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Essay

Models and Standards of Proof in Cross-Disciplinary Science: The Case of Arsenic DNA

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Of all the conflicts possible within cross-disciplinary studies, none are more confounding than those that arise from different standards that different disciplines require for "proof." Here, "proof" is not used as mathematicians use it, but rather it describes the collection of evidence sufficient to accept a discovery, stop experiments, and record a problem as "solved" (Davenas *et al.*, 1988). Experiments can easily produce evidence that is sufficient proof for one scientific discipline but sufficient only to create further controversy for another. Indeed, the identical experimental evidence that is conclusive for one community and controversial for another might cause a third community to reject the very same conclusion entirely. Failure to understand the differences in these standards of proof can allow cross-disicplinary activities to produce flawed science on one hand and reject genuine innovation on the other.

Astrobiology is quite cross-disciplinary. As such, astrobiology is expected to generate many such conflicts. As an example, a team of astrobiologists in 2010 reported evidence that they thought was sufficient to conclude that a microbe isolated from Mono Lake (GFAJ-1) contained DNA with some of its backbone phosphorus atoms replaced by arsenic atoms (Wolfe-Simon *et al.*, 2010). The referees who reviewed the paper in the "planetary science" track at *Science* enthusiastically recommended publication of this conclusion. Physicists, also a major community within astrobiology, also found that the paper's evidence strongly supported the conclusion of arsenate DNA (Kaku, 2010). However, biochemists and microbiologists, two other communities within astrobiology, found the very same data inadequate to conclude the presence of arsenic-substituted DNA (Redfield, 2010). Chemists went further, seeing in the very same data *disproof* of the hypothesized arsenic DNA (Drahl, 2010).

How could the same data be interpreted so differently by communities that must work together within the discipline of astrobiology to consider life in the Cosmos? While the specific experiments applied to GFAJ-1 have been much discussed, we believe that the study can be examined as a living illustration of how disagreements over the nature of proof confound cross-disciplinary studies. Such examination is necessary if cross-disciplinary fields are to contribute to their many constituent scientific communities. It allows us to address a larger question: What is proof?

We start with a simple aphorism: Only the unexpected needs explanation. Further, the more an observation is unexpected, the more explanations are needed. Captured in the aphorism of the late Carl Sagan ("extraordinary claims require extraordinary evidence") (Sagan, 1990), we cannot understand what a community finds "extraordinary," and what that community requires to meet a burden of proof, without understanding the expectations of the community.

Considering GFAJ-1, let us begin with chemists, who found the report of arsenate DNA most unexpected and most extraordinary. Over the past two centuries, chemists have examined millions of compounds. Each is associated with a molecular structure, a model that describes the arrangement in space of constituent atoms held together by bonds. Associated with each compound are also measurements, often quite detailed, of its physical properties and molecular reactivities.

These collections support "structure theory" in chemistry, which explains the properties and reactivities of *all* matter by making reference to molecular structures. Further, the structures in these collections are tightly and logically interconnected. Water is H_2O, not H_3O. If water were H_3O, then the difference propagates across the collection by force of deductive logic. Thus, any claim that water *is* H_3O is a claim that *many* of the structures in the entire collection must be wrong, and the observations that support *those* structures must also be revisited. That is, an enormous amount of data commonly viewed as true must be false if water turns out to be H_3O. This makes a claim that water is H_3O extraordinary, to a chemist.

The cross-validation of structures in the chemist's collection of compounds extends to reactivity. For example, modern databases of molecular structures contain many "arsenate esters," molecules containing an arsenic atom surrounded by four oxygen atoms, with one, two, or three of the oxygen atoms attached to carbon chains. The carbon chains are different in different arsenate diesters, but the species react analogously. All known arsenate esters hydrolyze rapidly in water at any temperature where water is liquid. This leads chemists to expect that *all* arsenate esters will hydrolyze rapidly in water, even those not yet examined. Indeed, if one draws a structure of an as-yet unknown arsenate ester, a chemist will anticipate how fast it will

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hydrolyze by analogy to arsenate esters whose structures are already known and whose hydrolysis rates have already been measured.

Chemists are so sure of the power of such analogies that they routinely reverse the logic. They often do not look at molecular *structure* just to predict molecular *properties*. Rather, they look at the *properties* of a molecule to infer its *structure*. In this example, if a compound does *not* hydrolyze rapidly in water, then the chemist infers that it is *not* an arsenate ester.

Wolfe-Simon *et al.* (2010) interpreted data collected from GFAJ-1 as evidence that its DNA contained arsenate esters. Indeed, they presented (their Fig. 2) an image of a gel and assigned a band on that gel as arsenate DNA that had been separated from other cellular components by procedures done in water and then resolved by electrophoresis in a gel containing water. Yet the putative arsenate DNA did not hydrolyze. Chemists, using their reverse logic and confident in the power of inference by analogy arising from the interconnectedness of their databases, concluded from the exact data published in *Science* that the DNA in GFAJ-1 is *not* arsenate-linked, because it did not hydrolyze in water, as arsenate esters should.

Of course, the *specific* molecule proposed by Wolfe-Simon *et al.* (arsenate DNA) is not in the database of known arsenate esters. Therefore, its instability in water has never been *specifically* measured. Thus, it might be argued (and was argued in the back-and-forth that followed from the 2010 report) that the chemists' databases did not *rule out* the possibility of the arsenate DNA structure being correct as hypothesized.

Fair enough. But the database of molecular structures of other arsenate esters and their associated reactivities made the claim for the arsenate ester structure for DNA extraordinary to the chemist, and demanding of extraordinary evidence. To a chemist, if the claim of a stable DNA containing arsenate ester linkages were to be found correct, then *all* the reactivities reported for *all other* arsenate esters in the chemist's collections must be revisited with the expectation that a substantial amount of data regarding those other arsenate esters, commonly held to be true, must be false. Wolfe-Simon *et al.* (2010) did not address this. Therefore, organic chemists dismissed their proposed molecular structure.

Geologists also do chemistry. However, the high temperatures involved in the formation of most minerals destroy most of the covalent bonds in molecular assemblages studied by organic chemists. The atoms are shuffled in molten rock, in supercritical water, and under other conditions that are far too harsh for most organic covalent bonds. As a consequence of this shuffling, atoms end up in arrangements determined less by their origins and more by the surrounding pressures and temperatures. They may be shuffled again as these change.

Accordingly, the Periodic Table provides a powerful and generally adequate guide for geochemists. Here, analogies are based on rows and columns. Elements in rows above in the table are often found substituted for elements below, and *vice versa,* in crystals of minerals that are otherwise idealized as single chemical mixtures at end points in a continuum of actual materials.

Chemical analyses based on the Periodic Table have worked well for geologists. For example, the existence of the element hafnium was predicted by the Periodic Table by its position below the then-known zirconium. Based on this prediction, hafnium was then discovered in zircons (zirconium silicate), a mineral that contains predominantly zircon. Here, individual hafnium atoms replace individual zirconium atoms in the mineral crystal lattice. If the zircons are formed in an environment rich in hafnium, then many of the zirconium atoms are replaced by hafnium atoms. Eventually, the mineral ceases to be called *zircon* and becomes *hafnon*.

From the perspective of the Periodic Table, phosphorus and arsenic should also be easily replaced by each other. And they are. The arsenate in many arsenate minerals is often substituted by phosphate if the arsenate mineral is formed in an environment rich in phosphate; the phosphate in phosphate minerals is often substituted by arsenate if the phosphate mineral is formed in an environment rich in arsenate. As an example of this type of geological exotica, yttrium phosphate (the mineral xenotime) forms a continuum in mineralogy with yttrium arsenate (the mineral chernovite). Students of mineralogy learn about these mineralogical "series" early in their studies and therefore cease culturally to regard them as extraordinary.

So why not have arsenate substitute for phosphate in DNA when the DNA is grown in an environment rich in arsenate, Mono Lake for example? To a geologist whose expectations are driven by the Periodic Table and their culture, nothing seems less exceptional. To that geologist versed in the Periodic Table, extraordinary evidence is *not* required to accept the proposal by Wolfe-Simon *et al.* With an unexceptional proposal on the table, an Occam's razor argument is sufficient and was exactly the argument that Wolfe-Simon presented. Here, the burden of proof was met by an argument that arsenate DNA is the simplest explanation for the data reported.

The physicist coauthor of this paper found this not particularly unreasonable. Physicists often seek to distinguish between two hypotheses, neither favored *a priori* by the evidence available. In these cases, the physicist seeks robust items of evidence favoring one over the other. For example, a distant star has an orbiting planet or it does not. *A priori,* neither alternative is favored. Therefore, a conclusion can be called by a series of spectra or by precision light curves having a community-acceptable level of statistical significance on the detection. The conclusion is then published, and the burden of proof shifts to those who wish to conclude the opposite.

The history of physics offers many examples of "definitive" experiments that the culture accepted as validation of theories of global import. For example, Eddington's 1919 measurement of deflected starlight by the Sun during a solar eclipe was viewed by the culture as strong support for general relativity; it shifted the burden of proof in the community to those seeking to deny general relativity. The detection of the relic 3 K cosmic microwave background radiation field was viewed by the culture as a landmark successful test in support of the Big Bang model of the Universe; it shifted the burden of proof to those seeking to deny that model.

This is not to say that it did not take time for the theories to undergo further tests to be fully accepted by the community or that physics regards all such theories as "proven." Alternatives to general relativity continue to be proposed. But the two items of data shifted the burden of proof away from general relativity and the Big Bang theory, respectively. Proponents of alternatives bear the burden of generating data.

From this perspective, physicists find familiar the challenge facing those who analyzed GFAJ-1. The hypothesis proposed that arsenate DNA could exist (Davies *et al.,* 2009; Wolfe-Simon *et al.,* 2009). To a physicist, either GFAJ-1 has arsenate DNA or it does not. Physicists expected Wolfe-Simon *et al.* (2010) to offer up items of data to distinguish these two *a priori* equivalent hypotheses. Wolfe-Simon and colleagues did. A higher arsenate:phosphate ratio was measured in the gel at the position where the arsenate DNA was expected than at other positions in the gel. Other data hinted that proteins and metabolites also had arsenate-for-phosphate replacements. Compared to the amount of data used to call the presence of an extrasolar planet, the data seemed abundant. The burden of proof seemed to move to those who doubted the presence of arsenate DNA.

What about the biologists, also important to astrobiology? Organismic biology is also information dense, like chemistry. However, the interconnectedness of a biological database is not based on deductive logic. Therefore, a claim cannot be ruled exceptional because it threatens the entire architecture standing behind biology, like a proposal for water being H_3O threatens all of chemistry. Further, much remains to be discovered in biology: life scientists can still be found who declare (with pride) that they do "hypothesis-free research" (Hunter *et al.,* 2008).

However, biologists operating within their scientific culture also have expectations that can cause them to regard a result as exceptional and, as a consequence, demand extraordinary data in its support. Their expectations are based on Darwinian theory, models for common ancestry of life on Earth, a broad overview of natural history, and if necessary, include databases from organic chemistry.

For example, when a report claimed the discovery of bacteria living at 250°C (Baross and Deming, 1983), biochemistry found it exceptional. Databases of proteins, DNA, and other biological molecules contained few measurements of the stabilities of such molecules at 250°C. However, analogies from measurements over the past century made strong the inference that these biomolecules would not survive at 250°C (Bernhardt *et al.,* 1984; Trent *et al.,* 1984; White, 1984). Thus, biochemists demanded extraordinary evidence to support this claim, evidence that was (in this case) not forthcoming.

Biology can also exploit historical context to decide what is extraordinary. A "universal" tree of life (named by biologists, with apologies to astronomers) interconnects all modern terran biology by kinship relations. Those kinship relationships reflect similarity at all levels, from chemistry to anatomy. Any deviation from this is unexpected, although not unprecedented. Thus, biologists observing an exotic new species, one perhaps different in structure from any observed before, might say first, "How marvelous!" They must then, however, ask, "What is its nearest kin?" If the structure of the new life-form does not fit within the relevant models for natural history, the exotic structure is extraordinary. For example, finding an ape-like jaw joined to a modern human skull in the Piltdown man was at most unexpected in 1912. By 1953, it was so extraordinary in light of the other family resemblances that extraordinary evidence was requested. That evidence was not forthcoming; the Piltdown fossil had been faked (Times Museum Correspondent, 1953).

From the perspective of natural history as understood by biologists, the hypothesis of arsenate DNA was certainly extraordinary. GFAJ-1 was reported to be closely related kin to bacteria that do *not* have arsenate DNA, some quite well studied in the biologists' databases. GFAJ-1 seemed to have biochemical features that made it not so different from its closest relatives. It was grown in the laboratory under quite standard conditions. Its cells did not appear to be markedly different from those of other arsenic-tolerant bacteria.

It was therefore natural for biologists to ask: "How can such a normal bacterium, with a natural history so closely related to bacteria we have studied thoroughly, have such abnormal DNA?" "How might the hypothetical arsenate DNA be biosynthesized in an otherwise unremarkable metabolism?" or "How can it have evolved from phosphate DNA, since its relatives (and their ancestors) appear to have contained phosphate DNA?" These questions alone are not reasons to reject the arsenate-DNA hypothesis, as chemists did. But they are reasons for biologists to demand extraordinary evidence in support of that hypothesis.

These questions attracted aggressive inspection of the data reported by Wolfe-Simon *et al.* (2010) to see if it met the exceptionality demanded by the Sagan dictum. The results of this inspection appeared in the formal literature (see Benner, 2011; Csabai and Szathmary, 2011; Redfield, 2011, among others) and the blogosphere (see for example Redfield, 2010). Some chemists and biochemists began experimental programs to apply their own standards of evidence to test the presence of arsenate DNA in GFAJ-1. Several studies concluded that, to the limits of detection, GFAJ-1 has normal, phosphate-based DNA and no relevant content of arsenate biomolecules (Reaves *et al.,* 2012). Still others have found alternative, and conventional, interpretations for all the data published by Wolfe-Simon *et al.* (Cotner and Hall, 2011; Forster, 2011; Elias *et al.,* 2012; Erb *et al.,* 2012). Today, the burden of proof has shifted in the eyes of many; GFAJ-1 appears to be an unexceptional example of an arsenic-tolerant organism with entirely standard phosphate DNA (Phung *et al.,* 2012).

The study by Wolfe-Simon *et al.* (2010) is not the only example of problems in cross-disciplinary science caused by differences in expectations and burdens in different scientific communities. Other examples relevant to astrobiology include hypotheses relating to the origin of life (Kaufmann, 1995; Dyson, 1999) and the possible biological origin of microscopic structures found in meteorites from Mars (McKay *et al.,* 1996). Others are found in cross-disciplinary studies in medicine (Davenas *et al.,* 1988; Hirst *et al.,* 1993; Bains, 2009).

The example provided by Wolfe-Simon *et al.* (2010) is, however, especially valuable for those who seek to study how cross-disciplinary science develops, as it has unfolded in real time. This allows the student to follow the conflicting standards of proof as they emerge. For this reason, the meta-analysis of the GFAJ-1 controversy is almost as interesting as the analysis of GFAJ-1 itself. This meta-analysis should not be ignored, as it can contribute to our general understanding of conflicts and their resolution in cross-disciplinary fields. We hope that this essay will stimulate the community of astrobiologists to discuss such conflicts and how conflicting standards of proof can be reconciled to enable revolutionary discoveries in the future.

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