CONCEPTS AND METHODS

PART I

CHAPTER

1

THE CONCEPT OF MICROBIAL SPECIES

Chapter contents

INTRODUCTION

What distinguishes microbiology from other disciplines of biology? This question no longer has a straightforward answer that can satisfy all biologists. The traditional answer focused on the extremely small size of organisms under investigation; however, this leaves little room for distinction on the basis of taxonomy because practically all organisms have a microscopic stage during their life cycles. Some organisms that are physiologically closely related to large macroscopic organisms spend their entire life span as microscopic organisms. Nevertheless, physical size remains a dominant conceptual framework for most practicing microbiologists, and most of the discussion in this book is presented from this perspective. Other responses have focused on unicellularity (as opposed to multicellularity) as the defining characteristic of microorganisms, however, viruses are acellular, and many investigators have argued that the so-called unicellular stage of bacteria, for example, is not a naturally occurring phenomenon. Some investigators have advanced the cellularity argument by invoking differentiation as the separating principle, but many "unicellular" organisms also go through developmental differentiation as in the case of sporulation and fruiting bodies. Finally, many investigators focused on the internal anatomy of organisms to identify unique characteristics of microorganisms that are not shared by macroorganisms. This response has been organized around the concept of karyology (pertaining to the organization of genetic material), with prokaryotes and eukaryotes as the main divisions of biological diversity. However, current understanding of endosymbiotic interactions with respect to the emergence of organelles that are usually attributed to the eukaryotes suggests that this response may not be stable over evolutionary time frames. The difficulty of defining the subjective aspects of microbiology is further compounded by the cosmopolitan adoption of incisive tools presented by molecular biology. Molecular analysis has been extremely influential in exposing major unifying concepts in biology, but at the same time, such analysis has revealed a remarkable level of diversity that has proven difficult to organize in discrete packets of information that are consistent with previously held concepts of diversity.

The concept of biological diversity implies consensus on the discrete nature of independent species and on the mechanisms that generate speciation. The recognition of differences and similarities among the discrete features of microorganisms is more challenging and less well understood than for large multicellular organisms. A thorough comprehension of the complexity inherent in the concept of microbial species is fundamental to the appreciation of microbial diversity, and to the understanding of processes that generate differences despite the influence of other ecological pressures that tend to produce similarity in a given environment. The balance of these two seemingly opposing processes (liberation of diversity and conservation of ecological function) has resulted in the emergence of homeostatic conditions that support perpetual phylogenetic lineages. The recognition of independent microbial species is based on an assumption of long-term stability of these homeostatic conditions. This chapter presents a discussion about the empirical foundation of the microbial species concept, including a balanced view of current controversies that have entangled the interpretation of phylogenetic categories according to recent data on phenotypes and molecular characteristics. The topics are selected to introduce important milestones and alternative perspectives that have been developed to explain the concept of microbial species. The following main points are explored:

1. The differences between the various species concepts; contemporary theories about speciation; and the relevance of these concepts and theories to microorganisms.
2. Comparative assessment of alternative models for speciation, with an explanation of how these models accommodate or ignore special genetic properties of microorganisms.
3. Linkage of the understanding of species concepts and mechanisms of speciation to the theoretical and methodological advances in the assessment of microbial diversity, and the relevance of such advances to the emerging understanding of Earth system processes.

OLD AND NEW CHALLENGES FOR ASSESSING MICROBIAL DIVERSITY

There is a plethora of convincing pieces of evidence that the Earth is a "microbial planet" in the sense that microorganisms predate other life forms, they are the most abundant—both in terms of numbers and distribution. In addition, microbial activities have profound influence on the integrity and functioning of global ecosystems. Despite the widely acknowledged importance of microorganisms on Earth, scientific knowledge of microbial diversity and function is scantier than for physically larger and scarcer organisms (Staley, 1997; Wilson, 1994). The term "diversity" in a biological context presumes a multiplicity of forms that may not necessarily be apparent without sophisticated observation by means of specialized tools. In no other discipline of biological sciences is this truer than in microbiology. To the untrained observer, microorganisms have much more in common than they can possibly have in differences. Therefore, the recognition of microbial diversity has always depended on the methods used for analysis. Early investigators such as Antoni van Leeuwenhoek (1632–1723; see Box 1.1) were limited to gross morphological differences in microbial cell shapes and colonies. It is now possible to recognize substantial differences between organisms at the molecular level, but this scale of analytical power has not yet provided a coherent solution to the persistent questions surrounding the concept of microbial speciation and diversity. These questions fall into three separate but interrelated categories, namely:

1. **Incomplete information on the number of existing microbial species**. The quantitative estimate of the number of microbial species has been limited by the inadequacy of techniques used for recovering, isolating, and cultivating microorganisms present in various ecosystems.
2. **Non-operational definition of "microbial niche"**. The biological concept of niche, as developed for macroorganisms, has not been very useful in microbiology. The niche refers to the multidimensional space where the coordinates are defined by parameters representing the conditions of existence of a given species. Niche is also used in reference to the ecological role of a species in a community. In microbiology the application of the niche concept has been limited by the difficulty of explaining the wide geographical, geological, and ecological ranges in which specific groups of microorganisms occur. In addition, correlations between microbial species diversity and ecosystem functions are very complex and difficult to reduce to any form of numerical modeling.
3. **Loose definition of strains and species**. The occurrence of intra-species and inter-species genetic exchange among microorganisms is recognized as a major driver of evolutionary innovation. The frequency of genetic exchange and the promiscuity of gene-transfer mechanisms have led to a questioning of taxonomic boundaries in microbiology.

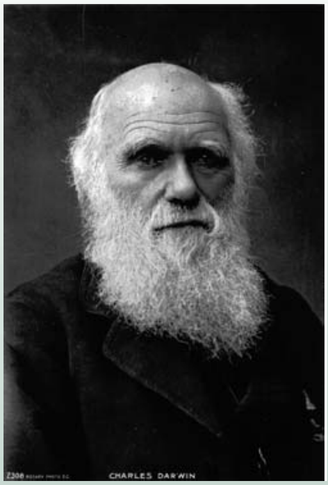
BOX 1.1

(a)

(a) The inventor of the microscope, Antoni van Leeuwenhoek (1632–1723), was the first to recognize microbial diversity, although his observations were mostly of eukaryotic organisms. Improvements on the simple microscope led to an expansion of the realm of microbiology to include many species of prokaryotic bacteria and viruses by the early twentieth century. Image by courtesy of Dr. Warnar Moll's private collection, Amsterdam, The Netherlands.

(b)

(b) Carolus Linnaeus (Carl von Linné; 1707–78) invented the basic structure of the system of phylogenetic classification that is still in use today. The Linnaean system was designed to classify large eukaryotic macroorganisms based on morphological differences and reproductive exclusion. Contemporary debates regarding phylogenetic classification have focused on the meaning of "species" as the fundamental unit of biological diversity. Early microbiologists adopted the use of morphological criteria (such as varieties of coccus and bacillus cell shapes) for species classification because of limited knowledge of microbial diversity. New molecular approaches for investigating microbial genetic structure and function have clearly demonstrated the limitation of using morphology as the basis for species categorization in the investigation of prokaryotes. The appreciation of Linnaeus's contributions to natural science is demonstrated by his image on Swedish currency. Image by courtesy of Thomas Hunt.



(c)

(c) Charles Darwin's (1809–82) theories of speciation revolutionized the understanding of the relationship between different "kinds" of organisms, or "species". Modern molecular biology produced an elegant biochemical mechanism that supports the Darwinian theory of evolution by natural selection, which applies to all phylogenetic categories, including microorganisms. However, the actual causes of speciation remain controversial inasmuch as the definition of species remains unresolved. Darwin's work illuminated two different but related conceptual pillars of biological evolution, one dealing with morphological transformation within lineages, and the other with the principle of diversity – leading to an increase in the absolute number of lineages. These concepts, although much better understood since their initial articulation, remain fundamental challenges for most accounts of microbial diversity and function. Image by courtesy of Henry Huntington Library, San Marino, California.

(d)

(d) Darwinian biologist, Ernst Mayr (1904–), developed the salient biological species concept that applies to most eukaryotic species. However, the relevance of the concept to microorganisms is doubtful. Nevertheless, most species concepts dedicated to prokaryotes are more or less variations on the themes that combine genetic and ecological criteria, which were developed for eukaryotes. Ernst Mayr rejected the three-domain universal phylogenetic tree based on comparative assessment of rRNA as proposed by microbiologists led by Carl Woese. In his view, the Archaea and Bacteria belong in the same prokaryote "empire", which is clearly distinguished from the only other domain, namely the eukaryotes. The apparent similarity of structural and morphological characteristics of Archaea and Bacteria cells outweighs the molecular similarity between Archaea and the eukaryotes (Woese, 1998b). Image by courtesy of Ernst Mayr Library, Museum of Comparative Zoology, Harvard University.

The exploration of these categorical questions has produced an expansion of the methodological basis for analyzing microbial diversity. Innovative methods have been used in environmental microbiology and microbial ecology to resolve practical questions while contributing to our understanding of evolutionary systematics. The major lines of methodological advances, namely microscopy, culture, molecular analysis, and phylogenetic bioinformatics are considered in subsequent chapters in this section of the book. These advances have contributed to a widening of the scope of microbiological research, but their applications for solving contemporary problems facing global biodiversity require a comprehensive understanding of what we mean by "species", how they are created, and how they become extinct.

TRADITIONAL CONCEPTS OF SPECIES

The invention of a systematic scheme for classifying organisms was necessitated by the recognition of an expansive biological diversity. Carolus Linnaeus (Swedish name: Carl von Linné; 1707–78) is credited with the first widely accepted hierarchical scheme, which consists today of seven categories, namely kingdom, phylum, class, order, family, genus, and species as the fundamental unit. For Linnaeus, and most of his contemporaries, this taxonomic scheme was immutable because the prevalent doctrine of creation at the time did not include evolution, and the scheme appeared to work well for animals and plants that then dominated the study of biology. Even so, Linnaeus did not explicitly define "species" and modern concepts of species probably include a mixture of attributes that Linnaeus separated into "genus" and "variety".

Charles Darwin (1809–82) argued in *The Origin of Species* that species are not real entities in nature. Since Darwin's time, two major schools of thought have emerged which define the concept of species in ways that are consistent with the emergent synthesis theory of evolution and contemporary understanding of molecular genetics. The **realists** have affirmed that species are real and are the actual units that evolve. The **nominalists** reject this tangible definition of species. One group of nominalists proposed instead that species are breeding populations, also known as **demes**, and are the evolving units. Another group of nominalists acknowledges the usefulness of defining a theoretical concept of species, but does not accept the existence of discrete species units in nature. Four conceptual frameworks have emerged in modern biology to resolve the challenges facing attempts to make the species concept useful. Briefly, these frameworks represent ways of classifying species concepts on the basis of whether they are:

1. **Prospective** with respect to consideration for the future evolution of populations, or **retrospective** by considering species as "dead-end" products of evolution.
2. **Mechanistic** with respect to an ongoing process of speciation, or **historical** through focusing on the outcome of the process.
3. **Trait-based** by focusing on observable characteristic defining traits of organisms with no reference to the inferred lineage of those organisms, or **genealogy based** by focusing on the historical relationships among organisms.
4. **Intrinsic** by invoking "self-imposed" barriers on species mixing through specifically evolved limitations on genetic exchange, or **extrinsic** through focusing on the external or flexible barriers to genetic exchange.

In view of these organizing frameworks, four major species concepts can be compared and contrasted, namely the **typological species concept**, the **morphological species concept**, the **biological species concept**, and the **evolutionary species concept**. There are several variations on these four concepts, and some of these are discussed in the context of their similarities to one or more of the major concepts. Mallet (1995) and Mayden (1997) provide a more detailed coverage of the overlap and trade-offs among the various species concepts.

Typological species concept

The typological species concept predates the Darwinian theory of evolution, and it is not consistent with evolutionist thinking. The concept defines species on the characteristics of a "type specimen". The concept is based on the Platonic and Aristotelian philosophical arguments of the existence of an organismal "archetype" (Plato referred to this as *eidos*). The observed diversity within an archetypal species represents the manifestation of imperfections in an eternal experimental strive to reproduce an immutable perfect state. In this context, species represents a static, non-variable assemblage of organisms that conform to a common morphological plan (Lincoln *et al.*, 1982). The explanatory power of the typological species concept has been seriously challenged by improved understanding of the patterns of variation within populations. Several characteristics are known to vary among members of a single population of interbreeding individuals. Since there are no generally accepted means of specifying the exclusive properties possessed by each and every member of a particular species, it is not possible to generate a complete catalog of "type" characteristics, and the true identity of the "type" species cannot be known. Additional problems with the typological species concept include the observation that apparently different organisms may share the same morphological traits during various segments of their life cycles. Given that the typological species concept is the oldest and simplest of the species concepts, it is perhaps not surprising that many investigators have difficulty accepting it as an operable concept. The shortcomings of the typological species concept led directly to a proliferation of other concepts aiming to improve upon it. However, no specific concept has yet successfully accounted for all the questions that can be raised on how to reconcile the logical products of evolutionary forces acting to maintain species integrity while generating diversity.

Morphological species concept

The morphological species concept considers anatomical (morphological) characteristics to be the primary discriminant function associated with species. It is a derivative of the typological species concept that is preferred by some plant taxonomists and investigators working on organisms that do not reproduce sexually. The concept has been usefully applied to the classification of large groups of undescribed species such as a collection of fossils. The problems associated with interpreting the morphological species concept result from the sometimes arbitrary nature of the evidence used for classification, which in many cases relies on expert opinion about morphological differences. The concept also cannot explain the occurrence of sympatric species that exist in the same habitat, look morphologically identical, but are reproductively isolated from one another. For example, several bacillary bacteria exist in soils and exhibit similar colony morphologies upon culturing, but are clearly physiologically and genetically unrelated when subjected to molecular analyses.

Additional concerns include the inability of the morphological species concept to distinguish among cryptomorphic sibling species, where substantial changes have been introduced into the genome of one species but the changes are expressed in ways other than morphological differences. The concept also does not address the observation of sexual dimorphism, where the male and female versions of the same species look different. In bacteriology, the production of conjugation plasmid-encoded sex pili cannot be explained by the morphological species concept. Finally, the morphological species concept cannot account for genetic polymorphisms that are not directly expressed as distinct morphological characteristics. In view of the fact that speciation at the level of genetic divergence necessarily precedes the expression of morphological differences, the concept focuses perhaps too much on the outcome of evolution at the expense of accommodating the mechanisms that underlie speciation. Overall, the strengths and weaknesses of the morphological species concept are better appreciated when compared to other concepts such as the biological species concept, which accounts for the mechanisms of speciation, including the potential for sexual reproduction.

Biological species concept

Ernst Mayr (1904–) is credited with developing the biological species concept, which defines species as "groups of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups" (Mayr, 1963). The biological species concept considers a species as the fundamental ecological and genetic unit, where consequential interactions occur only between species regardless of the fate of individual members. The species is genetically identifiable only through the population gene pool, which is in constant flux because of adaptive genetic exchange mechanisms. The biological species concept captures the significance of reproductive communities where specialized features prevent the dilution of the species gene pool through intra-specific genetic exchange. However, the phenomenon of natural transformation in which cells uptake “free” genetic materials from the environment is commonly observed in microbial communities. Therefore, species concepts that depend on a permanent reproductive isolation of a population do not fit prokaryotes. The biological species concept also fails to account for certain observations in populations of eukaryotic microorganisms. For example, in reviewing the taxonomy of free-living ciliated protozoa, Finlay and colleagues (1996) confirmed that these organisms have traditionally been identified on the basis of the extant morphological diversity, which is closely related to the natural functions of each of the 3,000 defined species. On this basis, the investigators rejected the biological species concept as inappropriate and impractical. Instead, they favor the morphological species concept for this group of microorganisms as more pragmatic.

Evolutionary species concept

The biological species concept does not accommodate organisms for which asexual modes of reproduction produce clonal species. To address this inadequacy, paleontologist George Gaylord Simpson (1902–84) proposed the evolutionary species concept, where the species is defined as “a single lineage of ancestor–descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate” (Simpson, 1951). This concept explicitly includes the evolutionary history or lineage of organisms as opposed to a focus on the recognition of current species. Therefore, it has been used extensively in the analysis of the fossil record, particularly in zoology (Ereshefsky, 1992). The evolutionary species concept has been formalized in various renditions of species concepts that focus on phylogenetic lineage, including the phylogenetic species concept in which individual members of a species are considered to be part of a monophyletic group haven descended from a single ancestral taxon (Wheeler and Meier, 2000). The concept does not fully account for genomic hybrids, where genes have passed from one taxon to another, and the genetic make-up of individuals can be traced to different phylogenies or genealogies. Assessments of completely sequenced microbial genomes have demonstrated that such hybrids are common. For example, 5% to 15% of bacterial species’ genomes can be attributed to acquisition from other species (Ochman *et al.*, 2000). This makes the evolutionary species concept, as originally proposed, practically irrelevant to prokaryotes.

Other concepts

At least 20 more species concepts have been described, primarily for eukaryotic organisms, but most are variations on themes explored by the four concepts described above. Among the newer concepts, two are particularly noteworthy, namely the **phylogenetic species concept** and the **ecological species concept**, because they are relevant to current attempts to formulate a species concept that is operational for prokaryotic organisms.

Willi Hennig (1913–76), a leader of the phylogenetic school of systematics, championed the phylogenetic species concept. The complex terminology used initially by adherents of this concept makes comparisons with previous concepts tedious; in short, phylogeneticists proposed that relationships among species should be interpreted strictly on the basis of genealogy as **clade** relations. Therefore, a species is defined as “a group of individuals, also known as character-bearing **semaphoronts**, which are interconnected by **tokogenetic** relationships that are strictly defined by the phenomenon of reproduction” (Hennig, 1966). The semaphoront (an individual at a specific period in its life cycle) subconcept was needed to broaden the meaning of an individual by emphasizing the importance of distinct developmental stages, including both phenetic and cladistic differences during the life cycle. Therefore, under this concept, the microbial spore and vegetative cell count as separate semaphoronts. A simpler version defines species as a complex of spatially distributed reproductive communities. This simplification highlights the closeness of the phylogenetic species concept to the biological species concept as defined by Ernst Mayr (Mayr, 1987). Proponents of the phylogenetic species concept, notably Joel Cracaft, Niels Eldredge, and Mary McKitrick considered that an evolutionary view of the Linnaean hierarchy would inevitably produce a nested set of clusters that are linked by shared derived characters (or **synapomorphous** traits). In this sense, a species represents an indivisible cluster of organisms at the base of the Linnaean hierarchy. Following this argument, a definition of species was presented as "a diagnosable cluster of individuals within which there is a parental pattern of ancestry and descent, beyond which there is not, and which exhibits a pattern of phylogenetic ancestry and descent among units of like kind" (Cracaft, 1983). Cracaft (1989) further refined the definition as "the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent." To deal with the problem of genealogical hybrids, the genealogical species concept was proposed by Baum and colleagues as a variant of the phylogenetic species concept by including a consensus of many estimated genealogies of different genes (Baum and Donoghue, 1995). Finally, the inclusion of ecological characteristics and reproductive isolation was added to the basic foundation of the phylogenetic species concept to produce a cohesion species concept (Templeton, 1989).

The ecological species concept is not very well connected to the intrinsic properties of organisms, such as molecular genetic characteristics. Instead it focuses on the occupation of adaptive zones by particular species. The adaptive zones are defined by resource distribution and the biotic and abiotic characteristics of specific habitats. Therefore, the ecological species concept is tied to the concept of ecological niche. The strength of the ecological species concept is that it attempts to categorize organisms by capturing the essence of phenotype as an expression of genomic information and environmental influences. However, it is difficult to consistently recognize ecological species because many organisms can occupy different ecological niches due to adaptation or developmental changes during the life course. The ecological species concept also precludes consideration of directionality in evolution, and it is not consistent with the hierarchical view of species diversity (Ereshefsky, 1992).

None of the traditional species concepts encompasses all groups of organisms. Furthermore, most of the concepts were developed without much thought given to usefulness in organizing the systematics of prokaryotic organisms. Table 1.1 presents a comparative assess-

Table 1.1 Comparative summary of major species concepts, and their relevance to prokaryotes.

Species concepts	Evaluation criteria								Does the concept apply to prokaryotes?
	Implications for the past or future status of populations		Conceptual grasp of process versus outcome of evolution		Basis for evaluating new organisms prior to classification		Barriers to genetic exchange		
	Retrospective	Prospective	Mechanistic	Historical	Traits	Genealogy	Intrinsic	Extrinsic	
Typological	*			*	*		*		No
Morphological	*			*	*		*		Not always
Biological		*	*		*		*		No
Evolutionary	*					*		*	Not always
Phylogenetic	*			*	*			*	Not always
Ecological		*	*		*			*	No

ment of six species concepts according to the four frameworks generally accepted as evaluation criteria for describing the coverage of these concepts.

The scarcity of a fossil record for prokaryotes, coupled with the recognition that most naturally occurring prokaryotes have not been described, posed intractable challenges for the application of species concepts developed for plants and animals to microbial systematics. These problems have led investigators toward proposals of species concepts that specifically consider peculiar characteristics of prokaryotic genetics and ecology. The main features of these "microbial species concepts" are described in the following section.

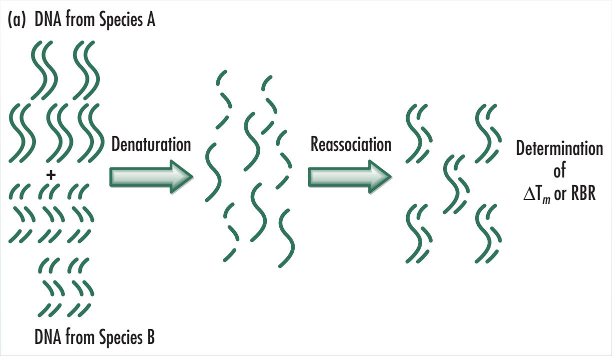
SPECIES CONCEPTS FOR PROKARYOTES

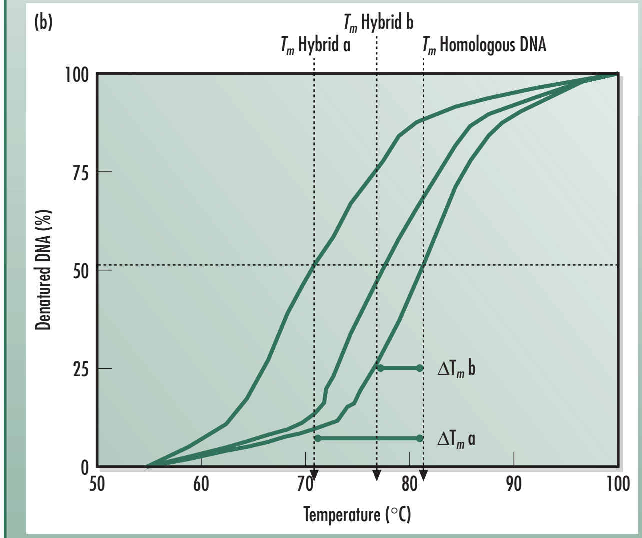
The Global Biodiversity Assessment program suspects that more than one million species of prokaryotic organisms exist in nature, but not more than 5,000 of them have been described (Rossello-Mora and Amann, 2001). Furthermore, there is currently no official definition of species for microorganisms, although several concepts have been proposed (Colwell *et al.*, 1995; Krawiec, 1985; Rossello-Mora and Amann, 2001; Ward, 1998). A number of criteria have been used to circumscribe existing microbial species categories, depending on the period of discovery and on the objectives of taxonomists working in different disciplines including medical, environmental, or industrial microbiology. The lack of agreement on a microbial species concept has led to an artificial amplification of the number of recognized microbial species because a single species can be identified with different names in different subdisciplines (Rossello-Mora and Amann, 2001).

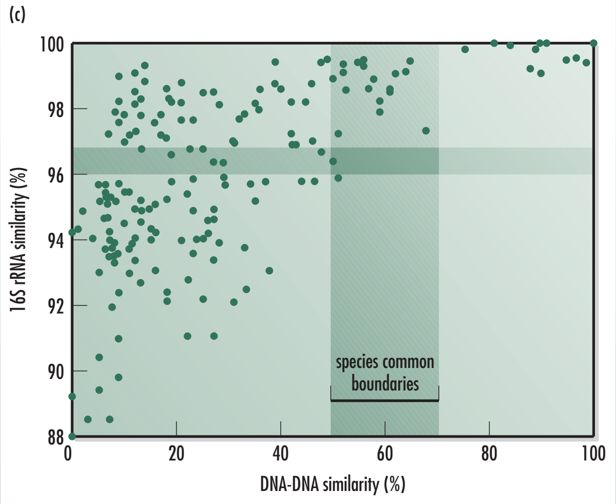
The conceptual understanding of microbial species has traditionally relied on criteria similar to those used to formulate species concepts for eukaryotic organisms. For example, Ravin (1960) struggled to apply the biological species concept to bacteria by defining the phenotypic clusters of mainstream bacterial systematics as "taxospecies". The taxospecies concept is based on numerical taxonomic methods and defines species as a group of organisms, including strains and isolates, with mutually high phenotypic similarity that forms an independent phenotypic cluster. This concept is analogous to the morphological species concept, but in addition to anatomical features, it includes consideration of physiological characteristics. Ravin also proposed a "genospecies" concept to define groups of bacteria that can exchange genes, but there was very little correlation between the groups of organisms described by the taxospecies concept, and those described as genospecies (Ravin, 1963). This incongruence provoked further dissatisfaction of microbiologists holding traditional species concepts. More recent attempts to make the biological species concept applicable to prokaryotes can be traced to the work of Dykhuizen and Green (1991) who proposed bacterial species as "groups of strains that recombine with one another but not with strains from other such groups." The recognition of historic events of genetic recombination was built into this definition because it had become feasible to reconstruct phylogenetic relationships according to molecular sequence data, which presumably can be used to delineate groups according to the genetic exchange criterion. However, this approach can be called to question on the basis of several observations that many bacteria are capable of exchanging genes both within and between the groups currently nominated as species.

Fred Cohan has argued persuasively that there is a fundamental misconception which limits the success of attempts to develop species concepts for prokaryotes through extrapolation from eukaryotic species concepts. For most eukaryotes, the species represents "a group of organisms whose divergence is capped by a force of cohesion; divergence between different species is irreversible; and different species are ecologically distinct" (Cohan, 2002). Whereas for bacteria, the characteristics of named species do not capture these universal properties, but instead bacterial "ecotypes" fit the definition of eukaryotic species. Ecotypes are defined as "populations of organisms occupying the same ecological niche, whose divergence is purged recurrently by natural selection." Bacterial ecotypes can be recognized by their molecular sequence signatures, and comparative assessments of these signatures have

BOX 1.2







Schematic representation of procedures for quantifying the degree of relatedness between two microbial isolates.

(a) DNA–DNA reassociation kinetics yields a thermal reassociation midpoint (ΔT_m) or the relative binding ratio (RBR). Either RBR or ΔT_m can be used to circumscribe species.

(b) The curves describing the denaturation kinetics of double-stranded DNA. At temperature T_m 50% of DNA in an aqueous solution becomes single stranded. Hybrid double-stranded DNA from two different species (heteroduplex) is less stable than homoduplex DNA from identical organisms. Therefore the T_m of hybrid DNA is lower than that of DNA from single species. The difference between homoduplex DNA and heteroduplex DNA is the ΔT_m. For example, in the graph, the T_m of homologous DNA from a single species is 82°C. The T_m for heterogeneous DNA hybrid "a" is 72°C. Therefore, the ΔT_m for heterogeneous hybrid "a" is 10°C. Similarly, the T_m for hybrid "b" is 76°C, and the ΔT_m for hybrid "b" is 6°C. According to the international committee on the reconciliation of approaches to bacterial systematics, species identities should be circumscribed by a range of ΔT_m lower than 5°C; or by a relative binding ratio of greater than 70% (for example, shaded area in (c)).

(c) The chart compares DNA–DNA and 16S rRNA based on a dataset of 180 values from 27 independent assessments for members of *Proteobacteria*, *Cytophaga-Flavobacterium-Bacteroides*, and Gram-positive bacteria containing a high proportion of guanine plus cytosine base pairs.

Diagrams are modified with permission from Raman Rossello-Mora and Rudolf Amann (see Rossello-Mora and Amann, 2001).

so far indicated that the most named bacterial species in fact contain many ecotypes, each of which exhibits complete attributes of the eukaryotic version of species. According to this line of argument, the groups currently recognized as individual bacterial species are more consistent with the genus level of classification in eukaryote systematics (Cohan, 2001). A version of the ecotype approach was formalized to a certain extent by David Ward who proposed a "natural species concept" for prokaryotes that is similar to the ecological species concept described for eukaryotes (Ward, 1998).

Modern renditions of Ravin's genospecies concept have emerged under the title of "genomic species concept", which defines a species as a group showing high DNA–DNA hybridization values (approximately 70% or greater DNA–DNA relatedness, and 5°C or less ΔT_m; see Box 1.2). In an attempt to anchor the genomic species concept within an ecological and evolutionary framework, Rossello-Mora and Amann (2001) proposed a "phylophenetic species concept" where species are defined as "a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity in many independent characteristics, and is diagnosable by a discriminative phenotypic property." This definition integrates character-based concepts that emphasize the presence of an apparent organismal attribute with history-based concepts that emphasize the degree of relatedness of a new isolate to previously characterized organisms. Furthermore, Sicheritz-Ponten and Andersson (2001) extended the application of the phylo-phenetic concept by developing a "phylogenomic" approach to microbial evolution where phylogenetic information is linked to the flow of biochemical pathways within and among microbial species. By constructing a complete set of phylogenetic trees derived from proteome databases, the phylogenomic approach facilitates the inclusion of horizontal genetic exchange events in the identification of microbial species (Ogunseitan, 2002; Sicheritz-Ponten and Andersson, 2001).

THEORETICAL MECHANISMS OF SPECIATION

Microbial diversity is a dynamic phenomenon, varying across both spatial and temporal dimensions in response to changes in the biotic and abiotic components of ecosystems. The emergence and extinction of species over long time frames are taken for granted according to the overarching theory of evolution. Unlike the case for eukaryotes, however, there is no direct evidence of microbial extinctions because fossil records cannot capture microbial characteristics in sufficient detail to determine species status. In fact, there are almost as many theories about the formation of species as there are concepts to define species (White, 1978). In view of the long time scale generally invoked for evolution by natural selection, most theories of speciation developed for large multicellular eukaryotes are necessarily based on the interpretation of historical evidence regarding the relationships among groups of organisms. To a limited extent, additional pieces of evidence for patterned speciation are based on the analysis of interactions among contemporary organisms and on the extent of similarity between independent genomes. In microbiology, however, the relatively short length of organism generation times and the availability of powerful techniques for genetic analyses and bioinformatics, are making it possible to investigate speciation and natural selection by means of empirical and computational methods (Arnold *et al.*, 2001; Elena and Lenski, 2003; Lenski *et al.*, 2003a and 2003b).

Speciation is the precursor of macroevolution, which leads to the generation of major taxonomic groups. Therefore, plausible theories about the mechanism of speciation need sufficient robustness to explain the outcome of both small and large changes in the parameters that modify phylogenetic lineages. As in the case of species definition and the development of a universal concept of species, most theoretical proposals on the mechanism of speciation are based on observations of eukaryotic organisms. The fossil record provides a particularly rich source of data enabling the discovery of change in eukaryotic lineages over long periods of time. These accessories have revealed two basic patterns that describe modifications in phylogenetic lineages. These patterns are formally known as theoretical frameworks for speciation, namely anagenesis and cladogenesis (Mayr, 2001). It is important first to explore the implications of these two basic patterns of speciation for theories that are directed more clearly toward macroevolutionary processes.

Anagenesis

Anagenesis is among the prominent speciation theories which hold that higher levels of specialization and/or organization are generated from primordial lineages through progressive evolution. As a pattern of evolutionary change, anagenesis involves the transformation of subpopulations to such extents that these subpopulations are sufficiently different from the ancestral population to warrant recognition as a separate species. There are no pre-established criteria for how much change must occur before a new species designation can be conferred. Therefore, the outcome of anagenesis is somewhat arbitrary. Anagenesis is sometimes referred to as phyletic speciation, implying that species are transformed along a phylogenetic lineage from a single progenitor without producing branches from the main trunk of the phylogenetic tree. This means that the total number of existing species remains conserved, excepting extinctions (Mayr, 2001).

Cladogenesis

Cladogenesis is a pattern of speciation that requires the branching of phylogenetic trees through the formation of species that are recognizably different from the parental lineage, which also continues existing. Unlike anagenesis, where species are transformed along a continuous lineage, cladogenesis actually increases the number of existing species, and as such, it leads to an expansion of biological diversity. It is possible to deduce from the fossil record that the cladogenesis pattern of speciation predominates, because the number of species within a taxon typically increases over long periods of time. The underlying mechanism of speciation in cladogenesis is either abrupt or gradual. Abrupt speciation, or “saltation”, is the discontinuity in a lineage that occurs through genetic mutations or chromosomal aberrations causing reproductively isolated individuals to establish a new species population. In proposing the theory of “punctuated equilibrium”, Stephen Gould and Niels Eldredge built upon the concept of “hopeful monsters”, first defined by Goldschmidt in the mid-twentieth century in support of abrupt speciation (Ayala, 1982; Eldredge and Gould, 1988; Gould, 2002).

The evidence for punctuated equilibrium is largely circumstantial in the fossil record. It has not been investigated among the prokaryotes, although laboratory experiments with *Escherichia coli* have provided valuable insights into the emergence of variants in microbial populations (Elena *et al.*, 1996; Rosenzweig *et al.*, 1994). In eukaryotes, polyploidy, the multiplication of chromosome sets, is a well-established mode of abrupt speciation, and by some estimates up to a third of all plant species resulted from polyploidy (Baum and Donoghue, 1995). Karyotypic fission, symbiogenesis, and lateral gene transfer are additional possible mechanisms of abrupt speciation. In contrast to abrupt speciation, gradual speciation is easier to infer from Darwin’s theories of evolution by natural selection. Random mutations, most of which have no impact on fitness, produce cumulative genetic divergence until reproductive isolation occurs to separate two or more distinct species (Mayden, 1997).

Macroevolution theories

Most biologists will agree that there is a qualitative difference between speciation as described by anagenesis or cladogenesis and major changes in organism forms and functions that lead to the emergence of completely different phylogenetic lineages. Such drastic change has been invoked in the emergence of the three domains, *Archaea*, *Bacteria*, and *Eukarya*. According to anagenesis and cladogenesis, members of a lineage can be traced back to ancestral organisms either through the fossil record or through structural and functional similarities maintained among a cohort of related species. However, it is more difficult to explain, for example, the emergence of complex structures that differentiate lineages as distant as bacteria are from plants or animals. The origins of multicellularity and complex organismal traits have long challenged evolution theorists. The traditional strategies for addressing these challenges have relied in part on arguments rooted in the relationship between ontogeny and phylogeny. That is, by comparing the size and function of features expressed during the course of growth and the development of an individual from gestation to maturity across phylogenetic groups, it is possible to make generalizations about the trajectory of anagenesis from the rudiments of complex structures.

More recently, researchers in the field of cellular automata—or computer-aided studies in evolution—have written computer software programs that simulate interactions among biological cell analogs to explore plausible outcomes of alternative scenarios in the emergence of multicellularity and the evolution of complex organs. Based on this approach, Pfeiffer and Bonhoeffer (2003) have argued that the benefit of clustering in populations of unicellular organisms includes the reduction of potential interactions with non-cooperative individuals. Furthermore, clustering can evolve as a biological, heritable trait for cells that cooperate to use external energy resources. Along the same lines, Lenski and colleagues (2003b) used digital organisms—computer programs that self-replicate, mutate, compete, and evolve—to argue for the relative simplicity of evolution toward the ability to perform complex logic functions that require the coordinated execution of many genomic instructions. In these quasi-experiments, complex functions evolved by building on simpler functions that had evolved earlier, as long as these functions were also favored by natural selection. Furthermore, no intermediate stages were required for the evolution of complex functions, and seminal genotypes with the ability to perform complex functions differed from their "wild-type" parents by just a few mutations. However, the genetic difference between these seminal genotypes and their distant ancestors was characterized by several mutations, suggesting that it may not be possible to track the lineage of biological complexity through the analysis of molecular sequences. In some cases, mutations that were deleterious when they first appeared served crucial functions in the evolution of complex features.

These results demonstrate that complex functions can indeed originate by random mutation and natural selection. However, it is important to explore organic versions of these experiments before firm conclusions can be reached about their implications for natural evolution. To place the experiments in the context of macroevolution, it is important to discuss earlier attempts to formulate theories that have been proposed to account for the discrete nature of speciation and gaps in phylogenetic lineages. Twelve major mechanisms of speciation are recognized under three theoretical categories, namely phyletic speciation, species fusion, and gradual speciation through populations. Not all of these mechanisms are relevant to speciation in prokaryotic populations but they are all presented to demonstrate the difficulty inherent in deriving a generalized theory of speciation that covers all categories of organisms (Table 1.2). Phyletic speciation or the transformation of existing species along a phylogenetic lineage is probably the best supported through evidence available from analysis of prokaryotes. Phyletic speciation occurs through anagenetic and cladogenetic pathways as discussed above, but there are no well-articulated ways through which these mechanisms can lead to the major transformations required to explain macroevolutionary events. However, species fusion and mechanisms of species multiplication can, in principle, explain major changes in species lineages. These theories remain controversial in microbiology because of the absence of concrete evidence suggesting that the theoretical mechanisms have actually contributed to the apparent diversity of microbial species.

Species fusion theory

The "species fusion theory" describes the formation of entirely new hybrid species from two or more pre-existing species where reproductive isolation mechanisms cease to exist as barriers. This can happen in cases where the reproductive isolation mechanisms are due to ecological factors as opposed to structural, physiological, or genetic factors. It is reasonable to argue in such cases that reproductive isolation was not strict, therefore the species preceding the hybridization are not independent, and instead are variants or strains. Predominance of species fusion over other mechanisms of speciation will result in reticulate evolution where repeated intercrossing between lineages produces a network of relationships in a series of related species. In this case, the phylogenetic map will resemble a net instead of a tree with branches (Hilario and Gogarten, 1993). The endosymbiotic theory as elaborated by Lynn Margulis and her colleagues is a representation of species fusion. Endosymbiosis among prokaryotic cells is the leading uncontested theory that is usually cited to explain

Table 1.2 Major speciation theories and underlying mechanisms.

Major categories of speciation theories	Subcategories of speciation theories	Mechanisms of speciation		Could the theory apply to prokaryotes?
Phyletic speciation (transformation of existing species)	Autogenous transformation (anagenesis and cladogenesis)	Random mutation and natural selection		Yes
	Allogenous transformation	Introgression from other species		Yes
Species fusion	Reduction in the number of species	Extinction of parental lineage due to competition with emergent (newly fused) species		Possible origin of Archaea
	Increase in the number of species	Both parental species coexist with newly formed (fused) species		Not documented
Species multiplication	Instantaneous speciation	Genetic	Single mutations	Yes
			Macrogenesis	No
		Cytological	Chromosomal mutation (e.g. transposition; translocation)	Possible
			Autopolyploidy	No
			Amphiploidy	No
	Gradual speciation	Sympatric speciation		Not documented
		Semi-geographic speciation		Not documented
		Geographic speciation		Not documented

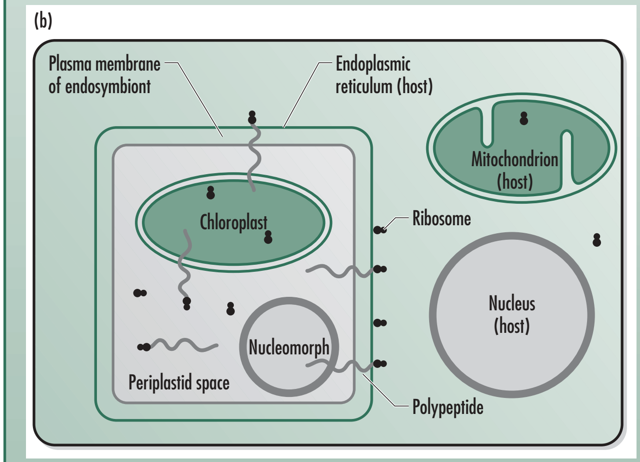
the origin of organelles such as mitochondria and chloroplasts. The contribution of endosymbiosis to the origin of the nucleus and, by default, the origin of the eukaryotic lineage, is more controversial. Nevertheless, endosymbiosis is at least as plausible as any other proposed mechanism used to explain the separation of the Eukarya branch from prokaryotic Archaea and Bacteria progenitors (see Box 1.3; Gray *et al.*, 1999; Margulis, 1996).

The endosymbiosis theory posits that the mitochondria found in all eukaryotic cells evolved from rickettsiae-like aerobic members of the α-*Proteobacteria* living within ancestral host cells (Emelyanov, 2001). In addition, algal and plant chloroplasts are presumed to have evolved from endosymbiotic cyanobacteria (Box 1.3(a)); whereas the cilia of eukaryotes evolved from endosymbiotic spirochetes (Box 1.3(b)). Furthermore, mitosis may have been invented through the creation of the mitotic spindle by basal bodies from which kinetosomes develop. These propositions of the endosymbiosis theory are supported by several lines of evidence, including observations that mitochondria and chloroplasts are not synthesized *de novo* by eukaryotic cells but instead they can only arise from pre-existing mitochondria and chloroplasts. These organelles also maintain independent genomes that bear remarkable semblance to prokaryotic genomes in the sense that they are covalently closed circular DNA molecules with no histones aiding in their packaging. The transcription and translation of mitochondrial and chloroplast genomes are also more related to prokaryotic rather than eukaryotic processes. Finally, the 16S ribosomal RNA (rRNA) signatures of these two organelles clearly demonstrate their affiliation with prokaryotes (Gray *et al.*, 1999; Margulis *et al.*, 1998). However, there are some contradictory pieces of

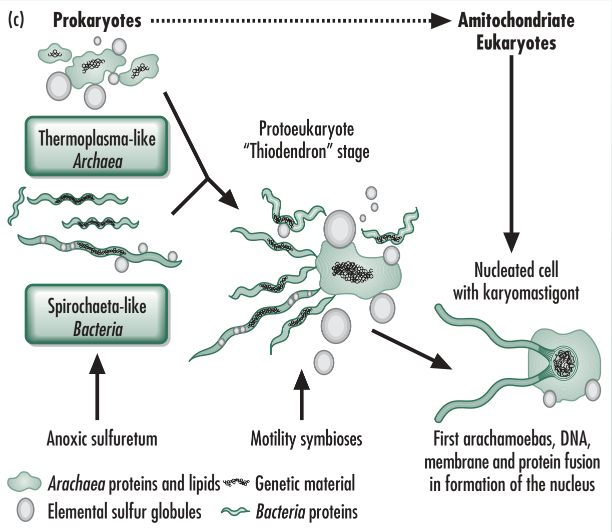
BOX 1.3

(a)

The endosymbiosis theory of speciation, as articulated by Lynn Margulis (a), is supported by substantial evidence in the prokaryotic origin of two classes of eukaryotic organelles: Mitochondria and the photosynthetic plastids. The relevance of the mechanism to other essential components of eukaryosis is less well understood, although there are indications that endosymbiosis resulted in the acquisition of prokaryotic features resulting in eukaryotic cell motility. Endosymbiosis also provides a plausible mechanism for the origin of viruses and plasmids, although the competing hypothesis based on excision of autonomously replicating units from microbial genomes is equally plausible. Like Mayr, Lynn Margulis rejects the three-domain phylogenetic tree proposed by Carl Woese in favor of a two-domain, five-kingdom system with the eukaryotes emerging from symbiotic interaction between the prokaryotic Bacteria and Archaea (see Margulis and Schwartz, 1998). The picture of Lynn Margulis is by courtesy of Jerry Bauer.



(b) Some of the autonomy maintained by mature endosymbionts such as the chloroplast, including protein synthesis machinery, and the vestigial nucleus (nucleomorph found in a limited number of protists groups). The diagram is by courtesy of John Kimball.



(c) The postulated pathway by which archaeal and bacterial cells enter stable endosymbiotic relationships is based on cell motility under environmental conditions that support sulfur metabolism. The illustration depicts a scenario where Thermoplasma-like Archaea species fuse with Spirochaeta-like Bacteria species in an anaerobic sulfur environment to create a motility symbiosis Proto-Eukarya species, which ultimately emerges as nucleated organisms with features similar to those associated with contemporary members of the Eukarya lineage. Circles represent genomes; triangles represent Archaea proteins and lipids; squares represent Bacteria protein and lipids; rods represent elemental sulfur globules. The diagram is modified by courtesy of Kathryn Delisle from Margulis *et al.* (1998).

(d)

(d) Carl Woese, a pioneer of molecular phylogenetic studies, focused on the analysis of microbial 16S rRNA to propose that the old kingdom "monera" should be recognized as consisting of two distinct phylogenetic lineages, the Archaea and the Bacteria. The cellular structure of the Archaea resembles the Bacteria, but aspects of their nucleic acid profiles are more like the Eukarya. The speciation process that splits the prokaryotic lineage into two major branches remains an unresolved research question. The Archaea and Bacteria are frequently observed to coinhabit similar ecological niches, and recent evidence of genetic exchange between the two branches may imply prehistoric interactions. Indeed, Radhey Gupta (1998a and 1998b) has shown through comparative analysis of protein sequences called indels that the fundamental phylogenetic distinction among prokaryotes is not the Archaea/Bacteria division because the Archaea show remarkable similarity to Gram-positive bacteria. Rather, prokaryotes may be delineated according to whether cells have a monoderm structure (surrounded by a single membrane, including members of the Archaea and Gram-positive bacteria) or whether cells have a diderm cell structure (surrounded by an inner cytoplasmic membrane and an outer membrane, including all true Gram-negative bacteria). The picture of Carl Woese is by courtesy of Bill Weigand and the University of Illinois at Urbana-Champaign.

evidence against the tempting generalization that all such organelles derive from prokaryotic endosymbionts. Martin (1999), while agreeing with the explanatory power of the endosymbiosis theory in accounting for the origin of mitochondria and hydrogenosomes, has argued that the endosymbiotic origin of the nucleus is not plausible, although the evidence appears incontrovertible for the endosymbiotic origin of the mitotic apparatus found in nuclei, the karyomastigont and other spindles (Margulis *et al.*, 2000). Also, the chloroplasts of some algae may have evolved through a different kind of endosymbiotic process (secondary endosymbiosis) involving the engulfment of pre-existing photosynthetic eukaryotes. In these cases, the organelle retains some eukaryotic properties such as a pseudonucleus (nucleomorph) (Archibald and Keeling, 2002). The endosymbiotic theory and its significance to the origin of eukaryotes are discussed further in Chapter 6.

Gradual speciation

The divergence of two or more populations of a single species to extents that they reach distinct status as independent species is called "gradual speciation". The population divergence has been postulated to occur either through geographical separation where gene flow is prevented by extrinsic factors (geographic speciation) or through the colonization of different ecological niches within the same geographical zone and the subsequent prevention of gene flow by intrinsic factors (sympatric speciation). Geographic and sympatric versions of gradual speciation agree on the significance of ecological factors, however, they disagree on the process by which ecological factors constitute a barrier to gene-pool mixing (Nixon and Wheeler, 1990; Templeton, 1989). In his searing criticism of sympatric speciation, Ernst Mayr evaluated several cases that have been proposed as examples in zoology, and reached the conclusion that the hypothesis is neither necessary nor supported by irrefutable facts (Mayr, 1970). Although there are no records of explicit tests of the sympatric speciation hypothesis in microbial populations, its marginalization of genetic dispersal, exchange, and recombination suggests that the hypothesis will be of little use in understanding microbial evolution and diversity. In contrast to sympatric speciation, and after periods of vigorous contestation, many zoologists and botanists now consider geographic speciation to be a universal mode of speciation. However, similarly vigorous explorations of the topic have not taken place in environmental microbiology (Zavarzin, 1994), although the recent development of powerful molecular tools for analyzing microbial populations and the research initiatives to establish microbial observatories may facilitate hypothesis testing in this direction (Petursdottir *et al.*, 2000).

Geographic speciation refers to an elaborate three-step mechanism by which new species are derived from established populations. The first step involves a niche division or spatial isolation caused by geophysical or chemical factors acting non-uniformly upon a habitat. Secondly, the environmental variation produces different selective pressures that favor certain mutations which would otherwise be neutral in the population. Thirdly, the resulting genetic and phenotypic differences are reinforced by reproductive isolation caused by geographic barriers. Geographic isolation can have three possible outcomes. **Character displacement** occurs when genetic differences between species reduce competition for limiting resources, and coexistence is maintained. **Competitive exclusion** may result from insufficient divergence in a recent speciation event, leading to the survival of a dominant species at the expense of a less fit species. The third alternative is the establishment of **hybrid zone** due to incomplete reproductive isolation of geographically adjacent species populations. A population of hybrids is thus established between the geographical zones occupied by the two species (Hull, 1997). Parapatric speciation has been postulated as a variation of geographic speciation in cases of sessile organisms, where physical geographical isolation is unnecessary to produce population isolation. When mutants in a population are able to exist in the same geographical space, but exploit different limiting resources, a contiguous niche is created that can further reinforce the speciation process.

One of the major challenges encountered in attempts to verify the importance of geographic speciation theory in microbial populations is the difficulty of accommodating the continuum of differences across populations and among species. For example, it is widely accepted to discuss morphological diversity as a crucial differentiating factor of microbial species. But so also is evolutionary history, biochemical diversity, behavioral diversity, or genetic diversity. Increasingly, genetic distance as measured by molecular sequence comparisons is emerging as the gold standard of microbial speciation. For example, Petursdottir and colleagues (2000) used allelic variation of 13 genes which code for polymorphic enzymes to explore the genetic diversity of 81 *Rhodothermus* isolates from different geothermal environments in Iceland. Their results revealed 71 distinctive multilocus genotypes with a mean genetic diversity per locus of 0.586 (on a scale of 0 to 1 where 1 represents maximum diversity). They concluded that the relatively high genetic variance observed within *Rhodothermus* isolates from different locations is most likely the result of genetic changes occurring independently in the locations studied. Furthermore, partial or whole sequencing of the 16S rRNA genes of the isolates confirmed that all the isolates belonged to the species *Rhodothermus marinus*. The results strongly suggest that despite phylogenetic and phenotypic similarity, genetic diversity within microbial species at different geographic locations may be very high.

MICROBIAL SPECIATION

One of the enduring debates in microbial evolution centers on the nature of the more important driving forces behind speciation: random mutation or genome acquisition? In an attempt to reinforce the endosymbiosis theory, Margulis and Sagan (2002) presented an argument against the dominant role of random mutation, partly through the recognition that the term "species" does not apply easily to prokaryotes because of the high frequency and extreme promiscuity that characterize genetic exchange in these organisms. They admit that random mutations are important for generating metabolic and genetic diversity in prokaryotes, but since there are no species *per se*, these mutations are irrelevant for speciation. In this view, the real question of speciation can only be posed to the eukaryotes—the animals, plants, protoctists, and fungi where the most secure pieces of evidence for speciation are linked, albeit controversially, to symbiotic genome relationships with microorganisms.

A comprehensive theory about the mechanisms of microbial speciation will likely await a more thorough assessment of microbial diversity with respect to the interactions between genetic and ecological factors that produce and maintain reproductive isolation. Speciation is driven by genetic variation, and in addition to random mutations that occur because of nucleic acid replication, prokaryotic organisms have evolved sophisticated ways for acquiring and losing genetic material. In prokaryotes, three major mechanisms of genetic exchange are well understood, and have been documented to exist in nature with major ramifications for the acquisition of new traits and speciation (Aravind *et al.*, 1998; Cohan, 1994; Ochman *et al.*, 2000; Ogunseitan, 1995). These mechanisms are discussed briefly in the following paragraphs, but a more detailed discussion is presented in Chapter 9.

Conjugation, the direct cell-to-cell transfer of DNA, is the closest process to eukaryotic sex-mediated genetic exchange. Conjugation is capable of moving large sizes of genetic material, including chromosomal genes and entire plasmids from one cell to another, but it is constrained within species. Therefore, it is likely to be very important in the creation of strains, but its role in actual speciation is not presently clear. Small segments of genetic material can also be transferred from one cell to another through the phenomenon of **transduction**, which is mediated by viruses. Virus infection is typically host specific, and the development of resistance is rampant. Therefore, the role of transduction in microbial speciation is also questionable. **Transformation**, the uptake of genetic material directly from the environment, is potentially an efficient mechanism for speciation because the source of transforming DNA can be from a variety of species. Therefore, transformation can introduce novel genes into a microbial population ultimately leading to niche specialization and speciation. However, the occurrence of microbial nucleases suggests that microorganisms have evolved protective measures against transformation, at least at high frequencies.

Given these effective mechanisms of genetic exchange among prokaryotes, it is pertinent to ask how much of microbial speciation can be explained by genome acquisition. Levin and Bergstrom (2000) considered that the genome acquisition theory of adaptive evolution in bacteria is a simple extension of theories developed for sexually reproducing eukaryotes, and that the modes of genetic recombination described above for microorganisms, especially bacteria, are quantitatively and qualitatively different from that of organisms for which recombination is an integral part of the reproduction process. These differences have substantial consequences for the evolution of accessory elements and their role in the adaptive evolution of bacteria as the major driving force for speciation. Lawrence (2001) attempted to correlate the rate of successful acquisition and integration of genetic material obtained through intra-specific lateral genetic transfer with the amount of **genetic headroom** present in recipient species. He defined genetic headroom as the codon usage bias and codon context bias that can be transiently sacrificed to allow a species population to experiment with functions introduced by gene transfer. In this context, Noble *et al.* (1998) also used a chaos-game representation scheme to identify tetranucleotide frequencies in microbial genomes as an index for the residence time of contiguous genomic segments in prokaryotes.

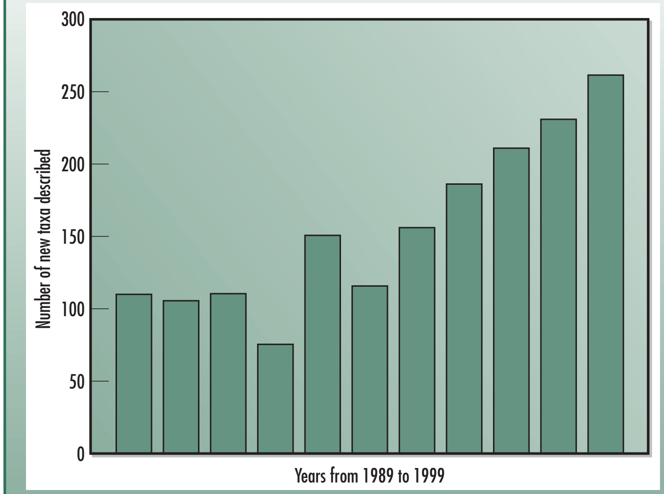
Similar observations regarding biases in codon utilization have led to a robust hypothesis on the differentiation of viral groups according to host range, the equivalence of prokaryotic speciation. By evaluating the relationships between the nucleotide composition of retroviral genomes, the amino acid composition of retroviral proteins, and evolutionary strategies used by retroviruses, Bronson and Anderson (1994) demonstrated that the genome of each viral lineage has a characteristic base composition and that the variations between groups are related to retroviral phylogeny. In experiments conducted with cultures of *E. coli*, several investigators have reported the development of genetic polymorphism arising from a single genetically pure strain (Finkel and Kolter, 1999; Souza *et al.*, 1997; Rosenzweig *et al.*, 1994). Such polymorphisms, when they are not silent (i.e. when they are expressed and confer a selective advantage), are consistent with the parapatric mode of speciation where polymorphic clones arise in a population, but coexist because of differences in niche specialization or resource utilization.

CONCLUSION: EMERGING CONCEPTS AND APPLICATIONS OF MICROBIAL DIVERSITY

Species and speciation are, respectively, the fundamental units and the determinants of microbial diversity. Firm understanding of the scientific bases for species concepts and proposed mechanisms of speciation will achieve more than simple provision of an internally consistent language for taxonomy and systematics. If achieved with confidence, the understanding of microbial speciation will facilitate the formulation and testing of hypotheses on the most important questions facing biology today. An introduction to the relevance of microbial diversity concepts to these questions is presented below. The research paradigms are changing rapidly on many fronts, and salient aspects of the changes are discussed in more detail in subsequent chapters of this book.

It is difficult to overstate the significance of the discovery of prokaryotic Archaea proposed as a third major branch of the universal phylogenetic tree, separate from prokaryotic Bacteria and eukaryotic Eukarya. The new phylogenetic tree is a major revision of the previous five-kingdom phylogenetic tree including plants, animals, fungi, protists, and the monera consisting of all prokaryotes. Many scientists contributed to this discovery, but Carl Woese was particularly influential in facilitating the comparative assessment of rRNA nucleotide sequence information for phylogenetic analysis (DeLong and Pace, 2001; Pace,

BOX 1.4



Aided by molecular phylogenetic methods, the number of new prokaryotic species described has steadily increased in the decade from 1989 to 1999. New requirements for polyphasic species identification may diminish concerns about basing speciation on single molecular signatures such as rRNA profiles. Data are from Rossello-Mora and Amann (2001).

1997; Woese, 1998a; Woese *et al.*, 1990). In addition to the proposal to regroup phylogenetic relationships among microorganisms, the use of phylogenetically conserved molecular sequence information allowed microbiologists to venture into the realm of identifying nonculturable organisms (Embley and Stackebrandt, 1997; Ward *et al.*, 1990). The extraction and analysis of nucleic acids from environmental samples contributed to the remarkable increase in the number of microbial genus and species discovered in the past decade (see Box 1.4). These molecular tools have raised new and important questions about the ecological context of microbial diversity. However, new doubts have arisen about the correlation between molecular identity and speciation, particularly with respect to the major domains of the universal phylogenetic tree (Brinkmann and Philippe, 1999; Gupta, 1998a and 1998b; Margulis *et al.*, 2000; Mayr, 1998; Palleroni, 1997; Woese, 1998a). Clearly, there is tremendous structural diversity and resilience in the microbial community. What is less clear is the correlation between the structural diversity and functional diversity. The problem is exacerbated by the impracticality of exhaustive inventories of microbial communities. Regardless of the technique used for defining species, an indulgence in statistical inference is essential for quantitative assessments of microbial diversity (Hughes *et al.*, 2001).

The prevalence of organisms belonging to the Archaea branch in extreme environments such as hot water springs, acidic lakes, and hyper-saline environments suggests that niche specialization plays a crucial role in microbial speciation (Stetter, 1996; DeLong and Pace, 2001). Conversely, the global distribution of the most described microbial species is not limited by geographical boundaries, indicating that geographic separation is not a major contributing factor of microbial speciation (Stoner *et al.*, 2001; Zavarzin, 1994). The deepest branches in the phylogenetic trees of the Archaea and those of the Bacteria (e.g. *Thermotogales* species and *Aquifex* species) are among thermophilic organisms, strongly suggesting that the progenitor of the phylogenetic lineage evolved in a hot environment and under conditions postulated to describe the early Earth environment (Doolittle, 1999; Koonin *et al.*, 1997; Stetter, 1996; Woese, 1998a). Therefore, investigations of microbial speciation and diversity are directly relevant to research on the origin of planetary life, and on how the physiological characteristics of prehistoric microorganisms contributed to the emergent signature of a co-evolving life and environment (Brown *et al.*, 2001; Martin and Müller, 1998; Rasmussen, 2000).

Improved understanding of microbial speciation and diversity can also shed light on the path dependence of the trends associated with biogeochemical cycles that are sensitive to industrial ecological problems such as global warming, toxic pollutants, and disease epidemics (Martin and Müller, 1998). For example, Lake and colleagues (1985) proposed a new group of photocytes as the progenitors of photosynthetic organisms. Photosynthetic prokaryotes are interspersed though most major phylogenetic lineages of Bacteria and Archaea, and they demonstrate a remarkable physiological diversity. An assessment of the diversity of photosynthetic light-gathering arrays in the microbial world can yield biotechnologically adaptive systems that are potentially relevant to the global energy crisis. Similarly, strong cases can be made for investigating the speciation processes underlying the ancient lineage of hydrogen-oxidizing bacteria such as *Aquifex*, and their contribution to models of early atmospheric composition on Earth. These and other cases of the contributions of microbial diversity to the understanding of contemporary global environmental problems are discussed in the final chapter of this book.

QUESTIONS FOR FURTHER INVESTIGATION

1 Find one example each, in the microbial world, of exceptions to the traditional species concepts discussed in this chapter.

2 Find four examples in the scientific literature of evidence to support microbial speciation through (a) genetic exchange mechanisms, (b) niche specialization, (c) geographical isolation, and (d) genetic drift.

3 The proposal to reconstruct the phylogenetic tree of life on Earth to include two main lines of prokaryotes and one line for eukaryotic organisms represented a paradigm shift in the understanding of speciation. Acceptance of this paradigm depends on the definition of species, and the driving forces of speciation, which differ across biological disciplines. All paradigm shifts in science are rigorously contested. Find one example each in the botany, zoology, mycology, and bacteriology literature where Carl Woese's revised phylogenetic tree is contested. Compare and contrast the rationale for each dissenting opinion.

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