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Genetic Transfer and Bacterial Taxonomy

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INTRODUCTION

Genetic information about bacteria has played a small role so far in bacterial systematics. With the increasing use of bacteria as subjects of genetic experiment, much information has become available in the last decade, but a great deal of it is fragmentary and scattered through the literature in papers that are not primarily concerned with taxonomy. The purpose of this review is to summarize the genetic evidence that bears on the

validity of genera and higher taxonomic groups of bacteria as this affects the bacterial taxonomist. In this we have considered in the main the evidence on gene transfer, although we have drawn on collateral data from studies on nucleic acid pairing and deoxyribonucleic acid (DNA) base ratios and also on numerical taxonomy and serology, each of which are now large areas that would require separate reviews of their own. The references from an earlier review (427) have been incorporated. Other reviews that cover much of the same ground are those of Martin (306) and Luria (291), whereas several articles deal with parts of the field (32, 302, 303, 318, 349, 438) together with critical discussion, particularly the article by Marmur et al. (303). A clear and readable introduction is that of Falkow (153). Although what we now may consider as gene transfer between bacterial genera was reported at least as early as 1927 (164), recent interest in the subject dates from the paper by Stocker (443) in 1955. Four groups of phenomena are involved: phage multiplication, plasmid transfer and establishment, integration of episomes, and integration of donor cell DNA. Three types of genetic material may be transferred: phage DNA, plasmid DNA, and chromosomal DNA. Ribonucleic acid (RNA) phages are an exception but they are of minor importance in this context. The taxonomist wishes to know what conclusions he may draw from genetic data about the taxonomic relationships of bacteria, and what gene transfers have been observed so far between bacterial species.

The word species is very liable to ambiguity. We use the convenient terms of Ravin (377) to distinguish three sorts of species where clarity requires.

A *taxospecies* is a group of strains that share a high proportion of similar properties; they thus form a tight phenetic cluster. This is the most usual sense in which the bacterial taxonomist uses the term species, as when he refers to *Escherichia coli* or *Bacillus subtilis* as species.

A *genospecies* is a group of organisms that can exchange genes. Thus, most of the *Enterobacteriaceae* form one genospecies, as will be seen from later sections. Similarly, many taxospecies of *Bacillus* (e.g., *B. subtilis*, *B. cereus*) can exchange genes, and so form one genospecies. Indeed, in those groups in which mechanisms for gene exchange are known, the genospecies in bacteria is more closely equivalent to the traditional bacterial genus than to the traditional taxonomic species category. Where such mechanisms are not yet known, the genospecies is a concept of limited value, and any given example may change considerably as wider genetic cross-reactions are discovered.

A *nomenspecies* is a group that bears a binomial name, irrespective of its claim to be a species on any other score. For example, one might unite the two nomenspecies *Xanthomonas hyacinthi* and *X. campestris* into one taxospecies if one believed they did not merit separate status as taxa.

It is evident that different criteria can be chosen for determining whether a group satisfies the definitions given above (e.g., the minimum similarity or gene exchange that is required). Nevertheless, terms such as these are essential to make clear the kind of taxonomic units that are being discussed.

TAXONOMIC RELATIONSHIP

Taxonomic relationship is a term that covers a number of different concepts. Genetic relationship (including gene transfer) is one of these, but before discussing this it is convenient to consider taxonomic relationship in the wide sense.

Phenetic and Phylogenetic Relationship

One can distinguish *phenetic relationships*, which are based on the properties of organisms as they are observed today, from *phylogenetic relationships*, which attempt to describe the evolutionary pathways that have given rise to the organisms and their properties. The most important kind of phylogenetic relationship is that expressed by a genealogy, and this is called cladistic relationship (68). Cladistic relationship does not necessarily parallel phenetic relationship in detail (owing to convergent evolution and other factors), although it is evident that there is a general correspondence between them, at least at higher taxonomic ranks. The cladistics of bacteria must necessarily rest on uncertain ground owing to the virtual absence of a fossil record and the present lack of alternative bases for making cladistic deductions, although newer methods in molecular biology hold some promise here. For the present, it is sufficient to note that most taxonomic work is in fact based on phenetic relationship and that this is also the case for much of the work that purports to describe phylogeny in bacteria.

Estimating Phenetic Relationship

The term relationship is generally applied to the overall resemblance between organisms, taking into account all of their observed properties, and it is this that is the main interest of numerical taxonomy. It is close to the usual meaning of taxonomic relationship when this is not further qualified. It will be seen later that some usages of the term genetic relationship are forms of phenetic relationship in the wide sense.

The growth of numerical techniques in system-

atics has focussed attention on the logical steps that are involved in taxonomy (426, 428, 431, 435). They may be summarized as follows. Numerical methods for estimating phenetic relationships are based on the observation of numerous characters of a collection of strains. At this stage, it is usual to give each character equal weight a priori, although for purposes of identification of the groups that are constructed by such methods different weights may be given a posteriori. The use of numerous phenotypic characters should give a reliable picture of the genotype even though it is not possible to determine all the characters of the organisms or to know what proportion of the genome is involved in a particular phenotypic expression. By comparing the characters of each strain with those of every other strain, a checkerboard table of phenetic resemblances is obtained. This is usually displayed as a triangular matrix of similarity coefficients. Genetic relationships (based, for example, on the degree of hybrid fertility) can also be expressed as a matrix of this kind. Although this is rarely attempted, it should be done wherever possible both to emphasize the formal analogy with a similarity matrix and to draw attention to the extent of missing information about the interstrain comparisons.

Estimation of relationship between strains is, however, only one step in the taxonomic process. It is now necessary to cluster the strains into groups consisting of bacteria that share many common properties. The results of this are often displayed as a treelike diagram (dendrogram) which is formally equivalent to a taxonomic hierarchy. It is quite possible to perform cluster analysis on a matrix of genetic relationships, though no systematic attempt has yet been made with bacteria. A subsequent and distinct step in classification is the making of deductions about the groups thus formed, and identification schemes belong here.

Taxonomic groupings ideally should reflect all that is known about the organisms—morphology, biochemistry, serology, genetics, and perhaps also ecology. The success of a taxonomy is judged largely by the consistency obtained when different classes of information are employed. It is agreement between these classes of evidence that carries conviction. If there is marked disagreement, the classification is placed in doubt and appeal to further evidence is needed. The samples of organisms or characters may be unrepresentative or the techniques faulty, but in some instances the discrepancy may point to some new biological phenomenon which merits investigation. Numerical methods for estimating concordance (congruence; *see* 431, 435) are useful here.

Because numerous characters are employed in numerical taxonomy, it is generally true that the results are more reliable than those based on very restricted expressions of the genotype. There are, however, theoretical grounds for thinking that nucleic acid base pairing has similar reliability, and, as noted later, there is excellent concordance between the two methods. Conclusions drawn from gene transfer must be expected to be less satisfactory because of a much weaker theoretical and empirical basis. Nevertheless, it will be seen that gene transfer evidence also agrees in broad outline with that obtained from other sources.

GENETIC RELATIONSHIP

Definitions

We use the term genetic relationship in a wide sense, corresponding to the usual dictionary definition of genetic, i.e., pertaining to the study of inheritance. This wide sense includes relations expressing cytoplasmic inheritance, genetic compatibility, and hybridization, as well as those that directly express similarity in DNA sequences. The inferences about similarity in DNA sequences, or about phenetic resemblance, that can be drawn from gene transfer data are limited, but we do not think it useful to restrict the meaning of wide terms such as genetic relationship. It would seem better to use a term like genomic relationship for similarity in DNA sequences. This point may be illustrated by the unwise attempts to restrict the word species (which originally meant merely a distinct kind) to a rigid genetic concept; after much sterile controversy over the species problem, we still need to distinguish different sorts of species concept, as noted above. We shall try to avoid confusion by discussing what conclusions from genetics may be drawn about taxonomic (i.e., ideally phenetic) relationship, rather than about genomic relationship. Genetic relationship is also used in a vaguer sense, in which it is not clear whether phenetic or cladistic relationship (or both) is meant.

New observations and concepts will require us to use clearer terminology in the future. They may impose stresses on our present concepts of bacterial taxonomy. Bacterial genes can be transferred across wide taxonomic gaps; no sharp distinction can be made between bacterial genes and genes of phages and plasmids; there is a growing view that the bacterial genome is best regarded as consisting of a number of different replicons (chromosome and plasmids) some of which are unstable and are continually merging (384). If these views are accepted, it can be seen that a strain X of taxospecies A, carrying a plasmid *p* derived from the genome of taxospecies B, has

multiple relationships to species B. These include genetic relationship (in the wide sense) due to the plasmid transfer. But X and B also have a genomic relationship (by virtue of the genes on the plasmid), a phenetic relationship (in respect of the phenotypic properties due to genes on *p*), and in addition a cladistic relationship due to the origin of *p*. Other strains of species A have close relationship to strain X, a relationship which is at least phenetic (but which may be assumed to be also genomic and cladistic). However, they have only a distant relationship of any kind to species B, except through the intermediacy of strain X. The taxonomic practices adopted by botanists for hybrids (particularly allopolyploids; *see* 119) will become increasingly pertinent to bacterial systematics, but additional conceptual problems would arise if replicons were present but not expressed as gene products; this would introduce incongruence between phenetic and genomic relationships, at least on the practical level.

Criteria of the Taxonomic Value of Genetic Data

The use of genetic data for taxonomic purposes may be justified on either theoretical or empirical grounds, but in neither case can such data be regarded as completely reliable indicators of taxonomic relations. Strictly, all conclusions from genetic evidence (or phenetic evidence, for that matter) can only be expressed as probability statements. It will become clear from subsequent sections that the theoretical bases of gene transfer evidence are extremely slender (although theoretical bases are strong for certain other classes of data, in particular DNA pairing). Bacteriophages and plasmids can multiply in hosts that are distant taxonomically, and it may be that only a few shared features are needed to permit cross-infection. Extreme examples of wide host range are shown by some viruses of eukaryotes. Certain plant viruses can multiply in insects (e.g., 88, 452), and it has long been known that arborviruses can multiply in both vertebrates and arthropods (*see* 160). There are reports that animal viruses may multiply in bacteria (2, 37), though further confirmation has not yet been forthcoming.

Despite these examples, gene transfer data are useful to the taxonomist. He wishes to know in particular the amount of phenetic information shared by two strains that is implied by a given instance of gene transfer between them. Although in theory it may be very little (reflecting perhaps only the presence on both strains of a surface receptor of simple structure), it might also be large; almost nothing is known about the frequency distribution of this shared phenetic information. In the majority of cases, the taxonomist will have no knowledge of the number of unit characters (in the phenetic sense) that are involved. He may therefore turn to empirical evidence to get some idea of the taxonomic relationship that is indicated *on the average* by some particular type of gene transfer.

The empirical evidence is reasonably firm: gene transfer is very much more frequent between strains that are closely related taxonomically than between strains that are distantly related. At the very least, the occurrence of gene transfer is a pointer for the taxonomist to organisms that he should study together. Most taxonomists feel, in addition, that they would place more weight on a gene transfer cross-reaction than upon a single phenotypic character such as the ability to ferment lactose. Presumably, this view is based on the belief that the cross-reaction does (as a rule) imply a considerable amount of phenetic resemblance. The empirical evidence favors this view, although we know of no special study upon this point.

It may be useful to draw a parallel with serology, which seems a sufficiently close analogy. Although some serological cross-reactions are known between extremely dissimilar organisms, in most serological systems the reactions are reasonably specific and useful, whether or not the immunochemistry is fully understood. The serologist will usually not know how much phenetic information is involved in a given cross-reaction, although in theory it may be very little. The cross-reaction, too, is formally more analogous to a resemblance measure between two strains than to a character of a single strain, and the same is so for genetic cross-reactions. Because of the difficulty of combining serological or genetic data directly with phenetic data, these three classes of taxonomic information are generally treated independently, in the hope that the conclusions from each will support the conclusions from the others.

Types of Gene Transfer

Three main classes of mechanism for gene transfer are known (although the borders between them may not be entirely sharp): those where genes are transferred as soluble DNA molecules, those involving transfer by a cellular particle, and those involving cell contact followed by transfer of the whole or part of the bacterial chromosome. In all cases (with the exception of RNA phages) the essential step is the transfer of DNA in some form, and except perhaps in the recombination systems of *Actinomycetales* it is effectively unidirectional. These mechanisms are only briefly described here; fuller considerations can be found in standard texts (e.g., 61, 203). The nomenclature of cellular particles is not yet entirely stabilized,

but it is now usual to refer to cellular particles that can multiply autonomously as plasmids, whereas those plasmids that can integrate into the chromosome of the host cell are referred to as episomes. The most familiar examples of episomes are the lambdoid phages and some fertility factors of the coliform bacteria. It is quite likely that many plasmids which have so far not been shown to integrate can do so occasionally; both episomes and plasmids are therefore conveniently considered together. With the exception of RNA phages, they consist of short lengths of DNA, and the evidence is that they normally exist intracellularly in the form of a ring of DNA (72, 324, 383). In many respects, they behave very much like small supernumary chromosomes. Extrachromosomal inheritance in bacteria has recently been comprehensively reviewed by Novick (339).

Transformation. Gene transfer as soluble DNA (either extracted from the cells or liberated spontaneously into the culture medium) is the phenomenon of transformation. The soluble DNA is taken up by the recipient cell and is then integrated into the chromosome of the recipient, where it is expressed as a changed phenotypic character. Recent reviews on transformation include those of Ravin (376), Spizizen et al. (438), and Schaeffer (404). Transformation is widely known in both gram-positive and gram-negative bacteria, and no very obvious pattern of occurrence is seen. It may well be that it can take place in almost any bacterial group if the right technical conditions are found, although some taxa are less amenable because of a poorly permeable cell wall or the production (externally or internally) of large amounts of deoxyribonuclease that rapidly destroys free DNA (376).

Transformation has commonly been demonstrated between different taxospecies and less frequently between different genera. Particularly in the gram-positive organisms, there are usually some strains that are easily transformed (competent strains) and others that are insusceptible, but in the gram-negative genera *Moraxella* and *Neisseria* this strain variation appears to be less marked (46, 80, 210). The state of competence (a term generally restricted to the early phase of DNA uptake) is determined partly by environmental conditions and partly by genetic factors, and also by specific substances that assist penetration or binding (competence factors); this subject has been extensively reviewed by Spizizen et al. (438). The uptake of DNA appears to be relatively nonspecific, and the most critical step is the integration into the recipient chromosome. For example, the number of transformants is much higher between strains of the same taxospecies of *Haemophilus* than between strains of different taxospecies (287, 288, 402, 403). The uptake of DNA, however, is almost the same in the two cases. This finding seems generally true in transformation, although the evidence in *Neisseria*, *Moraxella*, *Bacillus*, and *Streptococcus* is less clear-cut (85, 210, 230, 305, 359). It seems likely that the difficulty in integration is due more to differences in fine structure within genes (and perhaps absence of integrating enzymes; 185, 486) than to chromosomal rearrangements or different linkage relationships (86, 378; for further discussion *see* 87, 303, 349, 438). In theory, abortive transformation might allow gene expression without integration, but this (although known for transduction) does not seem to have been reported, although the findings of Catlin and Tartagni (83) bear some resemblance to this phenomenon.

Transformation is unlike the other gene transfer systems in several respects. The integration step is unique in that a single-stranded donor fragment is integrated into the double-stranded recipient genome. Surface compatibility is not involved; penetration mechanisms are presumably quite different. A particularly interesting observation (87) is that foreign DNA, even from mammals, will assist transformation of streptococci with streptococcal DNA, although the helping effect is greatest with DNA from the most closely related organisms. The full significance of this is still unclear, but it may be that the helping DNA saturates a cellular inactivator of unintegrated DNA. Since the size of DNA fragments in transformation is of the order of 0.01 of the genome [except for the large fragments reported by Kelly and Prichard (252)], only a few closely linked genes can be transferred by transformation at one time. Transformation is therefore not very convenient for studying the overall linkage maps of different bacteria, although better techniques may hold promise here (*see* 438).

The transmission of bacteriophages by infection of the recipient with phage DNA (transfection) is akin to transformation, and has been recently reviewed (438), but has not so far found application in taxonomy.

The empirical evidence on the concordance between frequency of transformation and taxonomic relationship has been discussed by Luria (291), Pakula (349), and Bøvre (51) and may be summarized as follows. The most useful measure is the ratio of the number of transformants obtained when a competent strain is treated with its own DNA to the number when DNA from another strain is used. The median ratios (not the mean ratios, which are greatly affected by aberrant values) are of the following orders of magnitude: for different strains of the same taxospecies

about 2×10^{-1}; for different species of the same genus about 10^{-3}; for different genera of the same family near or below the lower limit of detection usually about 10^{-6}. Although the same falling trend with increasing taxonomic difference is seen in all groups, the ratios in different groups differ by at least a power of 10. For example, the ratios between different species of *Streptococcus* are around 10^{-2}, but for different species of *Haemophilus* they are around 10^{-3}. It is not yet clear how far these discrepancies are accounted for by differing species criteria, or by the choice of close or distant pairs of species within any given genus. Nevertheless, it is notable that there is often a sharp decrease in ratios on passing from experiments involving species that are very close phenetically to experiments between more distant pairs of species of the same genus. Transformation evidently appears to be particularly sensitive to small taxonomic differences between donor and recipient strains.

Transduction. Bacteriophages can transmit genes of the host by transduction (for reviews *see* 348, 438) whereby the phage incorporates host genes by one of several mechanisms. On introduction into the recipient bacterium, the gene may become integrated into the chromosome. If it does not (abortive transduction) it may still be expressed, although special techniques are needed to detect this, so this has at present little relevance to taxonomy. Episomes can be transduced (233), and plasmids which are not themselves infective can at least sometimes be transmitted by transduction (340). There are two major forms of transduction: generalized transduction (in which the phage incorporates almost any gene, but with low frequency) and restricted transduction (in which the phage has a preferred combining site on the chromosome and incorporates only genes adjacent to this, but with very high frequency); of the two, the former has been of most interest in taxonomy so far.

Transduction is known in only a few groups of bacteria, notably the *Enterobacteriaceae*, pseudomonads, and *Bacillus*. It has not been much explored for taxonomic purposes but it is not often successful with different genera as donor and recipient. Little is known about how readily different strains of the same species can be transduced, but presumably the host range pattern of the transducing phages is the major determining factor. It has been suggested that the greater difficulty of transduction compared to transformation is because the DNA fragments involved in transduction are larger than those involved in transformation, and that larger fragments are less easily integrated into the recipient chromosome (143).

The small range of susceptibility to transduction would be expected on general grounds. The transducing phage must pick up genes from the donor chromosome; this apparently requires some form of pairing in the case of restricted transduction, but not in generalized transduction (*see* 231). The phage must be able to infect the recipient strain (although not necessarily able to lysogenize it) and, except for abortive transduction, the transferred gene must then integrate into the recipient chromosome. Failure at any step will effectively prevent transduction, and the last step probably implies fairly close relationship between donor and recipient bacteria. Like transformation, the number of genes transferred is small, and transduction is most useful for fine-scale mapping of closely linked genes.

Infection by bacteriophage. The inclusion as a gene transfer phenomenon of phage infection, with phage multiplication but without lysogenization, may occasion some argument. Several considerations have determined this course. No sharp distinction can be made between genes of prophage and bacterium; most of the literature of taxonomic interest does not allow one to say whether lysogenization took place. In bacteriophage infection, genes are transferred and are expressed even if they are not perpetuated in the lineage of the recipient. Those bacteriophages that are virulent do not have the ability to integrate with the host chromosome. Despite our ignorance of their origin, we think it plausible to treat them as special cases derived from temperate phages, in view of the numerous similarities in structure and behavior between temperate and virulent phage.

Specific surface receptors are necessary for the adsorption of phage onto the recipient cell (reviewed by Stent, 441). Such receptors may be carbohydrates, and these (as is well known from serology) may not be highly specific. In other cases [e.g., a phage that adsorbs to flagella (317)] the specificity of the receptors may be high. Certain small organic molecules are known to act as cofactors in adsorption (17, 251). More recent work (418, 419) supports the earlier evidence that the lysozyme associated with the phage tail plays an important part in penetration, but there is still uncertainty over the exact role and specificity of lysozyme in penetration and phage release (*see* 168). It is likely that the phage nucleic acid, after it has entered the cell, must become attached to a special site (possibly cell membrane) before it can enter the lytic cycle (*see* 45, 147, 397), but the specificity of this site is unknown. The phage may also be repressed if the recipient cell is carrying a homologous prophage (a common reason of phage resistance in those species with a high per-

centage of lysogenic strains) or it may be restricted enzymatically (20, 21, 316). There are no strong reasons for believing that there must be a considerable similarity between phage and host genes to allow phage multiplication. In summary, the ability of two strains to support the growth of a given phage may only reflect similarity in one or two host genes.

The empirical evidence for the value of phage cross-reactions for taxonomy is, however, more convincing. The host range of bacteriophages extends from those specific for a very few strains of one species to those that can lyse bacteria that belong to different families or orders in the present conventional taxonomy (427, 443). However, reports in the literature show that most phages will lyse a significant proportion of strains belonging to the same taxospecies as the propagating strain, but will less often attack strains of other taxospecies of the same genus. We have attempted to analyze some of the relatively few surveys that contain data of the requisite detail and abundance, in which the taxonomy of the bacterial groups seems satisfactory, and which span fairly evenly the better-known bacterial groups. These data (92, 95, 134, 135, 183, 230, 373, 392, 444) can be summarized as follows. The number of lytic cross-reactions with batteries of phages (that were, as far as one can tell, unselected for taxonomic peculiarities) was counted. These reactions were tabulated as reactions between different strains of the same species, different species of the same genus, and so on. The taxonomic relations were taken from *Bergey's Manual* (62). Out of a total of 26,681 attempted cross-reactions, the following proportions of positive lytic reactions were found. Some indication of the variation between different surveys is given by the ranges of the means observed in each survey, shown in parentheses: between different strains of the same species 1284/5453 = 23.5% (6.3–43.8%); between different species of the same genus 792/7050 = 11.2% (0–65.0%); between different genera of the same family 20/6751 = 0.30% (0–2.8%); between different families of the same order 0/534 = 0%; between different orders 5/6873 = 0.073% (0–0.28%).

As implied by the ranges of the means, the percentages for any of the above groupings varied considerably in different studies, but in almost every case the same falling trend with increasing taxonomic difference was evident. If surveys of *Actinomycetales* are included (e.g., 58, 272, 366) there is a rise in particular in the percentage of reactions between different families of the same order (from 0 to 2.5%); this is largely because of cross-reactions between genera like *Nocardia*, *Streptomyces*, and *Mycobacterium* that are conventionally placed in different families. The other figures are but little changed. Not too much reliance can be placed on the figures for different families and orders because of the uncertainty of the taxonomic validity of higher ranks in bacteria and the small number of experiments.

Bacteriophages are known from many genera of bacteria, probably in most genera in which they have been looked for. The occurrence of lysogeny is extremely variable, but lysogenic strains can be found in many, perhaps most, species.

Phage adsorption, killing, or lysis without phage multiplication are of uncertain taxonomic significance. Therefore, it is only phage multiplication and lysis (or lysogenization) which can be taken as evidence of taxonomic relationship. It may be that some of the unconfirmed records of phage cross-reactions are due to mistaking inhibition of growth, produced by simply killing or lysis by adsorbed phage, for true multiplication with lysis. This point, therefore, requires careful attention in experimental work. If concentrated phage preparations are spotted onto a lawn of the recipient organism, apparent areas of confluent lysis may be due to either cause. The production of isolated plaques with diluted preparations is the only reliable indication of phage multiplication. It would not be excessively laborious to confirm every reaction as due to phage multiplication; the formation of clearly recognizable plaques should be sufficient (*see* 57 for practical details). The host range of a phage may be determined to some extent by the last host in which it was grown [host-induced modification (*see* 20)], but major changes of host range due to this phenomenon do not seem to have been reported.

Lysogenization. The better-known type of lysogenization, as exemplified by λ phage of *E. coli* [and including the majority of temperate coliphages (*see* 231)], involves the integration of phage into the host chromosome. It is generally assumed that this entails extensive pairing between phage DNA and the chromosome, consequently implying a closer taxonomic relationship between donor and recipient than simple multiplication with lysis (318). Recent work by Signer et al. (415) suggests that there is very little base-pair homology between the attachment site of λ and the corresponding site on the chromosome. This, therefore, raises doubts about how closely related two organisms must be to support the integration of the same temperate phage.

In addition, Ikeda and Tomizawa (231) recently showed that the temperate phage P1 does not become integrated during lysogenization but exists as a separate plasmid; in this case lysogenization is more akin to episome infection. We do not know how common the P1 type of lyso-

genization is, but indirect evidence (transduction that does not involve covalently linked phage and donor DNA) suggests that certain transducing phages of *Bacillus* may behave like P1 (344).

The taxonomic significance of lysogenization may thus be less than is commonly supposed, although in practice it will necessarily show a narrower host range than will lytic infection because lytic multiplication must first be possible if lysogeny is to be detected. Taxonomic evidence is scanty; most studies do not record whether lysogenization occurred in addition to lysis. One has the impression from the literature that lysogenization after interspecies infections is quite common when it is looked for. Lysogenic conversion may be considered a form of gene transfer (*see* 29, 303), but so far it has not been of much taxonomic interest.

Plasmid transfer by conjugation. The F factor of *E. coli*, the colicinogenic factors of *Enterobacteriaceae* (and perhaps the bacteriocinogenic factors in general), and the resistance transfer factors (R factors) responsible for infective drug resistance in gram-negative bacteria all share many common properties. It is probable that similar factors of some other bacteria are much like them (40, 222). Although they are not liberated in the free form like phages, they can be transmitted by cell contact.

F factors mediate the intimate cell contact necessary for bacterial conjugation. They can integrate into the host genome and they then confer the Hfr property (high frequency of recombination) on the host cell. They can also incorporate genes from the bacterial chromosome, giving the F′ factors, and thus transfer such genes by infection. Similar fertility factors are known in *Pseudomonas* and *Vibrio* (40, 220, 290). Colicinogenic factors can also confer fertility (381, 401). R factors can act as fertility factors (356, 447) and probably can combine with the host genome in a similar way to F; they also have close relationships to phages and colicinogenic factors (216, 319, 322, 481–483). The R factors may consist of two parts, one which determines infectivity (transfer factor) and one which carries the genes for drug resistance, although the evidence on this point is conflicting (15, 16, 474, and *see* 339). Certain resistance genes are tightly linked to the transfer factor (15). Other resistance genes are not so tightly linked, and work by Falkow et al. (156) suggests that these genes are carried on small independent fragments of DNA, which can multiply autonomously. Presumably these unlinked genes (as well as the linked ones) are ultimately derived from bacterial genome, although no doubt often from some bacterium other than that in which they are first observed. The widespread and increasing occurrence of R factors in gram-negative bacteria of medical importance is shown by numerous recent studies (16, 116, 320, 322, 324, 333), and reports of in vivo transfer of R factors are now appearing (191, 472, 476). R factors were present, although apparently uncommon in pre-antibiotic days (421), but currently about 50% of coliform organisms isolated from clinical material carry them (1).

Plasmids may show as satellite bands in DNA density gradients if they have a different guanine plus cytosine (GC) ratio from the host (156, 158, 303, 304), and some satellites suggestive of as yet unknown plasmids have been observed in *Halobacterium* (245), *Serratia* and *Aeromonas* (296), and *Chromatium* and *Rhodopseudomonas* (450). It has been suggested that a plasmid of *Proteus* (whose cells have a GC ratio of 38–40%) originated in a bacterium with a GC ratio of about 50% (159). Although plasmids are known in gram-positive organisms such as staphylococci and perhaps streptococci (264, 340, 380, 383), they have not yet been shown to be transmissible by cell contact, although they can be transduced. There is now evidence that plasmids can be derived from bacteriophages (84, 308). There are a number of recent reviews on episomes and plasmids (16, 322, 324, 339, 401).

Plasmid transfer shows a range as wide or even wider than phage cross-reactions. This wide range is shown by at least some of the F′ factors (307), by R factors (201, 273, 284) and by colicinogenic factors (171). Some strains of a species are harder to infect than others, but so far we do not know very much about this strain variation. As Falkow (153) has well pointed out, a main reason for the wide range of plasmid transfer is that the genes carried by plasmids do not have to be integrated into the chromosome of the recipient, and hence they do not need to have a DNA sequence or GC composition similar to that of the host genome. They are instead perpetuated by the autonomous replication of the episome, which does not need to become integrated to replicate (unlike the fragments of DNA involved in transformation, for example). Susceptibility to plasmid transfer, like phage cross-reactions, may reflect only a surface receptor plus the presence of an internal attachment site. These may perhaps represent the activity of only a few genes, so that evidence from plasmid transfer cannot be taken as strong evidence of close taxonomic relationship. It has been noted that the frequency of transfer of R factors may parallel taxonomic relationship (e.g., 422), but quantitative data are still scanty.

The particular relevance of F′ and R factors for bacterial taxonomy is twofold: (i) they are very readily transmitted across wide taxonomic gaps,

and (ii) their transfer can be easily observed by using marker genes (e.g., for prototrophy or drug resistance) which can be powerfully selected for in the recipient organism. By such means, gene transfer has been demonstrated between genera that are usually considered only distantly related. It has recently been shown (321) that R factors in some strains are normally repressed in their ability to initiate conjugation and transfer but that derepressed mutants can be obtained which show much higher fertility. The competence of fertility factors may thus be of considerable importance for gene exchange by plasmid transfer and by conjugation with recombination.

Transfer of bacterial chromosome by conjugation. Transfer of bacterial chromosome following conjugation usually results in the production of partial diploids, which are nearly always unstable so that haploids are formed after recombination between donor and recipient chromosomes. In the conjugation system of the coliforms, if the F factor is integrated as Hfr, part of the F factor is transferred first and the other part is transferred last; usually the latter portion is not transmitted because complete chromosome transfer is infrequent. The normal mode of transfer is from male cells (F^+ or Hfr) to F^- female recipients, and F^- cells are not interfertile. Similar genetic systems have been observed in *Pseudomonas* (290), *Vibrio* (40), *Pasteurella* (282), and *Rhizobium* (214), A recent review of the mechanisms of conjugation is that of Curtiss (114).

The conjugation and recombination mechanisms in the *Actinomycetales* are less well known (412–414), and infective fertility factors have not been identified. It seems likely that recombination is due to the formation of transient heterogenotes by fusion of the parental cells, followed by segregation. There are indications that the heterogenotes may be stable for a considerable period (32).

Transfer of bacterial chromosome is not so frequently reported as other systems of gene exchange, and apparently it is usually only possible between closely related species or genera. Strain variation in ability to conjugate and transfer chromosome has been noted by most workers, and is often extreme. For successful conjugation, the cells must first possess surface compatibility and the necessary penetration mechanisms (or probably undergo cell fusion in *Actinomycetales*). Although in theory recombination of donor and recipient chromosome could occur by crossing-over at only two small pairing regions, the risk of loss of essential genes would be very high unless the linkage relations between genes were similar in donor and recipient. Indeed remarkable correspondence has been found between the gross chromosome structure and linkage positions in three genera that readily show recombination, *Escherichia*, *Salmonella*, and *Shigella* (398, 407), although the fine structure of homologous genes is detectably different (153). Recombination following conjugation thus implies some similarity over much of the genome, but to what extent this reflects similarity in the base sequence of DNA is still uncertain.

The extensive crossing over involved in recombination places this on a similar level to chromosome compatibility studies in the cytotaxonomy of higher organisms, although such studies have not been exploited for bacterial taxonomy. The reader may be referred to Davis and Heywood (119) for a general account of this in the taxonomy of higher plants. One further implication for taxonomy is that by this genetic mechanism two forms of intermediates are possible between classical taxospecies. First, the exchange of a large proportion of the genome (instead of a few genes, as is usual with the other gene transfer mechanisms) would allow the production of strains that are intermediate in most properties, rather than simply aberrant in a few characters controlled by the few transferred genes. Second, there is the possibility that stable diploids could exist, which would probably appear intermediate phenotypically but which would segregate various combinations of phenotypic characters as the diploids were from time to time reduced to haploids. Indeed, in streptomycetes there is some evidence for such stable diploids or heterokaryons (412).

Other Phenomena Associated with Gene Transfer Mechanisms

Phage adsorption or killing and lysis from without are phenomena of little interest for taxonomy. It is uncertain what degree of phenetic resemblance is implied by cross-reactions of this kind, but it is likely to be small because in many cases only a few cell receptors (and these perhaps of simple structure) are involved. It is difficult to assess from the literature the empirical evidence on how far these phenomena are correlated with the taxonomic difference between the strain yielding the bacteriophage and the recipient strain. It is, however, well known that phage adsorption is often of low specificity; for example, some staphylococcus phages adsorb to *Streptococcus* and *Bacillus* (371, 372), whereas bacteriophages of *Escherichia coli* may attack and kill *Pseudomonas* without multiplying (138).

Although sensitivity to bacteriocins is not strictly a phenomenon associated with gene transfer mechanisms (except in the sense that the colicins are produced by the colicinogenic factors), bacteriocin sensitivity is often studied to-

gether with phage sensitivity. It may, therefore, be briefly mentioned here for convenience. Like phage adsorption, bacteriocin sensitivity probably reflects only a few cell characteristics. The special claim of bacteriocins as taxonomic indicators is that they are defined as antibiotics that only attack closely related bacteria, but this introduces the danger of circular argument. Some bacteriocins have a very wide spectrum of action. Thus, certain colicins are active on *Pseudomonas* and *Xanthomonas* as well as on *Enterobacteriaceae* (196, 197, 199, 200). Similar wide cross-reactions are found in gram-positive genera, between *Bacillus*, *Streptococcus*, *Listeria*, *Lactobacillus*, and *Staphylococcus*, for example (65, 198, 381). Bacteriocin sensitivity should therefore be considered only an uncertain guide to taxonomy. There are several recent reviews on bacteriocins (171, 232, 381).

Taxonomic Significance of Steps Involved in Gene Transfer

Successful gene transfer requires a number of steps (not all of which are involved in any one genetic system) that are of unequal taxonomic significance, and the relevant points from the earlier discussion are summarized below.

Factors affecting entry of infecting material. In transformation, there does not seem to be any great limitation to the entry of specific DNA molecules. Penetration depends on the state of competence rather than the origin of the DNA. With bacteriophage infection, the major factor is the presence of the requisite surface receptors. In congugation, however, the mechanism is more complex. Curtiss (114) pointed out that there are two major steps: (i) the union of donor and recipient cells and (ii) the mobilization of the donor chromosome or plasmid. Factors important in the first step include the presence of fimbriae (pili) on the donor cell and the presence of specific components on the cell surfaces that serve to promote the union of donor and recipient cells. Less is known about the second step, and it is not clear to what extent this is affected by taxonomic differences between donor and recipient. In all the gene transfer systems, therefore, the factors affecting entry of foreign DNA appear to be of low taxonomic specificity.

Stability of the infecting material in the recipient cell. Once having entered, the genetic material must be sufficiently stable to allow it to become integrated into the host genome (or to multiply if it does so autonomously). Enzymatic restriction plays an important part in several genetic systems (20, 157, 316). Restriction is often strain specific, and Arber has suggested that it may provide bacteria with a mechanism whereby the expression or integration of foreign genetic elements can be prevented without hindering gene exchange between cells of the same strain (20). Single-strand DNA appears to be particularly vulnerable to destruction, and it may also be that the circular configuration of the DNA of plasmids is important in ensuring its stability (72, 324, 383).

Integration of transferred DNA into the host chromosome. Having entered the recipient cell the DNA must, in some genetic systems, integrate into the DNA of the host. This is necessary for successful transformation, transduction, recombination following conjugation, and the better-known (lambda) type of lysogenization. It may also occur subsequent to the establishment of a plasmid.

It is usually assumed that the pairing preliminary to crossing-over and integration requires substantial similarity in the base sequence of the donor and recipient DNA, and hence close genomic and taxonomic relationship. The assumption is made largely because of the lack of any plausible alternative; positive evidence is indirect. The efficiency of integration in interspecies transformation depends more on the adjacent DNA than on the selected marker (403). There is also some support from the work of Demerec and his colleagues (136, 137); integration of genes during transduction from hybrid donors is more frequent when the DNA fragments consist of genes derived from the same species as the recipient than when they are derived from a different species. It has also been noted that restriction does not wholly account for the low frequency of recombination in intergeneric crosses, so that presumably the remaining barrier to recombination reflects differences in nucleotide sequence between the organisms (157).

But even if base pairing is required it may perhaps not need to be extensive. The bacteriophage λ, although it integrates into the host chromosome with high efficiency, it may possibly do so without significant base pairing (415). It may thus be that two organisms do not need to be closely related to support the integration of the same temperate phage. Although specific enzymes may be needed to bring about integration, in the case of the lambda phages these are determined by the phage genome, not the host (185, 486). Less is known about the integration of other episomes, but in contrast to λ the in vitro pairing of F-factor DNA with chromosomal DNA suggests that integration of F does reflect extensive similarity in base sequence (155).

Integration may indeed be a useful criterion of taxonomic relationship, but this is based largely on the empirical evidence: taxa permitting gene transfer with integration are more closely related

on other criteria (DNA pairing in vitro, GC content, phenetic similarity) than are taxa that permit transfer without integration.

Replication of the transferred gene. The transferred gene must in general be replicated if its presence is to be detected, because most techniques (apart from those used in studying abortive transduction) require that it become established in the clone descended from the recipient. If the gene becomes integrated into the host genome, replication is effected by the host mechanism directly, whereas genes carried on plasmids are replicated by the autonomous replication of the plasmid. A specific nonchromosomal attachment site for the plasmid is probably required; the same may be true of virulent phages and those prophages (e.g., P1) that do not integrate, but at present we have no reason to assume that this attachment site is of high taxonomic specificity.

Gene expression. The gene must then be expressed in the recipient cell. This depends on various factors, on the production of messenger RNA and also possibly on modification of the RNA polymerase of the host, which may be mediated by cofactors (67, 267, 448, 465). Sufficient resemblance may have to exist between the controlling genes of donor and recipient, but control mechanisms are not very well known at this level of study so there may be some unsuspected barriers to the expression of foreign genes. It has been shown that foreign genes may sometimes be only weakly expressed (158, 424), although there is every reason to believe that an enzyme produced in a different taxon, as the result of transfer of the structural gene, has the same amino acid sequence as that enzyme in the donor strain (158, 307).

Although severally each of these steps may be controlled by only a few characters, taken together the steps are likely to reflect a significant number of taxonomic differences. Presumably this is the reason that the frequency of gene transfer is observed to be so strongly correlated with the closeness of taxonomic relationship between gene donor and gene recipient.

Summary of the Value of the Several Classes of Gene-Transfer Evidence for Indicating Taxonomic Relationship

It has been seen from the preceeding discussion that the ability to support the multiplication of a phage, and susceptibility to plasmid infection, may perhaps be determined by only a few genes, so that from the theoretical standpoint they may not be very reliable indicators of close taxonomic relationship. Those gene transfer mechanisms that involve integration of foreign DNA into the host chromosome may be safer indicators of taxonomic relationship, although the theoretical evidence is still uncomfortably slight. Nevertheless, the empirical evidence (that integration only takes place when donor and recipient are closely related) also supports the view that integration does imply closer taxonomic relationship between donor and recipient than is indicated simply by gene transmission. Gene transmission in turn usually implies closer relationship than is indicated by interference with metabolism by the byproducts of genetic phenomena such as phage adsorption; indeed, the significance of this type of evidence is too uncertain to be taken as more than a pointer for further work.

We can therefore arrange these three classes of phenomena with repect to taxonomic relationship as: (i) gene integration > (ii) gene transmission and expression > (iii) associated phenomena.

This may be illustrated for bacteriophages as follows. (i) Lysogenization of the recipient indicates closer taxonomic relationship than (ii) phage lysis, which in turn indicates closer relationship than (iii) phage adsorption and killing. The various phenomena in these three classes are as follows.

(i) Gene integration. Transformation; complete transduction; lambda type of lysogenization; presumably most cases of lysogenic conversion; episome integration; conjugation with chromosome recombination. Of these, the last appears to have the narrowest taxonomic specificity, and transformation seems to have the widest (at least on present evidence).

(ii) Gene transmission and expression. Bacteriophage infection and lysis; transfection (infection with phage DNA); episome or plasmid infection (including F factors, R factors, colicinogenic factors, and the P1 type of lysogenization); abortive transduction.

(iii) Associated genetic phenomena. Phage adsorption; phage killing including multiple-hit lysis; and though not properly belonging to this class, one may add that sensitivity to bacteriocins also has the low taxonomic value of this class of evidence.

The empirical evidence also allows the statement that, for any given gene transfer mechanism, the closer the taxonomic relationship between donor and recipient the higher is the usual frequency of gene transfer, although there are many exceptions. In addition, a single instance of gene transfer is not a reliable indicator for the taxonomist, although numerous transfers may allow reasonable certainty. If a collection of strains cross-reacted extensively with a battery of different phages, for example, there would be little doubt of the close relationship of the strains. We

are, however, some way from being able to give any satisfactory statistical basis to the reliability of gene transfer data for taxonomy. Perhaps the greatest weakness is that it is hazardous to say that two taxa are closer together than they are to a third taxon, because of the likelihood of unsuspected barriers to gene exchange with the third taxon.

Species Barriers in Bacteria

Ravin (375) has well discussed two main barriers to gene exchange between bacterial species, cell surface incompatibility and DNA pairing incompatibility. These may have very different significance in the different mechanisms of gene exchange, as noted earlier. Thus, in transformation surface incompatibility may often be slight but DNA pairing appears to be critical, whereas in plasmid infection DNA pairing is probably not involved in the replication of the plasmid but cell surface incompatibility may be a serious barrier. Probably the commonest barriers to gene transfer between strains of the same taxospecies are (i) lack of suitable surface receptors in the case of bacteriophage infection and conjugation, (ii) impermeability of cell wall, or enzymatic destruction of DNA in transduction, (iii) lack of suitable intracellular attachment sites, and repression or restriction in the case of phages and plasmids, and (iv) rarity of competent fertility factors for conjugation. These barriers operate, of course, also in gene transfer between different taxospecies, but there is the additional barrier of pairing incompatibility between donor and recipient DNA due to genetic differences between taxospecies.

Implications of Negative Findings

The other major point about gene transfer is that negative evidence is of little taxonomic value. This is, of course, true for higher organisms as well as bacteria. If two higher organisms cannot exchange genes, this provides little evidence of their taxonomic difference; they may belong to different families, or even to the same species if there are mechanisms to prevent self fertilization, as is common in higher plants. Close parallels are found in bacteria. Only when much experimental work has been fruitless is it justifiable to conclude that two forms are *unlikely* to be closely related, and then only when potentially competent gene transfer systems are known. For example, one might reasonably conclude that a given strain of coccus was *unlikely* to be a strain of *Staphylococcus aureus* if it was not lysed by any of numerous *S. aureus* phages, with the background knowledge that most strains of this species are lysed by many of the phages. It would, however, be unjustifiable to conclude this from cross-testing with culture supernatant fluids that one did not know contained any phages. Each case, however, would have to be based on empirical evidence largely derived from prior taxonomic studies, so that the capability of negative findings to advance taxonomic knowledge is obviously severely limited.

Other Forms of Genetic Relationship

Although DNA base pairing and the molar percentage of GC in DNA are outside the main scope of this review, we briefly discuss them here because we have included a good deal of evidence from these sources in the taxonomic sections that follow. A few comments on protein sequences have also been added.

Nucleic acid pairing. Nucleic acid base pairing (sometimes called hybridization or homology, although this is confusing in view of the different meaning of these terms in biology in general) is measured by the extent to which single-stranded nucleic acid molecules from different sources can associate to form double helices. Extensive pairing is due to extensive segments where the base sequence is very similar in the two nucleic acids. The pairing is therefore expressive of genomic relationship. Most often the extent of pairing between DNA from one strain and that from another strain is estimated and is expressed as a percentage of the pairing with DNA from the homologous strain as the control. DNA-RNA pairing can also be performed, employing messenger RNA which is treated as the equivalent of its parental DNA. Reviews and examples of application may be found in several recent articles (127, 130–132, 302, 303, 309).

Good pairing may indicate either (a) that many genes are almost identical (although some may be quite dissimilar) or (b) that most genes are fairly similar (but differ somewhat in DNA sequence). The physical form of the base pairing would differ in these two cases: in the first case there would be tight pairing in some regions but little in others, and in the second case there would be moderate pairing all along the molecules. It is not yet clear how far the pairing is of the one kind or the other, but recent work (354) shows that it is possible (in a comparison between a given pair of strains) to distinguish fractions of DNA that are highly similar in the two strains from fractions that are less similar. There is, therefore, some evidence in favor of the first case (a) above, although probably both kinds of pairing pattern occur to some degree in any given example. The cases (a and b) express somewhat different aspects of genomic relationship, and whether they have different implications for taxonomy does not appear to have been discussed. They certainly have different im-

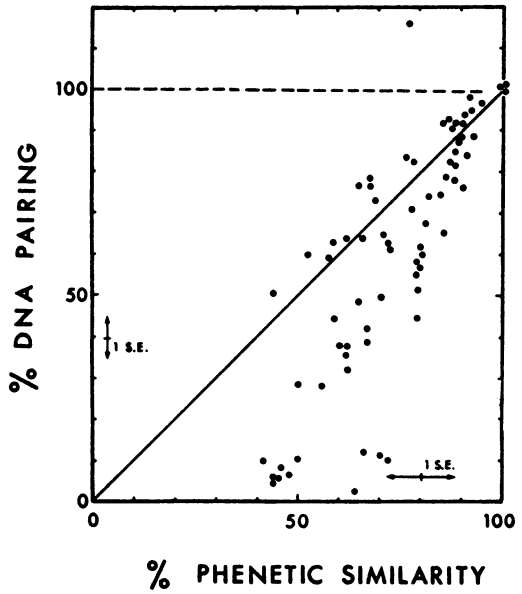

FIG. 1. *Congruence between DNA pairing and phenetic similarity (modified from reference 429). Circles show the per cent DNA pairing and the per cent phenetic similarity of comparisons between strains of the genera Pseudomonas, Xanthomonas, Agrobacterium, Rhizobium, Vibrio, and Chromobacterium. The DNA data are from De Ley et al. (132), Heberlein et al. (206), and Colwell (99), whereas the phenetic data are from Lysenko (294), Heberlein et al. (206), and Colwell (99), shown as closed circles, or are estimated from information of Dye (146, open circles). Although these studies are not strictly comparable, they appear from internal evidence to be reasonably so. Arrows show the standard errors attributable to experimental or statistical data (based on details given by the authors). It can be seen that the scatter is of the same order of magnitude as twice the standard errors (approximating to 95% confidence limits). The highest circle represents an anomalous reaction (greater pairing with a different strain than with the strain that was the source of the DNA).*

plications for cladistics, because pairing of the first type (a) could be used as evidence either (i) that the genes that were almost identical had been recently transferred from one bacterial lineage to the other, or (ii) that they represented unchanged ancestral segments of the genome (although it might be difficult to prove which explanation was correct).

DNA pairing is a direct expression of similarity in base sequences of the genetic material. The phenotype is also an expression of these sequences (although not necessarily an entirely faithful expression). Therefore, there are strong theoretical grounds for believing that DNA pairing and phenetic similarity should be highly correlated. The empirical evidence bears this out. It is a general rule that high pairing is always shown between strains of the same taxospecies: conversely, strains of the same taxospecies always show high nucleic acid pairing. No serious exceptions have yet been reported. Nucleic acid pairing and phenetic similarity estimated by numerical taxonomy are highly concordant (Fig. 1). Similar agreement is reported by De Ley (127) and Heberlein et al. (206) for somewhat different sets of data. The relationship between the two forms of resemblance, however, is not linear. There is very little nucleic acid pairing at phenetic similarities below 50*S*, and on theoretical grounds one might expect this (127, 128); isofunctional enzymes with similar amino acid sequences in the active centers but with different sequences in the rest of the molecules would be likely to give the same phenotypic feature, but their cistrons would not give good base pairing. The figure shows that the points nearly all lie within a band of approximately two standard errors, so that the agreement is as good as one could expect on the data available. It would probably be even better if deliberate comparative studies were made.

Certain technical problems require further study (127) and there are a few observations that need explaining. Thus, the pairing of DNA from *Streptomyces* spores with that from vegetative cells is lower than one might expect (151), and DNA-RNA pairing shows a similar phenomenon (140) in that vegetative cell DNA pairs less well with spore RNA than with vegetative RNA. There may be some nonspecific dependence on GC content (239).

Nucleic acid pairing can be regarded as a technique for estimating phenetic resemblance by physicochemical means. Although it does not provide direct evidence on phenotypic properties, nucleic acid pairing and the more usual phenetic techniques form an extremely powerful pair of methods which can be included in the general framework of numerical taxonomy. Like phenetic resemblances, the nucleic acid resemblances should be further processed by some form of clustering procedure, though it is not yet common practice to use numerical methods.

Protein sequences. Although few data are yet available (*see* 10, 11, 120), studies on protein sequences of bacteria are now growing in number. Comparison of the sequences of isofunctional proteins in different bacterial species can provide evidence of genomic relationship, by giving a reflection of the fine structure of the genes that code for the proteins. As data become available, one expects that similarities based on protein sequences and those from phenetics and DNA pairing will be found to be concordant, but it

should be noted that a single protein consists of only a very small portion of the genome, so that statistical sampling error must be considerable. Furthermore, different families of proteins show much variation in the absolute values of similarities. Protein sequences allow comparisons across very wide taxonomic gaps, where other data are difficult to obtain; for example, recognizable similarity can be found between sequences from organisms as different as man and yeast (120).

GC ratios. The taxonomic significance of the mean base composition of DNA expressed as the moles per cent of GC (%GC) has been discussed in several recent articles (127, 128, 302, 303). General reviews of the ratios observed in different bacteria are those of Hill (215), Rosypal and Rosypalová (394), and De Ley (128). Only *differences* in %GC are significant, and they indicate genomic difference. Similarity in %GC does not indicate genomic similarity; thus, the GC ratios of the pneumococcus, *Proteus mirabilis* and man are all about the same. De Ley (128) calculated the limits of %GC difference that can occur if two organisms share an appreciable number of identical or almost identical cistrons. He estimates that a difference of 20 to 30% in GC ratio must mean that there are practically no nucleotide sequences in common to the two bacteria. There is, therefore, a firm theoretical basis for the observation that highly similar organisms must have very close GC ratios; if the %GC difference is substantial, then the base sequence must be markedly different. Although a given protein may have identical functions and the same linkage positions on the chromosome of two organisms, if the organisms differ in %GC the amino acid sequences of the two proteins must be notably different (153).

Homogeneous phenetic groups are also homogeneous in %GC (e.g., 101, 103, 132, 416). The variation among strains of well-established taxospecies is quite small. It is usually reported as a range of about 2.5% (rarely greater than 4%), but would be better expressed as a standard deviation of the mean %GC of the DNA fragments (*see* 128, in which is discussed also the implications of the variation in GC content of the DNA fragments from one strain). Such standard deviations appear to be usually about 1% for strains of a taxospecies. As noted above, groups that are homogeneous in %GC may yet be heterogeneous phenetically. However, those groups that are heterogeneous in %GC are always also heterogeneous phenetically. Certain genera, such as *Vibrio* and *Flavobacterium*, have a very wide range of %GC, and this has been an extremely useful pointer to the need for taxonomic revision. In a number of cases, subsequent study has led to the splitting of the genus into several, and the work of Véron and Sebald provides one example (409, 469). The discovery in a set of strains of two subgroups with different GC ratios is a clear indication that there are at least two different phenetic groups. A difference in %GC of 5% usually implies at least a species difference, whereas a difference of less than 2% is seldom of taxonomic significance (128, 431).

GC ratios have implications for gene transfer, because if a gene is to integrate into the chromosome of the recipient it must have a sufficient similarity in nucleotide sequence with the corresponding region of the chromosome. This in turn requires a similar GC ratio. We should note, however, that in theory this similarity in sequence and %GC need only be quite local, and it may be that gene transfer mechanisms do from time to time lead to fragments of very different GC composition getting integrated as rare events.

It is seen from the data reported later that, in general, gene transfer is easier between organisms of very similar GC ratios than between those with different ratios, as expected. This does not hold so well for plasmid transfer as for transformation, which is not surprising because plasmid infection does not require integration. However, even with transformation, gene transfer can occur despite GC differences of up to 3.5% (81). It is not clear why bacteria differ so much in GC ratios compared with other organisms. Small but significant changes in %GC have been observed on mutation (125, 127), and Yanofsky et al. (480) have proposed a possible mechanism for systematic changes in GC composition based on the predominant direction of nucleotide base changes brought about by mutator genes.

TAXA CONSIDERED

Reports on genetic reactions between strains of the same taxospecies of bacteria are exceedingly numerous; therefore, we have restricted this review to reactions between species. We have noted, however, some reports on bacteriophages or genetic systems in groups that are not yet very well known, as these may be useful to workers wishing to extend genetical studies to new taxa. A very full bibliography of bacteriophages for diverse bacteria is that of Raettig (369), though it is now some years old. Bacteriophages of plant pathogens are reviewed by Okabe and Goto (342).

The lowest taxonomic unit considered, therefore, is the species category, and we have, in general, followed *Bergey's Manual* (62) in deciding what species are taken as valid, i.e., as taxospecies. There are some genera, such as *Xanthomonas*, *Pseudomonas*, and *Streptomyces*, within

which there are a great many named species of doubtful validity, and here (and also occasionally in other genera) the species considered are nomenspecies. Again, *Bergey's Manual* has been followed in general in the treatment of genera, but when these are clearly very heterogeneous more recent generic names have often been used. Certain generic names have been annotated as "in part," to indicate that only some of what may be a collection of unrelated organisms are involved in a reported genetic interaction; the species directly concerned in such cases are given in the most detailed of the figures, but to list all the species that plausibly belong to such a part of a genus would involve much taxonomic speculation, so the reader is referred to the original publication for further details.

CONVENTIONS AND SYMBOLS

Most of the evidence is presented in figures, which show in a compact form the bacterial taxa and the gene transfers that have been reported in the literature. When available, the GC ratios have been shown as percentages, and unless otherwise stated these are from existing reviews (128, 215, 394). The identity of named strains is sometimes in doubt, and when this seems probable annotations have been added; however, it is likely that a number of other misidentifications have occurred which cannot now be checked.

The numbers of the references in the bibliography are noted on the figures, together with a symbol to indicate the kind of gene transfer reported. The direction of transfer is shown by the arrowheads, and the symbols are listed below. (Tf) Transformation by soluble DNA (with integration into genome of the recipient presumed). (Td) Transduction of a host gene by a phage (and with integration into the host genome presumed). (P) Phage multiplication, i.e., lysis of the recipient strain. Multiple hit lysis, or killing without lysis, is excluded when this can be decided from the evidence presented. Unfortunately, such evidence is not always given, and bacteriocin action also cannot always be excluded. (L) Lysogenization of the recipient strain by phage when this is explicitly recorded. Very probably, many of the reactions labeled P also involved lysogenization, but no clear evidence is available in most of the reports. (E) Episome or plasmid transfer by infection (other than phages), including both the transfer of episomes or plasmids alone (F, R, or colicinogenic factors) and the transfer of a nonepisomal gene by an episome. In almost every case, no evidence is presented that the gene or plasmid becomes integrated into the host chromosome. (R) Recombination following conjugation, with transfer of at least some bacterial genes from donor to recipient. Integration is presumed, but it is possible that in a few cases the genetic mechanism was not conjugation with recombination but was gene transfer by a plasmid.

When several names are included in one box, this indicates that numerous cross-reactions have been reported between these organisms (which could not be shown by arrows without confusion). The details can be obtained from the references listed in the box. When fewer cross-reactions have been reported, the organisms are separated and connected by arrows.

The figures are arranged as follows. Figure 2 shows the broad outline of bacterial taxa as indicated by gene transfer data. Since this only includes those taxa in which genetic studies have been made, Fig. 3 shows our own idea of the broad outline of taxonomic groups in bacteria, in which additional genera are added although genetic evidence is not yet available for them. These figures are followed by more detailed figures of different areas of Fig. 2, and these form Fig. 4–20.

BROAD OUTLINE OF BACTERIAL GROUPINGS

Figure 2 shows a summary at the genus level of the gene transfer data which are discussed at greater length in the next section. Numerous isolated species have been omitted, because although they show intraspecies relationships (particularly cross-reactions of different strains with phage) no gene transfer has been reported between them and other species. The arrangement of the boxes is an attempt to reflect the major phenetic relationships and this is based on an earlier figure of this kind (429).

The genetic evidence shown in Fig. 2 may be placed in better perspective by comparing it with Fig. 3. This figure contains, in addition, most of the well-known genera of bacteria which, in our own view, satisfy the following two criteria: (i) there is reasonable evidence that they are homogeneous natural taxa, and (ii) there is sufficient evidence to allow them to be placed with some confidence in one of the general areas of the figure (although their detailed relations to near neighbors are often not known). This figure is based on numerous sources (mainly 62, 166, 428, 429, 432, with additional information in particular from 131, 132, 323, 327, 454, 463). It is not possible to show all the significant taxonomic relationships in two dimensions, so the positions should be taken only as a rough indication. We must re-emphasize that Fig. 3 represents our personal views, and is based on far less evidence than one would wish. Groups that are clearly

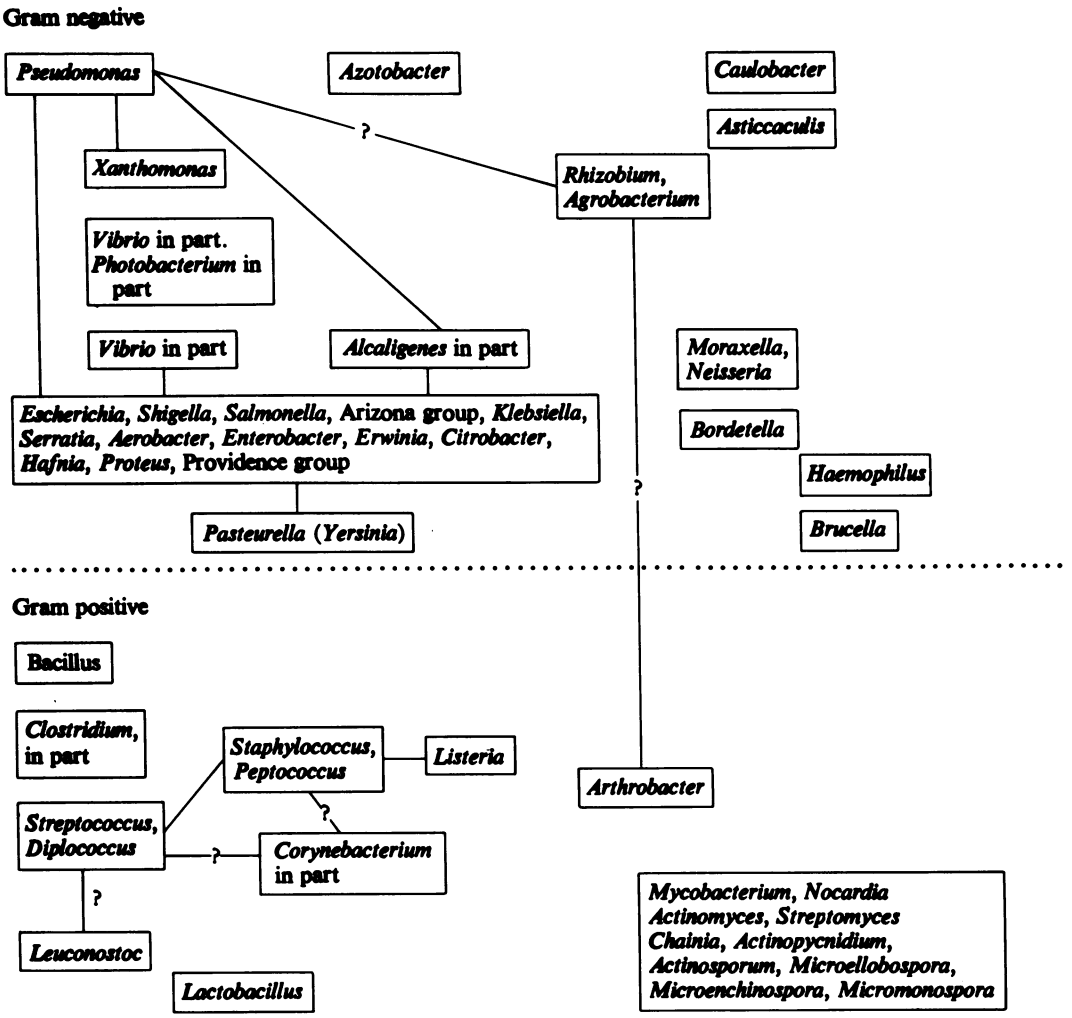

FIG. 2. *Major genetic groups of bacteria. The inclusion of several genera in one box indicates that numerous genetic cross-relations have been demonstrated among them. Lines joining boxes indicate that some genetic relationships have been found, although sometimes the evidence is dubious. The positioning of the genera is an attempt to reflect the main phenetic relationships between them. This, however, is often based on scanty evidence, and should be considered somewhat speculative.*

isolated and unlike the familar forms of bacteria (e.g., spirochetes) have been placed well to the right; however they are not necessarily closely related to one another.

A number of major groups have not been divided into genera because too little is known about their taxonomy. The *Mycoplasmatales* are shown as a separate order, as there is no firm evidence that they are permanent L-forms of more familiar bacteria (314, 436). The mycoplasmas are not all closely related on DNA pairing (382), though reasonably homogeneous in GC content [27 to 47% (167, 215)], and the same is so for *Leptospira* [GC 35 to 40% (194)].

Several genera (e.g., *Vibrio*, *Corynebacterium*) are quite heterogeneous, so that some species that are at present included in them may later be found to belong elsewhere in the diagram. In particular, the relationships between the gram-positive genera are obscure. The work of Barnes and Goldberg (31) suggests that Bacteroidaceae (e.g., *Bacteroides*, *Fusiformis*) requires reexamining in relation to *Clostridium*. The systematic position of *Listeria* (GC 38%) is also a puzzle, as it shares some properties of several gram-positive genera.

In the area of the *Pseudomonadales*, DNA pairing studies have helped to clarify the relations (132). *Acetobacter* (GC 54 to 64%) appears homogeneous on these grounds (124, 130), with several named species very close to one another,

Gram negative

Halobacterium (GC 55–68)
Thiobacillus (GC 58–70)
Methanomonas

Pseudomonas (GC 36–70)
Xanthomonas (GC 62–68)

Chromobacterium (GC 61–72)

Azotobacter (GC 54–66)
Beijerinkia (GC 54–59)
Derxia (GC 64–72)

Aeromonas (GC 51–63)
Vibrio in part (GC 44–46)
Photobacterium (GC 43–47)

Zymomonas
Acetobacter (GC 54–56)
Gluconobacter (GC 57–64)

Vibrio in part (GC 46–49)

Alcaligenes in part (GC 67–70)

Escherichia (GC 50–52) *Shigella* (GC 49–54) *Salmonella* (GC 50–54)
Arizona group (GC 53) *Klebsiella* (GC 52–59) *Serratia* (GC 54–63)
Aerobacter (GC 50–59) *Enterobacter* (GC 52–54) *Erwinia* (GC 50–57)
Citrobacter (GC 50–53) *Hafnia Proteus* (GC 36–53)
Providence group (GC 40–42)

Pasteurella (*Yersinia*) (GC 46–48)

Rhodospirillum (GC 60–67)

Caulobacter (GC 62–67)
Asticcacaulis (GC 55)

Rhizobium (GC 59–66)
Agrobacterium (GC 58–66)

Cytophaga (GC 31–42)
Flavobacterium in part (GC 32–42)

Acinetobacter in part (GC 41–43)
Neisseria (GC 40–52)

Moraxella (GC 39–45)

Actinobacillus in part (GC 41–43)
Pasteurella in part (GC 35–40)
Bordetella (GC 68–69)
Haemophilus (GC 38–42)
Brucella (GC 55–58)

Blue-green algae (*Cyanophyta*) (GC 39–51)

Myxobacteriales (GC 67–70)

Spirochaetales (GC 34–46)

Rickettsiales (GC 30–46)

Mycoplasmatales (GC 27–47)

Gram positive

Lineola (GC 42)			
Bacillus (GC 32–66)	*Sporosarcina* (GC 38–42)	*Staphylococcus* (GC 30–40)	*Micrococcus* (GC 62–74)
		Peptococcus	*Sarcina* in part (GC 54–73)
Clostridium (GC 25–45)	*Sarcina* in part		
	Streptococcus (GC 33–42)	*Listeria* (GC 38)	
	Leuconostoc (GC 39–42)	*Microbacterium* in part	
		Erysipelothrix	
	Lactobacillus (GC 28–64)		*Kurthia*
		Propionibacterium (GC 66–71)	
		Microbacterium in part	*Arthrobacter* (GC 62–64)
	Streptobacillus	*Corynebacterium* in part (GC 48–60)	*Corynebacterium* in part (GC 52–59)
			Mycobacterium (GC 62–73)
			Nocardia (GC 62–72)
		Actinomyces (GC 66–67)	*Streptomyces* (GC 67–78)
			Chainia
			Actinopycnidium
			Actinosporium
			Microellobospora
			Microechinospora
			Micromonospora

FIG. 3. *Salient phenetic relation between genera of bacteria. The GC ratios are taken from references 128, 215, and 394 with additional data from 38, 147, 259, 268, 323, and 332.*

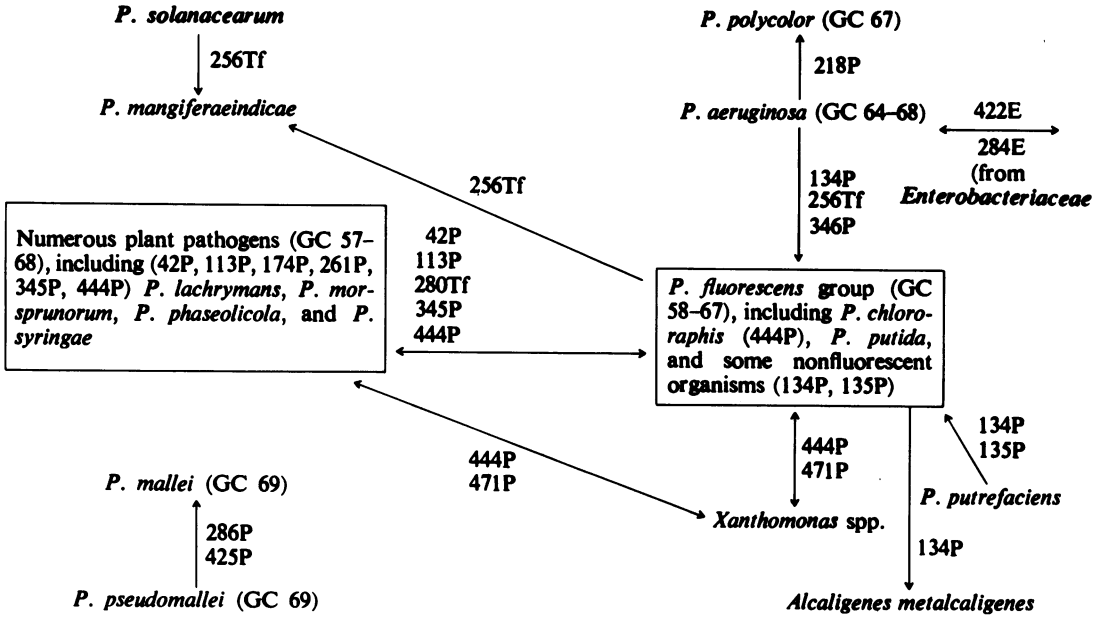

FIG. 4. *Pseudomonas*.

but *Gluconobacter* (GC 57 to 64%) is distinct though probably fairly closely allied to *Acetobacter*. A phage for *Hydrogenomonas facilis* has recently been reported (364). The rather scanty evidence on the photosynthetic bacteria suggests they are closest to the *Pseudomonadales*, but this is quite speculative.

The *Myxobacterales* are now arousing new interest (238, 240, 313). Their GC ratios vary widely (32 to 71%) and among them the cytophagas may be allied to some flavobacteria (163, 295, 323). Possibly *Cytophaga* and *Sporocytophaga* are closely allied (295). Phages for *Cytophaga* species and for *Chondrococcus columnaris* have been reported (12, 100).

The blue-green algae, also procaryotes, have been shown in Fig. 3 as well separated from the bacteria, but it is possible that they connect with them through colorless intermediate forms. DNA pairing studies are just beginning (112) in attempts to relate blue-green algae with bacteria and with chloroplasts of green plants.

Apart from the generally high GC ratios of the *Actinomycetales*, there is little obvious correlation of GC ratios with systematic position, though further correlations may be established with new work on the groups on the extreme right of Fig. 3. The *Actinomycetales* are unusual also in that they appear, at least superficially, to be phenetically rather variable, but despite this gene transfer occurs readily. There is very general agreement between genetic and phenetic groups where data of both kinds are available. Most genetic groups are good phenetic taxa, and when genetic systems are discovered in good phenetic taxa these taxa have usually also been found to be coherent genetic groups.

MAJOR GROUPS

The major groups in Fig. 2 are shown here in greater detail, with brief comments on each. The gram-negative groups are shown in Fig. 4–14, and the gram-positive groups in Fig. 15–20.

Pseudomonas (Fig. 4)

The genus *Pseudomonas* is somewhat heterogeneous (for general reviews, *see* 132, 294, 446), and genetic studies have only been made on a few of the major groups within it. The phytopathogenic named species (with the exception of some such as *P. solancearum*) are increasingly regarded (446) as forma speciales in the fluorescent group [itself not very clearly subdivided (236)]. The very close resemblance between plant pathogens and free-living forms is supported by the extensive cross-reactions with bacteriophage; phages usually do not distinguish between them, and there are few phages that are specific for particular plant forms of pseudomonads (113, 444).

DNA pairing shows close relationship between *P. multivorans*, *P. mallei*, and *P. pseudomallei* (389) and their GC ratios are also similar (394). Conjugation with recombination has been studied in *P. aeruginosa* (219, 222, 290), and transducing phages are reported for *P. aeruginosa* (221) and

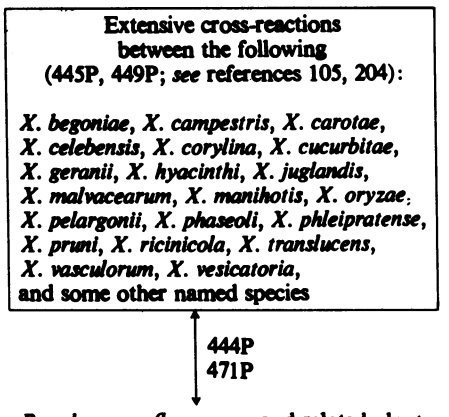

FIG. 5. *Xanthomonas* (*GC 62–68*).

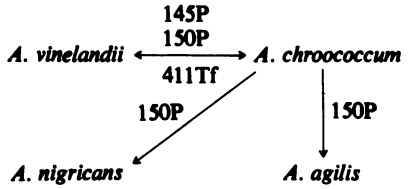

FIG. 6. *Azotobacter* (*GC 54–66*).

P. putida (223). DNA pairing shows a fairly close resemblance between *Pseudomonas* and *Xanthomonas*, and moderate resemblance between *Pseudomonas* and the genera *Azotobacter*, *Beijerinckia*, and *Derxia* (131, 132). *Beijerinckia* may be fairly close to the *Rhizobium-Agrobacterium* group (129). The transfer of R factors between coliforms and *Pseudomonas* (284, 422) is particularly interesting as being the first genetic evidence linking the two large areas of the pseudomonads and enterobacteria.

Xanthomonas (Fig. 5)

It is now widely considered that the many nomenspecies of *Xanthomonas* (named according to the host plant) should be reduced to one taxospecies with some varieties or forma speciales (132, 146, 445, 446), with a few exceptions such as *X. stewartii*. There is high DNA pairing between nomenspecies of this genus (132). *Xanthomonas* phages are sometimes specific for certain plant forms, but more usually they cross-react extensively (204, 445, 449). A preliminary account has appeared of resemblances based on phage susceptibility as the characters (105), and these resemblances show some correspondence with the resemblances based on other phenotypic characters. Transformation has been reported in this genus (108, 109). The organism known as *X. uredovorus* appears to belong to *Erwinia* (196, 197, 205, 445) and is included here with the *Enterobacteriaceae*.

Azotobacter (Fig. 6)

There is some evidence from DNA pairing that *Azotobacter* is a fairly homogeneous group (though *A. agilis* may be distinct), but that *Beijerinckia* and *Derxia* are more distant (131).

Caulobacteraceae (Fig. 7)

The position of the *Caulobacteraceae* is not very clear, but they seem on general properties to be closest to the pseudomonads. There are evidently a number of different genera which await more detailed study. Present knowledge is reviewed by Poindexter (362).

Vibrio (in part) and Photobacterium (Fig. 8)

The genus *Vibrio* is phenetically very heterogeneous (118) and its taxonomy is at present poorly known. The GC ratios vary widely, from 29 to 36% in the *V. fetus* group [sometimes called *Camplobacter* (*see* 469)] to 63 to 65% for the *V. alcaligenes* group (102, 409, 469). The type species of *Vibrio*, *V. cholerae*, is closely allied to the *Enterobacteriaceae* and is discussed there; the *V. cholerae* group will therefore retain the generic name *Vibrio*, whereas other groups will be allocated to existing genera or receive new generic names when the present genus is thoroughly revised. DNA pairing studies suggest that the *V. fetus* group is not very homogeneous (34).

The phage cross-reaction between *V. parahaemolyticus* and a luminous bacterium identified as *Photobacterium sepiae* (334) supports the growing view (22, 437, 467) that there is a natural group of organisms (perhaps allied to *Aero-*

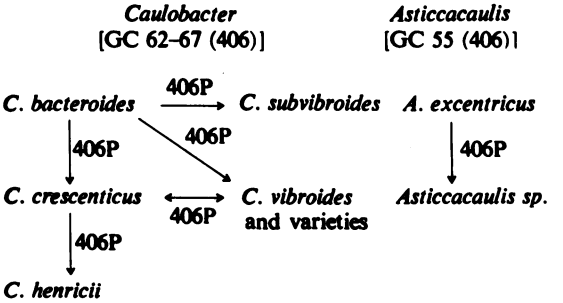

FIG. 7. *Caulobacteraceae*.

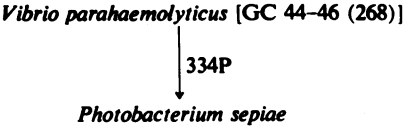

FIG. 8. *Vibrio* (*in part*) *and Photobacterium*.

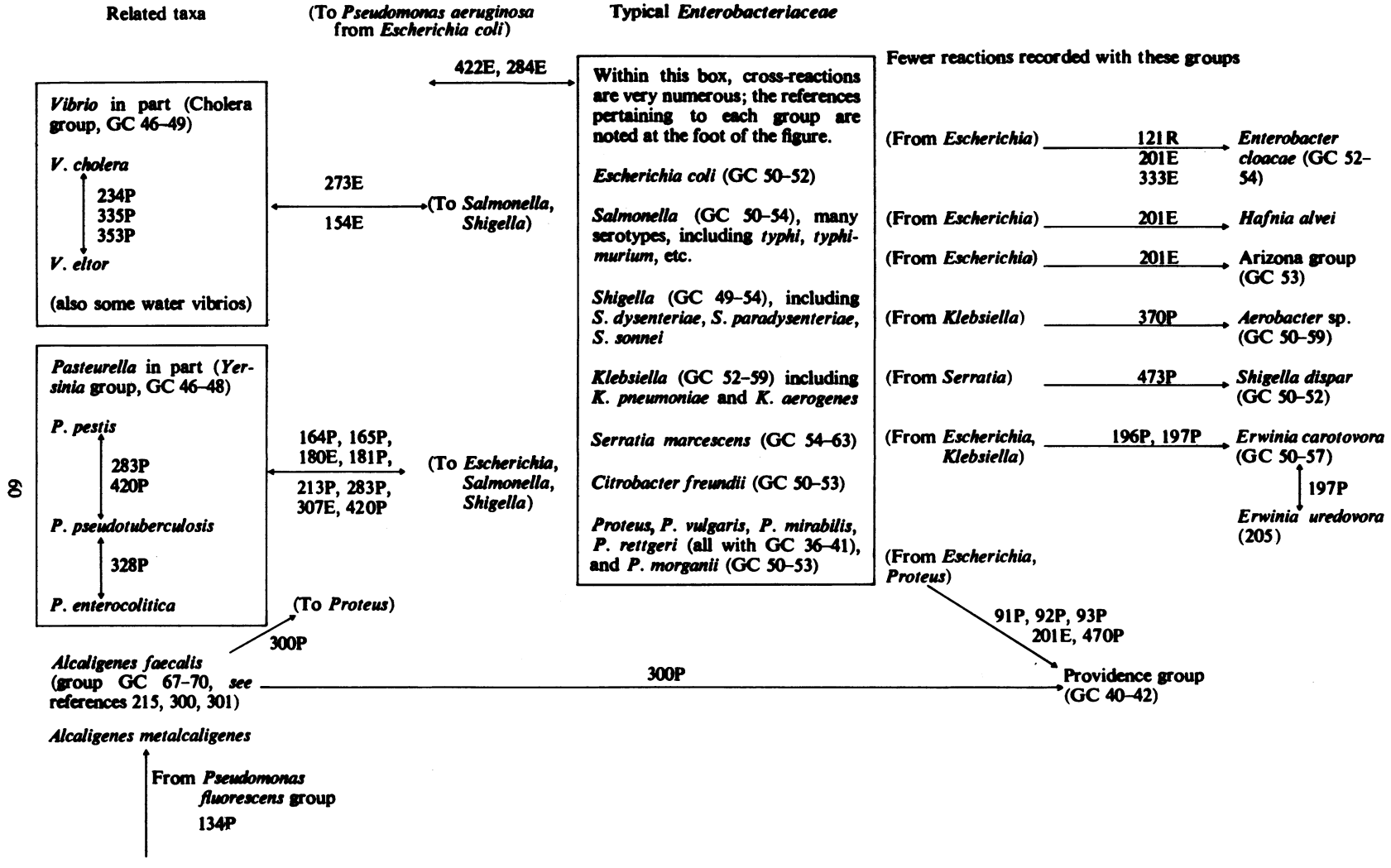

FIG. 9. *Enterobacteriaceae and related taxa. References pertaining to reactions between groups in the box labeled "Typical Enterobacteriaceae": Escherichia coli: 5P, 32R, 33R, 91P, 92P, 122E, 137R, 154E, 158E, 159E, 170E, 183P, 191R, 201E, 225P, 229P, 237R, 273E, 278R, 284E, 289Td, 292Td, 293R,P, 325E, 326R, 333E, 343Td, 352E, 370P, 407R, 472E, 473P, 477E, 478R, 484R; Salmonella: 33R, 115E, 137R, 154E, 158E, 159E, 183P, 191R, 201E, 229P, 237R, 273E, 284E, 326R, 343Td, 352E, 472E, 477E, 485Td; Shigella: 5P, 91P, 115E, 154E, 170E, 201E, 273E, 278R, 289Td, 292Td, 293R,P, 325E, 407R, 473P, 477E, 484R; Klebsiella: 90Td, 122E, 201E, 284E, 333E, 370P; Serratia: 5P, 90Td, 91P, 154E, 159E, 229P, 473P; Citrobacter: 183P, 201E, 325E, 333E, 352E; Proteus: 32R, 91P, 92P, 158E, 159E, 201E, 225P, 333E, 478R.*

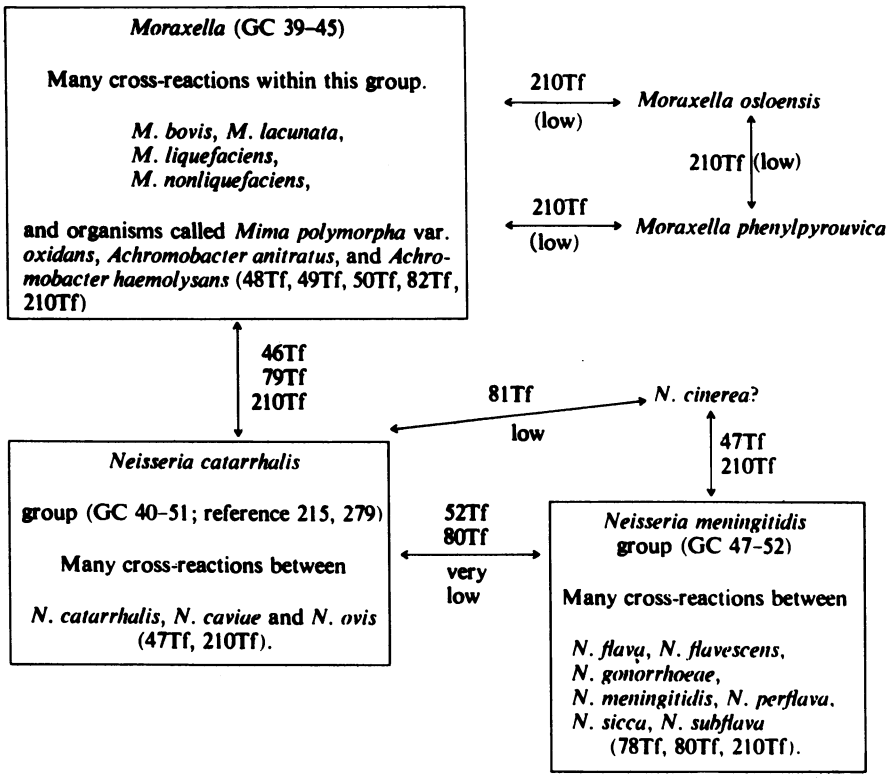

FIG. 10. *Moraxella and Neisseria.*

monas), some of which are luminous, and the relationship of these to other vibrios is problematic.

Enterobacteriaceae and Allied Taxa (Fig. 9)

It can be seen from Fig. 9 that gene transfer has been reported on numerous occasions between typical members of the *Enterobacteriaceae*, and there are many other reports which we have not cited that duplicate those noted in the figure. Genetic cross-reactions within the family have been reviewed earlier (303). It was noted in an earlier section that gene linkage maps are extremely similar in several genera. The family seems relatively heterogeneous on DNA pairing studies (63), though further work is needed here.

The genus *Erwinia* consists of two main groups, the group containing the type species *E. amylovora* (causing fire-blight, wilts, and dry necroses of plants) and the *E. carotovora* group (the soft-rot organisms, sometimes regarded as a genus *Pectobacterium*), and in addition some aberrant and poorly known forms (for discussion *see* 43, 439). Both groups have similar GC ratios around 50 to 58% (215, 394, 439). The reported gene transfers from *Enterobacteriaceae* have been to the *E. carotovora* group. Phages for *E. amylovora* have been found to lyse certain poorly known organisms similar to the nonpathogen *E. trifolii* (207). It was noted earlier that *X. uredovorus* is an *Erwinia*.

Of the other genera that are not at present usually included in the *Enterobacteriaceae*, the part of the genus *Vibrio* that contains the cholera bacillus and the part of *Pasteurella* that contains the plague bacillus (now commonly called *Yersinia*) might well be included in this family. The *Yersinia* group (GC 45 to 50%) contains the organisms usually called *Pasteurella pestis*, *P. pseudotuberculosis* and *P. enterocolitica*, and these three are similar phenetically (442) and on DNA pairing (387); these two studies support the relative closeness of *Yersinia* to the coliforms (and also their separation from the other main group in *Pasteurella*, which is discussed later in this section). *V. cholerae* possesses a conjugation system similar to that of the coliforms (40).

The taxonomy of *Alcaligenes* is not well known; some forms may be closer to *Vibrio* or *Moraxella* (460, 469). From details given in several papers (215, 300, 301), it seems that the form which shows phage cross-reactions with *Proteus* and the Providence group has the high GC ratio of 67 to 70%.

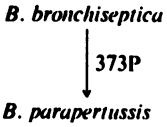

FIG. 11. *Bordetella* (*GC 68–69*).

Moraxella and Neisseria (Fig. 10)

The classification of the group of genera that include *Moraxella*, *Neisseria*, *Achromobacter*, and *Acinetobacter* is still confused, but recent work (35, 36, 211, 361, 463) suggests there are at least five main phenetic groups: (i) the *Moraxella lacunata* group (*M. lacunata*, *M. bovis*, and *M. nonliquefaciens*); (ii) the *M. lwoffi* group; (iii) the *Neisseria catarrhalis* group (*N. catarrhalis*, *N. caviae*, and *N. ovis*); (iv) the *Acinetobacter anitratum* group; and (v) the *Neisseria meningitidis* group (*N. meningitidis*, *N. gonorrhoeae*, and several other species). In addition, there are organisms such as *M. osloensis*, *M. kingii*, and *M. phenylpyrouvica* that are distict on serological and genetic criteria (210, 211, 224). Besides these, there are other organisms loosely referred to as *Achromobacter* or *Acinetobacter* (126, 128, 463). The identity of organisms known as *Mima* and *Herellea* is confused.

The close similarity between the members of the *M. lacunata* group is supported by serology, and they appear distinct serologically from the other groups (224). There are serological cross-reactions between *M. lwoffi* and *Acinetobacter anitratum* (224), and it is not yet clear how distinct these groups are from one another. The GC ratios of *Moraxella* species and *A. anitratum* are much the same (126, 128, 211, 215), ranging from 39 to 45%. The ill-defined genera *Achromobacter* and *Acinetobacter* also contain other organisms with very different GC ratios (53, 126, 128).

Genetic evidence supports the close relationship between the *M. lacunata* group and the *N. catarrhalis* group which is indicated by phenetic studies (35, 211, 463). DNA pairing studies (257, 258) show that the *N. meningitidis* group is homogeneous and distinct from the other major groups. Transformation is usually easiest between strains of similar GC content, but some exceptions have been noted (81). A transformation system and a transducing phage for *A. anitratum* have been described (250, 466), and phages are known for *Neisseria* (76, 360). Evidence has been adduced for low but definite DNA pairing between *Neisseria* and the psittacosis-trachoma organisms (259).

Bordetella, Haemophilus, Brucella and Pasteurella in part (Fig. 11–13)

These genera are conveniently taken together, as they seem to be rather similar. Serological cross-reactions have been reported between *Brucella* and *Yersinia enterocolitica* (6), so that their relations to one another and to the enterobacteria *Moraxella* and *Neisseria* require clarification. There has been much genetic work on *Haemophilus*, and the gene linkage order in various species has been investigated (336). *Brucella melitensis* is more difficult to infect with the phages available at present (70, 355) than the other *Brucella* species shown. DNA pairing shows that *B. melitensis*, *B. abortus*, *B. suis*, *B. neotomae*, and *B. ovis* are all closely related (226, 227).

Pasteurella has been mentioned as heterogeneous, with the *Yersinia* group closely allied to the *Enterobacteriaceae*. The other major group, containing *Pasteurella multocida* (GC 35 to 40%) and allied forms, may be closer to *Brucella*, *Bordetella*, and *Haemophilus*, though critical evidence is lacking. Phages have been described for this group (283, 385). Henriksen has described transformation in it, and his preliminary results (209) suggest that a very low rate of transformation may occur between *P. multocida*, *P. haemolytica*, *P. ureae*, and *P. pneumotropica* [which are close phenetically and on nucleic acid pairing evidence (41, 442)]. It is likely that *Actinobacillus lignierisi* is allied to this group (332). The positions of *P. tularensis* and *P. novicida* are uncer-

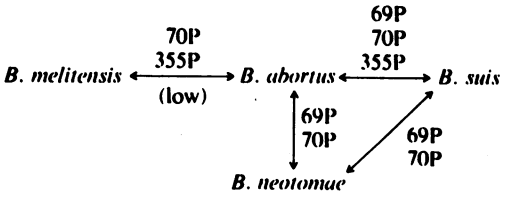

FIG. 13. *Brucella* (*GC 55–58*).

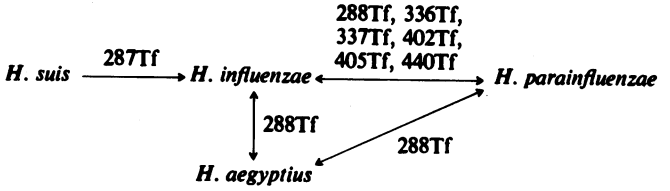

FIG. 12. *Haemophilus* (*GC 38–42*).

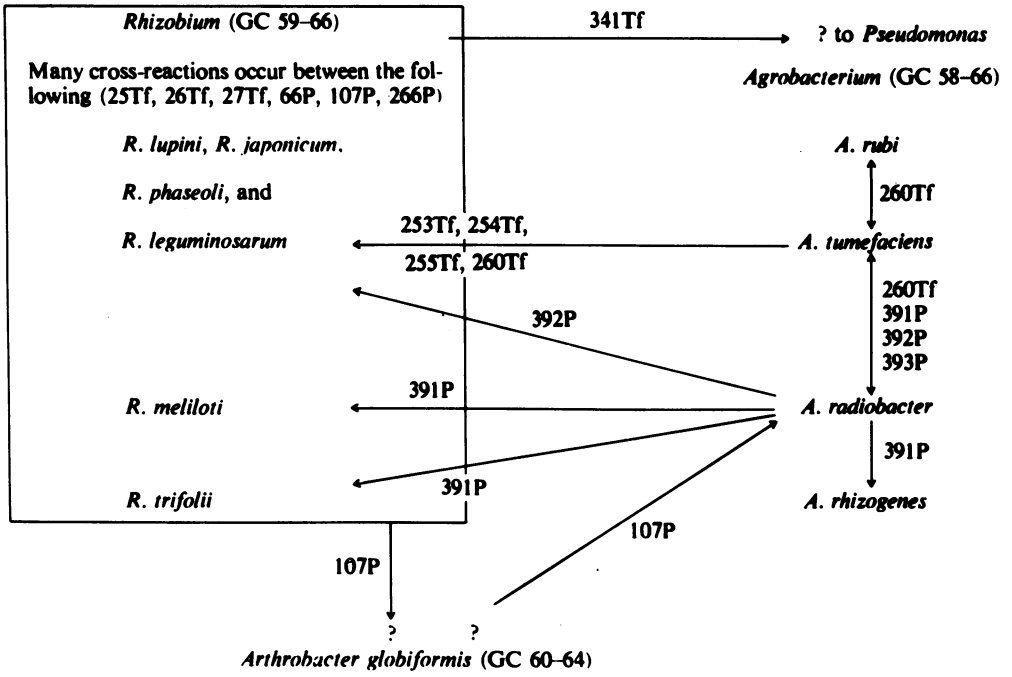

FIG. 14. *Rhizobium and Agrobacterium.*

tain; as judged by DNA pairing, they are close to one another, but not close to *P. multocida*, *Yersinia*, or the coliforms (387). Transformation has been reported in *P. novicida* (468).

Rhizobium and Agrobacterium (Fig. 14)

The close relationship of *Rhizobium* and *Agrobacterium* indicated by the genetic data is in agreement with phenetic and DNA pairing studies (129, 186, 206, 327), and some consolidation of the named species in both these genera is indicated, or possibly the merging of the two genera. There is some evidence from these studies that the forms known as *Rhizobium japonicum* and *R. meliloti* may be phenetically distinct from *R. leguminosarum* and other rhizobia (such as *R. trifolii*), but no clear distinction is seen from the phage and transformation work. However, *R. japonicum* has a slightly higher GC ratio than the other rhizobia (133), and DNA pairing also suggests it is somewhat different (206, 479). Transduction has been reported in *Rhizobium* (265).

The phage cross-reactions between the *Rhizobium-Agrobacterium* group and *Arthrobacter globiformis* (107) are the only ones so far recorded between gram-negative and gram-positive groups, and this requires confirmation. Several phages for *Arthrobacter* species are known (177, 388). Also in need of confirmation is reported transformation between *Rhizobium* and a soil *Pseudomonas* cited in the review of Nutman (341), of which we have not seen the original report.

Bacillus and Clostridium (Fig. 15 and 16)

The genus *Bacillus* is not very homogeneous as judged by nucleic acid pairing studies (140, 453). The sporing sarcinae (*Sporosarcina ureae*) appear closer to *Bacillus* than to the common sarcinae and micrococci as judged by DNA pairing (212). Another puzzling organism that probably belongs here is *Lineola longa* (38). Transducing phages appear fairly common in *Bacillus*. Species-specific phages for *B. polymyxa* have been reported (169). The data on some phage cross-reactions (390) are not very full, as the technique used only demonstrated inhibition of growth.

Little genetic work has been done with *Clostridium*, but phages have been described for *C. sporogenes* (39, 172), *C. tetani* (111), and *C. perfringens* (270). Early records on *Clostridium* phages were reviewed by McClung (312).

Streptococcus, Staphylococcus, Peptococcus, Leuconostoc, and Listeria (Fig. 17)

Streptococcal classification is currently undergoing considerable revision (97, 98), and the phenetic groups within the genus are not very clear. The pneumococcus is now generally included as *Streptococcus pneumoniae*. In vivo transformation of pneumococci has been reported

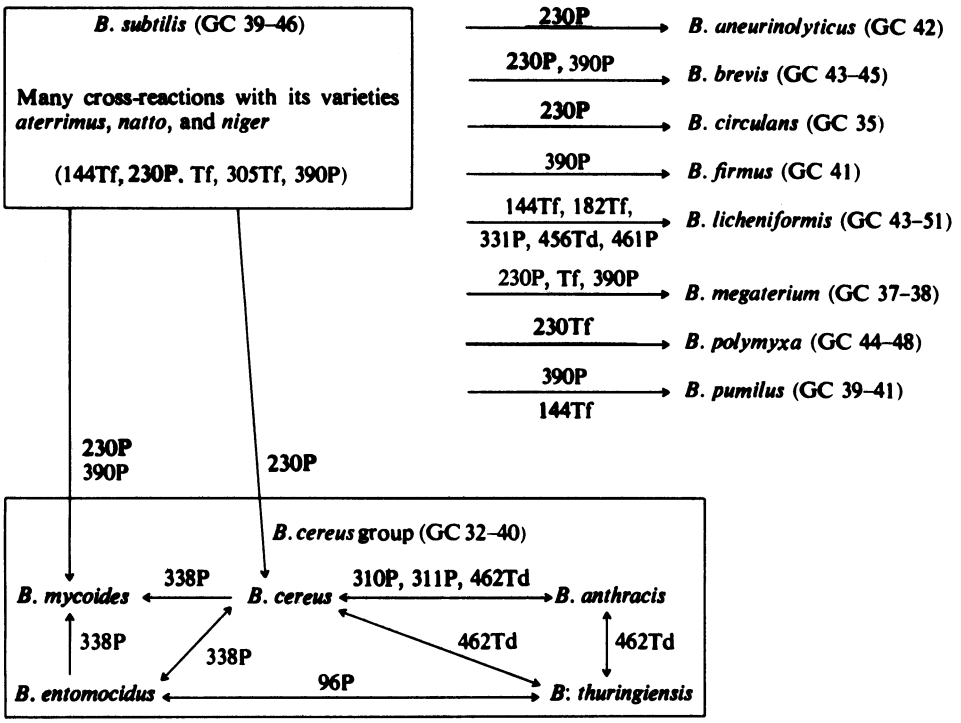

FIG. 15. *Bacillus*.

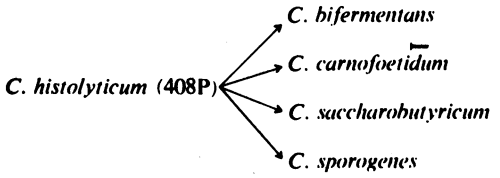

FIG. 16. *Clostridium (GC 25–45)*.

(106, 347), confirming the original work of Griffith (189). Transformation can readily occur between the traditional four main divisions of the streptococci, the haemolytic, viridans, enterococcus, and lactic groups. The identity of the strain called *Leuconostoc mesenteroides* (strain P60) is not certain (64) for it could be an atypical member of the enterococcus group (serological group D).

There is doubt about the identity of some of the organisms listed in *Staphylococcus*. It seems fairly clear that the organisms shown as *S. epidermidis* must have belonged to that species, although they were usually referred to as *S. albus* or coagulase-negative staphylococci in the original publications. The identity of the *S. lactis* (193) is not so clear, as there may be various organisms under this name (23). The peptococci are very poorly known; the named species reported (44) include organisms called *Peptococcus activus*, *P. aerogenes*, *P. asaccharolyticus*, *P. niger* (the type species), *P. prevotii*, and *P. saccharolyticus*, but we have not thought these sufficiently distinct to put these names in the diagram. Some anaerobic sarcinas have a low GC ratio (73) and may be related to *Peptococcus*.

The phage cross-reaction between *Staphylococcus aureus* and *Listeria monocytogenes* is of some interest because of other evidence relating *Listeria* more closely to the lactobacilli or corynebacteria (117, 187, 410). However, the staphylococcal phages were unable to lysogenize *Listeria* (Sword, cited in 410). Phages for *Listeria* have been reported (187, 451). Transduction is known (142, 330, 340) and transformation has been reported (178) in *Staphylococcus*; plasmids are known in this genus (340, 383) and perhaps also occur in *Streptococcus* (264, 380).

Lactobacillus and Corynebacterium (Fig. 18 and 19)

It can be seen from Fig. 18 that phage cross-reactions occur between members of all the three subgenera into which *Lactobacillus* is sometimes divided. These are (i) the homofermentative thermophils (*Thermobacterium*, GC 32 to 53%, e.g., *L. acidophilus*); (ii) the homofermentative mesophils (Streptobacterium, GC 43 to 48%, e.g., *L. plantarum*, *L. casei*); and (iii) the hetero-

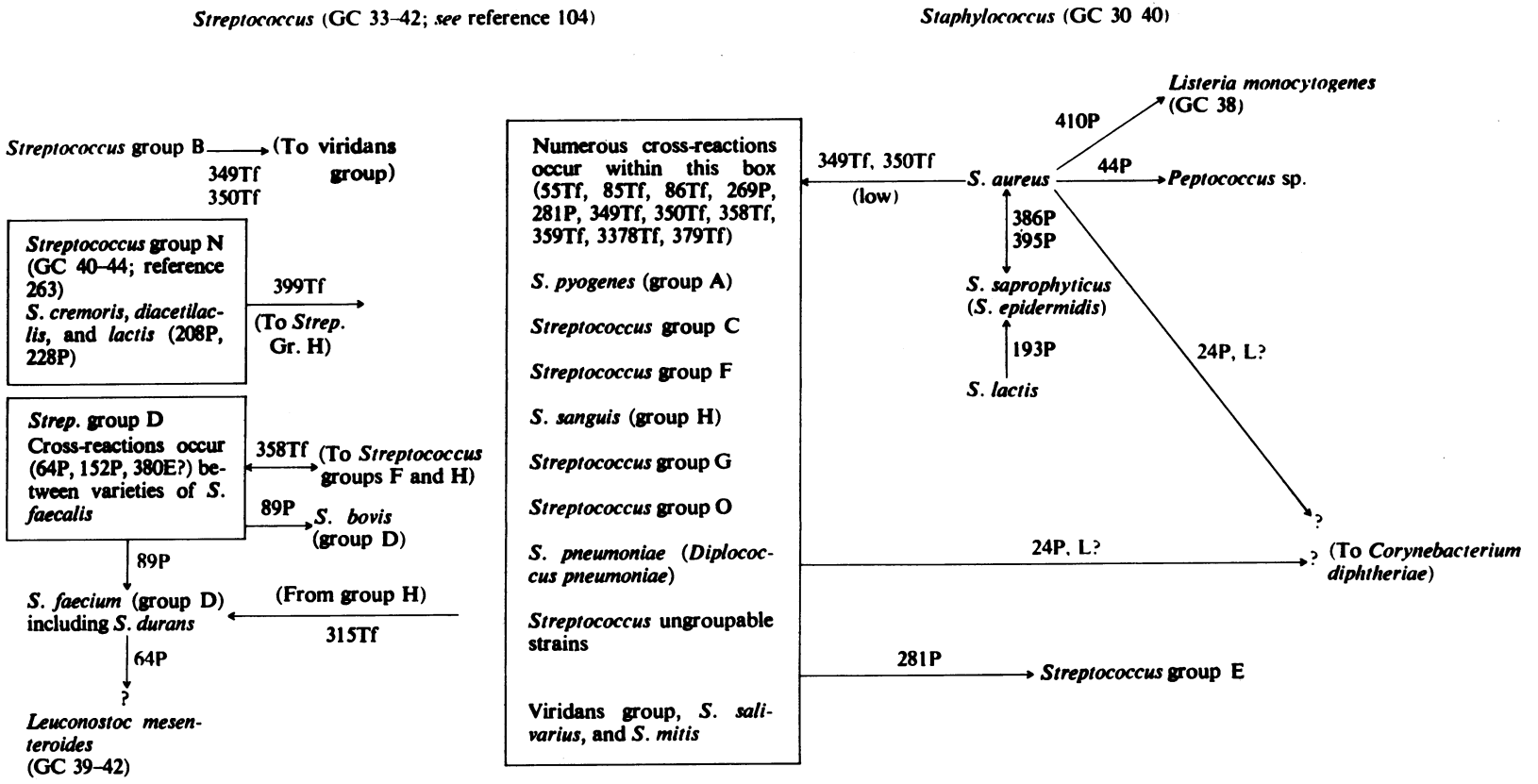

FIG. 17. *Streptococcus (including Diplococcus), Staphylococcus, Peptococcus, Leuconostoc, and Listeria.*

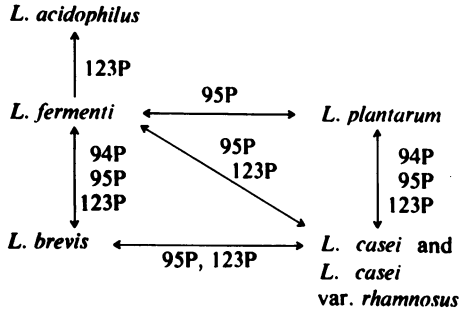

FIG. 18. *Lactobacillus* (*GC 28–64*).

fermentative group (*Betabacterium*, GC 42 to 58%, e.g., *L. fermenti*, *L. brevis*). It will be noted that the GC ratios vary widely (175, 394) but this does not seem to interfere with the phage cross-reactions. *L. bifidus* has higher ratios, and its relationship to other lactobacilli is still obscure.

The genus *Corynebacterium* is somewhat heterogeneous phenetically (202) though the GC ratios encompass a fairly small range. The type species is *C. diphtheriae*, and evidently a number of the animal corynebacteria are fairly closely related to it. *C. belfantii* may be simply a nitrate-negative form of *C. diphtheriae* var. *mitis* (192). The reaction between *C. pyogenes* and *C. diphtheriae* is of interest in that it connects the latter to the anaerobic coryneforms (such as *C. acnes*) which, it has been suggested, may be best regarded as members of the genus *Propionibacterium* (329). The gene transfer evidence does not support the hypothesis that *C. pyogenes* is particularly close to *Streptococcus* (30, 432). The phages of the diphtheria bacillus, together with their role in converting nontoxinogenic strains to toxinogenicity, have been reviewed by Groman and Lockhart (190). A report (24) suggesting that phages from streptococci and staphylococci can convert the diphtheria bacillus to toxinogenicity deserves following up. The two forms at the bottom of Fig. 19 are plant pathogens, and may well be only distantly related to the animal pathogens. There has been no confirmation of suggestions of close relationships between some coryneforms and some mycoplasmas (195, 436).

Actinomycetales (Fig. 20)

By both phenetic and genetic criteria, the genera *Actinomyces*, *Mycobacterium*, *Nocardia*, and *Streptomyces* appear to be fairly closely related (166, 243, 244). It is not yet clear whether the order *Actinomycetales* embraces part of the corynebacteria (and allied forms such as *Arthrobacter*). Rather little is known of the relations of *Actinomyces* (the anaerobes typified by *Actinomyces bovis*, not *Actinomyces*, as used by Russian authors, which are *Streptomyces*) although it is the type genus of the order, and reported phage cross-reactions (475) await confirmation. There seems to be a graded series from *Mycobacterium* through *Nocardia* to *Streptomyces*, as has been noted by various workers, and it can be seen that the genetic data support this (58, 242–244) as does serology (275, 276). Nevertheless, it seems doubtful to us whether any simple phylogenetic interpretations can be drawn from what is essentially phenetic evidence. DNA pairing (151, 457–459) shows that *Streptomyces* is more closely related to *Nocardia* than to *Mycobacterium*, so this also supports the order in which the genera are shown in the figure.

The genetic work on *Mycobacterium* shows cross-reactions between animal pathogens and saprophytes. The form known as *M. rhodochrous* appears to be an intermediate between *Mycobacterium* and *Nocardia*, but there is sometimes uncertainty about the identity of these strains (e.g., 298). *Jensenia canicruria* is regarded by Gordon and Mihm (184) as being *M. rhodochrous*. The reported transformation cross-reaction between *Mycobacterium* and a yeast (277) requires confirmation.

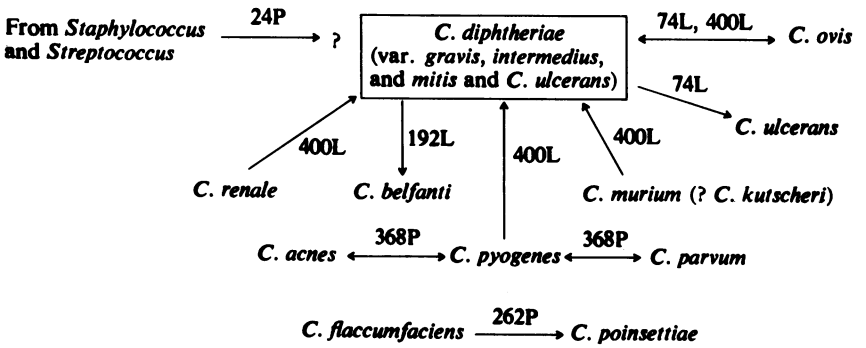

FIG. 19. *Corynebacterium* (*GC 48–60*).

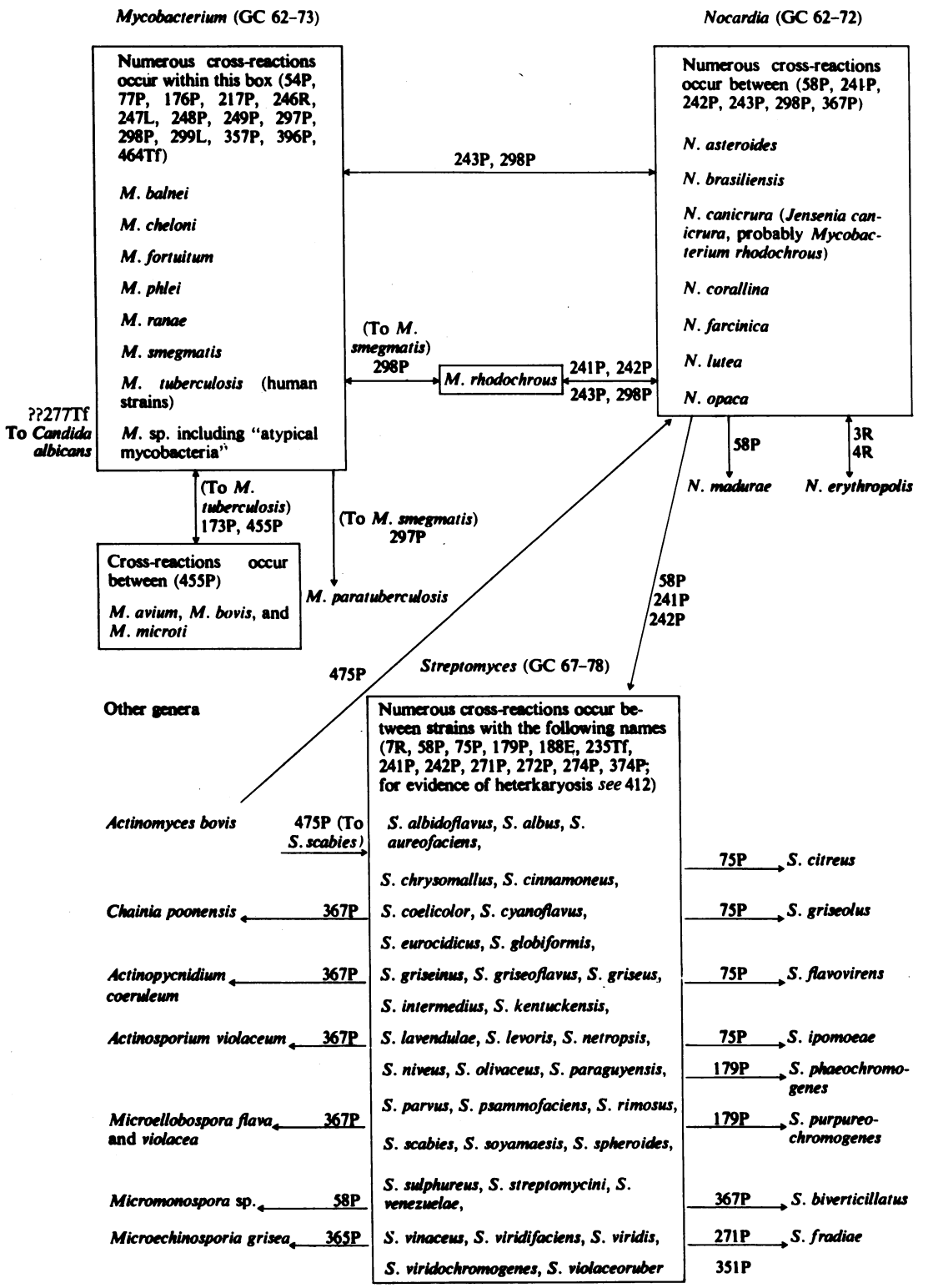

FIG. 20. *Actinomycetales.*

Within the genus *Nocardia*, no very clear taxonomic subdivisions are yet evident. The genus is in need of complete revision, and probably most named species will then be reduced to synonymy. Recombination has been reported in this genus (3, 4).

Streptomyces is a rather homogeneous group. The DNA pairing studies noted above show this, as well as the genetic and phenetic evidence; almost any named species can show phage cross-reactions with any other, and it is doubtful whether there are more than about 20 or 30 phenetic clusters within the genus (417, 430). Again, many of the named species are likely ultimately to be reduced to synonymy. Recombination systems are now being actively studied (e.g., 7–9, 60, 412). There is a good deal of doubt about the validity of several of the recently described new genera in the *Actinomycetales* (285, 365, 367). Some, such as *Microellobosporia* and *Actinopycnidium*, may be best combined with *Streptomyces* (59, 367).

DISCUSSION

The material presented shows that gene transfer can occur across wide taxonomic gaps. At present, there is no confirmed evidence that genes can be transferred between gram-positive and gram-negative organisms, but it may be that this too will prove possible. Indeed, it is not beyond all possibility that genes may be transferred across the whole taxonomic spectrum of bacteria, though doubtless they would need to be transmitted in steps from one genus to nearly related genera, in a chain-like fashion. The genera could behave, as it were, as stepping stones for the genes. At each step, it might be necessary to achieve integration into the recipient chromosome and this might well be the limiting factor (13). A gene that has too small a chance of integration when transferred by other genetic mechanisms might become integrated via an episome; its continuence is assured by the autonomous replication of the episome, so that integration could take place, as it were, at leisure.

Questions such as these are important to bacterial taxonomy, but before considering this a digression may be made into their implication for evolutionary studies. Of immediate interest is the potentiality of R factors to spread drug resistance; this is an active field (14, 16, 18, 139, 156, 264, 476), and it is clear that transmissible drug resistance is becoming increasingly widespread, involving not only the typical *Enterobacteriaceae* where it was first found, but also other gram-negative bacteria of medical importance. There is epidemiological evidence, although circumstantial, that this is mainly due to infection of genera that were once uniformly sensitive by R factors from genera in which resistance has arisen in some way as yet poorly understood. Perhaps the genes for resistance have come from non-pathogenic genera in which resistance occurred naturally in preantibiotic days; alternatively, resistance may have arisen by mutation and selection in the pathogenic organisms themselves. Evidently the whole process is largely dependent on the strong selection caused by the extensive use of antibiotics in many environments; this indirectly also selects for plasmids competent to transfer resistance. Selection, too, might result in the formation of R factors with several resistance genes from R factors carrying only one at a time (156).

Evidence presented by Falkow et al. (156) suggests that some R factors have a common origin, although epidemiological evidence (324) favors the view that they have arisen independently in different geographical areas. A great deal of controversy centers on the question of whether infective drug resistance arises in animals (consequent on the widespread use of antibiotics in animal feeding stuffs, etc.) and then spreads to man (16, 18, 19, 116, 322). This raises some unwelcome questions, for although it has been shown (476) that experimental transmission to resident strains of coliforms only occurs from large oral doses of strains bearing R factors, and the resistant strains do not persist long, yet the picture may be much changed in an environment in which antibiotics are widely used, as in hospitals. Spread of drug resistance is apparently taking place also in gram-positive organisms (106, 178, 264, 383). There is evidence that penicillinase in staphylococci did occur in preantibiotic days (363), e.g., *Staphylococcus aureus* associated with penicillin-producing dermatomycetes in hedgehogs (423). Richmond (383) discussed the particular relevance of transducing phages in transmitting plasmids that carry penicillinase genes, and also the possible role in this process of restricted transduction as opposed to generalized transduction

There is then a danger that the spread of drug resistance may lead to a new "Middle Ages" of preantibiotic days, for we should not too readily assume that the supply of antibiotics is endless. Whether resistance will, as a rule, disappear as a clinical problem if an antibiotic is no longer used (*see* 28) is an open question, or if it does, how long this will take. The danger would be yet more acute if drug resistance could cross from enterobacteria to gram-positive organisms [or even pass between gram-positive genera (e.g., 349), giving rise, for example, to widespread hemolytic streptococci clinically resistant to penicillin]. It

has been noted (116) that lack of knowledge of the flora of preantibiotic days, and of the epidemiology of infective drug resistance, is a serious barrier to understanding. Therefore, it would seem important for health authorities in many countries to collect at regular intervals, and preserve at very low temperature, numerous specimens from various sources (specimens rather than isolated strains, as one cannot foretell which organisms will prove of interest). This would allow retrospective studies of the spread of resistance to new antibiotics in different countries and environments, with the chance of finding where and how this arises. When fine structural detail of resistance factors (or of enzymes of carried genes) becomes known, this will greatly help in tracing the spread of resistance.

Although interest has centered on R factors and transduction, drug resistance might spread by other mechanisms. Transformation offers one possibility that is perhaps less likely with some other systems—that a gene might be transferred across very wide taxonomic gaps. There is no theoretical reason why a gene from a gram-positive organism should not be transferred in this way to a gram-negative one. The entering gene would have to become integrated into the recipient chromosome, and this is believed to require sufficient similarity between the corresponding segments of donor and recipient DNA to allow firm bonding as a preliminary to crossing over. This step would evidently be the most difficult one, but we know little about the minimal degree of bonding that is necessary for integration, and it may perhaps be small. Once a foreign gene was incorporated successfully, the transfer of variants of the foreign gene would become easier. In consequence, transformation might then appear to be restricted to one or two genes. Some indications of this kind have possibly been noted but not followed up (*see* 86, 378, 438). The initial integration of an extremely foreign gene would be a very rare event, and we have no very clear idea of how it would be most likely to happen. If it did happen, it is conceivable that the integration of a foreign gene would make the new genome more readily able to accept yet other foreign genes that were, in the donor, adjacent to the one that has already been transmitted. This would open the possibility of breakdown of the barriers between dissimilar bacteria, as increasingly large parts of the genomes become able to recombine (provided suitable selection pressures were maintained). Ready exchange of genes between dissimilar bacteria would have important applications in fields such as industrial microbiology in allowing the development of new strains of bacteria possessing certain desirable combinations of properties (*see* 56).

In considering the implications of gene transfer for bacterial evolution on a longer time scale, one may first note that among procaryotes, unlike eucaryotes, gene exchange generally involves only a small part of the genome. This may make it easier for genes to be transferred across taxonomic boundaries by making less stringent demands on integration and crossing over, with less chance of inviable products. This in turn could favor extremely reticulate modes of evolution, with numerous partial fusions of phyletic lines (i.e., involving only a few genes at a time; for general discussion on such points, *see* references 13, 191, 322, 426). It may well be that gene exchange is so frequent that the evolutionary pattern in bacteria is much more reticulate than is commonly believed and cannot be satisfactorily represented by the usual cladogenies that repeatedly branch with time, but show no fusions of branches (e.g., 71, 161, 434). Modern techniques, particularly in molecular biology, have reopened interest in the question of how far we can expect to be able to reconstruct the phylogeny of bacteria (127). For example, several workers (120, 141, 161, 162) have demonstrated the potential of protein sequences for reconstructing cladistics. Yet the introduction of reticulation in evolution presents severe conceptual and practical problems, which so far as we know have not been adequately discussed. The occurrence of highly similar proteins or DNA fractions in two different bacterial taxa may indicate recent gene transfer rather than recent common ancestry, as this is usually shown cladistically.

Phenetic patterns in bacteria are still poorly known, so that it is difficult to correlate them with genetic behavior. Views vary from the belief that all bacteria fall into distinct, well-separated species to the belief that they form a continuous spectrum of intergrading forms (110). This was recently discussed elsewhere (429, 431), but the evidence may be summarized briefly as follows. Some studies suggest that strains representing conventional taxospecies form compact clusters separated by empty gaps. Others, however, reveal another pattern which may be more prevalent, especially at higher ranks. This is where scattered single strains occupy phenetic space fairly evenly, while embedded in this are local concentrations of points which represent the compact clusters mentioned above. It may be that some of the isolated points represent single strains of forms that are common in restricted habitats, and on further study might later be replaced by tight clusters, but it seems unlikely that this is the whole explanation. It seems more probable that

at least some of the isolated strains are recent hybrids that have been produced by gene transfer between the compact clusters. Presumably such hybrids are at a selective disadvantage and do not persist long enough to give rise to dense clusters. This may not be so for bacteria that occupy habitats subject to marked human intervention (e.g., food and dairy products). In such habitats, one could envisage the replacement of the original flora by a hybrid form that possesses a selective advantage in the new habitat and consequently became very common and appeared as a compact and distinctive cluster of very similar strains. This could be happening with drug-resistant bacteria (*see* 322). The combination of widespread gene transfer, strong selection pressures, and rapid multiplication certainly provides a mechanism for swift evolution, but it may be that in natural habitats its rate is not very high. Little is known about the phenetic position of hybrid bacteria (*see* 291), but possibly stable diploids can exist (32, 412). Falkow (153) notes that such hybrids can raise problems of identification if they become common.

The evidence discussed earlier shows that there is a remarkable degree of concordance between genetic and phenetic evidence. Major discrepancies have not yet been observed, but if they arose they would pose some difficult problems. They could, of course, be due to technical points (inaccurate techniques, inappropriate sets of phenetic characters, misidentified strains, etc.). If, however, one found two organisms that were phenetically identical but had very different GC ratios, or low nucleic acid pairing, this would raise some basic genetic problems. Thus, a theoretical explanation, though unlikely, would be that the same phenotypic message (e.g., protein sequence) was represented by very different nucleotide triplets. Another possibility would be that substantial parts of the genome were not expressed phenotypically, and this may not seem so improbable when one reflects that the parts of the genome represented by prophage may perhaps be almost "silent" with respect to the usual taxonomic characters. The converse discordance (high DNA pairing with low phenetic similarity) would also be disturbing, and would suggest the presence in one of the strains of a mutation with numerous pleiotropic effects.

Certain special points arise in applying genetic work to taxonomy. It is usually not feasible to determine the genetic cross-reactions of all possible pairs of strains in a large survey (for an exception, *see* 105). Instead, it is usual to draw conclusions from the results of comparisons with only a few reference cultures. In effect, this leads to basing the conclusions on very incomplete tables of interrelationships, perhaps with atypical strains as the points of reference. Unfortunately, we know almost nothing about the tolerance of classifications toward gaps in data of this kind. In any case, it would seem unsafe to choose reference strains without supporting phenetic studies. This is not simply an academic point. The discovery of a close genetic relationship between apparently unrelated genera may be misleading if the genera are extremely heterogeneous or if the strains are misidentified. Thus, a reaction with a strain labeled *Flavobacterium* species is almost meaningless if no further detail is given because the strain could belong to one of ten quite different genera. Authentic and type strains of many bacterial species are listed in reference 433.

Is it possible that genetic criteria can provide reliable guidelines to taxonomic rank? DNA pairing would seem to have the most potential here. Protein sequence studies are only just beginning but they may in due course be useful. Gene linkage maps are also a possibility, though their numerical comparison will pose problems of homology similar to those in protein sequences. With gene transfer data, there are several difficulties: theoretical and empirical knowledge is still inadequate; negative findings have little or no meaning; and although general regularities may be found between frequency of gene transfer and difference in rank, the absolute values of the frequencies at a given rank vary a good deal from one group to another. For these reasons, we believe that gene transfer data are more useful as pointers to further taxonomic studies, or as confirmation of existing ones, than as criteria of taxonomic rank, except as very rough guides.

The most generally useful genetic systems for taxonomic work seem to be transformation, bacteriophages, and R factors. Mutants that do not produce extracellular deoxyribonuclease may be necessary for successful transformation (376, 383). Methods are needed for selecting phages capable of growing in two different hosts, perhaps by using alternate hosts during enrichment culture. Techniques for inducing lysis in lysogenic strains are obviously useful, and methods for selecting highly fertile R factors (149, 321) are worth trial. The heightened fertility of cells immediately after infection by a plasmid [due to derepression (*see* 16, 324)] might also be exploited in attempting intergeneric crosses.

What new work is particularly needed in this field? Several of the more uncertain findings cited in this review require more study. In particular, it would be important to explore the possibility of cross-reactions between gram-negative and gram-positive genera. It must be true that many potential experiments are not made because it

seems inconceivable that they could be successful, yet gene transfer has been shown between genera that were once considered quite unrelated. More work, too, is needed on gram-positive groups in the general area of coryneforms, and between *Actinomycetales* and coryneforms. The relations of myxobacteria to other forms, including blue-green algae, are also worth exploring, and also the relations of the rickettsia group to other bacteria. Planned studies of the quantitative relationships between the frequency of gene transfer and phenetic difference, between phenetic difference and nucleic acid pairing, etc., are also much needed. Such information is required before statistical treatment of genetic data can be used for taxonomic purposes (e.g., so that a given cross-reaction could be taken to imply that the probability was 90% that the two strains belonged to the same species, and 98% that they belonged to the same genus). In particular, the extent to which gene integration requires similarity in DNA base sequence, and whether this similarity need only be very local, is a question critical to the understanding of the taxonomic value of gene transfer data. Only when these problems can be answered will bacterial genetics show its full potential for taxonomic and evolutionary studies.

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