

Classification

What are the purposes of classifications? Why do we bother to arrange bacteria or higher organisms into groups? There are, in fact, at least three important answers to this question. The first concerns the transfer of information. Quite simply, a classification is a means of summarizing and cataloguing information about an organism. It follows that a classification is a form of database or information retrieval system containing a large amount of information about an organism which is collected and summarized by its place in the scheme. Its position in the system is denoted by the use of a name. For example, the generic name *Bacillus* indicates a group of Gram-positive bacteria which differentiate into endospores under aerobic conditions. More specifically, *Bacillus subtilis* refers to a group of strains with the general characteristics of the genus, which in addition secrete several extracellular enzymes such as amylases and proteases, use nitrate as a terminal electron acceptor under anaerobic conditions and are naturally competent for DNA-mediated transformation. This ability to predict properties of groups is a very important aspect of classifications, particularly in these days of computerized databases in which large amounts of information can be stored and easily accessed. Indeed, the more information in the classification the more useful it is.

A second purpose for classification is that organisms must be categorized into groups before identification systems can be devised for the recognition of new isolates. It is evident that without prior arrangements of individuals into groups it would be impossible to assign new isolates to a taxon, and without such order, we would not be able to conduct science.

The third purpose of classification may be considered to provide an insight into the origins and evolutionary pathways of bacteria and higher organisms. Indeed for some people, evolution is of over-riding importance and the evolutionary pathways and the classification are considered one and the same; but this need not necessarily be the case.

If classifications are to serve these purposes effectively, they should fulfil several criteria. Firstly, a classification should have high information content: essentially the greater the amount of information on which it is based, then the greater will be the 'predictivity' of the

classification and the more generalizations that may be made about the taxa involved. Secondly, classifications should be stable. This may seem obvious, but a classification, in which the composition and descriptions of taxa change frequently is confusing and unhelpful. Finally, it is important that the development of a classification should be empirical, reproducible and scientifically based.

1.1 DEFICIENCIES OF TRADITIONAL CLASSIFICATIONS

It is apparent to students of microbiology that bacterial classifications have, until recently, failed to achieve the necessary requirements of predictivity, stability and objectivity. One reason for this is that these aspects of a classification are intimately entwined and failure to achieve one is generally reflected in failure to satisfy all three. It is pertinent to enquire precisely what the early bacterial taxonomists were doing wrong so that we may be able to improve the situation. These shortcomings have been fully discussed by Cain (1962), but need to be briefly reviewed here because it is important to understand the reasons for the failure of early classifications.

Classifications have been traditionally based on Linnaeus's principles, which suggested that the process of classification should be conducted from 'above' and, by starting from the overall groups encompassing all living things, repeated divisions could be made until the species level was reached. In this system, species were recognized as being indivisible, i.e. the basic taxonomic unit, and at every rank, taxa were defined by specific features, which reflected the 'essential nature' of the groups. In the case of bacteria, these features might be the presence or arrangement of flagella, the morphology of the aerial mycelium in *Streptomyces* or the ability of *Escherichia coli* to form acid from lactose. The taxonomist had simply to discover these features and to distinguish the important from the unimportant to effect classification. This is regarded as an *a priori* choice of characters, since it supposes that the important feature(s) of a group can be chosen deductively. However, the process is purely subjective, since it cannot be known by intuition which features best reflect the 'essential nature' of the group or, for that matter, the organism. When such 'important' features are discovered, the reason is usually that the group has been already subjected to systematic study and useful diagnostic characters have been highlighted. The *a priori* choice of characters leads to serious problems because of disagreement between scientists. Characters considered by one worker to be of inestimable importance for defining groups may be totally disregarded by others. Thus, a lengthy discourse occurred earlier this century concerning the relative importance of

morphological characters, such as flagella pattern, cell shape, and physiological characters for classification. Indeed, this approach was taken to the extreme in the misguided assumption that a progression of characters existed that defined the hierarchy of taxa. It was considered that morphological characters defined genera, physiological characters defined species and serological features could be used to define sub-species.

If the criteria of a good classification as discussed above are considered, it will be apparent that the traditional approach failed in all respects. Since only a few so-called 'important' characters were used to construct the classification, it was based on little information and lacked predictivity. Very few assertions could be made about the taxa. Also, the classifications were unstable because the choice of important characters was subjective. Different taxonomists expressed contrasting views about the composition and defining features of taxa. This resulted in the continual revision of taxa with new descriptions and quite often new names. Finally, it may be argued that classification has not been conducted as an empirical science, because of reliance on subjectivity and intuition in the choice of defining characters. It was not repeatable because of the involvement of personal judgements by the scientists.

Having argued against the traditional approach to classification, it is necessary to provide a satisfactory alternative, but to precede this, consideration must be given to the kinds of classification that are available.

1.2 THE RANGE OF CLASSIFICATIONS

It is important to emphasize at this point that there is no single unifying classification of living organisms. Biologists are dogmatic in their belief that there is a single correct way to classify the individuals of a population. Sneath (1983) suggested that 'biology is so complex that underlying explanations are very difficult to detect: therefore there is a demand for a single dominating concept that will encompass all of its phenomena'. Alternatively, a view has been expressed that life may have started as a single event (whether caused by Creation or natural process) and consequently there has been a tendency to think of a single plan underlying all living organisms that can be used as a basis for classification. However, there is no reason to require a single classification; classifications are devised by man for various purposes. Since we have many purposes in mind there are many types of classification, and it is possible to classify them! Essentially there are three types of classification dependent on the nature of the relationships used in their construction.

1.2.1 Special-purpose classifications

Bacteriologists often use classification and identification schemes designed for their particular discipline. For example, food microbiologists or insect pathologists might use specific identification systems for the bacteria that they are most likely to encounter. Justifiably, these schemes ignore all other bacteria as irrelevant. They may be extremely useful for the specialist microbiologist, but are of little value to microbiology in general because most bacteria are excluded.

Special-purpose classifications are artificial in that they seldom display the 'natural' relationships among the organisms. The distinction between *Shigella dysenteriae* and *Escherichia coli* is a well known example. Strains of these taxa share considerable DNA sequence homology and are phenotypically very similar. From virtually every viewpoint they could be considered as a single species. However, the more serious pathogenicity of *S. dysenteriae* is of considerable importance to the clinician (and the patient!), and consequently, the separate taxa have been retained. Similarly, *Bacillus cereus* and *Bacillus thuringiensis* are virtually identical, but differ in one important respect. Strains of *B. thuringiensis* contain a large plasmid which carries a gene encoding a protein toxin which is lethal to various insect larvae. This bacterium is used to control insect pests of agricultural crops and therefore it is useful to indicate its single difference from *B. cereus* by using a different name. These artificial divisions obviously have their uses, and, in the two examples cited above, the needs of the specialist have been recognized by microbiologists in general, and these opinions are incorporated into the mainstream of microbiological classification. It is necessary for the clinical bacteriologist to distinguish *S. dysenteriae* from *E. coli* and for the insect pathologist to recognize the insect pathogenic bacilli; but we must also recognize the limitations of this approach. Artificial classifications are monothetic, in that a single feature (pathogenicity in the above examples) is deemed both sufficient and necessary for the placement of an organism in a group. Thus, for inclusion of a bacterium in the species *S. dysenteriae* it must cause dysentery in man. Non-pathogenic strains cannot be included in this taxon.

Monothetic classifications suffer from the serious disadvantage noted for traditional classifications in section 1.1. They are based on restricted information; pathogenicity in the cases mentioned above. They also tend to be unstable. Bacteriologists with different interests adopt different schemes, leading to considerable confusion. For example, the plant saprophyte *Erwinia herbicola* (classified and named by plant pathologists) is synonymous with an intestinal organism, *Enterobacter agglomerans* (classified and named by clinical microbiologists). After much debate about which is the 'correct' name, the latter won and the name *E. agglomerans* is used today. Finally, identification schemes

derived from monothetic classifications readily lead to misidentification. Because the group is based on a single or few features, the unknown organism need only be aberrant in that feature to be assigned to the wrong taxon. Relatively non-pathogenic isolates of *S. dysenteriae* would be placed in the genus *Escherichia*, non-toxic, plasmid-deficient strains of *B. thuringiensis* will be identified as *B. cereus*. Artificial classifications have their uses, but as a general system of value to all microbiologists their limitations are severe.

1.2.2 Natural (phenetic) classifications

The alternative to the special-purpose classification is the general-purpose classification, a system that is of value to all microbiologists whatever their discipline. Such a classification should encompass all bacteria and all aspects of these bacteria. Since special-purpose classifications are artificial so general-purpose classifications can be described as 'natural'. Natural in this sense can be attributed to Gilmour (1951) and was developed by Sneath (1983) to refer to relationships between organisms based on their overall similarity or affinity. Natural relationships embody all aspects of the organisms from molecular structure through physiology, to habitat. Such relationships are termed phenetic and refer to affinities based on the complete organism (genotype and phenotype) as it exists at present with no reference to the evolutionary pathways or ancestry of the organism. This contrasts with the term natural used in its evolutionary context (section 1.2.3).

In phenetic classifications, organisms are arranged into groups (phena) on the basis of high overall similarity using both phenotypic and genotypic characters. This approach encompasses all measurable features of the organisms so that the resultant classification should be useful to all microbiologists. Moreover, the taxa are polythetic rather than monothetic because they are defined as having a high number of features in common and there is no requirement for the presence of a particular attribute. Thus, individuals aberrant in a specific character can be accommodated by such groups. In a phenetic classification, *E. coli* and *S. dysenteriae* would be placed in the same species, since they have high genotypic and phenotypic similarity. Consequently, the distinction attributed to relative pathogenicity would not be given undue importance. This describes the natural relationships of these bacteria, and, although it may alarm the clinical bacteriologist, it is a sensible approach insofar as strains of *E. coli* carrying toxin and surface antigen-encoding plasmids are associated with serious diarrhoeal disease. This fact is often obscured by the artificial division of these organisms into 'pathogenic' and 'nonpathogenic' types.

It follows that phenetic classifications do not suffer the shortcomings of their artificial counterparts. Because the classification is based on

the overall properties of the organisms it has a high information content with the associated predictivity. The classification is also more stable, since the same names should be used to describe the same taxa regardless of the interests of the microbiologist. Finally, since phenetic classifications are always, in theory, polythetic, identification should be more accurate. Individuals are identified to groups using several characteristics and organisms that do not fully conform are still accommodated by such groups because identification requires only that the organism has the overall characteristics of the group. One favourite example is the black swan of Australia that is assigned to the swan species because it has the overall characters of the swan despite being the 'wrong' colour.

1.2.3 Natural (phylogenetic) classifications

For many biologists, particularly those who study animals and plants, the term 'natural' refers to an intrinsic quality of nature and natural classifications are based on 'species or groups of species that exist in nature as a result of a unique history of descent with modification' (i.e. evolution) (Wiley, 1981) or 'the true historical entities produced by the evolutionary process' (Cracraft, 1983). These natural classifications are based on phylogenetic (genealogical) relationships in that they attempt to trace the evolutionary pathways that have given rise to the organisms as we view them today, with the classification exactly reflecting the line of ancestry. This classification will be congruent with the phenetic classification if there has been no parallel or convergent evolution and the rate of change proceeds constantly in all lineages. The two classifications will differ, however, if convergent evolution or recent gene transfer gives rise to organisms that are phenetically similar but have different ancestry (Fig. 1.1). Much of what follows is more relevant to plant or animal biologists than microbiologists, but with the advent of macromolecule sequencing, bacteria are becoming more amenable to

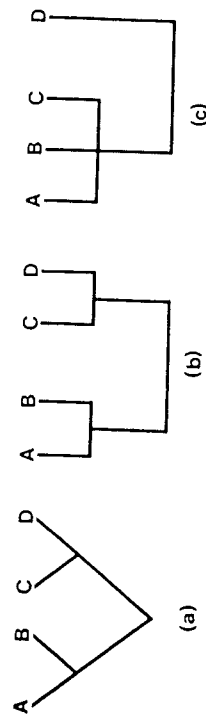


Fig. 1.1 Three dendrograms representing (a) a cladogram indicating the phylogenetic relationships of taxa A, B, C, D, (b) a phenogram of the same taxa in which evolution has been assumed to be divergent and at constant rate and (c) a phenogram in which convergent evolution or recent gene transfer has resulted in C being phenetically related to A, B.

phylogenetic analysis and therefore the major schools of phylogenetic thinking are described briefly below.

Among phylogeneticists, there is considerable controversy. The term cladistic refers to the branching pattern that describes the pathway of ancestry of a group of organisms. Hennig (1966) showed how cladistic relationships might be inferred. Hennigan cladistics involves the determination of monophyletic groups (or clades) that can be defined as groups distinguished by a set of characters inherited from an ancestor that are not shared by any other species outside that clade. In other words, all members of a monophyletic group possess a homologous character either in its primitive (earlier) or derived (later) form and it is the joint possession of this character by all descendants of the species that defines the group. In this choice of important characters, it can be seen that Hennigan cladistics differ completely from the pheneticist's view of a natural classification. As an example of Hennig's approach, Fig. 1.2 shows three organisms A, B and C classified on the basis of

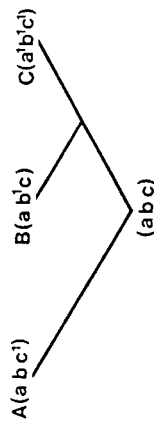


Fig. 1.2 A cladogram of species A, B and C. B and C constitute a monophyletic group defined by the shared derived character b^1 . A and B are not monophyletic because species C is excluded. Species A and C are not monophyletic because they have acquired character c^1 independently; it is not a homologous character (after Williams, 1985).

three characters a, b and c. B and C comprise a monophyletic group defined by the shared character b^1 derived from the primitive character b via an ancestor (not shown) at the upper branch point. b/b^1 is therefore a homologous character. A and B are not monophyletic because C, which does not share the primitive feature a, is excluded from the group. Moreover, A and C are not monophyletic because the only character which they have in common was acquired independently, i.e. is non-homologous. It is argued that by careful selection of characters, non-convergent homologous characters and monophyletic groups can be determined and built into a hierarchy that must reflect the evolutionary pathway. Strict adherence to this process, often termed evolutionary cladistics, can lead to some startling relationships. For example, birds and crocodiles form a natural clade because they share a common ancestor (Fig. 1.3). Crocodiles and lizards, on the other hand, do not form a clade because they share a common ancestor with the birds which is not included in the class Reptiles.

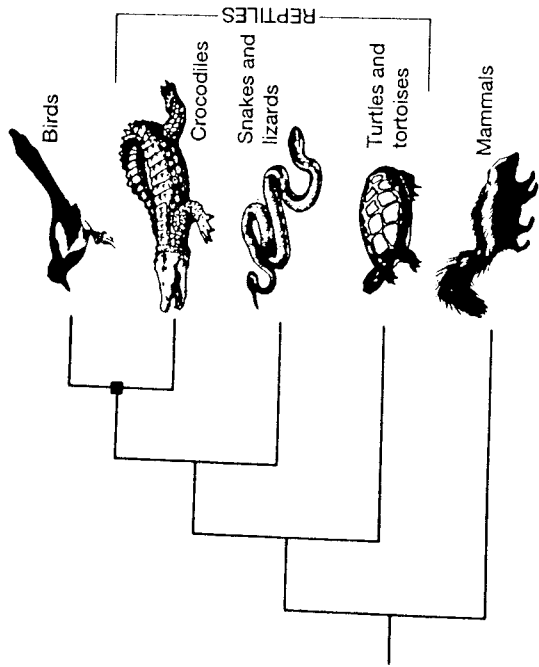


Fig. 1.3 Cladogram of birds, reptiles, and mammals. The reptiles do not constitute a natural clade because they share ancestors with the birds, which are not included in the Reptilia. Birds and crocodiles, on the other hand, constitute a natural clade (Archosauria) because they share a common ancestor (black box) not shared by any other organism (reprinted from Li and Graur (1991) with permission).

'Traditional evolutionists' avoid this problem by following the ideas of Simpson (1961) and Mayr and Ashlock (1991) and dilute phylogenetic relationships with an element of phenetic similarity when constructing the classification. Classification is practised with reference to the phylogeny, but without the requirement that all groups be monophyletic. The difference between Simpsonian and Hennigian cladistics has been neatly summarized by Williams (1985): 'According to Simpson (1961) the classification should be consistent with the presumed phylogenetic relationships, whereas for Hennig they are one and the same thing'.

To confuse the issue further, 'transformed cladists' or natural order systematists have drifted from Hennig's approach to such an extent that they are more akin to pheneticists and their views have no connection with phylogeny. They argue that evolution cannot be known and evolutionary homologous characters are unknown and can only be deduced from the classification itself. As emphasized by Sneath (1983) 'homologies cannot be recognized from the way character states are distributed in monophyletic groups because these groups cannot be constructed until the homologies have first been recognized'. The approach of the transformed cladists is therefore to assess the distribution of various character states among a group of organisms

and to arrange the organisms into a classification determined by just one criterion, maximum parsimony, that is the route which involves the minimal number of changes to arrive at the simplest possible arrangement (see Chapter 5). Thus the 'natural order' of taxa is revealed without recourse to any evolutionary theories other than that of maximum parsimony. Such arguments are fortunately of little relevance to mainstream microbiology because traditional characters useful for the construction of phylogenetic groups are few. Moreover, it is not possible to determine those characters which are primitive and those which are derived and attempts at constructing phylogenies of bacteria on the basis of morphological or physiological characters have been uniformly disastrous for this reason (see Chapter 5). However, recent developments in molecular biology have enabled phylogenetic relationships to be deduced on the basis of macromolecular sequence analysis. This approach has revolutionized much of our thinking about classification of bacteria and indicated how cladistic classifications can be constructed (see Chapter 5). Cladistic classification has therefore become an issue in microbiology and again caused lengthy debate about the relative merits of this approach compared with phenetics.

1.3 MERITS OF PHENETIC VERSUS PHYLOGENETIC CLASSIFICATIONS

Since it is commonly (but incorrectly) upheld by taxonomists that there is only a single 'natural' classification of organisms, there is obviously considerable controversy among the different schools of thought as to which approach should be adopted. In the case of bacteria, should classification be phenetic, based on overall similarities of genotype and phenotype as we view them today, or should it be cladistic, based on the evolutionary pathways deduced from macromolecular sequences? It will be useful to consider the relative merits of phenetics and phylogenetics in this context.

1.3.1 'Goodness' of the classification

The basic criterion of the phylogenetic classification is that it should precisely reflect the evolutionary pathway of the organisms. However, it is impossible to compare the cladogram with the true cladogeny because the latter is unknown (merely inferred from the cladogram) and probably can not be known.

The goal of the pheneticist is less well defined, but it could be argued that the classification should represent, as accurately as possible, the affinities between each and every organism. Various statistical measures have been developed to test the distortion within a hierarchical

classification and optimum procedures for constructing a phenetic classification have been proposed (see Chapter 2). However, because of the difficulties in defining the ultimate phenetic classification, we can not evaluate the accuracy of the classification. We must discuss the relative merits of phylogenetic and phenetic classifications on some other grounds.

1.3.2 Verifiability

Since the construction of a classification is a scientific exercise it should be a testable hypothesis or contain testable hypotheses. The phylogenetic approach is not verifiable in this sense because the only way to test a cladogram is with a second cladogram based on a different data set. As pointed out by Sneath (1983), if both cladograms have been derived using the same assumptions they may well be congruent, but this does not test those assumptions. It merely shows that the two databases are compatible and that there has been no lateral gene transfer but it remains impossible to compare the outcome with the actual evolutionary pathways. Moreover, all current theories of evolution may seem incorrect to the next generation of biologists.

Conversely, the phenetic classification, perhaps because its aims are less ambitious, is more accessible to verification. The operation of producing a phenetic classification involves gathering data, which are then analysed using established statistical techniques (see Chapter 2). This process is entirely objective and can be repeated (verified) by a second scientist. Moreover, phenetic classifications are independently testable. As new data are generated by advances in science so they can be included in the classification. If the original classification was correct, in that it accurately represented the affinities of the organisms, the new data should not alter it. If however, the new information does affect the classification it can be included and a composite, improved classification can be constructed. Phenetic classifications are therefore continually being updated and refined to produce the most accurate representation of the phenetic relationships under study.

1.3.3 Practicalities

It has been emphasized by Sneath (1989) that, if a phylogenetically coherent group was found to be phenetically heterogeneous then the cladistic group would be of little practical value. Conversely, if two phylogenetically independent lines converged due to strong selection pressure such that the organisms were phenetically similar, there would seem to be little practical value in separating them. Thus, the pheneticist aims for homogeneous taxa which can be readily identified. The argument of the cladist is that phylogenetic groups, once determined,

often prove to be phenetically coherent on further study. Indeed, were this not so, phylogenetic classifications would be of little value outside the specialist field of genealogy.

One of the principal aims of a classification is to provide a scheme whereby unknown organisms may be identified. For the microbiologist, simple, reliable, rapid tests are needed. Phenetic classifications can be analysed to select the most diagnostic characters for delineation of groups and to provide reliable identification schemes (Chapter 7). Phylogenetic classifications of bacteria rely largely on gene sequence data. Recent developments in hybridization technology are offering simple identification procedures based on nucleic acid probes which can be extremely specific and accurate. Although there remains a requirement for reasonably sophisticated technology, developments in this area are certain to continue to simplify the procedures and this is certainly one of the most important practical applications to emerge from molecular systematics (Chapter 7).

1.4 THE CHOICE BETWEEN PHENETIC AND PHYLOGENETIC CLASSIFICATIONS

The requirement is for a stable classification of high information content (predictivity) that lends itself to practical applications (e.g. identification of new strains; databases) and from which phylogenetic relationships can be inferred. Despite various claims in the literature, there is no firm evidence that cladistic classifications are any more predictive, congruent or stable than phenetic classifications (Sokal, 1985), and, in view of the comments made previously, it is more likely that phenetic classifications are superior on these grounds. Indeed, by changing the methods of analysis of sequence data, far-reaching changes in the branching patterns of cladograms can ensue (see Chapter 6). The choice therefore must be primarily for phenetic classification. It is important to note that many of the current approaches to bacterial classification based on ribosomal (*r*) RNA sequences and related techniques purport to be phylogenetic, but are actually phenetic measures of affinity with molecular sequences as characters. Inferences can be made about evolutionary patterns from these studies, but supposed evolutionary pathways are not necessarily shown in the classification. For such studies to show cladistic patterns the analyses must be made using phylogenetically based algorithms.

We agree with Jensen (1983) who suggested that what is needed are:

1. classifications that reflect what is known about the taxa, and
2. procedures for generating hypotheses about evolutionary relationships.

To this end, many systematists now agree that the two systems should be combined as far as possible (Stackebrandt, 1988). A classification framework derived from cladistic analysis of macromolecular sequences is of value to those interested in evolutionary patterns but the Hennigian reference system (containing branching patterns but the Hennigian general use. Phenetic studies (including both phenotypic and genotypic information) provide the practical databases but offer little insight into evolution. By combining both approaches, the framework can be derived from phylogenetic relationships and the detailed information from phenetics. Studying bacteria, we are fortunate that the items to be classified, the species, are largely the same whether defined phenetically or phylogenetically (see Chapter 6). It is only the arrangements of these units that will differ according to the relationships used for the classification, and in many areas the phylogenetic and phenetic are congruent. In several instances, the congruity was not obvious, for example the transfer of the thermoactinomycetes from the actinomycetes to the family Bacillaceae on the basis of rRNA sequence analysis at first seemed illogical. What could these branching filamentous bacteria have in common with the rod-shaped endospore formers? However, the relatively low content of guanine and cytosine in the DNA, the peptidoglycan composition of the cell wall and the formation of true endospores by thermoactinomycetes showed that these bacteria had considerable phenetic homology with the thermophilic bacilli. Perhaps this phenetic relationship would have been discovered earlier had the pheneticists not been clouded in their outlook by the 'importance' of morphological features, such as cell shape.

Problems arise when phylogenetic and phenetic arrangements differ and in these cases, we suggest that the classification should favour the phenetic. We see little value in a purely cladistic classification that does not accommodate the degree of phenetic relationship between species. Such is the case with higher organisms as we saw in Fig. 1.3. There seems little point in emphasizing the cladistics of the reptilia to the exclusion of the phenetic viewpoint and indeed most would agree that the phenetic approach, which groups the reptiles together and excludes the birds, is appropriate for most uses.

We shall investigate both aspects of bacterial taxonomy in the ensuing chapters and explore ways in which they can be integrated.

A simplified classification of the bacteria has been included in Appendix A, and illustrates the enormous complexity of the subject.

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