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What could arsenic bacteria teach us about life?

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Abstract In this paper, I discuss the recent discovery of alleged arsenic bacteria in Mono Lake, California, and the ensuing debate in the scientific community about the validity and significance of these results. By situating this case in the broader context of projects that search for anomalous life forms, I examine the methodology and upshots of challenging biochemical constraints on living things. I distinguish between a narrower and a broader sense in which we might challenge or change our knowledge of life as the result of such a project, and discuss two different kinds of projects that differ in their potential to overhaul our knowledge of life. I argue that the arsenic bacteria case, while potentially illuminating, is the kind of constraint-challenging project that could not—in spite of what was said when it was presented to the public—change our knowledge of life in the deeper sense.

Keywords Arsenic bacteria · Life · Weird life · Biochemical constraints · Origin of life

Introduction

In December 2010 NASA announced the discovery of bacteria that could substitute arsenic for phosphorus in their DNA and proteins. This was met with lively and largely negative commentary, on the web and in a series of followup papers published in Science. Critics took issue with the researchers' experimental protocol and interpretation of the data, and questioned the overall claims about the significance of these results. Regardless of the outcome of ongoing debate on the credibility of these particular results and the possibility of arsenic bacteria in

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general, the logic of this case is interesting and merits further attention.[1] In addition to being interesting from a sociology of science perspective, this case brings to the forefront important questions about the nature and extent of biological constraints. In particular, it presents a compelling opportunity to examine what we could really learn from looking for anomalous life forms that violate certain kinds of constraints on biochemistry.

The relevant debate about constraints in this case traces from a 1987 paper by Frank Westheimer entitled "Why Nature Chose Phosphates," to Felisa Wolfe-Simon and colleagues' 2009 paper entitled "Did Nature Also Choose Arsenic?" and their ensuing experiments on the alleged arsenic bacteria. This paper responds to that debate, using the 2010 experimental results and the surrounding claims as a starting point to examine how we could possibly extend our understanding of life by searching in the world for weird instances of it.

Arsenic bacteria: the story and the skepticism

The arsenic bacteria announcement was accompanied by significant media hype, with headlines claiming that the discovery "may redefine 'life'" (The Daily News, 2 Dec. 2010). NASA opened their press release with the claim that "NASA-funded astrobiology research has changed the fundamental knowledge about what comprises all known life on Earth," and quoted a NASA official as saying "the definition of life [had] just expanded" as a result of this discovery (Brown and Weselby 2010). In the following discussion, I examine more closely what these sorts of claims mean in the context of discovering life forms with fundamentally different biochemistry, and argue that NASA and others were entitled to these claims only in a particular and qualified sense.

The discovery of arsenic bacteria was not random; it represents a significant step in a decades-old debate about basic constraints on biochemistry. The background for this debate begins with Frank H. Westheimer's 1987 paper in Science, "Why Nature Chose Phosphates." Phosphorus is one of the six most important biological elements, along with carbon, hydrogen, nitrogen, oxygen, and sulfur. A phosphorus atom binds with four oxygen atoms to form a phosphate molecule (Fig. 1); these molecules play a crucial role in the biochemistry of all known life. Phosphates form the core structure of DNA and RNA, adenosine triphosphate (ATP) and other molecules involved in metabolism, and the phospholipid layers that make up all living cells' walls. Westheimer bases his argument for why nature chose phosphates on two main points. First, living things need to keep their metabolites inside their cell membranes. Negatively charged molecules are insoluble in lipids, and thus are ideal for staying inside of cell membranes. Phosphates are negatively charged; but so are many different kinds of potential structural molecules, so this first point alone is not sufficient to explain why nature chose phosphates. The second point has to do

[1] Since this paper was accepted, two new studies have been published indicating that these bacteria (1) are arsenate-resistant but nevertheless grow only on phosphate, not arsenate, and (2) do not, as claimed in the original paper, incorporate arsenate into their DNA (Erb et al. 2012; Reaves et al. 2012).

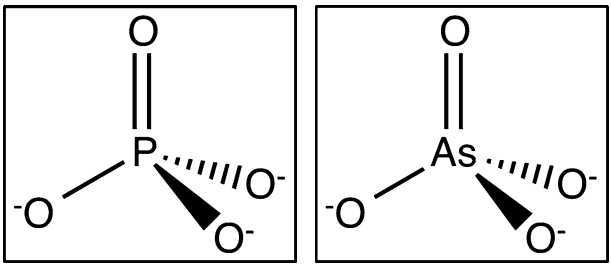

Fig. 1 Phosphate and arsenate

with molecular stability. Biomolecules, in addition to staying in a membrane, need to be stable in water for a long time. This is less important for metabolites, which living bodies make use of in a fleeting way, but much more important for molecules of genetic material. DNA needs to be stable enough to maintain its structure for long periods of time. Phosphates hydrolyze (break down in water) extremely slowly. Thus, Westheimer argues, they are the ideal candidates for optimally meeting both conditions: stability within a cell wall and longevity.

Westheimer considered the possibility that nature could have chosen alternative molecules, such as arsenate, to play this crucial structural role in biochemistry. Phosphorus and arsenic are chemical analogs, occupying the second and third positions in the nitrogen group of the periodic table, and an arsenic atom bound with four oxygen atoms forms arsenate, a molecular analog to phosphate (Fig. 1). So, why didn't nature choose arsenate, instead of or in addition to phosphate? Westheimer gives two reasons: First, arsenic is toxic. Precisely because of its close molecular similarity to phosphate, arsenate is easily taken up by living cells, where it damages their metabolism by blocking ATP production.[2] Second and more crucially, arsenate bonds are unstable. Biomolecules based on arsenate will break down much more quickly than those based on phosphate; Fekry et al. (2011) cite the half-life in water of the phosphate bonds in known DNA as approximately 30 million years, and estimate that the corresponding bonds in hypothetical arsenate-based DNA would have half-lives of less than one second. This is a problem, again, because organisms need certain key biomolecules like nucleic acids to stick around for a long time.

Westheimer's paper, which had over 550 citations when this paper was written, was an influential contribution to biochemical theory, explaining why phosphates are the (only) ideal molecules to play the role they do in living organisms. Along with other properties such as being carbon-based and having DNA or RNA, phosphate-based biochemistry has been regarded as a fundamental constraint on living

[2] While some microbes are known to be resistant to arsenic, or have developed mechanisms for coping with high levels of it, it is generally regarded as toxic to living organisms (Hughes 2002; Knowles and Benson 1983). Westheimer briefly mentions arsenic toxicity as a problem, and then moves on to focus on the bigger problem of molecular instability. It is worth noting that the point about toxicity is based on studies of arsenic response in organisms with phosphate-based biochemistry, i.e., the only organisms we know. A hypothetical organism with arsenate-based biochemistry would arguably not have the same problem with toxicity. In any case, even if toxicity were not an issue, molecular instability is still a serious problem.

organisms. In 2009, however, a team of astrobiology researchers proposed a theoretical challenge to this constraint. In their paper "Did Nature Also Choose Arsenic?," Wolfe-Simon and colleagues argue that hypothetical organisms equipped to overcome the toxicity and instability problems associated with arsenate could substitute arsenate for phosphate in their biomolecules. They defend this claim by analogy with known cases where similar elements can sometimes substitute for each other in biochemical reactions. This is possible because elements in the same group on the periodic table share properties relevant to their ability to interact with other molecules; one example is the known substitution of selenium for sulfur in certain amino acids, in spite of the former's lower stability (Rosen et al. 2011).

Westheimer argued that arsenate's instability in water made it a bad candidate for forming the structural basis of biomolecules like DNA and RNA. Wolfe-Simon and colleagues respond that this is not a decisive objection to the possibility of arsenate-based life. They postulate that an organism living in an environment with high enough arsenic concentration could replenish its arsenate supply instantaneously as biomolecules broke down, thus overcoming the instability problem. This supply of arsenate molecules could potentially come from the surfaces of arsenic-rich sulfide minerals, which are characteristic of environments like deep-sea hydrothermal vents (Wolfe-Simon et al. 2009, 71).

They also argue that the possibility of nature having chosen arsenate could shed light on our understanding of the origin of life. It is a well-regarded hypothesis that life began in "extreme" and arsenic-rich environments like hydrothermal vents. The authors propose that arsenate-based life could have gotten going in such environments and later evolved phosphate-based biochemistry once it spread to Earth's surface, where phosphorus is 10,000 times more abundant than arsenic. In fact, they argue, the higher reactivity of arsenate- as opposed to phosphate-based macromolecules might have been an asset in prebiotic systems or ancestral organisms. This is because less sophisticated, more primitive molecular machinery would be sufficient to get biochemical reactions going.

This was Wolfe-Simon and colleagues' theoretical challenge to Westheimer's argument justifying the ubiquity of phosphates in biochemistry. The following year, the same team produced organisms which they claimed realized this theoretical possibility. These were the so-called arsenic bacteria, discovered in Mono Lake, California, which is known for its high arsenic content. The researchers isolated the bacteria from the lake, took them back to their laboratory and found that their DNA contained a 16S ribosomal RNA gene. Amplifying 16S ribosomal RNA with primers and sequencing it is the standard means for phylogenetic analysis of bacterial samples; these particular bacteria were identified as a new strain of the Halomonadaceae family of the Gammaproteobacteria, strain GFAJ-1.

The researchers subjected GFAJ-1 to three different treatments, varying the contents of the bacterial growth medium (Wolfe-Simon et al. 2010). The first treatment contained added arsenate and no added phosphate (+As/−P), the second contained added phosphate and no added arsenate (−As/+ P), and the third contained neither (−As/−P). The third treatment was intended as a control to demonstrate that any phosphate impurities present in the +As/−P treatment would not be sufficient to support growth. They observed that the bacteria grew on +As/−P, grew faster on

−As/+ P, and did not grow on −As/−P. This was taken as evidence for arsenate-dependent growth; in other words, the bacteria grew well on phosphate, but also grew just fine on arsenate without phosphate. They observed phenotypic differences between the cells grown on the first and second treatments; the +As/−P cells appeared swollen and contained large internal cavities, which the authors hypothesized might exclude water or perform a more explicit arsenate-stabilizing function. They ran a number of tests showing that there was arsenic around in the parts of the cell where key biomolecules would be found, such as proteins, lipids, metabolites, and DNA. From these results, the authors concluded that the bacteria were substituting arsenate for phosphate in their biomolecules. They did not explain in detail the mechanisms by which arsenate was actually incorporated into the structure of the biomolecules, or how the arsenate-based biomolecules overcame the stability problems discussed above to functionally operate.

Significant backlash followed the publication of this paper, immediately on the web and later in a series of followup commentary papers in Science in May 2011. Several papers have also been published offering theoretical support for the possibility of arsenate-based DNA (Denning and MacKerell 2011; Tawfik and Viola 2011). But the response from the scientific community was for the most part negative. A number of criticisms were raised, targeted at aspects of the experimental setup itself and the researchers' move to the conclusion that the arsenic substitution was indeed taking place, which critics say was too hasty. I briefly overview several of the most forceful and recurrent criticisms here.

One line of criticism held that the data showed only that GFAJ-1 had a remarkable adaptation, namely, the ability to handle high arsenic levels and survive on trace amounts of phosphate, perhaps by scavenging it from their dead flask-mates or just adapting to very low phosphate levels (Benner 2011; Foster 2011; Kastnelson 2010; Redfield 2010, 2011). Wolfe-Simon and colleagues claimed that trace amounts of phosphate in the two −P treatments were not enough to support life, and that their control treatment demonstrated this. But critics argued that this is not the case, and that arsenate media added to the +As/−P treatment could be contaminated with enough further trace phosphate to support more growth than took place in the control (−As/−P), as was observed. Furthermore, Cotner and Hall (2011) point out a number of counterexamples to the researchers' claim that the extremely low phosphate content in the +As/−P treatment (0.02 %) was below the level that could support phosphate-based bacterial growth.

Another line of criticism attacked the researchers for using "low-tech preparation and… high-tech identification" methods to conclude that arsenic was incorporated into the bacteria's biomolecules. Microbiologist Rosemary Redfield said that the DNA samples were not properly purified prior to analysis via gel electrophoresis, and that the gel supposedly showing arsenic concentration in the DNA could just be showing arsenic carried over from the agarose plates the bacteria were grown on (2010, 2011). In this sense, the criticism goes, Wolfe-Simon and colleagues merely showed that arsenate was associated with the genetic material and other biomolecules—not that any actual substitution for phosphate was taking place (see also Borhani 2011; Kastnelson 2010; Oehler 2011; Redfield 2010, 2011; Tawfik and Viola 2011). As Sun and colleagues (2011) added, the fact that the DNA

extracted from GFAJ-1 remained stable in water would indicate that it contained phosphate bonds, not arsenate bonds, for the stability reasons discussed above.

Many other challenges and counterpoints were raised, some more complex and subtle in their scientific details.[3] Wolfe-Simon and colleagues published a response to these criticisms (2011), which offered further explanation and analysis of their 2010 results but no discussion of new experimental work or data. They invited the scientific community to take samples of GFAJ-1 and research its properties further for themselves. Redfield and colleagues and another team of researchers recently repeated the experiment and found no arsenate in the bacterial DNA, refuting the original results (Hayden 2012; Reaves et al. 2012).[4]

My aim is not to take a firm position on the credibility of the original results, or the ultimate force of these criticisms. In the rest of this paper, I set aside the questions of whether or not these particular bacteria really grew on arsenic, or whether or not arsenic bacteria could exist in general, and focus on what the upshot would be if they did. As a starting point for addressing this, I return to NASA's claim about this discovery "changing our fundamental knowledge of life on Earth" and expanding the definition of life, and look more closely at what this means.

Redefining life?

There is a rich and growing literature on the nature and definition of life. Scientists and philosophers have proposed various approaches and solutions to the question of how we should understand the difference between nonliving and living things, but the question is still open to debate (for collections of recent work in this area see Bedau and Cleland 2010; Synthese special issue on life, volume 185(1), March 2012). Recent contributions to the debate have included proposals to consider life as coming in degrees (Bedau 2012; Malaterre 2010), to focus on expanding what we know about life in the absence of a solid definition or theory (Cleland 2011), or to give up on the search for a definition of life altogether (Machery 2012). I think the project of trying to better understand the nature of life is worth pursuing, but for the purposes of this paper, I do not take a particular stance on how to best undertake this project or what its outcome should look like. The following discussion rests on the simple points that everyone intuitively takes the world to contain both living and nonliving things, and that it means something to say, as NASA did in their December 2010 press release, that the discovery of a novel organism could challenge or change our understanding of that former category. What this means, it turns out, is more subtle and complex than it might seem on the surface.

There are (at least) two different ways to think about what it might mean to change our fundamental knowledge of life. The literature on the nature and definition of life offers a useful reference point for thinking about such change: the

[3] For further discussion see the set of commentary papers in Science volume 332, published 3 June 2011, as well as Erb et al. (2012) and Reaves et al. (2012).

[4] Wolfe-Simon and colleagues maintain that more work needs to be done to validate the still-standing possibility that these are arsenic bacteria (Kaufman 2012), though it certainly looks more dubious than it did before following the publication of the two most recent studies mentioned above; cf. footnote 1.

notion of a "sample size of one" (Bedau 2010; Cleland and Copley 2005; Davies and Lineweaver 2005). All the living things that we are currently aware of comprise a single sample of life, in the sense that all organisms in the biosphere share a common ancestor.[5] This sample is of course extremely heterogeneous, but all of the organisms it contains came from the same origin event, and in this sense make up one unified biological sample.

The first way to change our fundamental knowledge of life is within the current sample, by which I mean broadening our knowledge of life by learning more about the entities that comprise the familiar biosphere. Minor change of this kind occurs, e.g., whenever a new species is discovered. More fundamental change of this first kind sparks revisions to biology textbooks. This involves, for example, major changes in our knowledge of organisms' physiological and morphological features, or the discovery of deep new branches in our phylogenetic classification scheme.

A notable example of change in our knowledge of life within the current sample is the discovery of Archaea in the late twentieth century. The paradigm of biological classification previously involved division of life into prokaryotes and eukaryotes, with the former lacking a nucleus and the latter having one, among other salient distinguishing features. This was overthrown when Woese et al. (1990) proposed a shift to a three-domain biological classification system to make room for Archaea. Archaea are microbes which share certain features with both eukaryotes and bacteria but in fact have their own independent evolutionary history; their discovery destroyed the phylogenetic significance of the traditional prokaryote/eukaryote distinction. This involved a revolutionary change in our knowledge of the relationships among living things, and an overhaul of our ideas about which branches delineate the tree of life. Another example of this first kind of change in our knowledge of life is the discovery of extremophiles, organisms that can thrive in environments not generally regarded as hospitable to life, e.g., with high concentrations of salt or noxious chemicals, or with no exposure to energy from the sun. Impressive extremophiles include Tardigrades, tiny multicellular eukaryotes that can survive remarkable perturbations like hydrostatic pressure of 600 megapascales, twice as high as the level where any other organism would die (Seki and Toyoshima 1998). Learning about new extremophiles can change how we think about the scope of possibilities for the forms, functions and life cycles of the organisms that comprise our biosphere.

The second way to change our fundamental knowledge of life would be beyond the current sample, which means expanding our knowledge of life explicitly beyond the familiar biosphere. This is the kind of change that comes to mind when we think of finding evidence of life on other planets, or multiple origins of life on Earth. There are no concrete examples of this second kind of change in our knowledge of life because

[5] There is debate about whether or not the root of the tree of life is a single ancestor, referred to as LUCA (Last Universal Common Ancestor), or a cluster of ancestors, sometimes called a "communal ancestor" (Glansdorff 2009, Malaterre 2010). This is an interesting question, and it is beyond the scope of this paper to get into the details of addressing it. In either case, the answer to that question does not affect the point I am making here, which is that all entities that comprise the known biosphere come from the same origin. Whether all life comes from a single organism, population of organisms, or heterogeneous mix of proto-organisms liberally exchanging genes, our familiar tree of life shares a common trunk. A genuine living exception to membership in the tree of life would not be a descendent of that common trunk, whatever its nature.

we have not achieved it yet; it would have to be initiated by finding a living thing that unambiguously shared no common ancestors with known life. One hypothetical example would be the successful creation of man-made living cells through bottom-up synthetic biology. Top-down synthetic biology re-engineers living cells or creates synthetic ones from pre-existing molecular building blocks. In contrast, artificial cells built entirely from nonliving materials or "from scratch," without shortcuts employing material from living things, would arguably represent a genuine second sample of life (cf. Rasmussen et al. 2009). Another in-principle example would be the discovery of life forms on another planet, if we were sure that they did not share a common ancestor with life on Earth.[6]

When we hear claims like NASAs about changing our fundamental knowledge of life, or redefining life, it seems that what they have in mind is what I am calling change beyond the current sample. But even if it were confirmed that the arsenic bacteria really were substituting arsenic for phosphorus, this could lead only to the first kind of change in our knowledge of life, change within the current sample. Arsenic bacteria—these particular ones, at least, which have been identified as members of a well-known bacterial family—are not the kind of discovery that leads to change in our knowledge of life beyond the current sample.[7] They might turn out to have physiological or morphological features unlike anything we have seen before. But they have DNA with conserved sequences in common with familiar organisms, and thus when we learn about them, we are learning more about organisms within our single sample of life.

There is a spectrum of different kinds of discoveries and projects that could, in principle, lead to change in our knowledge of life beyond the current sample. A number of papers in the last decade have discussed the possibility, and the conceptual and technical challenges, of searching for so-called "weird life" or a second sample of life on Earth (Cleland and Copley 2005; Davies 2011; Davies and Lineweaver 2005; Davies et al. 2009). An important lesson to draw from this literature is that there are a number of different ways of thinking about methodological approaches to discoveries that could change our knowledge of life.

A common thread in papers on weird life is the idea that life on Earth could have originated more than once, and there could be "shadow biospheres" of weird organisms that are so fundamentally different from familiar life that they live among us unrecognized. Searching for such life forms is a project related to, but distinct from, that of searching for life on other planets. The idea is that if we want to know more about the nature of living things and the possibilities for different kinds of life forms on Earth-like planets, we should look more closely at the most Earth-like planet we

[6] One hypothesis about the origin of life is that it began outside of Earth and arrived here, e.g., on a meteorite. While this is not the most popular hypothesis on the origin of life, it is worth pointing out that finding life on another planet would not automatically entail that that life shared no common ancestor with life on Earth.

[7] Of course, a discovery like arsenic bacteria could lead indirectly to change beyond the current sample. For example, the discovery that life can survive on arsenic without phosphorus, if validated, could motivate searching for life in previously ignored arsenic-rich environments, and this could in principle lead to a discovery of genuinely alternate life, expanding the sample size beyond one. But my point is that the arsenic bacteria themselves would not represent an expansion of the sample size.

know of: Earth itself. It is important to underline that these prospective alternate life forms are not just supposed to be living things whose existence we were previously unaware of. Genuinely weird life would not be just another organism to add to the familiar tree of life; the idea is to find something that genuinely challenges or changes our idea of what living things are. Motivations to search for weird life include learning more about the nature of life (i.e., expanding the sample size beyond one), the origin of life, and the physical and chemical conditions that can support life, which could be extrapolated beyond Earth to inform research in astrobiology and exobiology.

From this brief overview, it should be clear that anyone interested in searching for weird life is interested in change in our knowledge of life beyond the current sample. We are familiar with plenty of "weird" organisms, like Tardigrades, slime molds, and a host of other extremophiles. But these are not weird in the right way. As long as we can place them within our familiar phylogenetic classification scheme, learning about them expands our knowledge of life only within the current sample.

Discussions of searching for weird life sometimes lump under one heading projects that are different in interesting ways. Many examples are discussed in the literature on weird life of actual or proposed projects, tantalizing leads or hypothetical possibilities for finding candidate entities to expand our knowledge of life. These examples have included self-replicating RNA strands, organisms with oppositely-chiral amino acids, non-carbon-based life, and arsenic bacteria (e.g., Davies 2011; Davies et al. 2009). The discovery of Archaea has been discussed in this literature as an example of discovering a new form of microbial life (Cleland and Copley 2005); as discussed above, this had major implications for our understanding of life, but only within the current sample. The discovery of Archaea led us to update our views about the structure of the known tree of life, not to add a new tree to our ontology. It is important to be explicit about the difference between these two ways of changing our knowledge of life when discussing potential or actual discoveries of weird or novel life forms.

My overall argument is that arsenic bacteria, which challenge a particular material constraint on life, would not be candidates for changing our knowledge of life beyond the current sample; but this does not mean that they could not teach us something important about life. In the remainder of this paper I draw some distinctions regarding projects that search for weird or alternative life forms. These distinctions help to situate the arsenic bacteria discovery within this broader context, and to highlight the various possibilities for what we can and cannot learn about life from discovering different kinds of anomalies.

Looking for weird life

I suggest a distinction between two different kinds of project that look for evidence in the world that could challenge or change our fundamental knowledge of life: (1) challenging constraints on familiar life, and (2) investigating anomalies. While the distinction is not put in exactly these terms in the literature on searching for weird life, I think that it captures the range of possible projects and methodologies discussed there.

The first kind of project poses theoretical challenges to putative constraints on what living things are made of or what they can do, and then looks for organisms with the relevant departure from familiar biochemistry or physiology.[8] The arsenic bacteria case is a perfect example of such a project. Phosphate-based biochemistry was regarded as a constraint as defended in Westheimer's paper, Wolfe-Simon and colleagues proposed a theoretical challenge to this constraint, then sought evidence in the form of a living organism. Note that we could think about this discussion of constraints at more than one level: as a specific point about material realizations of biochemistry (i.e., the need for phosphate as opposed to arsenate), or as a more abstract functional point about stability (i.e., the need for a biomolecular structure that does not break down before metabolic or information-carrying molecules can do their job). Wolfe-Simon and colleagues' theoretical challenge in their 2009 paper, and their presentation of their experimental results, spoke only to the former way of thinking about constraints on life, in terms of particular materials.

Cleland and Copley (2005) and Davies et al. (2009) discuss a number of further examples of ways to challenge constraints on familiar life. These include searching for organisms that use alternative nucleotide bases in their genetic material. All known life uses the same bases (adenine, guanine, cytosine and thymine or uracil) in its DNA and RNA. This is an exceptionless feature of our current sample of life, but it is not an in-principle constraint. One could postulate the use of some other kind of base, and look for organisms that contain it. Other examples of challenges to constraints on known life could include looking for organisms that use a different genetic material altogether (e.g., peptide nucleic acid or PNA; Wittung et al. 1994), or that use alternative amino acids, or D- rather than L-amino acids.

The second kind of project, investigating anomalies, is the more open-ended search for novel life forms that challenge in unexpected ways our idea of what living things are made of or what they can do. Davies and colleagues (2009) say that this kind of project is the best place to start for detecting a second sample of life, but do not clearly distinguish between examples of projects that just challenge particular constraints on familiar life and those that investigate anomalies. These two kinds of project are importantly different in their methodology of inquiry. The former kind of project hypothesizes the violation of a particular putative constraint and then looks for evidence of it. This second kind of project, in contrast, begins with investigating anomalous entities or phenomena in the world which could potentially teach us something important about life.

Examples of this second kind of project include the investigation of rock varnish and nanobes. Rock varnish is a phenomenon where manganese-rich films appear on rocks, causing characteristic dark streaks in a zebra-like pattern. They are physically

[8] Here I focus on projects that, like the arsenic bacteria case, involve going out in the world and looking for previously unknown entities that could count as anomalous, "weird" or "alien" life. Another kind of project aimed at challenging putative constraints on life is bottom-up synthetic biology, which aims at constructing life forms or lifelike systems in the laboratory that have the potential to change our fundamental knowledge of life by manifesting it in different material or organizational forms (see, e.g., Rasmussen et al. 2009). For the scope of this paper I limit my discussion to the former kind of constraint-challenging project, but note that the latter has significant potential for changing our knowledge of life, also worthy of further discussion.

similar to stromatolites, layered sediments known to be caused by microbes; so they look like they should have a microbial origin, but none has been unambiguously identified. Recent papers (e.g., Northup et al. 2010) identify manganese-precipitating bacteria present in some rock varnish, but exactly which processes or organisms are responsible has not been settled yet; the possibility that bacteria from a second sample of life could be the source of rock varnish has been discussed (see Cleland 2007; Fleisher et al. 1999; Krinsley and Dorn 2009).

Nanobes are tiny cell-like blobs discovered in deep-sea rocks in Australia in the late 1990s. They are about one tenth the size of the smallest known living cells, and responded to DNA stains in the laboratory which suggests that they contain DNA, but this was not confirmed. There were a series of papers on nanobes around the turn of the century (Nanjundiah 2000; Uwins and Webb 1998), and they seem to have fallen off the scientific radar since.

In both cases, nanobes and rock varnish, an intriguing observation looked like it should be living or caused by living things, but there was no consensus on a purely biological or abiological explanation. Both of these cases started with an anomalous discovery that called for further explanation. Investigating anomalies can also be even more open-ended, in the sense of looking around with an open mind for weird life. But it is not clear how to do this in practice. A genuinely weird anomaly would differ from familiar biochemistry in important ways, but we do not have a clear grip on which constraints can and cannot be violated while remaining within the range of possible biochemistry and phenotypes of the organisms that comprise our familiar sample of life. This very fact is what allows us to pursue the first kind of project. So, it is hard to define what would constitute genuinely anomalous life in a more open-ended way. It is tempting to say "we'll know it when we see it." However, our current techniques for searching for life are limited by the biochemistry of familiar life. We detect and identify microbial life as such by collecting material from the environment and sequencing the genes found therein, using small-subunit ribosomal RNA (rRNA) sequences common to all known organisms. Currently available techniques—microscopy, culturing, and PCR amplification of rRNA—would be inadequate for identifying life whose biochemistry differed fundamentally from what we are familiar with (see Cleland and Copley 2005 for further discussion of this conundrum).

The second kind of project seems like a better candidate in principle for changing our fundamental knowledge of life in a way that goes beyond the current sample. As the papers cited above on the search for weird life optimistically point out, detecting the unexpected seems like the best route to uncovering truly alternative life forms. But there are major theoretical hurdles here, in addition to the technical hurdles involved in figuring out how to detect living things that differ from those we know in ways we are not yet aware of.

The theoretical challenges surrounding our lack of a clear theory of life and its constraints and borderlines do not really come up for projects of the first type, challenging constraints on familiar life.[9] We know how to do this kind of project;

[9] It might seem that we would need to have a clear idea of which purported constraints on life are frozen accidents, and which are universal constraints on life in principle (sample of one aside), to undertake the

regardless of its final outcome, the arsenic bacteria case is an in-principle example. But again, that case could have led only to change in our knowledge of life within the current sample. There was no ambiguity as to whether or not GFAJ-1 were biological entities, or about their membership in the Halomonadaceae bacterial family, i.e., their place in our familiar evolutionary classification scheme.

The extent to which projects of the first type could potentially expand our knowledge of life beyond the current sample is proportional to how hard it is to establish plausible common ancestry, or evolutionary linkage. In the arsenic bacteria case, even without the point that GFAJ-1 were definitely members of a known bacterial family, there was a story to be told about how phosphate-based life could have evolved from arsenate-based life. Wolfe-Simon and colleagues themselves outlined this possibility, as discussed above. As long as there is presumed or even tenable shared common ancestry, organisms that violate particular biochemical constraints are not the most likely candidates for changing our knowledge of life in the broader sense. Furthermore, the arsenic bacteria case challenged a particular, material constraint on biochemistry, the necessity of phosphates. The pertinent more high-level functional constraint—the need for a particular kind of stabilizing molecule—was not challenged by this case; rather, an environment where arsenic content would allow for the required level of biomolecular stability was postulated (cf. discussion of Wolfe-Simon et al. (2009) above). If projects that challenge constraints on familiar life are going to lead to change in our knowledge of life beyond the current sample, challenging more high-level, functional or organizational constraints seems like the more fruitful route.

Conclusion

I should underline in closing that even though arsenic bacteria could in principle lead to change in our knowledge of life only within the current sample, this is not to downplay the fact that the arsenate-phosphate substitution, if validated, would be a significant contribution to our knowledge of life. Critics accused Wolfe-Simon and colleagues' research of merely demonstrating an impressive adaptation to high levels of arsenic. But if the bacteria really were substituting arsenate for phosphate, they would represent more than just another extremophile to add to the list. This would overthrow the paradigm about constraints on biochemistry established by Westheimer's paper almost three decades ago, which would represent change in our fundamental knowledge of life in an interesting and important sense. But again, it is a change in our knowledge of the particular material constraints on life, not a challenge to our views on more high-level functional or organizational constraints.

Footnote 9 continued
project of finding life forms that challenge constraints on known life and determining whether or not they expand the sample size. But this is not necessarily the case. Cleland (2011) has argued that we can search for weird life by undertaking projects of both of the types I have identified without worrying about the answer to the above question. My point here is that we should not assume that any apparently anomalous discovery that comes from such a project will be a candidate for instigating change in our knowledge of life beyond the current sample.

It is not a change in the broader sense which NASA seemed to want us to subscribe to, and which literature on searching for weird life sometimes implies discoveries like this could lead to.

Precisely how to look for and achieve discoveries that lead to change in our knowledge of life beyond the current sample remains a huge and fascinating open problem. In conclusion, though, I hope to have clarified several different ways to think about the project of searching for anomalous life forms, and shown where the arsenic bacteria discovery fits into this picture. The distinctions drawn above regarding (1) different ways to change our knowledge of life and (2) different kinds of projects aimed at achieving such change do not solve this problem, but they do give us a reality check on how to evaluate the hypothetical and actual discovery of ostensibly weird or novel life forms. In particular, these distinctions highlight the subtleties and challenges involved in different ways of questioning constraints on life, and help in understanding when and why we should take claims like NASA's with a grain of salt.

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