

Epistemological issues in the study of microbial life: Alternative terran biospheres?

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Abstract: The assumption that all life on Earth today shares the same basic molecular architecture and biochemistry is part of the paradigm of modern biology. This paper argues that there is little theoretical or empirical support for this widely held assumption. Scientists know that life could have been at least modestly different at the molecular level and it is clear that alternative molecular building blocks for life were available on the early Earth. If the emergence of life is, like other natural phenomena, highly probable given the right chemical and physical conditions then it seems likely that the early Earth hosted multiple origins of life, some of which produced chemical variations on life as we know it. While these points are often conceded, it is nevertheless maintained that any primitive alternatives to familiar life would have been eliminated long ago, either amalgamated into a single form of life through lateral gene transfer (LGT) or alternatively out-competed by our putatively more evolutionarily robust form of life. Besides, the argument continues, if such life forms still existed, we surely would have encountered telling signs of them by now. These arguments do not hold up well under close scrutiny. They reflect a host of assumptions that are grounded in our experience with large multicellular organisms and, most importantly, do not apply to microbial forms of life, which cannot be easily studied without the aid of sophisticated technologies. Significantly, the most powerful molecular biology techniques available—polymerase chain reaction (PCR) amplification of genes augmented by metagenomic analysis—could not detect such microbes if they existed. Given the profound philosophical and scientific importance that such a discovery would represent, a dedicated search for “shadow microbes” (heretofore unrecognized “alien” forms of terran microbial life) seems in order. The best place to start such a search is with puzzling (anomalous) phenomena, such as desert varnish, that resist classification as “biological” or “nonbiological.”

Keywords: origins of life, microbial biodiversity, alternative biosphere, alternative life

1. Introduction

Despite its dazzling morphological diversity, life as we know it on Earth is remarkably similar in its basic molecular architecture and biochemistry. The assumption that all life on Earth today shares these molecular and biochemical features is part of the paradigm of

modern biology. Following the work of Cleland and Copley (2005), this paper challenges this assumption. In the first place, we know that life could have been at least modestly different at the molecular level and moreover that alternative molecular building blocks for life were available on the early Earth. If the emergence of life is, like other natural occurrences, highly probable given the right chemical and physical conditions then it seems likely that the early Earth hosted multiple origins of life, some of which produced chemical variations on life as we know it. These points are often conceded. But it is nevertheless maintained that any primitive alternatives to familiar life would have been amalgamated into a single form of life through lateral gene transfer (LGT) or alternatively been out-competed early on by our (presumably) more evolutionarily robust form of life, and in any case, the argument continues, if they still existed we would have found evidence of them. As I shall show, these arguments do not hold up well under close scrutiny. They reflect a host of assumptions about life that are grounded in our experience with large multicellular organisms and, most importantly, do not apply to microbial forms of life. They also ignore the fact that the tools currently used to explore the microbial world, viz., microscopy (even with sophisticated molecular staining techniques), cultivation, and molecular biology techniques such as PCR (polymerase chain reaction) amplification of rRNA (ribosomal RNA) genes, could not detect an alternative form of microbial life if it existed (Cleland & Copley 2005). In short, the possibility that the contemporary Earth is host to “shadow microbes” (as yet unrecognized forms of microbial life that differ in fundamental ways at the molecular level from familiar terran life) cannot be dismissed on scientific grounds. While it is not possible to assign a numerical probability to the hypothesis that such organisms exist,

considerations from molecular biology and biochemistry coupled with our current understanding of the origin of life and our growing knowledge of the complexity and dynamics of microbial ecosystems suggest that this hypothesis is worthy of serious scientific investigation.

2. What might an alternative form of terran microbial life be like?

It might be thought that recognizing an alternative form of life, microbial or otherwise, presupposes an understanding of the general nature of life. Two characteristics have traditionally been advanced as essential to life: (1) the capacity to self-organize and maintain self-organization for an extended period of time against both internal and external perturbations and (2) the capacity to reproduce and transmit to progeny adaptive heritable modifications. These characteristics are functional, as opposed to structural or compositional. They appear in rudimentary form in the writings of Aristotle, *De Anima*, Bk II, 412^{a13}-416^{b30}) (e.g., Mckeon 1941), who identified life with the capacities for “self-nutrition” and “reproduction”; writing two thousand years before Darwin and believing that the species are immutable, Aristotle did not touch on the idea of evolution.

In recent times, philosophers and scientists have tended to favor one characteristic over the other. This approach is clearly illustrated in modern scientific theories of the origin of life.¹ Soviet biochemist Alexander Oparin (1957), father of “metabolism first” theories, theorized that the first forms of life were simple self-organizing molecular systems interacting with their environments but incapable of replicating with fidelity. The Nobel Prize winning discovery that RNA has catalytic powers paved the way for “genes first” accounts of the origin of life. According to the currently dominant “RNA

world” hypothesis, the first living things were communities of self-replicating RNA molecules that eventually evolved the capacity to synthesize proteins and undergo metabolic cycles (Gilbert 1986). The tendency to favor one characteristic over the other is also illustrated by contemporary definitions of ‘life,’ which typically pick out one characteristic as essential. Autopoietic (e.g., Varela et al. 1974), cybernetic (e.g., Korzeniewski 2001) and thermodynamic (e.g., Kauffman 1995; Schulze-Makuch et al. 2002) definitions analyze life in terms of the first characteristic (the capacity to metabolize or, more generally, self-organize) whereas evolutionary definitions (e.g., Joyce 1994, Pace 2001; Bedau 1998) analyzing life in terms of the second (capacity to reproduce and undergo evolution, usually, but not always, by means of natural selection).²

Nevertheless, both characteristics are universal to life as we know it on Earth today and as such equally in need of explanation in terms of natural processes, regardless of whether one is, from some favored theoretical standpoint, more fundamental than the other. Molecular biology provides such an account. Rather than concerning itself directly with the question of which characteristic is more fundamental it provides a unified scientific account of how familiar terran life manages to realize both characteristics in a concrete physical system. Molecular biology has taught us that familiar life is based upon an exceedingly complex cooperative arrangement between two quite different types of macromolecule, proteins and nucleic acids. Proteins supply the bulk of the structural material for building organismal bodies as well as the catalytic (enzymatic) material for powering and maintaining them. Nucleic acids store the hereditary information required for reproduction and also for synthesizing the enormous

quantity and variety of protein required by an organism during its life span. The crucial process of coordinating these two functions—of translating the hereditary material stored in nucleic acids into proteins for use in growth, maintenance, and repair—is handled by ribosomes, minuscule but intricately structured molecular devices composed of both protein and nucleic acid (RNA). In other words, all known life on Earth utilizes the same molecular building blocks and core molecular architecture to realize the abstract functional characteristics traditionally held up as essential to life in a single physical system. Molecular biology thus provides us with a needed reference point--a definite physical framework--for thinking about alternatives to familiar life.

Admittedly we don't know how different life could be from life as we know it on Earth today because we don't know all the different ways in which a physical system might realize the abstract functions traditionally attributed to life. We also can't rule out the possibility that the most important characteristics of life have yet to be discovered. The characteristics traditionally held up as essential to life may be little more than potentially unreliable symptoms of more fundamental but as yet unknown properties. This has happened before in science. Lacking recourse to molecular theory, the alchemists, who were medieval chemists, chose solvency as the essential property of water, and as a consequence identified chemical substances, e.g., nitric acid, that we now know are not water as water (Roberts 1994); it is not an accident that they called nitric acid "*aqua*" *fortis*. Similarly, prior to the germ theory of disease, physicians identified most diseases in terms of their overt symptoms, which sometimes resulted in what we now recognize as the same disease (qua underlying infectious agent) being classified as distinct diseases; as an example, tuberculosis doesn't produce the tell tale "consumptive"

cough when it infects the lymph glands (vs. lungs). Biologists may someday discover that the functional characteristics that have historically been taken as essential to life are, like solvency and a consumptive cough, defeasible symptoms of a more fundamental underlying property. The problem is exacerbated in the case of life because, as will be discussed in subsequent sections, we have good reasons for believing that familiar Earth life descends from a common ancestor and moreover we know of at least some modest ways in which it could have been different at the molecular level. This suggests that we are dealing with a single, possibly unrepresentative, example of life. One cannot safely generalize from a single example of life to all life, wherever and whenever it may be found in the universe. Nevertheless, because it explains in detail how the most general characteristics exhibited by *familiar* Earth life are jointly realized in one kind of physical system, molecular biology allows us to adumbrate some definite, albeit circumscribed, possibilities for an alternative form of life, despite our ignorance of the extent to which these characteristics generalize to *all* life.

2.1 Alternative proteins?

How might an alternative form of life differ at the molecular level from familiar life? It is clear that some of the molecular building blocks of proteins and nucleic acids could have been modestly different without affecting their biological functionality. Although there are over 100 amino acids, familiar Earth life constructs its proteins primarily from the same 20.³ A protein typically consists of 50 to 1,000 amino acids joined together by peptide bonds into a long chain or polymer. Not just any sequence of amino acids will build a functional protein. The functionality of proteins crucially depends upon their

ability to fold into complex three-dimensional structures. The nature and order of the amino acids in the polymer determines (sometimes with the help of molecular chaperones) the three-dimensional structure of the protein. There must be a sufficient number of large, small, hydrophobic, hydrophilic and charged amino acids, and they must occur in the correct order; change the order and you change the functional properties, and hence identity, of the protein. But although this restricts the collections of amino acids that may be used to build a sufficiently diverse set of proteins, it by no means eliminates alternatives to the collection of 20 utilized by familiar terran life; see Benner (1994) for a review of work in this area.

Many molecules are asymmetrical and thus, like other asymmetrical three-dimensional objects, have the interesting geometrical property of handedness or (in chemical terminology) chirality; the stereotypical example of handedness is a left hand and its mirror image, an identical right hand, which while they have the very same size and shape cannot be superimposed by translation or rotation in three-dimensions. In organic molecules chirality commonly consists of two alternative asymmetrical arrangements of chemical bonds around a carbon atom. By convention one of these molecular arrangements is labeled "L" (levo) and the other "D" (dextro). Life as we know it on Earth today builds its proteins primarily from L-amino acids.

Chiral mixtures of amino acids do not build good protein structure. So it is hardly surprising that terran organisms utilize amino acids of the same chirality. The question is why L-amino acids as opposed to D-amino acids? Proteins have been chemically synthesized from D-amino acids, and they fold correctly and are functional (Milton *et al.* 1992; Zawadzke & Berg 1992; Fitzgerald *et al.* 1995; Canne *et al.* 1997). There is thus

no reason to suppose that life on Earth couldn't have used D-amino acids to build its structural and enzymatic material. Indeed, as will be discussed shortly, the abiotic processes that are thought to have supplied the early Earth with prebiotic amino acids produce chiral mixtures typically containing approximately equal amounts of L-amino acids and D-amino acids. Moreover, these processes also supply many varieties of amino acids that are not utilized by familiar Earth life. It is thus a mystery why L-amino acids predominate in the proteins of known Earth life.

2.2 Alternative nucleic acids?

Nucleic acids are long polymers composed of nucleotides. A nucleotide is a complex molecular unit consisting of three subunits, a phosphate unit bonded to a sugar (ribose or deoxyribose) unit that, in turn, is attached to a base unit. Familiar terran life constructs its nucleic acids exclusively from D-sugar molecules. Functional nucleic acids can be synthesized from L-sugars, however, and abiotic processes readily produce chiral mixtures of sugars. But as with proteins and D-amino acids, life as we know it on Earth today does not use the L-form.

Familiar Earth life employs two different kinds of nucleic acid, DNA and RNA. With the exception of some viruses, which use RNA, DNA is the repository of hereditary information and RNA supervises the intricate process of translating this information into proteins for immediate use by the organism. DNA is well suited for storing hereditary information. Each strand of its famous double helix consists of a sequence of individual nucleotides covalently bonded into a chain by their sugar-phosphate units. The two strands are held together by electrostatic hydrogen bonds between their respective bases.

Hydrogen bonds are weaker than covalent bonds, making it easier for strands to separate during replication, and yet are also strong enough to give the double helix structural integrity, making DNA a good permanent store for hereditary information.

The DNA molecule utilizes four standard bases—adenine (A), thymine (T), guanine (G), and cytosine (C)—to encode hereditary information. These bases pair off in a complementary pattern: C pairs with G and A pairs with T. As a consequence, each strand of DNA provides a template for reconstructing the other half. This arrangement provides an important source of redundancy in case of damage to one strand. Hereditary information is encoded on a single (the “coding”) strand of DNA by sequences of three consecutive bases. Each triplet of bases or codon specifies a specific amino acid or, alternatively, the initiation or termination of the construction of a chain of amino acids constituting a protein. With some minor exceptions, the genetic code is universal for all known life on Earth, with the same triplet of bases coding for the same amino acid.

In the context of the current state of our knowledge of biochemistry and molecular biology, a number of features of DNA could have been different. The four bases used by familiar life are not the only molecular possibilities for storing hereditary information on duplex DNA. Benner and co-workers (Piccirilli et al. 1990; Benner 2004; Benner & Swizer 1999; Geyer et al. 2003) have identified 8 viable alternative bases, yielding a total of six mutually exclusive base pairs (including the four bases used by our form of life) that could be accommodated by double stranded DNA. In addition, the genetic code seems somewhat arbitrary. It is redundant, with up to six different codons (e.g., UCU, UCC, UCA, UCG, AGC and AGU) coding for the same amino acid (serine), suggesting that some pairings could be dispensed with, and there is little reason to

suppose that amino acids couldn't have been paired with different triplets of bases.⁴ Furthermore, instead of triplets, life might have utilized a doublet or perhaps even a quadruplet or quintuplet coding scheme. A triplet coding scheme is the most efficient available for 4 bases and 20 amino acids since $4^3=64$ possible distinct coding sequences. But there are alternative combinations of amino acids and bases for which this is not obviously true. Moreover, there is little reason to suppose that life couldn't have utilized a mixed coding scheme, e.g., triplets and doublets.⁵

2.3 Wilder Possibilities: Neither proteins nor nucleic acids?

What about the backbone structure of DNA: Could it have been different? The important features of the backbone are its ability to form an extended, redundant, stable structure that is insensitive to the precise order of the bases, allowing it to encode an enormous amount of information through variations in base sequence. Efforts to construct alternative backbones having all of these features have not been very successful (Huang et al. 1993; Richert et al. 1996; Eschenmoser 1999; Benner & Hutter 2002). But it is important to keep in mind that these efforts are typically top down, involving modest changes to the structure of familiar DNA, e.g., replacing deoxyribose with a different sugar. The possibility that these features could be realized by an alternative macromolecule has not been adequately explored. In this context it is important to keep in mind that the DNA molecule is not the sort of thing that a chemist (who was unfamiliar with it) is likely to have dreamt up on her own or stumbled upon in an experiment. It is a truly bizarre molecule, huge and extremely complex, built from large numbers of several different chemical elements (carbon, nitrogen, phosphorous, oxygen,

and hydrogen). Indeed, when compared to the typical molecule found in nature, DNA seems highly improbable! Even today no one knows how to produce it abiotically under natural conditions from basic chemical building blocks; see Shapiro (2000) for a discussion. Who knows whether carbon has the capacity to form, as yet unencountered, complex alternative macromolecular structures with the functional capacities of nucleic acids or, for that matter, proteins?

It is often claimed that silicon, the only other chemical element known to form extended structures analogous to those formed by carbon, is incapable of forming polymers of the complexity, stability, and versatility required of biomolecules. But such judgments seem premature. As Schulze-Makuch and Irwin (2006) discuss, there is reason to believe that silicon is capable of forming more promising molecular structures under chemical and physical conditions quite different from those on the contemporary Earth, more specifically, in environments with temperatures far below the freezing point of water, little oxygen, a scarcity of water, etc. Furthermore, while carbon forms extended physical structures in nature such as graphite, diamond, and fullerenes (which weren't discovered until 1990!), none of them comes close in molecular size and complexity to nucleic acids. The upshot is that intelligent, silicon-based life from a very un-earth-like environment might well draw an analogous conclusion about the possibilities for carbon-based life, namely, that carbon isn't capable of forming macromolecules with the requisite degree of complexity, stability, and versatility. Indeed, scientists still do not know how to produce polypeptides and oligonucleotides (or even just nucleotides), let alone proteins or nucleic acids, from naturally occurring abiotic processes. These points are particularly salient when viewed in light of the difficulties faced by in situ and robotic

investigations of planets and moons in our solar system in explaining, not to mention predicting, the ways in which the laws of nature work themselves out in unfamiliar physical and chemical environments. Many phenomena observed on Mars and Titan not only defied initial expectations but remain difficult to explain in terms of our current understanding of chemistry and physics. In short, our failure to identify molecular alternatives to nucleic acids and proteins provides much less support for the claim that they don't exist than is often thought.

In any case, however, my less extravagant point remains: Although we don't know all the chemical and physical possibilities for realizing a living system, we do know of some fairly modest ways in which life on Earth could have been different, and these include utilizing amino acids and bases differing in number and/or kind to construct proteins and nucleic acids. The question is why does life as we know it on Earth today use the particular combination of molecular building blocks that it does?

The best explanation, given our current understanding of chemistry and molecular biology, is that these building blocks are the result of physical and chemical contingencies present on the early Earth. This is true not only for proteins and nucleic acids but also for ribosomes. Because they physically realize the translation of abstract hereditary information into functioning, self-maintaining organisms, ribosomes lie at the very heart of the molecular architecture of familiar life. Their unique structural characteristics are almost certainly the product of their own set of historical contingencies as well as those responsible for the molecular compositions of the proteins and nucleic acids (RNA) that comprise them. In addition, it is very unlikely that the highly sophisticated ribosomes found in modern cells represent the only possibility for

translating hereditary information stored on nucleic acids into proteins, let alone the original mechanism utilized by the first terran protocells. It seems clear: Had conditions on the early Earth been different, life on Earth would have been different too.

3. Alternative proto-organisms on the early Earth?

While we don't know the specific chemical and physical processes that gave rise to the earliest forms of terran life, we do know that substantial variations in the molecular building blocks of life were available on the early Earth. Asteroids and comets supply a large variety of simple organic molecules. Indeed, over 70 amino acids (only eight of which are utilized by known life on Earth today) have been identified in meteorites, and although some studies suggest that there may be a slight excess of L-amino acids, D-amino acids are nonetheless quite common; for a general discussion, see Andersen and Haack (2005). Other hypothesized sources of simple organic molecules include electrical discharges through various mixtures of simple gases (Miller, S.L. 1953; 1955), hydrothermal processes in oceanic volcanic vents (Holm & Andersson 1998; Martin & Russell 2003), and geochemical processes involving mineral surfaces (Wächtershäuser 1988; Cairns-Smith et al. 1992; Cody 2004). These processes produce a large variety of small organic molecules, including many that are not utilized in the biomolecules of familiar Earth life. It would thus be very surprising if the chemical resources for building proto-organisms were the same in every incipient "cradle of life" on the early Earth. If conditions conducive to the emergence of life were present at multiple locations and these locations contained, as they surely did, variations in the basic molecular building blocks of life, then one would expect the earliest life forms—perhaps primitive genetic

systems such as communities of self-replicating RNA molecules (Eigen 1992; Gilbert 1986), or, alternatively, primitive autocatalytic metabolic cycles constituted by polypeptides either encapsulated in proto-cells (e.g., Oparin 1957, Dyson 1985) or bound to mineral surfaces (Cairns-Smith et al. 1992, Wächtershäuser 1992)—to reflect these differences in their molecular composition. In other words, the hypothesis that the early Earth hosted multiple origins of life and that some of these origins produced molecular variations on familiar life is consistent with our current chemical and biological understanding of life.

One cannot, of course, eliminate the possibility that life originated just once as a result of an extraordinarily improbable combination of chance occurrences. It is important to keep in mind, however, that there is no evidence for this claim other than our current ignorance of the chemical and physical conditions under which an ensemble of nonliving molecules turns into a primitive living system. Ignorance about how life actually got its start on Earth cannot—as some scientists (e.g., Mayr 1982, Crick 1981) seem to assume—support the claim that the emergence of life is (in essence) a scientific miracle. For the only claim that ignorance can support is the trivial claim that we just don't know. Furthermore, to the extent that science operates under the guiding principle that natural phenomena are explicable in terms of specifiable natural processes, appeal to cosmic coincidences as an explanation for ignorance is self-defeating, placing the emergence of life beyond the reach of scientific investigation just as surely as would an appeal to supernatural creation; for further discussion, see Fry (1995). If the emergence of life is, like other natural phenomena, highly probable under certain, as yet unknown, chemical and physical conditions *and* these conditions were present on the early Earth,

then it seems more likely than not that the early Earth hosted alternative forms of primitive life. The second conjunct in the antecedent of this conditional is crucial. The origin of life may require conditions that were not present on the early Earth, in which case one could not infer the consequent. Although most scientists believe that terran life originated on Earth, some think that conditions on Mars may have been more conducive to life than those on Earth in the early Solar System. Furthermore, given our limited scientific understanding of the emergence of life, we cannot rule out discovering that part or even all of the synthesis of nucleic acids or proteins require exotic conditions that were never present on Earth--just as astronomers discovered that the heavy elements are synthesized in second and third generation stars. This would not, however, amount to embracing the view that the origin of life is a scientific miracle. The origin of life would still be highly probable under the “right” chemical and physical conditions. They just wouldn’t be found on the early Earth.⁶

4. Could the present-day Earth be host to as yet undiscovered alternative forms of life?

While many biologists are willing to concede that the first terran protocells may have exhibited significant molecular variability, few are willing to take the next step and seriously entertain the possibility that their microbial descendants might still be with us today. In the following subsections, I discuss and reject some reasons that might be advanced for dismissing this intriguing possibility.

4.1 The vast and uncharted microbial biosphere

It is undoubtedly true that if there were large alternative forms of terran life, analogous to

plants or animals, we would have stumbled upon them by now. The situation is quite different in the case of microbes, however.

The term ‘microbe’ is used loosely to encompass a large and diverse group of tiny organisms that, with a few exceptions, cannot be seen without the aid of a microscope. Microbes include (true) Bacteria (also known as Eubacteria), Archaea, algae, yeasts, and protozoans. With a few exceptions (e.g., acellular viruses, and microscopic invertebrates such as rotifers⁷), the organisms classified as “microbes” in microbiology texts are single-celled, although many unicellular microbes participate in multicellular communities that may be visible to the naked eye. While the typical microbial community (e.g., a biofilm) consists of cells with a variety of different genomes, the cells of a few (e.g., swarms of myxobacteria) resemble “true” multicellular organisms (fungi, plants and animals) in having the same genome and producing specialized cells for performing specific functions.

Unicellular microbes have traditionally been divided into two groups, prokaryotes and microbial eukaryotes, on the basis of the gross internal organization of their cells. The distinctive feature of the prokaryotic cell plan is the lack of membrane-enclosed, subcellular structures, most importantly a nucleus encompassing their chromosomes. In contrast, the chromosomes of eukaryotic cells are enclosed by membranes; most have additional membrane-bound organelles such as mitochondria or chloroplasts as well. Of the three domains of life (the highest taxonomic level currently recognized), Bacteria and Archaea have prokaryotic cells while Eukarya have eukaryotic cells. The Eukarya include unicellular microbes—protists, algae (excluding cyanobacteria), and yeasts—as well as all “true” multicellular organisms. As will be discussed in subsequent sections,

the morphologically based prokaryotic-eukaryotic distinction has been shown to lack phylogenetic significance.⁸

The metabolic diversity and environmental range of terran microbes far exceeds that of large multicellular terran organisms. This is particularly true of prokaryotic microbes, and to a lesser extent of unicellular eukaryotes. Microbes from all three domains prosper under conditions in which no large eukaryotic organisms are found. Bacteria and Archaea, however, are the real stars, being found in highly acidic streams, boiling hot springs, several kilometers beneath the Earth's crust, and the coldest regions on Earth. Prokaryotic microbes also exploit virtually every reliable potential energy resource on Earth. Like plants, some photosynthesize and, like animals, some metabolize organic material. But unlike plants and animals, which are limited to extracting energy from light or organic material, some prokaryotes exploit inorganic materials such as ammonia, hydrogen sulphide, ferrous iron, and even hydrogen gas. Similarly, some are able to metabolize organic material under anaerobic conditions by utilizing a wide variety of oxidants other than oxygen.

The first proto-organisms were almost certainly microbial. There is intriguing evidence suggesting that life on Earth goes back at least 3.5-3.8 billion years (Mojzsis et al. 1996; Schopf 1992) and, moreover, that microbes were the only form of life on Earth for 2-3 billion years; the earliest fossil traces of large organisms (the mysterious Ediacara fauna) date back only 650-543 million years. In addition, the genetic diversity of the microbes exceeds that of multicellular Eukarya, strongly suggesting that they are much older. Despite their striking morphological similarities, Bacteria and Archaea differ genetically and biochemically from one another more than Archaea differ from Eukarya

(Woese et al. 1990). Finally, it has been argued (Ward & Brownlee 2000) that the presence of large multicellular organisms on Earth may be the result of the confluence of a large number of unusual conditions peculiar to our solar system and home planet, e.g., nearly circular planetary orbits, outer gas giants protecting inner terrestrial planets from meteorite impacts, a huge moon stabilizing the Earth's tilt, and a series of global glaciation events. Indeed, of the three domains of familiar terran life, only the Eukarya evolved into large multicellular organisms. These considerations, coupled with their limited metabolic diversity and environmental range, strongly suggest (but of course do not prove) that large organisms analogous to plants and animals may be the exception rather than the rule in the universe.

Even today, microbes are the dominant form of life on Earth. Their biomass exceeds that of large multicellular Eukarya (DeLong & Pace, 2001), and no one knows by how much. As a point of reference, the number of microbial cells in the human body is estimated to exceed that of human (somatic and germ) cells by a factor of 10 to 1 (Savage, 1977; Berg, 1996). Although the concept of biological species is problematic for microbes, it is estimated that there are as many as 10^7 - 10^{12} distinct varieties of Bacteria and Archaea, less than 1% of which have been cultured or identified by PCR analysis of ribosomal rRNA genes (Pace 1997). Indeed, Pace (1997) estimates that a handful of soil contains billions of different "species." In short, our knowledge of the inhabitants of the microbial world is still exceedingly limited, much more limited than our knowledge of macroscopic terran organisms.

In this context, it is worth noting that Archaea, Bacteria, and microbial Eukarya aren't the only minuscule biological entities with a potential claim to the title "microbe."

Viruses, which are commonly lumped in with prokaryotes and microbial Eukarya in texts on microbiology, provide a particularly salient example. Consisting of little more than protein-coated DNA or RNA molecules, viruses cannot be characterized as cellular because they are not divided into an inside and an outside by a cell membrane. Indeed, debate still simmers over whether they even qualify as living things. Nevertheless viruses have some of the characteristics thought to be essential to life. Although they don't metabolize, they are nonetheless fairly stable, maintaining their structural and functional capacities against environmental perturbations for extended periods of time. Viruses also direct their own reproduction by literally invading a host cell and commandeering its reproductive machinery. Last but not least, viruses are subject to Darwinian evolution, mutation and natural selection.

Viruses raise the question of what constitutes a living individual in the microbial realm. Shapiro (e.g., 1998) and O'Malley and Dupré (2007) have explored this question in one direction, arguing that microbial communities consisting of different varieties of microbe ought nevertheless to be construed as multicellular organisms, despite the lack of a shared genome. But the question can also be explored in the other direction, below the scale of the cell. Moreover, viruses aren't the only mobile, acellular molecular units meeting some of the putative requirements of life. Plasmids and prions provide two more examples. Consisting of extra-chromosomal DNA, plasmids are found in bacteria. They encode "information" that is useful (e.g., antibiotic resistance) but not indispensable to normal cell function, and some are capable of moving between cells (Lewin 2004) while maintaining their identity as plasmids.⁹ Prions, in contrast, are infectious proteinaceous particles found in eukaryotic cells. In many ways the protein counterparts of viruses,

they cause diseases such as scrapie and bovine spongiform encephalopathy in mammals but are also found in unicellular yeasts. Traveling between cells, prions propagate by interacting with normal proteins and converting them, in template like fashion, into more of themselves. Prions are remarkably stable, maintaining their identities (infective protein conformations) under high temperatures, harsh chemicals, and ultraviolet irradiation (Wickner 1997).

Let me hasten to add that my purpose here is not to convince the reader that viruses, plasmids, and prions are living acellular individuals; for one thing, they require the machinery of a cell in order to reproduce.¹⁰ My point is only that the microbial world displays a greater diversity of structures having life-like functions than is sometimes appreciated. Our current cell-based ontology for parsing it into individuals (unicellular and multicellular) suppresses certain distinctions that might otherwise prove fruitful for theorizing.¹¹ And this brings us to an important and sometimes overlooked point: There are no guarantees that all microbial forms of life are cellular. Indeed, some theories of the origin of life (Cairns-Smith et al. 1992, Wächtershäuser 1992) postulate that the first primitive organisms (qua self-maintaining and self-reproducing individuals) consisted of complexes of organic compounds intimately bonded to mineral surfaces. We cannot dismiss the possibility that the contemporary microbial realm includes as yet undiscovered acellular forms of life that (unlike viruses, plasmids, and prions) do not rely upon the detailed mechanism of a cell in order to reproduce and maintain themselves as individuals.

In summary, the absence of large, easily identifiable, alternative forms of life doesn't count much against the possibility that microbial descendents of an alternative

origin of life may still be with us today. As discussed, the earliest forms of life were almost certainly microbial and there are good reasons for thinking that the evolution of large multicellular forms of life is the exception rather than the rule. Furthermore, it is clear that our knowledge of the microbial world is still very limited. It undoubtedly holds many more surprises for us. Perhaps an alternative form of life, cellular or acellular, is among them!

This brings us to three more technical objections to the possibility that the present-day Earth is host to an alternative form of microbial life: (1) lateral or (as it is also known) horizontal gene transfer would amalgamate different forms of microbial life into a single form of life; (2) any alternative form of microbial life would be eliminated by our form of life in the ruthless Darwinian competition for vital resources; (3) if such microbes existed, we would have discovered them by now or at least encountered telling signs of them using the sophisticated tools available to contemporary microbiologists. As we shall see, none of these arguments stands up under close scrutiny.

4.2. Objections based on lateral gene transfer:

The phenomenon of lateral gene transfer (LGT) might be thought to preclude the possibility of an alternative form of microbial life. LGT occurs when genes are transferred directly from one microbe to that of another, instead of being transferred vertically from parent to offspring during the process of reproduction. The main mechanisms are (1) uptake and incorporation of DNA fragments released into the environment from dead cells (transformation), (2) exchange of DNA between living cells (bacterial conjugation), and (3) insertion of genetic material directly into a host cell's

genome as a result of viral infection (transduction). LGT is very common among prokaryotic microbes, providing them with a source of heritable variation other than mutation. It occurs to a lesser extent among microbial eukaryotes and is rarer still among multicellular eukaryotes, which not only have various barriers (e.g., nuclear membranes) to the first two modes of LGT but also specialized germ cells for reproduction, decreasing the possibility that genes that are integrated into the DNA of an arbitrary cell by means of viral transduction will be inherited by their offspring; nevertheless the genomes of multicellular eukaryotes contain lots of heritable retroviral DNA. Importantly for the argument under consideration, while much less common than intra-domain LGT, cross-domain LGT nonetheless occurs, and it occurs among microbes from all three domains.

The pervasiveness of LGT in the microbial world might be thought to preclude the possibility of an alternative form of microbial life. The basic idea is that LGT would eventually amalgamate all forms of microbial life into a single form of life. The problem with this scenario is that LGT presupposes significant similarities in the molecular machinery for replication, transcription, and translation, which explains why it is most common among microbes from the same domain and rarer among microbes from different domains. Indeed, cross-domain LGT is possible only because organisms in all three domains share important elements of their core molecular machinery. No microbe from any of the three domains could incorporate genes from an even modestly different form of life—one that utilized bases differing in either identity or number, for instance—into its genome, or vice versa. And even supposing that such an event were to occur, the gene could not be replicated or used to make protein.

Not unsurprisingly, LGT is thought to have played a key role in the evolution of modern microbes from their primitive precursors. Carl Woese (1998, 2004), who has written more extensively about this than perhaps anyone, speculates that LGT was the primary mode of gene transfer available to the earliest forms of Earth life. According to Woese, the progenitor of familiar life was not, as commonly supposed, a single lucky microbe but rather a diverse community of primitive cellular entities. Lacking the genetic apparatus of modern cells, these proto-cells could not evolve by Darwinian evolution. Instead they developed as a loosely confederated community, subject to extensive mutation and rampant LGT, reshuffling their contents as they split and merged in haphazard ways. Eventually, according to Woese, this chaotic process hit upon the critical precursors for the complex cooperative arrangement of proteins and nucleic acids that characterize life as we know it today, and the process became increasingly orderly. On this scenario, any primitive alternative forms of life would have been amalgamated by LGT into a single form of life before the emergence of the first modern microbial cells with their sophisticated genetic apparatus.

But this argument fares little better than the earlier one. On Woese's account, the LGT engaged in by the first primitive cell-like entities is significantly different than modern LGT, involving a free exchange of "most if not all cellular componentry" (Woese 2004, p. 18). It is difficult to envision how the complex cooperative arrangement between proteins and nucleic acids that characterizes life as we know it today could emerge from a disorganized exchange of primitive biomolecules, and not at all obvious that the particular combination of molecular building blocks characterizing familiar life is the only possibility. In addition, Woese contends that microbes from the three domains

achieved the transition to modern cells separately, with Bacteria crossing the “Darwinian threshold” first, followed by Archaea and finally microbial Eukarya. But if this is so, why couldn’t Woese’s communitarian “progenote” have produced novel forms of microbial life, differing at the molecular level in some of the ways discussed earlier, that crossed the Darwinian threshold independently of the Bacteria, Archaea, or unicellular Eukarya? Finally, nothing in Woese’s account rules out molecularly distinct progenotes arising in different environments on the early Earth, particularly if geographical isolation occurred. In other words, Woese’s account of the origin of modern microbes is consistent with the emergence of alternative forms of microbial life differing in some of their basic molecular building blocks from familiar life.

4.3 Objections based on Darwinian evolution:

It might be maintained that once the ancestors of our highly successful form of life crossed the Darwinian threshold—either in three waves, as Woese speculates, or in one fell swoop—no other form of life could withstand competition with them. Much of the plausibility of this argument undoubtedly derives from the manifest ubiquity and diversity of familiar microbial life, coupled with the fact that we haven’t discovered any alternative form of life.

This argument is undermined, however, by what microbiologists have learned in recent years about the structure and dynamics of microbial communities. Microbial communities are highly organized, well integrated systems that modify their environments, both chemically and physically, in significant ways, and moreover maintain these modifications, creating an extensive collection of fairly stable ecological

niches that wouldn't otherwise exist. The phylogenetic diversity of microbial communities is staggering; they do not at all resemble pure laboratory cultures. Some varieties of microbe are represented in huge numbers whereas others are represented in very small numbers. The number of microbes occupying a given niche reflects its "size," i.e., the pertinent physical and chemical resources made available by the community. Being a rare microbe is not an evolutionary disadvantage because rare microbes occupy different ecological niches than common microbes, producing or utilizing material that is utilized, produced or ignored by other varieties. Indeed, recent studies suggest that rare microbes supply the bulk of the phylogenetic diversity of many microbial communities (Baker et al. 2006; Sogin et al. 2006). Depending upon its nature, environmental degradation of an ecosystem may have a more deleterious effect on a common variety of microbe than on a rare variety.

Importantly for our purposes, many microbial communities are extremely diverse, containing representatives from all three domains of life, as well as viruses. Admittedly alternative forms of microbial life couldn't "swap" genes with familiar microbes. But even familiar microbes within the same domain vary in the degree to which they participate in LGT; some bacteria participate little if at all (Dykhuizen & Baranton, 2001). There is thus little reason to suppose that microbial descendants of an alternative origin of life couldn't participate with familiar microbes in a microbial community even supposing that they were present in small numbers and couldn't exchange genes with most members of the community.

But even supposing for the sake of argument that it couldn't survive in environments swarming with familiar microbes, an alternative form of microbial life

might have survived by (in essence) “removing” itself from deleterious interactions with familiar microbes. They might have become adapted to environments (e.g., extremely dry deserts, deep subsurface environments) that are less hospitable to familiar microbes, perhaps participating in microbial communities in which they are the only or at least the dominant form of life. Alternatively, those forms of microbial life that differed the most from our ancestors would have had an evolutionary edge. Finding them more difficult to metabolize, and hence a poorer source of nutrition, our ancestors might have left them alone. They might not only have survived but evolved their own parallel, interdependent microbial eco-systems of predator-prey relations, a shadow biosphere having little to do with our own but nevertheless co-existing along side it.

In this context another commonly held assumption needs airing, namely, the belief that there are no primitive forms of microbial life on Earth today. As above, a Darwinian argument might be mounted: Our form of life would have voraciously ingested any remnants of the origin of life, driving them rapidly to extinction. But this argument is suspect for the same reasons as the previous argument. Primitive forms of life might have been winnowed by the process of natural selection to those differing the most from familiar life in their basic molecular building blocks or banished by natural selection to places that are inhospitable to familiar life. Furthermore, as several authors (Shapiro (1986), Wächterschäuser (1992), Benner et al. (2004)) have suggested, one cannot eliminate the possibility that primitive life is still emerging on Earth. And this brings us to my central point thus far: Our theoretical understanding of the origin and evolution of microbial life isn’t good enough to settle the question of whether the Earth is currently inhabited by an alternative form of life, primitive or otherwise. For every

argument purporting to establish that such organisms couldn't exist, there is a surprisingly plausible argument suggesting that they could exist.

4.4 Empirical objection: If they exist, why haven't we found them?

The most scientifically compelling objection to shadow microbes—heretofore undetected alternative forms of microbial life—is probably empirical: If they existed we would have discovered them by now or at least encountered telling signs of them using the sophisticated tools available to contemporary microbiologists. The problem with this objection is that it ignores the difficulties involved in discriminating between shadow microbes and familiar microbes. Detecting salient differences among microbes requires sophisticated instrumentation. The most commonly used tools for identifying novel microbes in environmental samples are: (1) microscopy, (2) cultivation, and (3) molecular biology techniques such as PCR amplification of rRNA genes. As originally discussed in Cleland and Copley (2005) and expanded upon below, these tools couldn't detect microbes that differed even modestly from familiar life in their basic molecular composition or core molecular architecture.

The utility of microscopy for identifying novel microbes is exceedingly limited. Molecular biology has taught us that superficial similarities in gross cellular morphology can hide crucial differences at the molecular level. The Archaea provide a particularly salient example. Because they have the prokaryotic cell plan, they look similar to Bacteria under a microscope, and both look quite different from unicellular Eukarya. Yet, as mentioned earlier, Archaea differ genetically and biochemically from Bacteria more than they differ from Eukarya. Analogously, shadow microbes with a prokaryotic

cell plan couldn't be discriminated from Archaea and Bacteria under a microscope. This is not a minor concern. As is well known, evolutionary pressures sometimes produce similar adaptations from different biological building blocks. A good example is the wings of insects, birds, and bats, which do not share a common ancestor with wings. Just as conditions on the later Earth favored the independent development of wings in insects, birds and bats so the prokaryotic cell structure of Archaea and Bacteria may represent independent adaptations to conditions on the early Earth, which could explain its somewhat surprising lack of phylogenetic significance. In other words, one cannot, as is sometimes supposed, conclude that every microbe having the prokaryotic cell morphology is either an Archaeon or a Bacterium. It is possible that we have already viewed shadow microbes under a microscope but, being misled by their gross cellular morphology, failed to recognize what they represent!

Our ability to visualize microbes under a microscope has been extended in recent years by the development of specialized molecular staining techniques for viewing specific cellular components, including, most importantly, biomolecules such as nucleic acids and lipids. These stains are unlikely to be very useful in discriminating morphologically similar shadow microbes from familiar microbes, however. As an example, DAPI (4',6-dimidino-2-phenylindole), which is commonly used for detecting microbes in environmental samples, intercalates into double stranded DNA in living cells, causing it to fluoresce. But DAPI would probably stain modest molecular variants on familiar DNA, e.g., DNA utilizing different bases to encode hereditary information. Lipid stains are designed to detect the presence of lipid membranes but the presence of a lipid membrane tells us nothing about proteins and nucleic acids. Lastly, hybridization

techniques such as FISH (fluorescence *in situ* hybridization) cause specific regions of chromosomes within intact cells to fluoresce by hybridizing them with a fluorescently labeled, complementary (oligonucleotide) probe. The utility of FISH is thus limited to cells whose DNA base sequences are complementary to the probe being used. DNA consisting of a different sequence of bases or, for that matter, bases that are not utilized by familiar life would not hybridize with the probe, and hence would not fluoresce under a microscope. In other words, even when enhanced by high-tech staining techniques such as DAPI and FISH, microscopy cannot eliminate the possibility that the present-day Earth is host to modestly different, let alone very different, forms of microbial life; these stains can only reveal cellular components having the “right” molecular composition.

Our most detailed knowledge of microbes has been achieved through cultivation, i.e., growing many copies of a single microbe in a laboratory apparatus such as a petri dish. Cultivating a microbe yields large quantities of essentially identical microbes, providing biologists with enough material to perform extensive analyses of a microbe’s genetic, structural, and enzymatic composition. Our ability to cultivate microbes is still exceedingly limited. As mentioned earlier, it is estimated that less than 1% of what can be seen under a microscope has been cultivated. This is somewhat surprising when one considers that many of these microbes co-exist with microbes that have been cultivated; one would think that both would thrive under similar conditions.¹² On the other hand, many uncultivated microbes thrive under extreme (from our point of view) environmental conditions (pressures, temperatures, pH, etc.) and exploit a wide range of energy and nutrient resources. It is hardly surprising that the latter cannot be cultivated since it is difficult to fully identify let alone replicate these conditions in a laboratory setting. This

problem is exacerbated when one considers trying to cultivate shadow microbes, which are even more likely to require unanticipated growth conditions; shadow microbes co-existing with readily cultivated microbes under “normal” conditions would also face this problem since they are likely to have nonstandard nutritional requirements. Thus the fact that no colonies of chemically deviant microbes have been found growing in petri dishes does not count much against their existence.

Of the three tools, molecular biology techniques are the most powerful. Circumventing the need for isolating and cultivating microbes, these methods extract genetic material directly from the environment and propagate it. Gene sequences are then determined and compared to databased sequences, allowing microbiologists to infer the kinds of organisms present in the sample. Two basic strategies are used to isolate genetic sequences. The oldest is PCR (polymerase chain reaction), which amplifies single genes such as those encoding ribosomal RNAs. Metagenomic analysis is newer and more comprehensive. It augments PCR by allowing microbiologists to clone larger genomic sequences, including protein-coding genes, and analyze their functional properties. Not surprisingly, estimates of microbial diversity are based on genomic analyses.

There are two features of PCR that make it a very poor tool for detecting even a modestly different form of microbial life. PCR is typically used to target rRNA genes because, as Woese (et al. 1990) argued, ribosomes are found in all cellular organisms and their complexity and integration tends to suppress LGT; rRNA genes are thus a better source of information about microbial diversity than other, less conserved, genes. Insofar as it is used to amplify ribosomal genes, however, PCR couldn't detect a form of life that lacked ribosomes even supposing that it had DNA like ours.

The second problem with PCR is that it requires “primers,” small pieces of synthetic DNA to delimit the genomic segment to be copied; the primer initiates DNA replication of the target gene. As a consequence, PCR presupposes prior knowledge of a genomic sequence in order to amplify it. Universal primers have been developed for rRNA genes of all three domains of life, and they are frequently used in environmental assays. But it is unlikely that any of these primers would “recognize” rRNA genes from an alternative form of life, and the same goes for any other “alien” genes that one might try to amplify by means of PCR. Indeed, a recent metagenomic analysis indicates that standard PCR based surveys of microbial communities are missing large numbers of low-abundance, genetically divergent Archaeon microbes (Baker et al. 2006). The situation would of course be even worse for shadow microbes. Even supposing that they had ribosomes, their rRNA would be too different to be amplified by any of the “universal” primers currently in use. It is thus very unlikely that PCR analyses would detect shadow microbes.

Metagenomic analysis does not fare much better when it comes to identifying shadow microbes. Its trademark technique utilizes “vectors”—small units of DNA derived from bacteria that can be inserted into “model organisms,” typically *Escherichia coli*—to propagate fragments of DNA extracted directly from the environment. But even supposing that DNA from a shadow microbe could be combined with a vector, it is unlikely that the genetic machinery of the cell into which it is inserted could clone let alone express it. It follows that our failure to detect an alternative form of microbial life in environmental samples using molecular biology techniques does not provide a good reason for believing that they do not exist.

In summary, none of the tools currently used by microbiologists for identifying novel microbes in environmental samples could detect an alternative form of microbial life that differed even modestly from familiar life at the molecular level. Thus the fact that we haven't found any using such techniques does not count much against their existence.

4.5 Blinded by a paradigm?

Even so, someone might argue, we should have seen *some* sign of such organisms if they exist. For life invariably modifies its environment, converting energy and material into compounds for growth, maintenance, and repair, and releasing waste products. An alternative form of microbial life would be no different. It would leave traces (a.k.a. shadows) in its environment. But we haven't found any signs whatsoever of such organisms. Therefore they don't exist.

The first issue to keep in mind when evaluating this argument is the difficulty involved in discriminating traces produced by shadow microbes from those produced by familiar microbes. More importantly, however, the default assumption when faced with a perplexing, seemingly biological, trace is that it was produced either by familiar life or has a non-biological origin. The possibility that an unfamiliar form of life produced it is not seriously entertained. In other words, the belief that there is only one form of life on Earth today is part of the paradigm of contemporary biology.¹³ This assumption makes it unlikely that biologists would recognize traces of shadow microbes for what they represent even if they encountered them.

The history of the discovery of the Archaea illustrates how a paradigm can blind

scientists to new possibilities. As discussed earlier, mid-twentieth century biologists divided all terran organisms into “prokaryote” or “eukaryote” on the basis of their gross cellular morphology. These categories were taken as fundamental—as reflecting crucial transitions or saltations in the evolution of terran life. On the prokaryote-eukaryote paradigm Archaea aren’t distinguishable from Bacteria since they both share the prokaryotic cell plan. In hindsight, however, it is clear that there were telling signs that the prokaryotes did not constitute a natural phylogenetic category. But because microbiologists were working under the prokaryote-eukaryote paradigm these signs went unrecognized for what they represented. What are now understood to be important chemical differences in the membranes of Archaea and Bacteria were interpreted as mere adaptations of “bacteria” to extreme environments. As a late 1970s textbook on thermophilic bacteria counsels the reader:

“The fact that *Sulfolobus* and *Thermoplasma* have similar lipids is of interest, but almost certainly this can be explained by convergent evolution. This hypothesis is strengthened by the fact that *Halobacterium*, another quite different organism, also has lipids similar to the acidophilic thermophiles” (Brock 1978, p. 178)
[Author’s note: all three of these microbes are Archaea!]

With the mid-twentieth century development of molecular methods for isolating and sequencing nucleic acids, the prokaryote-eukaryote paradigm began to break down. As the database of ribosomal RNA sequences grew increasingly large, it became clear that some prokaryotes cluster together phylogenetically to the exclusion not only of eukaryotes but of other prokaryotes as well, placing the theoretical utility of the prokaryote-eukaryote dichotomy in jeopardy. As we now know, Archaea translate DNA

into proteins utilizing machinery that is more similar to that of Eukarya than Bacteria and the chemistry of their cell walls is strikingly different from both Bacteria and Eukarya. The discovery that Archaea are so different from Bacteria revolutionized biological taxonomy, splitting the old category “Bacteria” in two, and replacing the traditional five “kingdoms” of life—Animalia, Plantae, Bacteria, Fungi, and Protocista—with three “domains” of life, namely, Archaea, Bacteria (originally known as Eubacteria), and Eukarya (which incorporates animals, plants, fungi, and protists) (Woese 1998, 2004).¹⁴

Just as earlier microbiologists encountered telling traces of a new kind of microorganism, Archaea, but failed to recognize their significance because of the paradigm under which they were working, so it is possible that contemporary microbiologists have already encountered telling traces of shadow microbes and failed to recognize their significance because of the assumption that there is only one form of life on Earth today. And this brings us to a serious problem, namely, determining when atypical traces are a sign of something new and important.

As Thomas Kuhn (1970) emphasized, anomalies are the driving force behind scientific revolutions. Kuhn believed, however, that evidence could not be seen as anomalous within the framework of a well entrenched scientific paradigm. It is beyond the scope of this paper to explore this important issue. But even supposing for the sake of argument that Kuhn is right, which I doubt, the belief that there is only one form of life on Earth is not a central part of the contemporary biological paradigm; for as I have argued, we know of ways in which life could have been at least modestly different at the molecular level, and the arguments from Darwinian evolution and lateral gene transfer do not hold up well under careful scrutiny. Even more importantly, however, there are some

good candidates for traces of what just might turn out to be an alternative form of terran microbial life.

Desert varnish, a mysterious hard dark coating found on rock in arid regions, provides perhaps the best candidate. There is no scientific consensus on how it is produced despite the fact that geologists have extensively studied it; even Darwin found it puzzling (Darwin 1871, pp. 12-13). Consisting of extremely thin chemical and mineralogical layers, it bears an uncanny morphological resemblance to stromatolites, primitive microbial mats that still exist on Earth today, the fossilized remains of which date back at least 3.5 billion years. Even more provocatively, varnish coatings are enriched in manganese and iron despite the fact that the rocks on which they are found are not. To many geologists this intriguing combination of microstructural and chemical features suggests a microbial origin, particularly since bacteria and algae commonly produce manganese or iron as by-products of metabolism. Yet microbes are infrequently found on varnish surfaces, as they are on living stromatolites, and PCR analysis of 16S rRNA genes extracted from coatings and surrounding rocks and soils vary from region to region, suggesting that no one group of bacteria, let alone single variety, is responsible for the coatings (Perry et al. 2002). Furthermore, attempts to produce varnish-like coatings in laboratory settings using bacteria and algae have been unsuccessful. The question of whether desert varnish has a biological origin is thus still hotly debated.¹⁵

Desert varnish isn't the only example of a puzzling natural phenomenon that resists classification as "biological" or "nonbiological." Some are so outlandish that they are pretty much dismissed out of hand, e.g., Philliipa Uwins's "nanobes" (Uwins et al. 1998) and the red rain of Kerala, India (Louis & Kumar 2005), whereas others are so

modest that the suggestion that they might represent something new and important is rarely taken seriously, e.g., the inability of microbiologists to cultivate microbes co-existing under moderate conditions with microbes that are easy to cultivate. Phenomena of both kinds are potential candidates for what may someday be regarded in hindsight as telling signs of an alternative form of microbial life. They are thus well worth exploring with this possibility in mind.

5. Conclusion

Is it very likely that an alternative form of microbial life exists on Earth today? In a recent paper, Davies and Lineweaver (2005) estimate the probability of an alternative origin of terran life to be 90%, and moreover contend that descendents of such an origin are likely to have survived to the present day. Unfortunately their argument glosses over important chemical and biological details, and relies upon extremely speculative quantitative reasoning such as the assignment of a 50% probability for the emergence of life on Earth over a 100 million year period. We know far too little about the chemical and physical processes responsible for the emergence of life, not to mention conditions on the early Earth, to confidently assign a numerical value to probabilities of this sort (Cleland and Copley 2005).

On the other hand, as I have argued, we also know that the existence of such microbes is compatible with our current biological and chemical understanding of the origin and nature of terran life. So even if we can't in good conscience assign a numerical value to their probability, they are still well worth taking seriously. Furthermore, our failure to discover such microbes can't be construed as evidence that

they don't exist because the tools currently used to investigate the microbial world couldn't detect them if they did exist. Similarly, theoretical arguments to the effect that they couldn't survive in environments swarming with familiar microbes are undermined by what we know of the processes involved in microbial evolution and the complexity and diversity of microbial communities. This doesn't mean of course that shadow microbes exist or for that matter ever existed. The point is only that the objections commonly raised against them do not bear up well under close scrutiny.

The discovery of a shadow terran biosphere would be profoundly important, both scientifically and philosophically. It is clear that life as we know it on Earth today has a common origin, and hence represents a single example of life. Logically speaking, one cannot generalize on the basis of a single example. If we are ever to achieve a satisfactory understanding of the nature of life we need examples of unfamiliar forms of life. Although there are good theoretical reasons for believing that life could be at least modestly different in its basic molecular building blocks and core molecular architecture, we don't know how different it could be. It is thus important that we do not prematurely constrain our thinking about life on the basis of a limited and possibly very misleading sample. Given that a shadow biosphere cannot be ruled out either on theoretical or empirical grounds and the profound importance that such a discovery would represent, a dedicated search for such microbes seems in order. The best place to start is with puzzling phenomena, such as desert varnish, that resist classification as "biological" or "nonbiological."¹⁶

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¹ There are exceptions, of course, such as Freeman Dyson's "double-origin theory" (1985), but they have not been as popular among scientists.

² For a remarkably thorough survey of definitions of 'life', see Popa (2004 Appendix B), and for a discussion of problems with not only particular definitions but the whole project of defining 'life', see Cleland and Chyba (2000, 2007)

³ Some modified amino acids, e.g., seleno-cysteine, are found in proteins. Also, microbes produce a variety of nonstandard amino acids that are utilized for purposes other than protein synthesis.

⁴ Shelley Copley and coworkers (2005) have shown that at least one of the associations may not be as arbitrary as once thought, however.

⁵ As John Wilkins has pointed out to me, we almost have this already in cases where methyl groups pair up with existing RNA bases, creating modified bases.

⁶ This point is sometimes glossed over by advocates of panspermia, some of whom assume that if life originated outside the solar system it must have always existed or alternatively be the product of a celestial event so improbable that it will never occur again; see Kamminga (1982) for a discussion of the former and Fry (1995) for a discussion of the latter.

⁷ Texts in microbiology sometimes include microscopic invertebrates under the rubric "microbe" in their introductions; they do not, however, discuss them in any detail, leaving that task to texts on invertebrates. Part of the reason that microscopic invertebrates are sometimes classified as microbes is that (even in biology) the term "microbe" is more of a common term than a technical term; originally denoting organisms that couldn't be seen without the aid of a microscope, it became associated with unicellular organisms when most microscopic organisms turned out to be unicellular, and was then extended to large unicellular organisms. The other reason for including microscopic invertebrates as microbes is that they participate with unicellular microbes and acellular viruses (which are discussed at length in texts on microbiology) in microbial communities. There just doesn't seem to be a good rationale for excluding them and including viruses unless one wants to explicitly define "microbe" as "unicellular organism," which would of course render the extensive discussions of viruses in textbooks on microbiology inappropriate.

⁸ Norman Pace (2006) contends that the distinction is so misleading that the term "prokaryote" should be expunged from the vocabulary of biology. Indeed, as discussed in Section 4.5 of this paper, it hindered the recognition that Archaea are fundamentally different from (Eu)bacteria!

⁹ Plasmids are not the only mobile acellular genetic units affiliated with cells, but they are particularly good candidates for purposes of this discussion.

¹⁰ I might add, however, that they strike me as providing better candidates than certain computer simulations, e.g., Thomas Ray's digital organisms (Ray 1994), which have been touted as instances of life.

¹¹ Unlike O'Malley and Dupre, however, I do not believe that pluralism is inevitable. Our inability to settle on a convincing ontology is more likely a result of an inadequate theory of life. A truly satisfactory theory of life, which we currently lack (for many reasons, not the least of which is an inadequate sample of life), is likely to unveil an ontology of natural kinds that is significantly more fruitful for explanatory and predictive purposes than the others.

¹² NASA microbiologist Chris McKay made this point to me at a recent meeting.

¹³ The term "paradigm" is, of course, associated with the work of Thomas Kuhn (1970). While Kuhn's use of the term has been justifiably criticized as ambiguous, the intuitive idea of a paradigm nevertheless contains an important insight that is pertinent to our concerns in this paper. Paradigms (qua holistic units of scientific commitment) amount to more than reigning theories. They include accepted methods, standards for solutions, and subsidiary assumptions about a subject matter. As such, paradigms provide invaluable tools for scientific research, guiding the construction of hypotheses, design of experiments, and interpretation of results. But they may also hinder the exploration of nature, blinding us to important possibilities by discouraging certain avenues of investigation and biasing the

ways in which data are interpreted. As a consequence, important scientific discoveries and the theoretical advances that wait upon them may be delayed for years.

¹⁴ Although microbiologists still employ the term “kingdom,” imposing the 3 domains as a higher order taxonomic structure upon them, it is important to appreciate that the meaning of the term has changed very radically. There are no longer just five “kingdoms” and some of the old “kingdoms” have been subdivided and separated. More specifically, the old kingdom “Bacteria” has been subdivided into many kingdoms, and they are organized differently, with some coming under the domain “(Eu)Bacteria” and others coming under the domain “Archaea”; see Doolittle (1999) for a discussion.

¹⁵ The most recent contribution to this debate (Perry et al. 2006) postulates a very complex series of inorganic chemical reactions but still can’t explain the presence of so much iron and manganese in environments that otherwise contain little.

¹⁶ Acknowledgments: I would like to thank Maureen O’Malley, John Wilkins, and Daniel Frank for helpful comments on an earlier version of this paper. I am also grateful to Norm Pace for helpful discussions on many of the issues addressed in this paper; I hasten to add, however, that my thanking him should not be taken as indicating that he agrees with what I say, and I’m sure that my provisional use of the term “prokaryote” early on in the paper is bound to offend him! This work was supported in part by a NASA grant to the University of Colorado’s Astrobiology Center.