Searching for an Alternative Form of Life on Earth

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Abstract: Biologists tacitly assume that all life on Earth descended from a common origin. This assumption is based on biochemical similarities and gene sequencing, which enables organisms to be positioned on a common tree of life. However, most terrestrial organisms are microbes, and it is impossible to deduce their biochemical nature from morphology alone. The vast majority of microbes remain unclassified, leaving open the possibility that some of them might be an alternative form of life, arising either from an independent origin, or representing a hitherto overlooked very ancient branch of the known tree. Thus there may exist an extinct, or even extant, shadow biosphere. I discuss various research proposals for locating and identifying "alien" organisms on Earth, both ecologically separate and ecologically integrated.

Keywords: biogenesis, arsenate, shadow biosphere, impact reseeding

1. Multiple biogenesis events

The origin of life is one of the great unsolved problems of science. Nobody knows how, where or when life originated. About the only certainty is that microbial life had established itself on Earth by roughly 3.5 billion years ago. In the absence of hard evidence of what came before, there is plenty of scope for disagreement. The simplest known autonomous organisms are already exceedingly complex. If they arose by random self-assembly of basic organic molecular building blocks then the transformation process would have had a vanishingly small probability and so was unlikely to have been repeated within the observed universe. This "chemical fluke" theory was the prevailing view among scientists a generation ago, and exemplified by Monod[1].

The opposite position is that life forms easily under earthlike conditions and is therefore widespread in the universe (given the high expectation of a large number of earthlike planets). It is a point of view called biological determinism by Robert Shapiro[2], and is sometimes expressed by saying that "life is written into the laws of nature." A strong proponent of biological determinism is de Duve[3], who describes life as "a cosmic imperative." It is a founding tenet of the astrobiology program, and has gained considerable popularity in recent years.

There is thus a vast spectrum of opinion, from the conservative view that life's origin was a freak event to the claim that life emerges more or less automatically under earthlike conditions. How can this spectrum be narrowed? The most direct way is to seek evidence for life on another planet, such as Mars. If life originated from scratch on two planets in a

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single planetary system, it would decisively confirm biological determinism. Unfortunately there is a complication. The bombardment of Mars and Earth by comets and asteroids has resulted in large amounts of ejected material from Mars falling on Earth, and a lesser, but still significant, quantity going the other. It seems very likely that at least some microbial life will have hitched a ride in rocks traded between the two planets, resulting in natural cross-contamination[4]. If the biospheres of Earth and Mars have become intermingled in this way, it will complicate any attempt to demonstrate that life has started independently on both planets. In any case, it may be a long time before Mars missions are sophisticated enough to study putative Mars biota at that level of detail.

An easier test of biological determinism may be possible, however. No planet is more earthlike than Earth itself, so if life does emerge readily under terrestrial conditions then perhaps it formed many times over on our home planet[5,6]. The orthodox view is that if life on Earth originated more than once, then one form would come to predominate and eliminate the others, for example, by appropriating all the resources, or genetically out-competing. A key part of this argument is that genes are regularly transferred between organisms, especially micro-organisms, so that successful traits acquired by one organism can spread through the biosphere. However, two very different domains of micro-organism, bacteria and archaea, have peacefully co-existed for billions of years without one domain eliminating the other. Moreover, this mutual success has taken place in spite of the fact that the genes for a very successful trait, namely methanogenesis, seem not to have been exchanged with bacteria. Methanogenesis is widespread among archaea, from deep-sea vents to the human gut, and is therefore presumably a basic property, yet it has not spread to bacteria or eucarya. An additional point is that alternative forms of life may occupy non-overlapping environments, or require different resources, and so would in that case not directly compete anyway. These deliberations raise the fascinating question of whether there may be traces of a second or subsequent genesis. If there are, what might we look for?

One possibility is that alternative forms of life flourished on Earth in the remote past, but have not survived to the present day. For example, the epoch prior to about 3.8 billion years ago was marked by fierce bombardment from space. Some impacts may have been severe enough to temporarily sterilize the entire planet from the heat released[7]. This may have provided an opportunity for life to begin again. Would the annihilated life forms have left any markers? Evidence for life comes both directly, in the form of fossils, and indirectly, from chemical alterations of the planet, the classic example being the build up of oxygen in the atmosphere from the effects of photosynthesis. Unfortunately, the Earth's surface has been so thoroughly reprocessed by tectonic activity it is likely that all traces of very ancient life have been obliterated. There is a faint chance that we may one day discover very ancient terrestrial rocks on the moon, bearing the imprint of an earlier form of life, but this remains a distant prospect.

A more likely scenario is that material flung into solar orbit by these huge impacts could have found its way back to Earth millions of years later, and any micro-organisms cocooned therein that remained viable would have re-seeding our planet with earlier life forms[8,9]. There would thus be old and new forms of life present on Earth at once. Even if all but one form of life eventually died out, there might still be markers of extinct biology in the geological record. If alternative life had a distinctively different metabolism, it may have altered rocks or created mineral deposits in a way that cannot be explained by the activity of known life. There may even be "alien" bio-markers in ancient micro-fossils.

A more exciting, but also more speculative, possibility is that alternative forms of life have survived to the present day and are extant in the environment, constituting a sort of shadow biosphere. At first sight this idea seems preposterous. Surely scientists would have discovered it already? It turns out that the answer is no. The vast majority of organisms are microbes, and it is almost impossible to tell simply by looking what they are. Only a tiny fraction of observed microbial life has been characterized by microbiologists, for example, using gene sequencing. It is very likely that all life so far studied descended from a common origin. Known organisms share a similar biochemistry and use an almost identical genetic code, which is why biologists can sequence their genes and position them on a single tree of life. But there is an obvious circularity here. Organisms are analyzed using chemical probes carefully customized to life as we know it. These techniques may well fail to respond meaningfully to a different biochemistry. If shadow life is confined to the microbial realm, it is entirely possible that it has been overlooked. I shall assume that shadow life is at least carbon-based, while leaving open the possibility that one or more of the remaining key elements H, N, O, P, S may be substituted for others.

2. Ecologically separate shadow biosphere

There may be some terrestrial environments in which conditions are too harsh for regular life to survive, but nevertheless a more resilient shadow life thrives there. It therefore makes sense to extend the search for life in extreme environments to even more hostile locations than those studied hitherto. The question then is how shadow life might betray its presence in such locations. This question was confronted many years ago in the context of the Viking missions to search for an unknown form of life on Mars.

Signatures of life divide into two broad categories: structure and function. Structural evidence for life can take many forms – for example, mineral alteration (chemical concentrations, fossils, stromatolite-like structures). Extant life may be most readily identified by the appearance of a pattern in the distribution of organic molecules away from equilibrium[10]. A crucial proviso is that shadow life and regular life produce distinctively different patterns, otherwise the products of shadow life might be indistinguishable from contamination by regular life. Note that we need to be sure not only that regular life does not mimic the distribution of shadow organics, but that none of the breakdown organic products of regular life confuse the signal either. Even if shadow life occupies a separate ecological niche from regular life, the organic detritus from the latter could still invade the former as a result of water or wind transportation. If shadow life is present at very low levels, its organic signature could be swamped by the breakdown products of much more abundant regular life, creating a major signal-to-noise problem. Conversely, we might detect trace products of shadow life that had diffused or been transported into the regular biosphere.

Evidence for biological function might come from signs of metabolism. Viking looked for signatures of carbon cycling (on the assumption that we would at least be dealing with carbon-based life)[11]. If shadow life is metabolizing, there should be a distinctive chemical disequilibrium and a throughput of matter and energy. It would therefore be worth designing a "mission to planet Earth," involving a "super-Viking" suite of experiments. In spite of their post-Viking sophistication, these experiments will be far cheaper than their Viking counterparts because they do not have to be transported to another planet, nor do they need to be sterilized, since by assumption super-Viking will be sampling environments lethal to known life.

Interestingly, there are examples of profoundly isolated microbial ecosystems, although none of them is a candidate for shadow life. These systems are seemingly sustained without access to light or external organic material, or free oxygen. The primary energy source in two cases is H_2 released from rock reactions with water, which is then metabolized to form methane[12,13] :

$$H_2 + CO_2 \rightarrow CH_4 + H_2O$$

A third system derives its energy from radioactivity[14].

Regular life depends on a "habitable window" in several parameters, the most notable being temperature, pressure, salinity, pH, concentration of metals and other potentially toxic substances, and background radiation. There is thus a multi-dimensional parameter space the boundary of which delineates the outer reaches of known life. An ecologically separate shadow ecosystem could in principle occupy an overlapping region of parameter space, but simply be physically isolated from the known biosphere. However, known life seems to have invaded every available terrestrial niche in the permitted region of parameter space, so consider the possibility of alternative life occupying a disconnected region of parameter space. A clear signature of shadow life would be if regular life reached a limit as a particular parameter is varied, and then, after a gap, some additional evidence for life is found. For example, as the temperature is raised, it seems likely that regular life ceases at about 130C. If no life is found in the range 130 – 180C and then there is evidence for life at, say, 180 – 200C, it would constitute strong evidence for hyperthermophilic shadow life.

A systematic search strategy would also consider variations in two or more parameters simultaneously. We might, for instance, seek life at extremes of both acidity and temperature, or metalicity and radiation (e.g. in the tailings of uranium mines).

Environments contiguous with the known biosphere that nevertheless seem to have exceeded the outer limits for regular life include ultra-dry deserts, such as the core of the

Atacama, ice sheet plateaux at high latitudes, highly elevated mountain tops, the upper atmosphere, hot ocean vents above a maximum temperature and the subsurface of the Earth's crust below a maximum depth (perhaps 3 km). If carbon-based shadow life has taken up residence in one of these locations, we might at the very least expect to find carbon concentrated there. A more careful study may then show carbon cycling. Not all the suggested locations have yet been studied systematically. One that has is the Atacama desert[15], and so far at least, no evidence for abundant carbon or indigenous organic material has been found there. (Some organic material falls in from the atmosphere, but it is rapidly destroyed by the highly oxidizing desert soil.)

A complement to this approach is to create an artificial environment in which regular life cannot survive, introduce material from candidate shadow ecosystems, and then test for residual biological activity. This was in fact how the highly radiation resistant organism *Deinococcus radiodurans* was discovered, growing in a radiation "sterilized" environment.

3. Ecologically integrated shadow biosphere

By far the most exciting, yet speculative, possibility is that shadow life and regular life interpenetrate in both geographical and parameter space, and might to some extent compete for resources. If a fraction of microbial life "under our noses" (or beneath our microscopes) is life as we don't know it, how can the "alien" organisms be identified? The challenge is to find the signal in the face of the noise of the regular biosphere.

Again, a search could target either structure or function. An alternative biochemistry will use at least some distinctively different organic molecules. One strategy would be to identify potentially biologically useful molecules that are not incorporated in regular life or produced in its decay products, and seek evidence for them in the biosphere. Among the very large number of amino acids, known life uses a restricted subset of 21. Yet a broad class of amino acids forms readily in nature: the Murchison meteorite contains several dozen. Alpha-methyl amino acids, for example, are not used by any known organism. If they were found in conjunction with biological activity such as carbon cycling, they would be a strong indicator of alternative biochemistry. A good place to begin such a search is in oil deposits, which may preserve molecular remnants of an ancient shadow biosphere.

An indirect sign of a shadow biosphere might come from the discovery of viral parasites in the general environment that are clearly maladapted to known life. Because viruses are easily transported, they might be indicative of either ecologically separated or integrated shadow biospheres. A shotgun analysis of seawater, which is teeming with vast numbers of different viruses, might be an efficient way to proceed.

Another class of molecules that serve as a signature of known life is sugars. Because sugars are relatively simple and easy to form chemically, and because they play such a fundamental role in biochemistry, both structural (in DNA) and functional (in intercellular communication), we might expect them to be present in alternative life too. A systematic search for sugars in the environment that are not used by known life would also be worthwhile.

Mono-chirality is a universal feature of known life. Although there is no agreement on its origin, it is likely that the specific chiral signature of left-handed amino acids and right-handed sugars is a frozen accident. If life were to start over again, there would be a 50 per cent chance that the opposite chirality – "mirror life" – would emerge next time. The abiotic formation of amino acids tends to produce racemic mixtures, and organic remnants of living organisms racemize over time, so the mere presence of opposite chirality amino acids in the environment cannot be taken as evidence for mirror life. More convincing would be the discovery of mirror versions of steranes, which have no known abiotic origin. Attempts to culture micro-organisms in "anti-soup" – a nutrient broth containing D amino acids and L sugars – has the potential to act as a filter for known life, and thus to identify rare examples of mirror life buried in a mixture or regular micro-organisms. However, the procedure is complicated by the existence of organisms that can metabolize opposite chirality molecules[16].

A possible alternative biochemistry is one that uses a different set of elements. For example, arsenic is chemically closely similar to phosphorus and was more abundant in soluble form in the anoxic conditions of the early Earth. We can conceive of life in which arsenic substitutes for phosphorus and plays a similar role: as an energy source (ATAs replacing ATP), as part of the backbone of DNA, and in arseno-lipid cellular membranes.[17] Although the oxygenation of the biosphere has made arsenate less favorable than phosphate for modern biochemistry, there remain niches that are arsenic-rich and phosphorus-poor. Known examples are deep ocean volcanic vents, Yellowstone hot springs and desert varnish. It is possible that an ancient biochemical system lives on in one or more of these niches. A targeted search currently in progress at Arizona State University is seeking organisms with arsenic systematically incorporated into key organic molecules. Another approach is to try to culture organisms from high-As environments and measure their metabolic or reproductive rate as a function of As concentration. If the rate declined as the As concentration was reduced to zero, it would be a strong indicator of As having a crucial biochemical function.

4. Biological anomalies: has alien life already been discovered?

It is conceivable that the presence of alternative microbial life has already been noted. A gram of soil typically contains a million different species of microbes, of which only a tiny fraction have been sequenced or even characterized. If a shadow biosphere is ecological integrated with the known biosphere, the respective member organisms will be intermingled, and it is unlikely that morphological differences alone would constitute a distinctive signature. Alien organisms would presumably resist attempts at standard culturing and gene sequencing, and for this reason may have been shrugged aside as "uncooperative." But any microbial species that fails to respond to standard biochemical techniques is a candidate for alternative life, and should be scrutinized for novel chemical content. A simple procedure is to use mass spectrometry to determine the amino acid inventory of "recalcitrant" organisms. Any departure from the regular set of 21 would suggest alien biochemistry.

Another way in which hypothetical alien organisms might announce their presence is if they are anomalous in some conspicuous property. Desert varnish has always been something of a mystery. This substance coats rock surfaces in many deserts. It has a high mineral content (e.g. manganese), but it also contains microbial communities. Opinions differ over whether the microbes create the varnish or whether the coating is a mineralogical and weathering phenomenon and the microbes have merely colonized it opportunistically[18].

Another possible biological anomaly is the alleged existence of organisms too small in physical size to be life as we know it[19-21]. Variously called nannobacteria or nanobes, these are cellular structures with dimensions typically no greater than a few hundred nanometers, which is too small to contain ribosomes. They have been reported in environments as diverse as oil wells and human blood, although it is far from clear that they are actually autonomous biological organisms. If they are in fact living, they would be good candidates for a radically alternative form of life, perhaps one in which proteins are made some other way, or not used at all.

5. When is a tree really a branch? Defining biogenesis

In the event that a new form of life is discovered, a major challenge will be to determine whether it is derived from a genuinely independent biogenesis, i.e. it represents a second tree of life, or whether it is simply a hitherto undiscovered side branch on the known tree of life. The more the new life differs biochemically from known life, the more plausible it is that we would be dealing with multiple genesis events. For example, it is very likely that the familiar triplet genetic code has evolved from a simpler and more primitive precursor, perhaps a doublet code. It is conceivable that some ancient micro-organisms have survived using the earlier doublet code. These would not be a genuinely new form of life; rather, they would be "living fossils" occupying a new, deep branch on the known tree of life, having bifurcated from the main branch before the establishment of the modern code (presumably before the split into bacteria, archaea and eucarya). On the other hand, the discovery of "mirror life" would be strongly indicative of an independent origin, because it is hard to imagine an earlier achiral form of life that split into left and right handed versions, as achiral molecules lack the necessary complexity to build living organisms.

A complicating factor is the possibility that a shadow biosphere might not only be ecologically, but also biochemically, integrated with the known biosphere. The power of evolutionary convergence might be strong enough for independently-originating life forms to “discover” the same biochemical solutions to some problems. Organisms on sufficiently convergent biochemical paths might reach the point where they are able to swap some genes. This would progressively obliterate the distinctive evolutionary pathways of the two forms, making their provenance hard to discern. This possibility is supported by the work of Freeland[22], which suggests that the modern genetic code is not a frozen accident, but a system that optimizes efficiency, suggesting that it has evolved over time under the action of selection pressure. If so, similar selection pressures on a shadow biosphere might lead to the evolution of a similar code – a necessary prerequisite for successful lateral gene transfer.

A deep issue of principle that underlies the entire discussion is the tacit assumption in what we have discussed so far that the origin of life was a discrete and readily-identifiable event, similar to a phase transition in physics. Biogenesis might come about, for example, when a system of autocatalytic chemical cycles crosses a certain threshold of complexity.[23] It is then clear what one means by the term “second genesis.” But a case can be made that there is no clear dividing line between non-life and life, between complex chemistry and biochemistry. If so, the distinction between a tree and a branch becomes indistinct too.

If the pathway from non-life to life is in fact extended, the possibility arises that we may discover intermediate forms that fall short of the complete autonomy of living cells, but nevertheless are players in the overall life story. It is possible that prions and viruses are members of this class, representing hangovers from an early protein or RNA world from which regular life subsequently evolved. It is then a moot point as to whether these entities represent an alternative biology, a deep branch on the known tree of life, or do not count as life at all. It is conceivable that nanobes fall into the class of “missing links” – forms intermediate between the realm of chemical complexity and full autonomous organisms such as bacteria. A shadow nanobe biosphere might be sustained into the present epoch if it was sufficiently biochemically integrated with the known biosphere, in the same way that the viral biosphere is a parasitic outlier of the known biosphere.

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