor. Reduction or total loss of eyes or wings are examples of such unreliable *regressive characters*. Both of these examples are known to have occurred a number of times independently in many groups. To link taxa because they have, for example, a reduced number of ommatidia in a compound eye might well lead to the creation of a polyphyletic group. Differences in the relative value of characters from 'good' to 'unreliable', and in all their intermediates, have given rise to the concept of *character weighting*. A 'good' character, according to some taxonomists, is one to which extra weight should be given in considerations concerning the construction of classifications.

especially if these have any pretensions of reflecting phylogenetic relationships. Characters to which they would assign high weight are those with so-called high *information content*. Characters whose states are restricted to particular taxa and are of little intra-taxon variability come into this category. Further comments on weighting will be made under the heading of numerical taxonomy (pp. 53, 57). In addition to unreliable characters, there are also some that are totally unacceptable or *inadmissible*. An example of this is the use of characters that are known to be completely correlated and therefore should be regarded as one. Examples are seen in an insect's ability to fly and the presence of fully functional wings; albinism and the lack of normally present pigmentation; and the red colour of blood in vertebrates and the presence of haemoglobin. During the synthetic phase of taxonomy when species are arranged in hierarchical categories to form a classification rarely, if ever, are single characters employed even though they may exist in a number of possible states. This is also true, but to a lesser extent, in the construction of keys for identification. Procedures employing single characters are said to be *monothetic* and those using a number of different characters at the same time are known as *polythetic*. It is the latter that is an essential aspect of numerical taxonomy but it is by no means confined to it.

2.2 Types of taxonomic character

Taxonomic characters are of two main types: qualitative and quantitative. The former are sometimes difficult to express precisely, especially when employed in numerical taxonomy. However, this is not always the case for such characters are often discontinuous as in the A, B, O blood groups. Discontinuous qualitative characters are generally the result of unifactorial inheritance, that is to say they are controlled by a single or a few genes, the environment having little or no effect on their expression. Quantitative characters, on the other hand, are usually more easily expressed numerically but may provide considerable difficulty when their occurrence is more or less continuous such as in the stature of man. Continuous variation of this sort is usually associated with determination by a number of genes (polygenic or multifactorial inheritance). These characters are also frequently subject to considerable phenotypic (i.e. environment related) variation. The use of continuously variable quantitative characters involves the sub-division of the continuum into definable sub-units. In the case of size, for example, one may have a character state from 15–25, from 25–35 and from 35–45 mm. Divisions of this sort are never entirely satisfactory. Quantitative characters of another type, known as *meristic*, are much more useful as they depend on countable features which may differ precisely from one taxon to another as, for example, the number of setae on a well-definable area or part of an arthropod's body, or the number of spots on the wing cases of a ladybird. Even meristic characters vary within a single taxon and must be used with caution – the number of vertebrae and the number of fin-rays in some fish vary with the temperature of the water so that specimens taken along a north-south axis of distribution may provide a more or less continuous variation or *cline* in the numbers of these structures. Specimens of the Pacific herring (*Clupea pallasii*) possess high vertebral and fin-ray numbers off the coast of Alaska, both

gradually decline as their distribution approaches southern Californian waters.

The sources of taxonomic characters used in modern studies are too numerous to be covered satisfactorily in this book and a selection only of some of the more important ones is provided in the following chapters. Before proceeding with them, however, brief mention will be made of two areas of investigation that are still in a comparatively early stage of development.

2.3 Electron microscopy in taxonomy

In recent years, since the innovation of the electron microscope, ultrastructure and fine structure have provided useful data. Scanning electron microscopy (SEM) is used by taxonomists to a much greater extent than transmission electron microscopy (TEM). The great advantage of SEM lies not only in the greater magnification and resolution that it provides, but also because it gives a depth of focus about three hundred times as great as that of light microscopy. Useful magnifications of up to about $\times 80\,000$ are possible and with biological

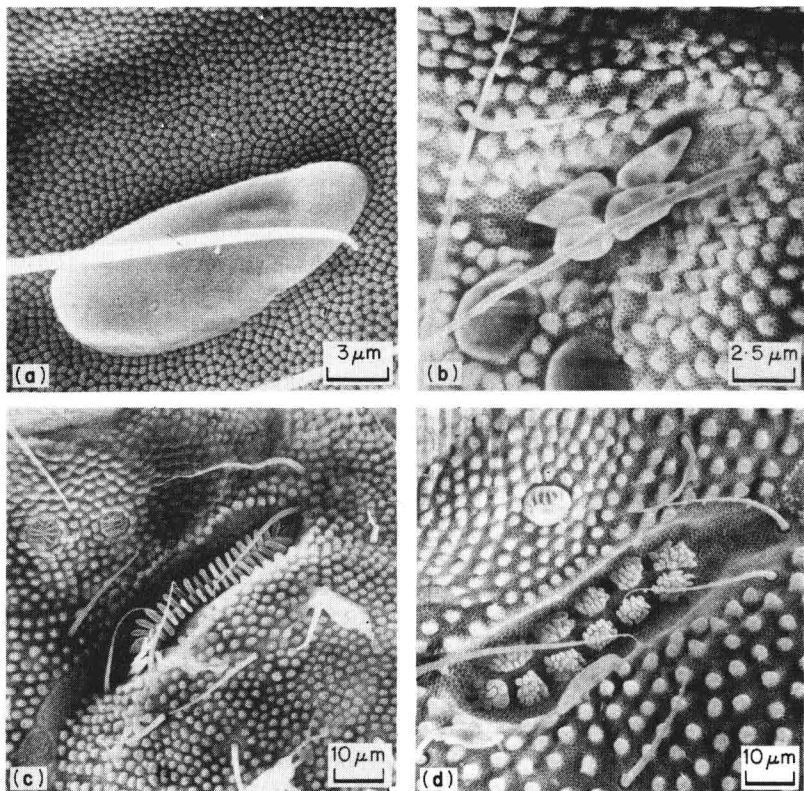


Fig. 2-1 Scanning electron micrographs postantennal organs of some Collembola: (a) *Folsomia candida*; (b) *Hypogastrura denticulata* (c) *Onychiurus flavidulus* and (d) *Onychiurus sinensis*.

material a resolution of about 12 nm can be obtained. Unlike TEM, scanning electron microscopy provides an almost three dimensional picture of the surface of a specimen. This is achieved by appropriate treatment of secondary electrons emitted from the material (usually gold) coating the specimen when bombarded by the beam of electrons scanning the surface of the structures under examination. The specimen can be moved and, to some extent, manipulated, whilst under observation on a television screen. Electron micrographs, such as those shown in Fig. 2-1 can be produced by photographing the image on the screen. Specific differences in fine structure can often be found in this way.

TEM studies are based on very thin sections cut with an ultramicrotome, 'stained' with electron opaque materials and viewed with a transmitted beam of electrons. Much greater magnifications can be achieved in this way than with either light microscopy or SEM.

TEM, although used to a lesser extent, also has its place in modern taxonomic studies. There is a genus of Protozoa, related to *Amoeba*, known as *Thecamoeba* whose species are not uncommon in damp leaf litter. They differ from *Amoeba* in having a shape more or less fixed by a pellicle and being without pseudopodia. They have been divided on the basis of their external appearance into two groups, those with smooth and those with rugose surfaces. The two groups have, by some authors, been placed not only in different genera but even in distinct suborders. Other studies based on features of the nuclear division have suggested another classification that does not coincide with that established on the smoothness or rugosity of the surface. F. C. Page and S. M. Blakey (1979) have examined sections of the surface with TEM. Their findings do not support the division of *Thecamoeba* according to either of these systems. They have found, however, that one of the species they studied, *T. granifera*, differed so markedly in surface structure and chemistry that they proposed it should be placed in a new genus *Dermamoeba*.

It is in groups such as the Protozoa where surface features appear to be few that TEM studies may be used to great value. Nevertheless they can play a very useful role in some other groups. Gremigni (1979) has studied the fine structure of cytoplasmic inclusions of the ovarian oocytes of planarian Turbellaria (free-living flatworms). His results support the classification and phylogenetic views put forward, on the basis of a number of criteria, by Ball (1974; 1977) and not those of other workers.

2.4 Recognition characters

The best types of specific characters are those used by the animals themselves to distinguish one another, especially in mate selection – the biological species concept being one of a naturally interbreeding population. The assumption is nearly always made that animals perceive each other with the same sensory limitations that we ourselves possess. It has been known for many years that not all animals are sensitive to the same wavelengths of light or hear the same frequencies of sound. It is only comparatively recently, however, that this knowledge has been put to use in taxonomy. Most vertebrates, especially those which are diurnal, are blind to ultraviolet light because it is absorbed in the lens

of the eye. This is not true however of all animals. Among the arthropods both the simple ocelli and compound eyes are, at least in some cases, sensitive to UV radiation. The wing patterns of butterflies so familiar to us, may be seen quite differently by the insects themselves. It is now known that UV reflection patterns are present on the wings and that these sometimes bear little or no relation to those seen by humans. Such patterns have been used as taxonomic characters but at present only in a very limited number of taxa. Y. P. Nekrutenko (1964), for example, has employed UV reflection patterns in studies on the taxonomy of palaearctic species of the pierid butterfly genus *Gonepteryx* (brimstone butterflies and their relatives) and more recently R. E. Silberglied (1972) has carried out similar studies on *Colias* (clouded yellow butterflies) in the same family. Polymorphism in these patterns has been observed, so has geographical variation and sexual dimorphism. We have a good example of the need for making taxonomic observations on as many individuals as possible within a given taxon. There is no doubt that, at least in some cases, the UV reflection patterns are used by the butterflies themselves in intraspecific recognition and can thus act as a prezygotic barrier to interspecific mating (see MAYR, 1970). This character therefore fulfils the requirement of the best type of specific character mentioned above.

In a similar way, ultrasonic sounds emitted in one way or another by a number of animals are now known to be used in both intra- and interspecific signalling. Taxonomic use of these sounds has been made to a very limited extent but may in the future give us characters of value equal to those provided by the 'hidden' UV reflecting patterns. In future studies UV patterns and ultrasonic sounds may be found to be of importance in the discovery of sibling species as well as in the separation of 'difficult' ones and also in the delimitation of higher taxa and their classification.

3 Chemotaxonomy

For many years it has been known that there are demonstrable differences in the biochemical composition of different organisms. The earliest to be noted were naturally those between higher taxa and not between species, genera or even families.

In 1871, E. R. Lankester wrote '*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 could clearly grasp the difference of the molecular constitution and activities of different kinds of organisms, we should 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 considerations.*'

Since Lankester's time comparative biochemistry has progressed a long way and his prediction has proved to be well founded.

In 1958, F. H. C. Crick wrote that it could be argued that the amino acid sequences in the protein molecule '*are the most delicate expression possible of the phenotype of the organisms and that vast amounts of evolutionary information may be hidden away within them*'.

It is not surprising therefore that little chemotaxonomic work with animals has been carried out with substances other than protein, peptides and amino acids. There are, however, some exceptions. For example, there have been the studies on the distribution of different phosphagens, the indoles and imidazoles in primate blood and urine and bile salts.

Proteins are all very similar in chemical properties and it is difficult to isolate them by classical methods without denaturing them. For this reason the techniques most commonly used, in what is sometimes termed *protein taxonomy*, are those of chromatography, electrophoresis, immunology and various combinations of these. Included in such studies are conjugated proteins and enzymes. It is however the protein fraction of a conjugated molecule that nearly always provides the significant data (e.g. in haemoglobin studies).

Differences of two main kinds can be found in studies of protein taxonomy.

1. *Relative quantitative differences* between amounts of different proteins and, more important, of different constituent amino acids of the macromolecule. Such data are liable to vary according to the physiological state of the individual especially in relation to free amino acids in the cytoplasm and body fluids. Consequently the material must always be standardized as far as possible.

2. *Qualitative differences of three types exist*

- (a) Different constituent amino acids – this is not so useful because most amino acids occurring in animal proteins can be present even in distantly-related or unrelated organisms, and very closely-related organisms may differ qualitatively

in one or more amino acids if one studies, as one often does, not a complete protein molecule but a particular peptide. For example glycine is present at three positions in fibrinopeptide B in ox blood but is absent from that of some other artiodactyls, e.g. the dromedary camel where it is replaced by glutamine, arginine or aspartic acid.

(b) Of much greater significance is the relative order in which these amino acids are arranged along the length of the macromolecule. Studies on the structure of fibrinogen from different species have provided valuable information on mammal classification.

B. Blombäck and M. Blombäck (1968) studied the amino acid sequences in the fibrinopeptides split off from the parent fibrinogen molecule by the action of thrombin in 34 different species, mainly mammalian.

The fibrinogen molecule consists of three polypeptide chains (α , β and γ) linked by disulphide bridges (Fig. 3-1). The proteolytic enzyme thrombin splits off two fibrinopeptides, one from the α and one from the β chain. It is in these two, usually known as fibrinopeptide A and B, that the sequences have been worked out.

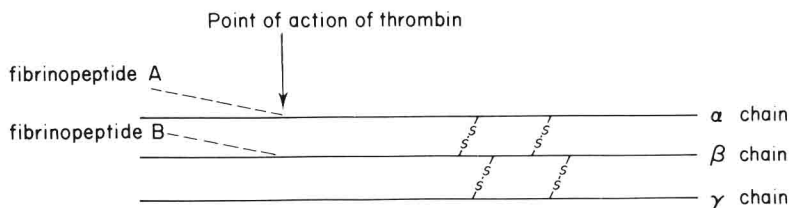


Fig. 3-1 Diagrammatic representation of a fibrinogen molecule showing formation of A and B fibrinopeptides.

Fibrinopeptides seem to be especially suitable for taxonomic studies as they constitute genetically equivalent parts of the fibrinogen from different species.

Many protein sequences have now been worked out (R. V. Eck and M. O. Dayhoff, 1966- ; L. R. Croft, 1973-).

The classification of species according to their amino acid sequences in the peptides agrees in general with accepted schemes based on morphology; and phylogenies, such as that illustrated in Fig. 3-2, can be constructed from the data so obtained.

Cytochrome C is present in cells of all living organisms. It contains some 110 amino acids. The sequence of these is now known for many organisms – our knowledge of these already fits in fairly well with current classifications.

(c) Variation in *shape* of the protein molecule. The chains of the macromolecule are held by weak electrical forces in certain definite shapes. These electrical forces are derived from hydrogen bonding. The shape of the macromolecule is important wherever it has to be matched or complemented to another molecule as in antibody formation and, for example, in the association between the prosthetic group (in this case haem) of the haemoglobin molecule with the protein fraction. Maintenance of the shape of the haemoglobin molecule is