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The structure of microbial evolutionary theory

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Abstract

The study of microbial phylogeny and evolution has emerged as an interdisciplinary synthesis, divergent in both methods and concepts from the classical evolutionary biology. The deployment of macromolecular sequencing in microbial classification has provided a deep evolutionary taxonomy hitherto deemed impossible. Microbial phylogenetics has greatly transformed the landscape of evolutionary biology, not only in revitalizing the field in the pursuit of life's history over billions of years, but also in transcending the structure of thought that has shaped evolutionary theory since the time of Darwin. A trio of primary phylogenetic lineages, along with the recognition of symbiosis and lateral gene transfer as fundamental processes of evolutionary innovation, are core principles of microbial evolutionary biology today. Their scope and significance remain contentious among evolutionists.

Keywords: Microbial evolution; Microbial phylogeny; Procaryote; Superkingdoms; Symbiosis; Lateral gene transfer

1. Introduction

The evolutionary synthesis of the first half of the twentieth century crafted a sterile conception of evolution: one without microorganisms. It was confined to plants and animals whose histories at best cover 20% of the total evolutionary time on earth. Accordingly, the historical works on that synthesis make scant reference to microbes, if at all (e.g. Mayr & Provine, 1980; Mayr, 1982). Certainly, evolutionists assumed that plants and animals evolved from the 'lower' or 'primitive' organisms that microbes were conceived to be. The evolution of the microbial world was, however, largely beyond the evolutionists' purview and outside the structure of classical evolutionary thought. Biologists knew little more about the natural history of microbes, their relationships to one another and to other organisms, than they did in the time of Pasteur and Koch.

The emergence, of microbial phylogenetics, based on macromolecular sequencing, has brought great change to biology, revitalizing the study of evolution and extending it over the span of some three and a half to four billion years. In so doing, it has sown several fundamental concepts in the field of evolution: the three primary domains of life (Archaea, Bacteria and Eucarya); the role of symbiosis in the origin of the eucaryotic cell; and the ubiquity of lateral gene transfer among bacteria (between 'species'). Each of these represents a profound theoretical shift; together they provide the basic structure for microbial evolutionary thought today. Still, their paradigmatic parameters, their scope and significance, remain subjects of

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controversy. The Archaea and Bacteria are widely taught in textbooks as fundamental phylogenetic lineages. Should the procaryote–eucaryote dichotomy be discarded? It is accepted today that mitochondria originated from alpha proteobacteria, and chloroplasts from cyanobacteria. Did the nucleus also arise from another symbiosis? It is recognized now that lateral gene transfer may occur across the bacterial phylogenetic spectrum. Is it so intense as to completely confound bacterial phylogenetics? Does Darwin's genealogical framing of evolution actually hold true in the microbial world? To elucidate how these issues have arisen, I begin with an historical overview of one of the basic dichotomies of biology: that between the procaryote and the eucaryote.

2. Superkingdoms of legend

Biological thought is profoundly affected by classifications and dichotomies; it therefore becomes of some importance to deconstruct them. We have all been taught a basic bifurcation of life: that between the procaryote and the eucaryote, typically presented as the superkingdoms *Procaryotae* (or Monera) and *Eucaryotae*. The story about these superkingdoms begins with an extraordinary legend consisting of three vital components. The first is that in the 1920s or 30s (it is not certain when) French protozoologist Edouard Chatton coined the terms 'procaryote' and 'eucaryote' with 'singular prescience', articulated their differences, and formulated them as the basis for classifying organisms into two taxa at the highest levels. Secondly, in 1962, when Roger Stanier and C. B. van Niel reintroduced the terms to English readers and further defined those differences, they too argued for two fundamental taxonomic domains, and thirdly, they defined procaryotes as organisms lacking a cytologically definable nucleus. Recent scholarship has dispelled all three features as erroneous historical artifacts (Sapp, 2005b; 2006a), but because they have become integral parts of the foundation of microbiology, it is vital to understand their roots.

The tale of Edouard Chatton, embellished to the present day, originated in Stanier and van Niel's famed paper of 1962, 'The concept of a bacterium'. The authors lamented that,

> Any good biologist finds it intellectually distressing to devote his life to the study of a group that cannot be readily and satisfactorily defined in biological terms; and the abiding intellectual scandal of bacteriology has been the absence of a clear concept of a bacterium. (Stanier & van Niel, 1962, p. 17)

There had been several unresolved issues about the organization of bacteria and blue-green algae over the previous eighty years: Was it true that bacteria and blue green-algae lacked a nucleus? Did they possess plastid-like entities? Stanier and van Niel aimed to define the anatomy of bacteria and blue-green algae in a way that unequivocally distinguished them from other organisms. They proclaimed,

> It is now clear that among organisms there are two different organizational patterns of cells, which Chatton (1937) (sic) called, with singular prescience, the eucaryotes and procaryotic types. The distinctive property of bacteria and blue-green algae is the procaryotic nature of their cells. It is on this basis that they can be clearly segregated from all other protists (namely, other algae protozoa and fungi), which have eucaryotic cells. (Ibid., pp. 20–21)

Since the 1960s, biologists who have proposed that prokaryotes and eucaryotes be given the highest taxonomic rank of superkingdoms have gone further and suggested that Chatton had articulated an organizational and taxonomic distinction. Ernst Mayr wrote,

> Although foreshadowed by suggestions made by earlier authors, by far the most important advance made in our understanding of the living world as a whole was the realization by Chatton (1937) (sic) that there are two major groups of organisms, the procaryotes (bacteria) and the eucaryotes (organisms with nucleated cells). This classification was confirmed and made more widely known by Stanier and van Niel, and it was universally accepted by biologists until recently. (Mayr, 1998, p. 9720)

Actually Chatton did not propose this dichotomy as a basis for classification, nor did Stanier and van Niel. I will explain why momentarily. First, it is important to note that Chatton wrote very little about it, and he referred to bacteria interchangeably as protists or protozoa. He used the terms 'procaryote' and 'eucaryote' in two diagrams in 1925, and in his only published statement about it, in 1938 (typically miscited as 1937), he wrote,

> Protozoologists agree today in considering the flagellated autotrophs as the most primitive of the Protozoa possessing a true nucleus, Eucaryotes, (a group which also includes the plants and the Metazoans) because they alone have the power to completely synthesize their protoplasm from a mineral milieu. Heterotrophic organisms are therefore dependent on them for their existence as well as on chemotrophic Procaryotes and autotrophs (nitrifying and sulfurous bacteria, Cyanophyceae). (Chatton, 1938, p. 50; my translation)

Although Chatton wrote nothing else about this, and despite statements made about his 'singular insight' and 'prescient generalization', others before him did articulate a distinction between two kinds of cellular organization, but their views about that dichotomy differed. For some, bacteria and blue-green algae possessed a primitive nucleus comprised of chromatin material, without a nuclear membrane separating it from the surrounding cytoplasm. For others, bacteria lacked all traces of a nucleus with hereditary determinants of any kind.

In his *Generelle Morphologie* of 1866, Ernst Haeckel proposed the kingdom Protista in which he included

primitive non-nucleated organisms that he grouped together as Monera. In biology textbooks today, Monera are often presented as one of five kingdoms, the remaining four being eucaryotic: Plantae, Animalia, Protista, and Fungi (Whittaker, 1969; Whittaker & Margulis, 1978). Yet here too there is historical and conceptual confusion, in effect another myth, for the nature of the organisms that Haeckel imagined to be Monera were remarkably different from those referred to decades later under the realm of that kingdom. Monera, as Haeckel (1866) conceived of them, were pre-cellular entities lacking all trace of hereditary determinants comparable to other organisms. Indeed, his postulation was in direct opposition to those who insisted that bacteria and blue-green algae possessed a primitive nucleus, and like other cells contained chromatin and hereditary determinants. Haeckel's more primitive Monera were the fruit of his monist philosophy which required the elimination of explanatory boundaries separating life and non-life.

Most of the creatures Haeckel assigned to the group in 1866 were later shown either to be nonexistent or to possess a nucleus. Later in his *The wonders of life* of 1904, he claimed bacteria and chromacae (blue-green algae) to be true to the definition. 'The whole vital activity of the simplest monera', he wrote, 'especially the chromacae is confined to their metabolism, and is therefore a purely chemical process, that may be compared to the catalysis of inorganic compounds' (Haeckel, 1904, p. 208). Bacteria and chromacae lacked any trace of what he regarded as the most 'the first, oldest, and most important process of division of labour' of the nucleus, which 'discharges the functions of reproduction and heredity, and the *cytoplasm* of the cell body [which] accomplishes the metabolism, nutrition and adaptation' (ibid., p. 35). The difference between monera and any higher organism, he said was 'greater in every respect than the difference between the organic monera and the inorganic crystals. Nay, even the difference between unnucleated monera (as cytodes) and the real nucleated cells may fairly be regarded as greater still' (ibid.).

Microbes had been understood predominantly from a medical perspective: the concept of germs as the agents of killer diseases signified the nineteenth-century transition to modern medicine. Indeed, the history of bacteriology is predominantly depicted from the perspective of pathology, germ theory and practice. Led by the pioneering work of Pasteur and Koch, pathological and public health laboratories expanded and multiplied as pathologists developed antitoxins, anti-sera and vaccines. To be sure, germ theory encompassed a wide range of processes, such as putrefaction and fermentation, as well as animal and human diseases. Life did not result from decayed organic matter, but was its cause. This was the conclusion of the debates and experiments over 'spontaneous generation' of the 1860s and 1870s (Farley, 1974; Geison, 1995; Strick, 2000).

Despite those achievements, virtually nothing was known of the origin and evolution of the microbial world; bacteria remained undefined biologically. Microbes generally had been referred to as Infusoria since the days of Linnaeus because they were readily found in infusions of decaying organic matter, but it became common place from a disease based perspective in the late 1870s to call them 'germs'. Joseph Lister referred to the 'theory of germs' in a letter in 1874 to Pasteur who, two years later, used the 'expression in print instead of 'theory of organized ferments'. In 1878, the term 'microbe' was introduced by Charles Sédillot, and was used interchangeably with 'germ' (Carter, 1991). The word 'bacteria' (from the Greek meaning little rod or staff) was frequently employed to embrace the smallest of germs, 'all those minute, rounded, ellipsoid, rod-shaped, thread-like or spiral forms' (e.g. Woodhead, 1891).

Biology had been divided into two ancient kingdoms: plants and animals (as it continued to be throughout most of the twentieth century). Linnaeus had classified all Infusoria as animals, but by the mid 1850s bacteria were usually considered to be plants (and still are when we refer to the 'flora' in our gut) and studied by botanists in universities who referred to them as *Schizomycetes* (fission fungi), and when they were grouped with blue-green algae, they were called as *Schizophyta* (fission plants) (Cohn, 1872). Haeckel argued that bacteria had nothing in common with fungi, and that the only real comparison between chromacea and plants was with the chromatophores (chromatella or chloroplasts). Thus, he suggested that chloroplasts had evolved as 'a symbiosis between a plasmodomonous green and plasmophagus not-green companions' (Haeckel, 1904, pp. 195–196).

Did bacteria and chromacae really lack nuclei? Observations were far from straightforward. Bacterial internal organization remained below the resolution of the light microscope. It did seem evident that bacteria lacked a nucleus that was enclosed by a membrane, but many bacteriologists reported clusters of granules that stained with dyes used to stain chromatin of other cells. 'For these reasons', wrote Edmund Beecher Wilson in *The cell in development and inheritance* in 1900,

> most observers . . . regard them as true chromatin granules which represent a scattered or distributed nucleus not differentiated as a definite morphological body. If this identification is correct, such forms probably give us the most primitive condition of the nuclear substance, which only in higher forms is collected into a distinct mass enclosed by a membrane. (Wilson, 1900, p. 40)

Still these issues remained unresolved, and diverse theories about bacterial anatomy were presented well into the 1940s (Dobell, 1911; Dubos, 1945).

In 1938, when Herbert Copeland proposed that Haeckel's name Monera be given to a fourth kingdom in addition to Protista, Plantae and Animalia, his argument was based on two assumptions: that bacteria and blue-green algae are 'the comparatively little modified descendants of whatever single form of life appeared on earth, and that

they are sharply distinguished from other organisms by the absence of nuclei' (Copeland, 1938, p. 386). In 1941 Stanier and van Niel followed Copeland in assigning the bacteria and the blue-green algae to the kingdom Monera (Stanier & van Niel, 1941). They also expanded the characterization of the group by adding to the absence of nuclei two additional and equally negative criteria: the absence of plastids, and the absence of sexual reproduction. But that would be the last time that they would refer to monera, or to bacteria as non-nucleated organisms.

At that time it was still not certain if bacteria (and viruses) possessed genes. In *The evolution of genetic systems*, British cytogeneticist, Darlington referred to 'asexual bacteria without gene recombination' and of 'genes which are still undifferentiated in viruses and bacteria' (Darlington, 1939, p. 70). In *Evolution: The modern synthesis*, Julian Huxley conceived of bacteria (and viruses) in the same way as did Haeckel at the turn of the century:

> Bacteria (and *a fortiori* viruses if they can be considered to be true organisms), in spite of occasional reports of a sexual cycle, appear to be not only wholly asexual but pre-mitotic. Their hereditary constitution is not differentiated into specialized parts with different functions. They have no genes in the sense of accurately quantized portions of hereditary substances; and therefore they have no need for accurate division of the genetic system which is accomplished by mitosis. The entire organism appears to function as soma and germplasm, and evolution must be a matter of alteration in the reaction-system as a whole. (Huxley, 1942, pp. 131–132)

The difference between bacteria and other cells became uncertain with developments in genetics and election microscopy following the Second World War. In 1946, Joshua Lederberg and Edward Tatum launched bacterial genetics when they experimentally demonstrated genetic recombination in *Escherichia coli* (Lederberg & Tatum 1946). Viruses were conceptualized as naked genes, as had been suggested by H. J. Muller (1922) earlier in the century. Both bacteria and their viruses (phages) were domesticated as vital biotechniques for molecular biology.

When in 1955, van Niel reconsidered the organization of bacteria and blue-green algae anew, in this new light he renounced the Monera kingdom on the same three grounds on which he and Stanier had avowed it in 1941. New evidence based on electron microscopy seemed to suggest that bacteria possessed nuclei, and that the photosynthetic bacterium, *Rhodospirillum rubrum* possessed plastid-like entities. Moreover, he argued, demonstrations of recombination in mixed cultures of bacteria by Lederberg and Tatum in 1946 had demanded 'a healthy scepticism with regard to the earlier belief that sexual phenomena do not occur among the bacteria'. His conclusion was decisive. 'It is clear', he said, 'that the criteria for a kingdom of organisms without nuclei do not apply to the bacteria and blue-green algae. This does not mean, however, that the notion of establishing a separate kingdom for these organisms should be abandoned' (van Niel, 1955, p. 93).

3. An organizational concept

Importantly, Stanier and van Niel made no mention of a separate kingdom for bacteria when they introduced the 'procaryote' and 'eucaryote' to English readers in 1962. Theirs was an organizational distinction, much like that made five years earlier by André Lwoff who distinguished the organization of the virus from that of the smallest bacteria. The virus did not reproduce by division like a cell; it contained either RNA or DNA enclosed in a coat of protein, and it possessed few if any enzymes, except those concerned with attachment to and penetration into the host cell. 'Viruses should be treated as viruses', Lwoff concluded, 'because viruses are viruses' (Lwoff, 1957, p. 252) There were no known transitional entities between a virus and a bacterium.

Lwoff recommended the terms procaryote and eucaryote which his former mentor Chatton had coined decades earlier (Chatton, 1925). And Stanier and van Niel employed them to characterize the bacteria and blue-green algae, using organizational terms, just as Lwoff had done with the virus. Chatton may have said little about that organizational dichotomy, but in effect he had said just enough. 'Procaryote' was a neutral term that could be molded to contemporary science. Stanier and van Niel explained that a satisfactory description of the bacteria and blue-green algae could be articulated only after advances in microscopy, molecular biology and genetics that followed the Second World War. Still, they could do little more than define the 'procaryote' negatively in relation to the eucaryote.

Eucaryotes possessed a membrane bound nucleus, a cytoskeleton, an intricate system of internal membranes, mitochondria that perform respiration, and in the case of plants, chloroplasts. The nucleus of bacteria lacked a membrane separating it from the cytoplasm, it divided by fission not by mitosis, and its DNA was never organized into individual chromosomes:

> The principle distinguishing features of the procaryotic cell are: 1. absence of internal membranes which separate the resting nucleus from the cytoplasm, and isolate the enzymatic machinery of photosynthesis and of respiration in specific organelles; 2. nuclear division by fission, not by mitosis, a character possibly related to the presence of a single structure which carries all the genetic information of the cell; and 3. the presence of a cell wall which contains a specific mucopeptide as its strengthening element. (Stanier & van Niel, 1962, p. 21)

The dichotomy was unmistakable; there would be no transitional forms between the procaryote and all other organisms. Stanier, Michael Douderoff and Edward Adelberg declared the next year in *The microbial world* that, 'In fact, this basic divergence in cellular structure, which separates the bacteria and blue-green algae from all other cellular

organisms, represents the greatest single evolutionary discontinuity to be found in the present-day world'. (Stanier et al., 1963, p. 85).

4. A natural order for bacteria?

Stanier and van Niel had aimed to make the distinction between the procaryote and eucaryote unequivocal, but they did not assign taxonomic value to them. To understand why, we need to situate their paper in the methodological debates during the first half of the twentieth century, over whether or not one could have a classification of bacteria that reflected their evolutionary relationships (Sapp, 2005a).

'All true classification is genealogical', Darwin wrote, and 'that community of descent is the hidden bond which naturalists have been unconsciously seeking, and not some unknown plan of creation, or the enunciation of general propositions, and the mere putting together and separating objects more or less alike' (Darwin, 1964 [1859], p. 420). When constructing genealogical trees, one had to distinguish relatively trivial traits from fundamental traits at the core of organisms. Adaptive characters (those that were most closely related to the habits of the organisms) were the least useful because they would be relatively recent developments characteristic of the species or variety. Constructing genealogical trees required comparisons of highly conserved ancient traits those that were far removed from everyday life. Such a phylogenetic classification among plant and animals could be based on comparative anatomy, comparative embryology and an ever improving fossil record.

Bacteria lacked complex morphological traits, developmental histories, and a fossil record. Although they did exhibit enormous physiological or biochemical diversity, one could not discern which traits were old and which were recent adaptations, and thus distinguish convergent traits from those that might have phylogenetic meaning. The relationships of bacteria to each other, and their mechanisms of inheritance, had been subjects of recurrent discussion, debate and speculation among bacteriologists prior to the Second World War (Sapp, 2005a). Constructing a 'natural', that is genealogical, classification of bacteria seemed to be impossible, and by the early 1920s, many bacteriologists had given up on phylogeny. They opted for a reasonably stable, determinative taxonomy, based solely on utility, like the organization of library books.

Stanier, van Niel and their colleagues were exceptions in that they strove for a taxonomy that would reflect genealogical relations (Kluyver & van Niel, 1936). It would be based on increased morphological complexity, and on physiology. They reiterated the arguments for a phylogenetic classification in 1941 when they assigned the bacteria and the blue-green algae to the kingdom Monera (Stanier & van Niel, 1941). After years of frustration, they finally conceded that a phylogenetic classification was not possible, and they abandoned the kingdom Monera (van Niel, 1955; Stanier & van Niel, 1962). When they defined the procaryote they therefore dissociated it from any taxonomic implication. 'But even though we have become sceptical about the value of developing formal taxonomic systems for bacteria', they wrote, 'the problem of defining these organisms as a group in terms of their biological organization is clearly still of great importance, and remains to be solved' (Stanier & van Niel, 1962, p. 17).

Nevertheless, bacteriologists and taxonomists of the 1960s were quick to decree superkingdoms, *Procaryotae* and *Eucaryotae* (Sapp, 2005a). Yet, when understood taxonomically and defined largely in negative terms, 'procaryote' would be comparable to the grouping 'invertebrate', which includes such diverse creatures as insects and worms. Stanier and his colleagues emphasized in the second edition of *The microbial world* that 'the ultimate scientific goal of biological classification cannot be achieved in the case of bacteria' (Stanier et al., 1963, p. 409). Though they denied that one could have a genealogical classification based on their structure, and even though the procaryote was proposed, in 1962, as an organizational concept only, they had little doubt that procaryotes did actually constitute a genealogically coherent group, based on their structure. 'All these organisms share the distinctive structural properties associated with the procaryotic cell . . . and we can therefore safely infer a common origin for the whole group in the remote evolutionary past' (ibid., p. 409).

Bacteria had typically been defined in negative terms: they lacked a membrane-bound nucleus, lacked mitosis, and lacked sex. For Stanier, however, the procaryote–eucaryote distinction seemed somehow to resolve the problem, when he referred to superkingdoms in 1982:

> Indeed that was the catch about it. As recently as 40 years ago, Stanier and van Niel (1941) could do little better, in an attempt to define collectively these two groups. The issue was at last resolved (at least, to the author's satisfaction) by the discovery of a major evolutionary discontinuity, at the cellular level, amongst all biological systems. I allude to the distinction of two super-kingdoms, eucaryotes and procaryotes. I think it is profoundly significant that the fundamental difference between eucaryotes and procaryotes could not be rigorously formulated prior to approximately 1960. (Stanier, 1982, pp. 9–10)

No one doubted the monophyly of procaryotes for fifteen years, until the development of molecular methods of classification. Molecular evolutionary biology constructed a new kind of character at the molecular level of organization. The new microbial taxonomy by molecular methods was experimental, quantitative, and 'natural', concerned above all with genealogies. A single molecule, the small subunit ribosomal RNA (SSU rRNA) led the way, and won approval as the favored molecular chronometer. It transformed bacterial taxonomy from a descriptive non-evolutionary practice, to an experimental science and a phylogenetic order of things. In so doing, it challenged

the venerable procaryote-eucaryote dualism and it confirmed the symbiotic origin of the eucaryotic energy-generating organelles, mitochondria and chloroplasts.

5. Birth of bacterial phylogenetics

The field of molecular evolution emerged in the 1960s (Dietrich, 1994, 1998; Morgan, 1998), and molecular approaches to taxonomy commenced. Instead of comparative anatomy and physiology, one could construct family trees on differences in the order of amino acids of proteins and nucleotides of genes. Genetic mutations that either have no effect or that improve protein function accumulate over time. As two species diverge from an ancestor, the sequences of the genes they share also diverge, and as time advances, the genetic divergence will increase. Because nucleic acids and proteins are digital in nature and are typically hundreds to thousands of residues long, the space of possible sequences was considered to be so vast that extensive similarity could effectively never be the result of convergence. Homology for any gene or protein was readily recognizable. One could therefore construct genealogies and make phylogenetic trees by comparing their sequence divergence.

By 1955, British chemist Frederick Sanger and his colleagues had succeeded in determining the complete sequence of insulin (Sanger et al., 1955), for which he was awarded the Nobel Prize in chemistry in 1958. Comparative molecular morphology for taxonomic purposes was predicted by Francis Crick that year, a few years before the genetic code was cracked:

> Biologists should realize that before long we shall have a subject which might be called 'protein taxonomy'—the study of amino acid sequences of proteins of an organism and the comparison of them between species. It can be argued that these sequences are the most delicate expression possible of the phenotype of an organism and that vast amounts of evolutionary information may be hidden away within them. (Crick, 1958, p. 142)

Emile Zuckerkandl and Linus Pauling pioneered the comparative study of amino acid sequences of hemoglobin to infer primate phylogeny (Zuckerkandl & Pauling, 1965). Walter Fitch and Emmanuel Margoliash compared amino acid sequences of cytochrome c, to infer phylogenetic relationships among diverse eucaryotes, from horses, humans, pigs, rabbits, chickens, tuna, and baker's yeast (Fitch & Margoliash, 1967). Cytochrome c is the terminal enzyme in the respiratory chain, and is located in the inner membrane of mitochondria, the respiratory organelle of eucaryotes. Cytochrome c is also present in bacteria that respire oxygen (aerobes), but many bacteria live in the absence of oxygen.

To explore deep bacterial phylogeny, Carl Woese looked to the translation machinery. He did not look to proteins, however, but rather to those RNAs which together with proteins comprised ribosomes, the ancient organelles in which translation from nucleic acid to protein ocurred. The choice of ribosomal RNA (rRNA) for phylogenetic purposes was obvious both from a conceptual and a technical standpoint. Ribosomes were ancient organelles present in all organisms from bacteria to elephants; they were at the core of the organism, and presumably far removed from adaptive traits, and because there were thousands of ribosomes per cell, rRNA was relatively easy to abstract. The basic techniques were announced in 1965, when Sanger and his co-workers published a method for sequencing and cataloguing short RNA nucleotide sequences (Sanger et al., 1965).

Beginning in 1970, Woese and his colleagues at the University of Illinois focused on comparisons of the small subunit ribosomal RNA (16S rRNA) oligonucleotides (short fragments of five to twenty or more nucleotides). Although it was not possible to sequence the larger RNA outright in those days, it was possible, by using specific ribonucleases (such as T1 ribonuclease) to cleave a large RNA into oligonucleotides with a lengths of five to twenty bases. These then could be experimentally sequenced and catalogues made, quite like the parsing of a book into its individual words. Matching oligonucleotides in different bacteria could be compared to one another to determine how closely the organisms were related. Woese and his co-workers made catalogue collections of oligonucleotide sequences: 'dictionaries' characteristic of organisms in various taxa.

By 1980, Woese's group had created catalogues of the 16S rRNA fragments for almost 200 species of bacteria and eucaryotes. Their results often contradicted the standard classification of bacteria. But none of their reports caught the attention of biologists more than did their announcements of a newly discovered form of life, or 'urkingdom', which Woese and his post doctoral fellow George Fox referred to as 'Archaebacteria'. They were a group of microbes that were phylogenetically no more related to typical bacteria (which they called eubacteria) than they were to eucaryotes (Woese & Fox, 1977).

The first organisms identified as members of the group were methane producing organisms. Methanogens are typically chemoautotrophs; they derive their energy from carbon dioxide and hydrogen. They were found to lack most of the 'signature' sequences characteristic of all previously characterized procaryotic 16S rRNA. Over the next two years, Woese's group expanded the archaebacterial urkingdom to include other organismal phenotypes, organisms that also inhabited extreme environments: the extreme halophiles which are found in brines several times more salty than the oceans, and the thermophiles, *Sulfolobus* and *Thermoplasma* found in geothermal environments that would cook other organisms.

During the late 1970s, all of these organisms were shown by biochemists and molecular biologists to have certain unusual phenotypic traits in common (Fox et al., 1980): the lipids in their cell membranes had a structure that differed sharply from the lipids of eubacteria (Tornabene & Langworthy, 1979). The cell wall chemistry of these

organisms was also shown to be of special phylogenetic importance. In 1977, Otto Kandler at the University of Munich, reported that the cell walls of methanogens lacked the complex molecule peptidoglycan (or murein) typical of bacteria, (and indeed part of Stanier and van Niel's definition of procaryotes) just as did the cell walls of halophiles. He and his collaborators subsequently showed that the same was true of the other organisms that Woese's lab had grouped together as archaebacteria (see Kandler, 1994). Kandler became a key champion of the archaebacteria concept. At the Max Planck Institute, in Martinsried, Kandler's former student Karl Stetter and Wolfram Zillig reported that the structure of transcription enzymes—the DNA dependent RNA polymerases of *Halobacterium halobium*, *Sulfolobus acidocaldarium* and Methanobacteria—differed characteristically from their counterparts in typical bacteria and closely resembled the transcription enzymes of eucaryotes (see Zillig et al., 1982).

6. Confronting the procaryote

Woese and Fox (1977a) met the procaryote concept head on when they announced the 'archaebacteria' and distinguished them from true bacteria or eubacteria. They declared,

> Dividing the living world into *Procaryotae* and *Eucaryotae* has served, if anything, to obscure the problem of what extant groupings represent the various primeval branches from the common line of descent. The reason is that eucaryote/procaryote is not primarily a phylogenetic distinction, although it is generally treated so. (Woese & Fox, 1977, p. 5088)

In their scheme, procaryotes did not lead to the eucaryotes; all three lineages, archaebacteria, eubacteria and eucaryotes, diverged early from hypothetical proto cells, 'progenotes', which would have been in the throes of evolving their translation mechanisms in terms of precision and speed (Woese & Fox, 1977b; Woese, 1998). The characteristic differences between archaebacterial and eubacterial cell walls, membranes and transcription enzymes suggested that these features might have been in the process of development at the progenote stage of evolution. One thing was certain: there was no monophylogenetic group that could be called procaryotes (Woese, 2004, 2005; Pace, 2006).

Microbial phylogenetics grew in the 1980s, as techniques for sequencing RNA and DNA dramatically improved (Maxam & Gilbert, 1977; Sanger et al., 1977). Comparisons of the nucleotide sequences of whole genes was greatly enhanced with the development of the means to clone DNA, that is, to make many copies of sequences from minute samples. The invention of the polymerase chain reaction (PCR) further revolutionized microbial phylogenetics (Mullis et al., 1986; Judson, 1992; Rabinow, 1996).

The tree of life was widely branching and had deep roots. In 1989 two groups independently used ancient gene duplications for proteins to root the tree of life (Gogarten et al., 1989; Iwabe et al., 1989). Although the molecular markers and algorithms differed, the two studies reached the same conclusion: the root of the universal tree appeared to be located between the Bacteria on the one side and the Archaebacteria and Eucaryotes on the other. The next year, Woese, Kandler and Mark Wheelis proposed the name Archaea for the archaebacteria to further emphasize that 'procaryotes' do not share a common ancestry and to counter the notion that they were 'just bacteria' and they made a formal taxonomic proposal for three 'domains' of life: Bacteria, Archaea and Eucarya (Woese et al., 1990).

The announcement of the archaebacteria had already signaled the great depth and diversity to be explored in the microbial world. But there were technical problems to the study of that diversity. Knowledge of bacteria (and their niches) depended on studies of pure cultures in the laboratory. Yet, the great bulk of the organisms seen microscopically could not be cultivated by routine techniques. Norman Pace and his collaborators developed means to get around these limitations (Pace et al., 1985; Pace, 1997). They sought to make an inventory of microbes by sequencing rRNA genes obtained from DNA isolated directly from the environment. Those who worked on the evolution and phylogeny of Bacteria and Archaea suggested that they possessed greater biological diversity than plants and animals combined (Pace, 1997, p. 734). A handful of soil contains billions of them; most life in the ocean is microbial. The orders of magnitude of the different types remain unknown. Bacteria, it was announced at the end of the last century, not only have the greatest diversity but constitute the greatest biomass on earth (Whitman et al., 1998). Arguing that biologists' understanding of the makeup of the microbial world is rudimentary, Pace called for a representative survey of the Earth's micro-biodiversity with the use of automated sequencing technology (Pace, 1997).

7. Preserving an essentialist dichotomy

Today, the three domains are widely accepted, and 'prokaryotes' are generally recognized not to represent a monophyletic group. Still, whether the term should remain in biology is hotly debated. Woese (1994, 1998) and Pace (2006) have suggested that the word be expunged from the biological lexicon, because of its misleading phylogenetic connotations and a negative organizational definition. In their view, the procaryote–eucaryote dichotomy concealed much more than it revealed about the evolution and diversity of the microbial world.

Classical evolutionists have objected that the three domain proposal obscures the phenomenal morphological differences between procaryotes and eucaryotes. They have insisted instead on maintaining the superkingdoms, or 'empires', *Eucaryotae* and *Procaryotae* (Mayr, 1991; Margulis & Guerrero, 1991; Mayr, 1998). They placed the 'archaebacteria' and 'eubacteria' as kingdoms or subkingdoms within the later, and they see no problem

with a negative definition for the procaryotic group. The question concerning the lack of a nucleus is at most only a semantic issue today. Although Stanier and van Niel (1962) defined the procaryote as possessing a nucleus, today it is typically stated that the procaryote lacks a nucleus. Though the bacterium lacks a nuclear membrane, it does possess a 'nuclear body' or 'nucleoid' where DNA is localised. Both Bacteria and Archaea, they argue, lack the structural eucaryotic cell complexity as expressed in its cytoskeleton, and membrane enclosed, energy generating organelles.

The issues underlying these differences in perspectives were encapsulated in an almost decade long public debate between Mayr and Woese (e.g. Mayr, 1998; Woese, 1998b). Mayr (1991) perceived their differences in terms of competing kinds of taxonomy. One of these dated back to Linnaeus, taking a phenetic approach to classification, by grouping species based on overall phenotypic similarity; the other followed cladism and classified strictly on the basis of genealogy or branching points. These two approaches did not conflict when phenotypic grouping based on similarity reflected groupings based on genealogical relationship. Darwin had recognized that classification had to reflect two distinct characteristics of the evolutionary process: genealogy (branching order) and 'degree of modification' (divergence). There were instances, however, in which phenetic and genealogical analyses led to two different conclusions. The reptiles, for example, are a grouping that genealogically included birds and mammals. How to deal with phenotypically defined groups that are genealogically incomplete (paraphyletic) was a trying issue that caused a great schism among taxonomists.

For Mayr, genealogy, or common origin, was not a necessary condition of classification, at least for microbes. It did not matter to him that 'the procaryote' was a polyphyletic group, or that it was paraphyletic, having given rise to a group not included among them: the Eucaryota. The amount of evolutionary change that had occurred with the emergence of the eucaryote was all that mattered. Thus he accepted procaryotes as an 'empire' regardless of genealogy, just as he accepted fungi as a kingdom based on their physiological differences from plants, regardless of genealogical considerations (Mayr, 1982). It was sufficient to classify based on degree of modification, no matter how the genealogies played out. Put differently, genealogies at best may inform phenetic classification, which is primary. This was his perspective when it came to the microbial world. And for Mayr, utility—the organization of an effective storage system—should be the aim of taxonomy just as it was for Stanier and van Niel's adversaries in the first half of the last century. The reference system he supported was 'based on the traditional principles of classification which biology shares with all fields in which items are classified, as are books in a library or goods in a warehouse' (Mayr, 1991). For Woese, on the other hand, degree of modification was not a sufficient condition for delineating taxa. Both common origin (genealogy) and degree of modification were necessary conditions, just as they had been for Darwin. Genealogy was primary, and phenotypic differences could at best corroborate taxonomy based on phylogenetic, molecular characteristics.

Woese did not see himself as a cladist. The conflict with Mayr is better understood as one between the differences in the aims and methods of morphologists and classical naturalists, and those of molecular evolutionists. Their taxonomic differences were intricately interwoven with differences in the technical capacity of their scientific fields, in what counted as diversity, and what counted as taxonomic traits. Mayr conceived of Woese as a newcomer and an interloper from molecular biology, ignorant of the intricacies of taxonomy and older debates that Mayr believed that he himself had resolved. For both it was a struggle for authority. Molecular evolution brought with it a wholly new approach to taxonomy that was experimental and based on a new kind of character and a finer level of organization: the quantifiable macromolecular sequence. Woese (1998b) contrasted that with what he called the qualitative 'eye of the beholder' approach of the classical taxonomists based on morphology—an approach that could not be applied to bacteria in any case. Indeed, microbial systematics had long tried and failed to produce a natural microbial system based on classical systematic concepts.

All molecular evolutionists agreed that microbial genealogies could be determined only at the molecular level. Phenetic or 'organismal traits' were at best 'confirmatory indicators of prior grouping determined by use of molecular characters' (Wheelis et al., 1992, p. 2932). On the other hand, those who classified based on morphology tended to see molecular structure and sequences, at best, as just one more kind of taxonomic characteristic, which certainly could not be privileged over morphological ones any more than genealogy itself could be (Margulis & Guerrero, 1991). One could not compare the differences between the Archaea and the Bacteria at the molecular level to the great morphological differences that distinguished a giant Sequoia or an elephant from all bacteria *sensu lato*. Thus the debate centered over the direction of biology. Was classification going to be based on genealogies and degree of modification or not? Was it going to be experimental and quantitative or morphological and qualitative? For both Woese and Mayr there would be no intermediate position that could ever reconcile their differences.

The search for a phylogenetically based classification was the shared goal of all molecular evolutionists, an aim that had been technically impossible for microbial taxonomists before them. Still, even some molecular evolutionists who sought a genealogically based classification made an exception in the case of the procaryote–eucaryote dichotomy. While recognizing the fundamental phylogenetic trio of the primary domains, and that the genetic informational systems of Archaea and Bacteria differ radically in terms of replication, transcription and translation, they continued to defend the procaryote–eucaryote dichotomy and add molecular features to organizationally conjoin the Archaea

and Bacteria. Walsh and Doolittle have pointed to their lack of introns, which are central to eucaryotic cell differentiation and complexity. They characterize Bacteria and Archaea as 'procaryotic domains', as possessing 'a typically (but not always) circular chromosome(s); absence of spliceosomal introns; organization of many genes into operons (sometimes with homologous genes in the same order)' (Walsh & Doolittle, 2005, R238). Martin and Koonin (2006) have added the coupling of transcription and translation as the key positive common character of procaryotes.

By pointing to the transcription and translation mechanisms as key common features of the procaryotes that distinguish them from eucaryotes, they have produced a paradox. For, as they recognize, those very traits are radically different in Archaea and Bacteria at the molecular level. Together with hundreds of other signature gene clusters, they represent the fundamental differences in the organization that distinguish the three major cell types, archaeal, bacterial, and eucaryotic (Graham et al., 2000). There seems to be no indisputable way to define the concept of procaryote.

8. Symbiosis, convergence, and genealogy

When Stanier and van Niel defined the bacterium in 1962, they made no mention of either plasmids in bacteria or organellar heredity in eucaryotes. That year, DNA was demonstrated in chloroplasts, and the following year in mitochondria. Those organelles were also shown to have their own transcription machinery distinct from that of the nucleus. The notion that mitochondria and chloroplasts originated as engulfed symbionts re-emerged anew (Margulis, 1970, 1981).

Symbiotic conceptions of cellular organelles had clung to the edge of biology throughout the twentieth century, but like bacterial phylogeny itself, had been generally dismissed as idle speculation (Sapp, 1994, 2003, 2006b). The idea that symbiosis is a source of evolutionary innovation is as old as the term itself; it evolved in the 1880s with evidence of the dual nature of lichens (as fungi and algae), of nitrogen-fixing bacteria in the root nodules of legumes, and of mychorrizal fungi in the roots of forest trees. Those findings were coupled with evidence of cellular organelles that seemed to reproduce by division. Andreas Schimper first suggested that chloroplasts originated as symbionts when he coined the word chloroplasts in 1883. That idea was advocated by Haeckel (1904) and then developed, most prominently by Constantin Merezhkowsky between 1905 and 1918. Merezhkowsky also claimed that the nucleus and cytoplasm were symbiotic partners, and he coined the word 'symbiogenesis' for the synthesis of new organisms by symbiosis (Sapp et al., 2002). Symbiotic conceptions of nucleus and cytoplasm had been proposed since the 1890s, but such ideas were dismissed as fanciful speculation outside the purview of experimental science (Sapp, 1994, 2006b).

In his book, *Les symbiotes*, French biologist Paul Portier at the Institut Océanographique de Monaco, developed an elaborate theory about mitochondria as symbionts (Portier, 1918). Then, during the 1920s, the notion that mitochondria were symbionts was reworked by Ivan Wallin at the University of Colorado. In his book *Symbionticism and the origin of species*, he proposed that the inheritance of acquired bacteria was the source of new genes and the primary mechanism for the origin of species and cellular differentiation (Wallin, 1927).

The evolutionary importance of symbiosis was emphasized by Félix d'Herelle who in the mid 1920s discussed the perpetuation of mixed cultures of bacteria and their viruses (which he named bacteriophages) in terms of symbiosis. He referred to the bacteria which harbor viruses (lysogenic bacteria) as microlichens. The morphological and physiological changes resulting from symbiosis led him to assert in 1926 that 'symbiosis is in large measure responsible for evolution' (d'Herelle, 1926, p. 320). At that time Paul Buchner had begun his systematic investigations of the morphological and physiological effects of microbes transmitted through the eggs of many species of insects (Buchner, 1965; Sapp, 2002). Buchner divorced himself from the claims of Portier and Wallin, that mitochondria were symbiotic bacteria, which he saw to be a liability to his own more empirical work. Instead, he focussed on a more basic struggle: to change the prevalent view that microbial symbiosis in the tissues of animals was a rare phenomenon.

The nature of symbiotic relations, analogies and anthropomorphisms, from 'mutualism' and 'consortia' to 'parasitism' and 'slavery', need not concern us here (but see Sapp, 1994, 1999, 2003, 2004). Suffice it to say that no matter how symbiosis was conceived, from the nineteenth to the late twentieth century, the evolutionary effects of interspecies integration due to microbial infections remained close to the margins of 'polite biological society'. As such, symbiosis research offers a most critical standpoint from which to view the development of biology.

First and foremost, symbiosis was overshadowed by a medical perspective of microbes as agents of infectious disease. That bacteria played any beneficial role in the tissues of plants and animals was in virtual conflict with the basic tenets of the germ theory of disease. Bacteria had no natural history, and were defined largely as disease causing germs and portrayed as the 'the enemy of Man'. Bacteriologists only searched for infectious microbes when tissue was diseased, not in healthy tissue, and for many it was ridiculous to suggest that bacteria living in tissue could be part of the physiological well being of animals.

Portier's work on *Les symbiotes* was framed by the opposition between symbiosis and germ theory. Rather than viewing microbes from 'the window of medicine', he looked at 'microbiology from the window of comparative physiology' and envisaged 'a new form of bacteriology: physiological and symbiotic bacteriology' (Portier, 1918, p. 294). His ideas were met with strong resistance from

bacteriologists and histologists at the Institut Pasteur who rejected, and effectively Pasteurized all of his claims about bacteria living in healthy animal tissue (Sapp, 1994, pp. 93–109). The contrast between a symbiotic perspective and that from pathology was echoed in the United States by Wallin:

> It is a rather startling proposal that bacteria, the organisms which are popularly associated with disease, may represent the fundamental causative factor in the origin of species. Evidence of the constructive activities of bacteria has been at hand for many years, but popular conceptions of bacteria have been coloured chiefly by their destructive activities as represented in disease. (Wallin, 1927, p. 11)

Indeed, the relatively meagre evidence of the beneficial effects of bacteria was no match for the evidence of their destructive effects. Wallin emphasized that bacteria lacked biological characterization and that the concept of disease was often embedded in their very definition. His theory was attacked by those concerned about the negative effects it might have on the aetiology of disease (Sapp, 1994, pp. 116–119).

Studies of symbiosis as a source of evolutionary change also conflicted with nucleo-centric genetics, a view of the organism in terms of one pure germplasm, and an experimental study of life in isolation. Thus the inheritance of acquired symbionts was relegated to the field of pathology and outside the boundaries of genetics and heredity, as contamination. American geneticist E. M. East put it succinctly:

> there are several types of phenomena where there is direct transfer, from cell to cell, of alien matter capable of producing morphological changes. It is not to be supposed that modern biologists will cite such instances when recognized, as examples of heredity. But since an earlier generation of students used them, before their cause was discovered, to support arguments on the inheritance of acquired characteristics, it is well to be cautious in citing similar, though less obvious, cases as being illustrations of non-Mendelian heredity. (East, 1934, p. 431)

This view of life, which dismissed the inheritance of acquired symbionts as being merely due to parasites of no significance to heredity, persisted among leading geneticists throughout the decade following the Second World War, in debates over the scope and significance of non-Mendelian, cytoplasmic heredity (see Lederberg, 1952; Sapp, 1987).

The role of symbiosis as a source of evolutionary change also conflicted with central tenets of the evolutionary synthesis of the 1930s and 40s, which focussed on hereditary differences between individuals in interbreeding populations. That synthesis was based on the concept that sexual recombination and mutations of genes in the cell nucleus were the fuel of evolutionary change. The creative effects of microbial symbiosis were also eclipsed by illustrations of conflict and competition: a view of nature that, it had long been argued, only reflected views of human social progress.

Symbiotic theories of mitochondria and chloroplasts found a new footing when DNA was discovered in plastids and mitochondria (Sapp, 2006). The paradigm began to turn over rapidly. Lynn Margulis proposed that the reach of symbiosis be extended to account for the origin of centrioles/kinetosomes and therefore for the origin of protistan motility and mitosis (Sagan, 1967; Margulis, 1970, 1981). Her argument hinged on a morphological analogy with spirochetes, at one time actually mistaken to be cilia, attached to the protist *Mixotricha paradoxa* which lives in the hind gut of termites (Cleveland & Grimstone, 1964).

In the genealogy of ideas, no less than in phylogenetics, we need to be cautious of convergent properties. Though on the surface they appear similar, the theories of symbiosis after 1970 differed dramatically from some of those proposed before the second world war. Wallin's concept of mitochondria and their origins was very different from the ideas of the 1970s and 1980s (Sapp, 1994, 2006b). Like several others of his generation, he held mitochondria to be the principal organs of cellular differentiation and morphogenesis. He believed that chloroplasts and centrioles, golgi bodies and other cell organs were products of mitochondria. In his scheme, mitochondria were not organisms that had entered some primitive microbes eons ago; they were constantly being added to the germplasm in the course of evolution. In effect, ontogeny would be the recapitulation of a symbiotic phylogeny. Wallin also claimed that he had cultured mitochondria to prove their actual bacterial nature.

In the new concepts of endosymbiosis of the 1970s, mitochondria were not repeatedly added to organisms, reflecting the course of phylogeny and ontogeny, and nor were they organisms that could be cultured outside the cell. Mitochondria and chloroplasts were held to have each originated once from bacteria in the remote past (and in some cases plastids were acquired secondarily by engulfment of a photosynthetic protist, as long had been suggested). Mitochondria were not organelles of morphogenesis. Biochemists showed that they were the energy generating organelles of the cell, responsible for oxidative phosphorylation, and containing enzymes of Krebs cycle. The question for cell evolutionists centered on explaining what was held to be the greatest evolutionary discontinuity in the living world: that between a procaryote and a eucaryote.

Crucial evidence for the symbiotic hypothesis was lacking. In the 1960s and early 1970s, it remained possible that both chloroplasts and mitochondria emerged endogenously by differentiation and compartmentalization within the cell (Allsopp, 1969; Raff & Mahler, 1972; Uzzell & Spolsky, 1973). Their similarities with bacteria would then simply be a case of convergent evolution. That possibility was strengthened with evidence that the genetic bases of those

organelles are highly integrated into the nuclear chromosomal system; only a small fraction of the genes needed for mitochondrial and chloroplast functions are actually located in those organelles themselves.

The origin of organelles could not be rigorously tested without genealogical methods. Comparing ribosomal RNAs of chloroplast, mitochondrial, and nuclear origin with each other and with different kinds of bacteria provided the rigour and closed the main controversy about their origin (Zablen et al., 1975; Gray & Dooolittle, 1982; Yang et al., 1985; Gray, 1992). In the mid 1970s the rRNA technology was exported by Woese's technician Linda Bonen from Urbana to the laboratories of Ford Doolittle and Michael Gray at Dalhousie University, Halifax, Canada. Doolittle's laboratory and Woese's laboratory focused on chloroplast rRNA, whilst Gray's focused on mitochondrial rRNA. Collectively, their results indicated that chloroplasts and mitochondria had independent origins from each other and from nuclear derived rRNA. Chloroplasts descended from the photosynthetic blue-green bacteria (cyanobacteria), and the mitochondrial ancestor was traced to the alpha-proteobacteria. There were no comparable data to test whether centrioles/kinetosomes arose as symbionts. Centriolar structure and function had remained uncertain since the nineteenth century (Sapp, 1998). The evidence for DNA in those organelles had been on-again, off-again since the 1960s, until it was refuted by evidence from electron microscopy and molecular hybridization, which indicated that genes affecting centriolar/flagellar function are located in the nucleus (Hall & Luck, 1995).

What about the eucaryotic cell nucleus? Did it also have a symbiotic origin? Woese, Fox and their collaborators had raised that issue in 1980. Perhaps the nucleus of the eucaryote had emerged from a chimeric mixture of eubacterial and archaebacterial genes. That possibility was strengthened in the 1990s. Genomic comparisons of ancient genes indicated that the nucleus was comprised of three phylogenetically distinct groups of genes: information transfer genes, concerned with transcription and translation, which were closely related to those of the Archaea; bacterial genes thought to be transferred to the nucleus from the mitochondria; and bacterial genes, whose functions were not obviously related to mitochondria (Bell & Jackson, 1997; Brown & Doolittle, 1997; Doolittle, 1996).

Several interpretations have been offered for these data (Roger, 1999). The first, most popular hypotheses is that the nucleus arose from an engulfed Archaeon symbiont in a Bacterial host (Lake & Rivera, 1994; Lake et al., 2005; Gupta & Golding, 1996; Gupta, 2005; Moreira & Lopez-Garcia, 1998; Horiike et al., 2001; Melinsky et al., 2005). Because the genes affecting the cytoskeleton, which conditions phagocytosis in eucaryotes, are found in no existing bacterial lineages, several researchers have suggested that the eucaryotic nucleus was formed from an engulfment of an archaeon by an extinct microbe (Sogin, 1991; Doolittle, 1995; Hartmann & Fedorov, 2002). The second hypothesis is that many of the ancient 'non-mitochondrial' genes in the present eucaryotic nucleus may have been derived from the ancestor of mitochondria which had entered an archaeon that subsequently evolved the nucleus (Martin & Müller, 1998). A third option is that 'non-mitochondrial' bacterial genes in the nucleus could have been imported from symbionts acquired and lost after the primordial eucaryotic cell was formed (Doolittle, 1996; Roger, 1999). After all, hereditary symbionts are ubiquitous among Protists (Margulis & Fester, 1991). Fourthly, non-mitochondrial bacterial and archaeon genes in the nucleus of the eucaryotic lineage could have been acquired by lateral gene transfer before the rise of the three domains in keeping with the concept of the progenote as a population of pre-cellular entities with under developed, error prone replication and translation machinery (Woese, 1998). Accordingly, before the development of the modern translation apparatus, evolution would be driven by a different mode and tempo; there would be no individual organisms as such and intense gene mutation and lateral gene transfer would generate enormous diversity very quickly. The fifth and final suggestion is that those non-mitochondrial bacterial (and archaeon) genes in the eucaryotic nucleus might have been acquired by lateral gene transfer after the eucaryotic lineage emerged and the nucleus developed therein. There was growing evidence beginning in the late 1990s that such lateral gene transfer between bacterial taxa was pervasive.

9. Phylogenomics and lateral gene transfer

In 1995, researchers at The Institute for Genomic Research (TIGR) headed by Craig Venter published the sequence of *Haemophilus influenzae* (Fleischmann et al., 1995) and the following year Woese and Gary Olsen together with researchers at TIGR published the complete sequence of the first archaeon, *Methanococcus jannaschi* (Bult et al., 1996). By 2005, 260 complete genomes had been sequences (33 eucaryotes, 206 eubacteria and 21 archaeons) and more than 1000 genome projects are in progress (Delsuc et al., 2005).

Gene phylogenies for various functions often indicated different organismic genealogies than those based on rRNA. For example, while comparisons of 16S rRNA placed the microsporidia low on the phylogenetic tree, comparisons of the gene for the enzyme RNA polymerase placed the microsporidia higher on the tree with the fungi (Doolittle, 1999). Significantly, the new gene phylogenies disagreed not only with the rRNA-based phylogenies, they also conflicted among themselves. Those gene histories were so convoluted that the only reasonable answer was lateral gene transfer, a phenomenon whose scope and significance had been greatly underestimated.

The first generation of bacterial geneticists had recognized that bacteria had various means for exchanging genes. Bacteria (*sensu lato*) comprise a main circular DNA genome or genophore, and typically various other bits of DNA in the form of bacteriophage, as well as small

circular pieces of DNA, called plasmids. In bacterial conjugation, genetic material of the 'male' plasmids, (and sometimes parts of the main chromosome), is transferred to the 'female' recipient, and some genes may recombine with the female's chromosome. Whole plasmids and fragments of the genophore can be transferred between different species of bacteria by conjugation. Bacterial genes can also be transferred by the uptake of DNA fragments from dead bacteria: transformations. Viruses can also act as vehicles to transfer genes between bacteria. Lederberg and Norton Zinder coined the term 'transduction' for that phenomenon (Lederberg, 1952; Lederberg, 1956).

Because of lateral gene transfer (LGT), a bacterium of one strain could acquire one or several genes from a completely unrelated organism (Bushman, 2002). Therefore, similarities and differences in some genes may not be a measure of genealogical relationship. For example, if organism type A and organism type B carry the same gene for a protein, it may not be because they both belong to the same taxonomic group, but that one of them acquired that gene (by 'infection' or passive uptake) from a third type of organism, C, which is not ancestral to them. Lateral gene transfer could potentially scramble the phylogenetic signal.

The significance of LGT for antibiotic resistance had been well known for decades, and the importance of LGT had been considered from the outset of molecular evolutionary studies (Anderson, 1966; Jones and Sneath, 1970; Stanier, 1971; Reanney, 1976; Fox et al., 1980; Dickerson, 1980; Woese et al., 1980). Sorin Sonea speculated that because of LGT the whole bacterial world was comparable to a super-organism (Sonea & Panisset, 1983). By the end of the twentieth century, analyses of complete genome sequences suggested that lateral gene transfer occurred far more widespread than previously appreciated.

The ease with which genes seemed to be interchanged among bacteria reinforced long standing views that 'the biological species concept' (in the general sense of a genetically isolated interbreeding group) did not apply to bacteria (Ochman et al., 2000; Eisen, 2000; Ward, 1998; Gogarten et al., 2002). Certainly, many bacteriologists of the 1950s and 1960s had also recognized that the concept of species did not apply to bacteria, but that was not because bacteria could exchange genes between taxa, but rather because laboratory studies indicated that sexual reproduction was a rare event for bacteria, as indeed it was for most microbes (Lwoff, 1958; Schaeffer, 1958). 'The microbial species does not exist', Samuel Cowan declared; 'it is impossible to define except in terms of nomenclatural type; and it is one of the greatest myths of microbiology' (Cowan, 1962, p. 451).

Lateral gene transfers between taxa would make genealogies resemble more a web than the tree with which we have envisaged evolutionary descent since Darwin (Doolittle, 1999; Gogarten et al., 2002). Conjecture about the nature and intensity of lateral gene transfer have led some bacterial phylogeneticists to suggest that a phylogeny of bacteria may be impossible. Others emphasize, as they have since the 1970s and 1980s, that while lateral gene transfer is ubiquitous for adaptive functions, strong phylogenetic signals persist. This includes those genes for rRNA, far removed from the everyday life of the bacterium, at the core of its cellular fabric and interacting with many cellular components (Woese et al., 1980; Woese, 1998, 2000).

10. Transcending Darwinism

Microbial evolutionary biology moves us outside the confines of traditional Darwinian thought, and beyond the evolutionary dynamics that Darwin and his followers had envisaged to define evolution. It represents a synthesis of its own, distinct from the neo-Darwinian synthesis of the 1930s and 1940s. It emerged from an interdisciplinary fusion brought on by technical innovations acquired laterally as individuals from molecular biology crossed over to explore the history of the microbial world. That union of molecular biology and taxonomy has resulted in a new era in microbiology in which unquestioned assumptions have been exposed and fresh possibilities revealed by new coherent experimental research programs based on macromolecular sequencing. The resulting changes were saltational, both methodologically and conceptually.

An evolutionary taxonomy of bacteria could only be achieved by divorcing itself from the classical morphological approaches used in plants and animal systematics. The transformation of bacterial taxonomy from a deterministic classification based on utility to evolutionary biology relied on a new kind of character, a new measure at the macromolecular level of organization. Comparative studies of SSU rRNA led the way in sorting out old groupings and organizing phylogenetically coherent ones.

The great diversity and the evolutionary order determined by that approach culminated with the proposal of three fundamental forms of life. The procaryote grouping is universally recognized as being polyphyletic, but whether the procaryote–eucaryote dichotomy should remain in biology as a taxonomic distinction remains a cathectic issue.

That same comparative molecular anatomy led to the phylogenetic verification of the symbiotic origin of chloroplasts and mitochondria. The conjecture that the nucleus may have also arisen from a separate symbiosis remains underdetermined by molecular data. Subsequent discussions of the scope and significance of lateral gene transfer in the age of genomics, resonate against the conceptual background of the three domains, and the verification of the symbiotic origin of mitochondria and chloroplasts.

Present day microbial evolutionary theory distinguishes itself from classical evolutionary theory by its recognition that acquired genes and genomes trafficked between taxa are fundamental forces in evolution. They are consequences of vital evolutionary mechanisms in addition to alterations by random gene mutation and interspecies recombination. The ubiquity of lateral gene transfer among bacteria has exacerbated attempts to develop a bacterial

species concept. And lest we forget, lateral gene transfer is by no means restricted to microbes, bacterial or protistan. Genomic studies today indicate that 8% of the genes in our own nuclear genome are retroviruses (Ryan, 2006). Indeed, all eucaryotes are chimeric superorganisms comprised of organellar DNA, and that of other symbionts and viruses; all are polygenomic. The boundaries of the individual super-organism, the 'symbiome', extend well beyond the cell (Sapp, 2003). We have left aside in this overview developmental symbiosis and the role of bacteria in the development of plants and animals. As detailed elsewhere (Sapp, 2003), the transfer of genes between species and the inheritance of acquired symbionts—as fundamental processes of evolutionary change—contradict several tenets of the classical evolutionary synthesis, crafted as it was on the belief that gene mutations and intraspecific gene recombination were the exclusive fuels for evolutionary change.

Symbiosis and lateral gene transfer more generally are still not taught as central principles of evolutionary biology. The inheritance of acquired symbionts as a source of evolutionary innovation has continued to be trivialized or ignored by both the champions of the neo-Darwinian evolutionary synthesis as well as by some of its most recognized critics. In his *Wonderful life*, Stephen J. Gould regarded the symbiotic origin of mitochondria and chloroplasts as 'entering the quirky and incidental side' of evolution (Gould, 1989, p. 310). Symbiosis is not mentioned in *The structure of evolutionary thought*, and bacteria are mentioned on three of 1432 pages (Gould, 2002).

In understanding the place of symbiosis in evolution, we should consider too that much of evolutionary biology has focussed on the origin of species, often taught as population genetics, and typically as zoocentric as embryology texts. The origin of species, in the classical Darwinian population sense of gene pools, neither addresses the organism as a whole, nor the processes underlying the major transitions in evolution. It does not deal with the evolution of complexity: the origin of the code, the origin of life, the origin of eucaryotes, and the origin of multicellular organismic organization. These are aspects of emergence in which symbiosis and lateral gene transfer are important processes.

The extent to which symbiosis as a source of evolutionary change and lateral gene transfer is taught in class rooms today may also reflect a reductionist one germplasm–one organism conception of individuality, as well as a ('selfish') gene's eye view which confronts a microbe's eye view of evolution. Indeed, leading neo-Darwinians who have attended to theories about the great transitions in life have insisted that the inheritance of acquired bacteria is a rare exceptional phenomenon in plants and animals. John Maynard Smith and Eörs Szathmáry asserted that 'transmission of symbionts through the host egg is unusual' and that hereditary symbionts are best regarded as captive slaves (Smith & Szathmáry, 1999, p. 107). That statement is based on their assumptions about the evolution of associations and about the gene as the primary unit of selection (Sapp, 1999).

Contrary to such theoretical expectations, hereditary symbiosis is indeed prevalent, especially among the insects. All aphids carry bacteria of the genus *Buchnera* in their cells; those symbionts are inherited through the host egg. Surveys based on molecular phylogenetic techniques reveal that bacteria of the genus *Wolbachia* are inherited through the egg cytoplasm of about 75% of all known insect species, including each of the major insect orders (Werren, 2005; Zimmer, 2001). Their complete distribution in arthropods and other phyla are yet to be determined. *Wolbachia* are alpha proteobacteria, and far from being 'slaves'; they are specialists in manipulating their hosts' reproduction and development. They cause a number of profound reproductive alterations, including cytoplasmic incompatibility between strains and species, parthenogenetic induction, as well as femininization. They can also convert genetic males into reproductive females (and produce intersexes). Sometimes, as in the case of weevils, *Wolbachia* are inherited along with other bacterial symbionts that provide vitamins and energy, and enhance the insect's ability to fly (Abdelaziz et al., 1999). *Wolbachia* are of considerable evolutionary interest today, especially as a mechanism of speciation.

The effect of the industrial–medical complex in fostering studies of disease over symbiosis and evolution has long been noted by symbiosis advocates. René Dubos expressed the problem aptly fortyfive years ago, when discussing the creative evolutionary effects of viral infections, and how it was scientifically 'unfashionable' to search for integrative processes. Microbiologists, he lamented, maintain themselves as 'pour cousins in the mansion of pathology' (Dubos, 1961, p. 204). 'The time has come', he declared, 'to supplement the century old philosophy of the germ theory of disease with another chapter concerned with the germ theory of morphogenesis and differentiation'. Thus he prophesied, 'there would soon develop a new science of cellular organization, and indeed perhaps a new biologic philosophy' (ibid, p. 204).

The study of symbiosis, and integrative processes of evolution, heredity and development, continue to be confronted by the medicalisation of biology departments, as well as the obvious dangers of biowarfare and emergent diseases. Sociopolitical analogies and militaristic metaphors also permeate seminars and writings in biology, as microbes, symbiosis, and co-evolution are discussed in anthropocentric terms. Expressions of evolutionary processes reflect the two way traffic of ideas about human society and nature as much today as they did in Darwin's day.

Finally, we need to consider the neo-Darwinian retrenchment evident in our times as Darwin is held up as a sacred totem against advocates of 'intelligent design'. As a result, evolution itself, descent with modification, has become conflated with classical Darwinian theory. To speak of non-Darwinian mechanisms is seen as heresy by some evolutionists and pernicious in so much as it lends itself to abuse by those seeking to substitute evolutionary biology with super-natural conceptions of nature. This state of affairs is reminiscent of the controversy in the Cold

War when the Lysenkoist movement, originating in the Soviet Union, attacked and dismissed Mendelian chromosomal genetics in the West. Research on non-Mendelian, cytoplasmic inheritance and evidence for the inheritance of acquired characteristics in the West was caught in the middle (Sapp, 1987). It is a microbial world; the fundamental role of the inheritance of acquired genes and genomes in evolution is a biological reality that is both beyond Darwinism and the socio-political trappings of our times.

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References

Abdelaziz, H., Grenier, A.-M., Khatchadourian, C., Charles, H., & Nardon, P. (1999). Four intracellular genomes direct weevil biology: Nuclear, mitochondrial, principal endosymbiont, and Wolbachia. *Proceedings of the National Academy of Sciences USA*, *96*, 6814–6819.

Allsopp, A. (1969). Phylogenetic relationships of the procaryota and the origin of the eucaryotic cell. *New Phytologist*, *68*, 591–612.

Anderson, E. S. (1966). Possible importance of transfer factors in bacterial evolution. *Nature*, *209*, 637–638.

Bell, S. D., & Jackson, S. P. (1997). Transcription and translation in Archaea: A mosaic of eukaryal and bacterial features. *Trends in Microbiology*, *6*, 222–228.

Brown, J. R., & Doolittle, W. F. (1997). Archaea and the procaryote-to-eucaryote transition. *Microbiology and Molecular Biology Reviews*, *61*, 456–502.

Buchner, P. (1965). *Endosymbiosis of animals with plant microorganisms*. New York: Interscience Publishers.

Bult, D. J., White, O., Olsen, G. J., & Woese, C. R. (1996). Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. *Science*, *273*, 1058–1073.

Bushman, F. (2002). *Lateral DNA transfer: mechanisms and consequences*. New York: Cold Spring Harbor Laboratory Press.

Carter, K. C. (1991). The development of Pasteur's concept of disease causation and the emergence of specific causes in nineteenth-century medicine. *Bulletin of the History of Medicine*, *65*, 528–548.

Chatton, E. (1925). Pansporella perplexa: Reflexions sur la biologie et la phylogénie des protozoaires. *Annales des Sciences Naturelles Zoologiques, 10è série*, *VII*, 1–84.

Chatton, E. (1938). *Titre et travaux scientifique (1906-1937) de Edouard Chatton*. Sottano, Italy: Sette.

Cleveland, L. R., & Grimstone, A. V. (1964). The fine structure of the flagellate Mixotricha paradoxa and its associated micro-organisms. *Proceedings of the Royal Society of London B*, *159*, 668–686.

Cohn, F. (1872). Untersuchungen über Bacterien. *Beiträge zur Biologie der Pflanzen*, *1*, 127–222.

Copeland, H. F. (1938). The kingdoms of organisms. *The Quarterly Review of Biology*, *13*, 383–420.

Cowan, S. T. (1962). The microbial species: a macromyth. In G. C. Ainsworth, & P. H. A. Sneath (Eds.), *Microbial classification, 12th Symposium of the Society for General Microbiology* (pp. 433–455). Cambridge: Cambridge University Press.

Crick, F. H. C. (1958). The biological replication of macromolecules. *Symposia of the Society for Experimental Biology*, *12*, 138–163.

Darlington, C. D. (1939). *The evolution of genetics systems*. Cambridge: Cambridge University Press.

Darwin, C. (1964). *On the origin of species: a facsimile of the first edition with an introduction by Ernst Mayr*. Cambridge, MA: Harvard University Press. (First published 1859)

Delsuc, F., Brinkmann, H., & Philippe, H. (2005). Phylogenomics and the reconstruction of the tree of life. *Nature Reviews Genetics*, *6*, 361–375.

d'Herelle, F. (1926). *The bacteriophage and its behavior* (G.H. Smith, Trans.). Baltimore: Williams and Wilkins.

Dickerson, R. (1980). Evolution and gene transfer in purple photosynthetic bacteria. *Nature*, *283*, 210–212.

Dietrich, M. (1994). The origins of the neutral theory of molecular evolution. *Journal of the History of Biology*, *27*, 21–59.

Dietrich, M. (1998). Paradox and persuasion: Negotiating the place of molecular evolution within evolutionary biology. *Journal of the History of Biology*, *31*, 85–111.

Dobell, C. (1911). Contributions to the cytology of the bacteria. *Quarterly Journal of Microscopical Sciences*, *56*, 395–506.

Doolittle, R. F. (1995). The origins and evolution of eucaryotic proteins. *Philosophical Transactions: Biological Sciences*, *349*, 235–240.

Doolittle, W. F. (1996). Some aspects of the biology of cells and their evolutionary significance. In D. M. Roberts, P. Sharp, G. Alderson, & M. A. Collins (Eds.), *Evolution of microbial life: 54th Symposium of the Society for General Microbiology* (pp. 1–26). Cambridge: Cambridge University Press.

Doolittle, W. F. (1999). Phylogenetic classification and the universal tree. *Science*, *284*, 2124–2128.

Dubos, R. (1945). *The bacterial cell*. Cambridge, MA: Harvard University Press.

Dubos, R. (1961). Integrative and creative effects of infection. In M. Pollard (Ed.). *Perspectives in virology* (Vol. 2, pp. 200–205). Minneapolis: Burgess.

East, E. M. (1934). The nucleus–plasma problem. *The American Naturalist*, *68*, 289–303 (402–439).

Eisen, J. (2000). Horizontal gene transfer among microbial genomes: New insights from complete genome analysis. *Current Opinion in Genetics and Development*, *10*, 606–611.

Farley, J. (1974). *The spontaneous generation controversy from Descartes to Oparin*. Princeton: Princeton University Press.

Fitch, W. M., & Margoliash, E. (1967). The construction of phylogenetic trees: A generally applicable method utilizing estimates of the mutation distance obtained from cytochrome c sequences. *Science*, *155*, 279–284.

Fleischmann, R. D., Adams, M. D., White, O., Clayton, R. A., Kirkness, E. F., Kerlavage, A. R., Bult, C. J., Tomb, J. F., Dougherty, B. A., Merrick, J. M., et al. (1995). Whole-genome random sequencing and assembly of Haemophilus influenzae Rd. *Science*, *269*, 496–498 (507–512).

Fox, G. E., Stackebrandt, E., Hespell, R. B., Gibson, J., Maniloff, J., Dyer, T. A., Wolfe, R. S., Balch, W. E., Tanner, R. S., Magrum, L. J., Zablen, L. B., Blakemore, R., Gupta, R., Bonen, L., Lewis, B. J., Stahl, D. A., Luehrsen, K. R., Chen, K. N., & Woese, C. R. (1980). The phylogeny of procaryotes. *Science*, *209*, 457–463.

Geison, G. L. (1995). *The private science of Louis Pasteur*. Princeton: Princeton University Press.

Gogarten, J. P., Doolittle, W. F., & Lawrence, J. G. (2002). Procaryotic evolution in light of gene transfer. *Molecular Biology and Evolution*, *19*, 2226–2238.

Gogarten, J. P., Kibak, H., Dittrich, P., Taiz, L., Bowman, E. J., Bowman, B. J., Manolson, M. F., Poole, R. J., Date, T., Oshima, T., Konishi, J., Denda, K., & Yoshida, M. (1989). Evolution of the vacuolar H+-ATPase: Implications for the origin of eucaryotes. *Proceedings of the National Academy of Sciences, USA*, *86*, 6661–6665.

Gould, S. J. (1989). *Wonderful life: The Burgess shale and the nature of history*. London: Hutchison Radius.

Gould, S. J. (2002). *The structure of evolutionary thought*. Cambridge, MA: Harvard University Press.

Graham, D. E., Overbeek, R., Olsen, G. J., & Woese, C. R. (2000). An archaeal genomic signature. *Proceedings of the National Academy of Sciences, USA*, *97*, 3304–3308.

Gray, M. (1992). The endosymbiont hypothesis revisited. *International Review of Cytology*, *141*, 233–257.

Gray, M. W., & Dooolittle, W. F. (1982). Has the endosymbiont hypothesis been proven?. *Microbiology Reviews 46*, 1–42.

Gupta, R. (2005). Molecular sequences and the early history of life. In J. Sapp (Ed.), *Microbial phylogeny and evolution* (pp. 160–183). New York: Oxford University Press.

Gupta, R., & Golding, G. B. (1996). The origin of the eucaryotic cell. *Trends in Biology*, *21*, 166–170.

Haeckel, E. (1866). *Generelle Morphologie der Organismen* (2 vols.). Berlin: George Reimer.

Haeckel, E. (1904). *The wonders of life: A popular study of biological philosophy* (J. McCabe, Trans). New York: Harper and Brothers.

Hall, J. L., & Luck, D. J. (1995). Basal body-associated DNA: In situ studies in Chlamydomonas reinhardtii. *Proceedings of the National Academy of Sciences, USA*, *92*, 5129–5133.

Hartmann, H., & Fedorov, A. (2002). The origin of the eucaryotic cell: A genomic investigation. *Proceedings of the National Academy of Sciences, USA*, *99*, 1420–1425.

Horiike, T., Hamada, K., Kanaya, S., & Shinozawa, T. (2001). Origin of eucaryotic cell nuclei by symbiosis of Archaea in Bacteria is revealed by homology-hits analysis. *Nature Cell Biology*, *3*, 210–214.

Huxley, J. (1942). *Evolution: The modern synthesis*. London: Allen and Unwin.

Iwabe, N., Kuma, K., Kasegawa, M., Osawa, S., & Miyata, T. (1989). Evolutionary relationships of Archaebacteria, Eubacteria and Eucaryotes inferred from phylogenetic trees of duplicated genes. *Proceedings of the National Academy of Sciences, USA*, *86*, 9355–9359.

Jones, D., & Sneath, P. H. A. (1970). Genetic transfer and bacterial taxonomy. *Bacteriological Reviews*, *34*, 40–81.

Judson, H. F. (1992). A history of the science and technology behind gene mapping and sequencing. In D. J. Kevles, & L. Hood (Eds.), *The code of codes: scientific and social issues in the human genome project* (pp. 37–80). Cambridge, MA: Harvard University Press.

Kandler, O. (1994). Cell wall biochemistry and the three domain concept of life. *Systematic and Applied Microbiology*, *16*, 501–509.

Kluyver, A. J., & van Niel, C. B. (1936). Prospects for a natural system of classification of bacteria. *Zentralblatt für, Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abt. II*, *94*, 369–402.

Lake, J., Moore, J. E., Simonson, A. B., & Rivera, M. A. C. (2005). Fullfilling Darwin's dream. In J. Sapp (Ed.), *Microbial phylogeny and evolution* (pp. 184–206). New York: Oxford University Press.

Lake, J., & Rivera, M. (1994). Was the nucleus the first symbiont? *Proceedings of the National Academy of Science, USA*, *91*, 2880–2881.

Lederberg, J. (1952). Cell genetics and hereditary symbiosis. *Physiological Reviews*, *32*, 403–430.

Lederberg, J. (1956). Genetic transduction. *American Scientist*, *44*, 264–280.

Lederberg, J., & Tatum, E. (1946). Gene recombination in *Escherichia coli*. *Nature*, *158*, 558.

Lwoff, A. (1957). The concept of virus. *Journal of General Microbiology*, *17*, 239–253.

Lwoff, A. (1958). La notion d'espèce bactérienne à la lumière des découvertes recentes. *Annales Institutes Pasteur*, *94*, 137.

Margulis, L. (1970). *The origin of the eucaryotic cell*. New Haven: Yale University Press.

Margulis, L. (1981). *Symbiosis in cell evolution*. New York: W. H. Freeman.

Margulis, L., & Fester, R. (Eds.). (1991). *Symbiosis as a source of evolutionary innovation: speciation and morphogenesis*. Cambridge, MA: MIT Press.

Margulis, L., & Guerrero, R. (1991). Kingdoms in turmoil. *New Scientist*, *23*, 46–50.

Martin, W., & Koonin, E. V. (2006). Introns and the origin of nucleus–cytosol compartmentalization. *Nature*, *440*, 41–45.

Martin, W., & Müller, M. (1998). The hydrogen hypothesis for the first eucaryote. *Nature*, *392*, 37–41.

Maynard Smith, J., & Szathmáry, E. (1999). *The origins of life: From the birth of life to the origin of language*. New York: Oxford University Press.

Mayr, E. (1982). *The growth of biological thought: diversity, evolution and inheritance*. Cambridge, MA: The Belknap Press of Harvard University.

Mayr, E. (1991). More natural classification. *Nature*, *353*, 122.

Mayr, E. (1998). Two empires or three? *Proceedings of the National Academy of Sciences, USA*, *95*, 9720–9723.

Mayr, E., & Provine, W. B. (Eds.). (1980). *The evolutionary synthesis: Perspectives on the unification of biology*. Cambridge, MA: Harvard University Press.

Maxam, A. M., & Gilbert, W. (1977). A new method for sequencing DNA. *Proceedings of the National Academy of Sciences, USA*, *74*, 560–564.

Melinsky, H., Rainey, F. A., & Margulis, L. (2005). The karyomastigont model of eucaryosis. In J. Sapp (Ed.), *Microbial phylogeny and evolution* (pp. 261–280). New York: Oxford University Press.

Moreira, D., & Lopez-Garcia, P. (1998). Symbiosis between methanogenic Archaea and alpha-Proteobacteria as the origin of eucaryotes: The syntrophic hypothesis. *Journal of Molecular Evolution*, *47*, 517–530.

Morgan, G. L. (1998). Emile Zuckerkandl, Linus Pauling, and the molecular evolutionary clock, 1959–1965. *Journal of the History of Biology*, *31*, 155–178.

Muller, H. J. (1922). Variation due to change in the individual gene. *American Naturalist*, *56*, 32–50.

Mullis, K. F., Faloona, S., Scharf, R., Saiki Horn, G., & Erlich, H. (1986). Specific enzymatic amplification of DNA *in vitro*: The polymerase chain reaction. *Cold Spring Harbor Symposia for Quantitative Biology*, *51*, 263–273.

van Niel, C. B. (1955). Classification and taxonomy of the bacteria and blue green algae. In *A century of progress in the natural sciences, 1853-1953* (pp. 89–114). San Francisco: California Academy of Sciences.

Ochman, H., Lawrence, J. G., & Groisman, E. A. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature*, *405*, 299–304.

Pace, N. (1997). A molecular view of microbial diversity and the biosphere. *Science*, *276*, 734–740.

Pace, N. (2006). Time for a change. *Nature*, *441*, 289.

Pace, N. R., Stahl, D. A., Lane, D. J., & Olsen, G. J. (1985). Analyzing natural microbial populations by rRNA sequences. *American Society of Microbiology News*, *51*, 4–12.

Portier, P. (1918). *Les symbiotes*. Paris: Masson.

Rabinow, P. (1996). *Making PCR: a story of biotechnology*. Chicago: University of Chicago Press.

Raff, R. A., & Mahler, H. R. (1972). The non-symbiotic origin of mitochondria. *Science*, *177*, 575–582.

Reanney, D. (1976). Extrachromosomal elements as possible agents of adaptation and development. *Bacteriological Reviews*, *40*, 552–590.

Roger, A. (1999). Reconstructing early events in eucaryotic evolution. *The American Naturalist*, *154*, S146–S163.

Ryan, F. (2006). Genomic creativity and natural selection: A modern synthesis. *Biological Journal of the Linnaean Society*, *88*, 655–672.

Sagan, L. (1967). On the origin of mitosing cells. *Journal of Theoretical Biology*, *14*, 225–274.

Sanger, F., Brownless, G. G., & Barrell, B. G. (1965). A two-dimensional fractionation procedure for radioactive nucleotides. *Journal of Molecular Biology*, *13*, 373–398.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, USA*, *74*, 5463–5467.

Sanger, F., Thompson, E. O., & Kitai, R. (1955). The amide groups of insulin. *Biochemistry Journal*, *59*, 509–518.

Sapp, J. (1987). *Beyond the gene: Cytoplasmic inheritance and the struggle for authority in genetics*. New York: Oxford University Press.

Sapp, J. (1994). *Evolution by association: A history of symbiosis*. New York: Oxford University Press.

Sapp, J. (1998). Freewheeling centrioles. *History and Philosophy of the Life Sciences*, *20*, 255–290.

Sapp, J. (1999). The evolution of complexity. *History and Philosophy of the Life Sciences*, *21*, 215–226.

Sapp, J. (2002). Paul Buchner (1866–1978) and hereditary symbiosis in insects. *International Journal of Microbiology*, *5*, 145–160.

Sapp, J. (2003). *Genesis: The evolution of biology*. New York: Oxford University Press.

Sapp, J. (2004). The dynamics of symbiosis: An historical overview. *Canadian Journal of Botany*, *82*, 1–11.

Sapp, J. (2005a). The bacterium's place in nature. In J. Sapp (Ed.), *Microbial phylogeny and evolution* (pp. 1–52). New York: Oxford University Press.

Sapp, J. (2005b). The procaryote–eucaryote dichotomy: Meanings and mythology. *Microbiology and Molecular Biology Reviews*, *69*, 292–305.

Sapp, J. (2006a). Two faces of the procaryote concept. *International Microbiology*, *9*, 163–172.

Sapp, J. (2006b). Mitochondria and their host: Morphology to molecular phylogeny. In W. Martin, & M. Müller (Eds.), *Origin of mitochondria and hydrogenosomes* (pp. 57–83). Heidelberg: Springer Verlag.

Sapp, J., Carrapiço, F., & Zolotonosov, M. (2002). Symbiogenesis: The hidden face of Constantin Merezhkowsky. *History and Philosophy of the Life Sciences*, *24*, 413–440.

Schaeffer, P. (1958). La notion d'espèce après les recherches récente de génétique. *Annales de l'Institut Pasteur*, *94*, 167–178.

Sogin, M. L. (1991). Early evolution and the origin of eucaryotes. *Current Opinion in Genetics and Devolepment*, *4*, 457–463.

Sonea, S., & Panisset, P. (1983). *The new bacteriology*. Boston: Jones and Bartlett.

Stanier, R.Y. (1971). Toward an evolutionary taxonomy of the bacteria. In P. Miravete, & D. Peláez, (Eds.), *Recent advances in microbiology: International Congress for Microbiology*, *Vol. 7* (pp. 595–604). Mexico.

Stanier, R. Y. (1982). Foreword. In N. G. Carr, & B. A. Whitton (Eds.), *The biology of cyanobacteria* (pp. ix–x). Berkeley: University of California Press.

Stanier, R. Y., Doudoroff, M., & Adelberg, E. A. (1963). *The microbial world* (2nd ed.). Engelwood Cliffs, NJ: Prentice-Hall Inc.

Stanier, R. Y., & van Niel, C. B. (1941). The main outlines of bacterial classification. *Journal of Bacteriology*, *42*, 437–466.

Stanier, R. Y., & van Niel, C. B. (1962). The concept of a bacterium. *Archiv für Mikrobiologie*, *42*, 17–35.

Strick, J. (2000). *Sparks of life: Darwinism and the Victorian debate over spontaneous generation*. Chicago: University of Chicago Press.

Tornabene, T. G., & Langworthy, T. A. (1979). Diphytanyl and dibiphytanyl glycerol ether lipids of methanogenic archaebacteria. *Science*, *203*, 51–53.

Uzzell, T., & Spolsky, C. (1973). Origin of mitochondria. *Science*, *180*, 516–517.

Wallin, I. E. (1927). *Symbionticism and the origin of species*. Baltimore: Williams and Wilkins.

Walsh, D. A., & Doolittle, W. F. (2005). The real domains of life. *Current Biology*, *15*, 237–240.

Ward, D. M. (1998). A natural species concept for procaryotes. *Current Opinion in Microbiology*, *1*, 271–277.

Werren, J. H. (2005). Heritable microorganisms and reproductive parasitism. In J. Sapp, (Ed.), *Microbial phylogeny and evolution* (pp. 290–315). New York: Oxford University Press.

Wheelis, M. L., Kandler, O., & Woese, C. R. (1992). On the nature of global classification. *Proceedings of the National Academy of Sciences, USA*, *89*, 2930–2934.

Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Procaryotes: The unseen majority. *Proceedings of the National Academy of Sciences, USA*, *95*, 6578–6583.

Whittaker, R. H. (1969). New concepts of kingdoms of organisms. *Science*, *163*, 150–160.

Whittaker, R. H., & Margulis, L. (1978). Protist classification and the kingdoms of organisms. *Biosystems*, *10*, 3–18.

Wilson, E. B. (1900). *The cell in development and inheritance*. New York: Macmillan.

Woese, C. R. (1994). There must be a procaryote somewhere: Microbiology's search for itself. *Microbial Reviews*, *58*, 1–9.

Woese, C. R. (1998a). The universal ancestor. *Proceedings of the National Academy of Sciences, USA*, *95*, 6854–6859.

Woese, C. R. (1998b). Default taxonomy: Ernst Mayr's view of the microbial world. *Proceedings of the National Academy of Sciences, USA*, *95*, 11043–11046.

Woese, C. R. (2000). Interpreting the universal phylogenetic tree. *Proceedings of the National Academy of Sciences, USA*, *97*, 8392–8396.

Woese, C. R. (2004). A new biology for a new century. *Microbiology and Molecular Reviews*, *68*, 173–186.

Woese, C. (2005). Evolving biological organization. In J. Sapp, (Ed.), *Microbial phylogeny and evolution* (pp. 99–118). New York: Oxford University Press.

Woese, C. R., & Fox, G. E. (1977a). Phylogenetic structure of the procaryote domain: The primary kingdoms. *Proceedings of the National Academy of Sciences, USA*, *75*, 5088–5090.

Woese, C. R., & Fox, G. E. (1977b). The concept of cellular evolution. *Journal of Molecular Evolution*, *10*, 1–6.

Woese, C. R., Gibson, J., & Fox, G. E. (1980). Do genealogical patterns in purple photosynthetic bacteria reflect interspecific gene transfer? *Nature*, *283*, 212–214.

Woese, C. R., Kandler, O., & Wheelis, M. L. (1990). Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences, USA*, *87*, 4576–4579.

Woodhead, G. S. (1891). *Bacteria and their products*. London: Walter Scott.

Yang, D., Oyaizu, Y., Oyaizu, H., Olsen, G. J, & Woese, C. R. (1985) Mitochondrial origins. *Proceedings of the National Acadamy of Science, USA*, *82*, 4443–4447.

Zablen, L. B., Kissil, M. S., Woese, C. R., & Buetow, D. E. (1975). Phylogenetic origin of the chloroplast and procaryotic nature of its ribosomal RNA. *Proceedings of the National Academy of Science, USA*, *6*, 2418–2422.

Zillig, W., Schnabel, R., Tu, J., & Stetter, K. O. (1982). The phylogeny of archaebacteria, including novel anaerobic thermoacidophiles in the light of RNA polymerase structure. *Naturwissenschaften*, *69*, 197–204.

Zimmer, C. (2001). Wolbachia: A tale of sex and survival. *Science*, *292*, 1093–1096.

Zuckerkandl, E., & Pauling, L. (1965). Molecules as documents of evolutionary history. *Journal of Theoretical Biology*, *8*, 357–366.