Alternative Biochemistries of Life Making Universal Constraints Testable

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Abstract

Synthetic biology defies traditional views about the scope of biological inquiry (Hull 1978; Waters 2007; Weber 2013;). Some authors are skeptical about the epistemic value the field has to the rest of the life sciences (Keller 2009a). This paper demonstrates how artificial biochemistries of life serve an essential epistemic function as semifactual models in biology (Knuuttila and Koskinen 2021). As semifactual models, alternative biochemistries of life facilitate the testing of hypotheses about universal biological constraints. Importantly, clarifying this role also unveils further research questions that would lend greater rigor to synthetic biology research programs.

1. Introduction

A common view in the philosophy of biology states that biology is distinctive from the other physical sciences in its focus. Biology does not seek to explain what might be physically or chemically possible, but instead what actually occurs in living systems as the result of natural, contingent processes (Hull 1978; Weber 2013; Waters 2007). Yet, the new emerging area of synthetic biology defies this consensus. Synthetic biology's idiosyncratic focus has prompted some authors to express skepticism about what epistemic value this research has for traditional areas of biology (Keller 2009a; 2009b).

The purpose of this paper is to make a case for an epistemic payoff that comes from efforts to make and study alternative biochemistries of life. While the field of synthetic biology may, indeed, generate a wealth of knowledge for non-traditional areas of biology – notably, biomedicine and bioengineering – I argue that alternative biochemistries of life also have epistemic value for theorizing about the origin and evolution of the universal, system of heritable information.

Few traits in the biological world are as invariant as the biochemistry that supports the flow of heritable information in living organisms. The molecular structure and chirality of DNA, RNA, nucleotides, and amino acids as well as the genetic code are commonly described as universal, biological constraints (Maynard-Smith 1985; Wimsatt 2007; Šponer et al. 2012; Firnberg and Ostermeier 2013). Biological constraints possess several distinguishing features, which this paper outlines (Ross 2023; Wimsatt 2007; Love 2015; Brigandt 2015).

A crucial strategy for testing hypotheses about biological constraints involves showing that the range of variation observed in a lineage is a subset of what is expected. In evolutionary-developmental (evo-devo) biology, phylogenetic analysis is a principal method for arriving at

estimates of the expected range of a trait for a given set of lineages. However, this method is not available for universal biological constraints (Schwenk 1995; Alberch 1989; Schwenk and Wagner 2003; Schwenk and Wagner 2001). How can biologists set their expectations of what range of variation is to be expected when there is no variation to observe in living organisms?

I argue that alternative biochemistries of life should be understood as semifactual models that aid understanding of the origin and evolution of the universal system of heritable information (Knuuttila and Koskinen 2021; Ijäs and Koskinen 2021; Koskinen 2019). Alternative biochemistries of life serve an analogous epistemic function that naturally evolved lineages serve for the evo-devo researcher. In what follows, I show how alternative biochemistries of life can be a crucial puzzle piece in testing hypotheses about universal biological constraints. The analogy with evo-devo also brings into relief further research questions that synthetic biologists should explore to make theorizing more rigorous.

The paper proceeds by presenting (Section 2) a traditional view in philosophy of biology and how the research effort to make alternative biochemistries of life defies this consensus. Next (Section 3), I outline several distinctive features of biological constraints, and how the standard, universal system of heritable information meets them. In Section 4 I describe the crucial role phylogenetic analysis plays in evo-devo research for testing hypotheses about biological constraints and I explain how this reasoning strategy is unavailable for theorizing about the universal system of heritable information. In Section 5 I argue that alternative biochemistries of life provide scientists with essential semifactual models for conducting an analogous form of reasoning when it comes to universal components of the system of heritable information. In Section 6 I conclude.

2. Alternative Biochemistries of Life – Defying Philosophical Consensus

Nearly all of life is characterized by the same biochemical components that constitute a system of heritable information. DNA and RNA contain a sugar-phosophate backbone running along one side with sequences of nucleic acid bases running along the other. Watson-Crick base pair rules ensure that a sequence of nucleic acid bases can be copied with a high degree of accuracy to produce complimentary strands of DNA or RNA. Protein coding genes are sequences of nucleic acid bases in DNA that are transcribed into messenger RNA, which in many cases is processed by alternative splicing factors and then translated by ribosomes and transfer RNA (tRNA) into sequences of amino acids according to a universal set of translation rules – i.e., the genetic code. Across all of life, the biochemistry of these components is the same. The same nucleotides are found in DNA and RNA, the same set of amino acids are found in proteins and are specified by the same set of rules.[1] In this paper, I will use the expression "universal system

[1] A myriad of molecular processes are responsible for subtle exceptions to the rules of protein synthesis (see Griffiths and Stotz 2013 for a comprehensive discussion). For example, many protein coding genes encoded in eukaryotic genomes undergo alternative *cis*-splicing. Additionally, some microbial genomes encode 22 instead of the canonical 20 amino acids.

of heritable information" to refer to the universal biochemical mechanism and its components that facilitates the flow of heritable information.

A research effort in synthetic biology seeks to design, synthesize, and study alternative biochemical components of the universal system of heritable information – such as nonstandard nucleobases and backbone structures, orthogonal transfer RNA and aminoacyl-tRNA synthetases, alternative codon-amino acid assignments, and unnatural amino acids (Rackham and Chin 2005; Freier and Altmann 1997; Liu and Schultz 2010). Work to develop alternative biochemistries of life are at odds with what several philosophers of biology have identified as the principal research focus of the biological sciences.

In many ways, biology is a historical science. The actual history of populations from the level of local communities to Life on Earth governs the phenomena including developmental entrenchment, species categories, and the 'frozen accidents' of life's genetic code. For many authors, not only does the actual history of life determine the target of biological research but delimits the scope of biology as a discipline – its explanatory focus, the structure of its explanations, and its conception of what is possible. Following David Hull (1978), philosophers of biology have predominantly argued that actual history must delimit the scope of its concepts. This position has been buttressed by several authors who argue that the scope of biological explanation is narrower than the scope of explanation in the physical sciences. For example, C. Kenneth Waters argues that biological explanation focuses on the 'actual difference makers' (as opposed to 'potential difference makers') of biological traits (Waters 2007). Similarly, Marcel Weber argues that biologists are interested in explaining biologically normal interventions – interventions that (1) could also be due to natural processes, and (2) that are compatible with the life of the system (Weber 2013).

The research program to make and study alternative biochemistries of life represents a focus on living systems that have no natural history, that are (prior to their actualization) merely potential, and that are feats of human construction. Indeed, this research program is driven by an interest not in how life actually is, but how it could have been different with alternative physical and chemical elements. The apparent strangeness of this research has prompted some authors to question synthetic biology's epistemic value to the rest of the life sciences. For example, Eveyln Fox Keller writes,

"[i]t is not clear what, if anything our expertise in synthetic biology contributes back to an understanding of traditional biology. Its object is rather to expand our known universe to include living entities not as they have evolved but as we design them…engineering not as a part of science, but as an alternative: making not as knowing, but as an alternative (or replacement for) knowing" (Keller 2009a, 338; also see Keller 2009b).

The purpose of this paper is to explain and defend one way in which the above research program is epistemically valuable to biology. While much of synthetic biology is aimed at manipulating living systems and their components to develop novel technologies for real world applications,

the goal of this paper is to defend synthetic biology's role in developing semifactual models as a strategy to help scientists reason about the universal constraints of life. Theorizing about the universal system of heritable information spans billions of years including a period of time when there was multiple, competing systems of heritable information. Questions remain as to why the universal system of heritable information is the way it is and what processes led to it becoming universal. It's likely that several processes led to the universal system we have today. Ongoing research seeks to fill in the details of which processes contributed to the universal system's origin and evolution. To what extent, if any, did the laws of chemistry, historical contingency, frozen accidents, and selection play in the universal system's origin and evolution? This is an area of research characterized by significant epistemic uncertainty. However, the contention of this paper is that semifactual models can help scientists make some modest progress in this area.

3. The Universal System of Heritable Information as Biological Constraint of Life

The universal system of heritable information and its various components are frequently characterized as biological constraints. Maynard Smith et al. (1985) mention numerous examples of biological constraints, including the chirality of amino acids. The sugar-phosphate backbone structures of DNA and RNA have been said to constrain "the conformational space accessible to the bases (Šponer et al. 2012);" and the organization and redundancy of the genetic code has been said to constrain "the adaptive exploration of sequence space" in proteins (Firnberg and Ostermeier 2013)." In this section, I identify several, interrelated features of biological constraints and illustrate how some of the components of the universal system of heritable information exemplify these features.

The first noteworthy feature of biological constraints is that they structure, limit, or bias the range of variation that is possible in a population (Ross 2023; Love 2015; Hansen 2015; Smith et al. 1985; Schwenk and Wagner 2003). Consider the sugar-phosphate backbone of RNA. This molecular structure limits the range of conformations RNA molecules can take. The sugar-phosphate backbone is what explains why it is impossible for RNA molecules to have a very rigid structure. The backbone of RNA enables it to carry out various functions, such as the capacity to maintain a floppy structure despite variability in the nucleobases, which in turn enables complimentary binding with other biomolecules.

The standard genetic code constrains the flow of heritable information in two distinct ways. The standard genetic code consists of all possible nucleic acid triplets (or codons) and 22 naturally occurring amino acids.[4] For the most part, each codon specifies one of the 22 amino acids (with the exception of some "stop codons" which typically do not specify any amino acid). The genetic code is characterized by two organizational features that contribute to error-minimization during protein synthesis. The genetic code has (i) a relatively high degree of redundancy. Redundancy appears to minimize the chances that a different amino acid will be inserted into a polypeptide as the result of a misreading the third base of a codon. Additionally, (ii) biochemically similar amino acids appear to be specified by codons with similar nucleic acid triplets. Features (i) and

(ii) arguably bias the flow of heritable information to possible mutations likely to be maintained in a lineage (Firnberg and Ostermeier 2013). The genetic code's organization explains why some alleles are rare in a lineage. Yet, another sense in which the genetic code acts as a constraint has to do with the type of amino acids the standard genetic code encodes into proteins. It is widely theorized that the genetic code is "frozen" in place (Crick 1968). This is what explains why proteins containing nonstandard or noncanonical amino acids are ruled out as possible polymers synthesized in living organisms.

Importantly, biological constraints also facilitate evolvability (Brigandt 2015). While biological constraints might restrict, limit, or rule out some types of variation, they also make some evolutionary outcomes more probable (Brown 2014). Scientists commonly describe the internal structure of the universal genetic code as facilitating the evolvability of proteins. Conditions (i) and (ii) previously mentioned increase the probability that when single point mutations produce substitutions in amino acids, the substitutions will be very subtle biochemically. While subtle biochemical changes are unlikely to severely disrupt the functions of a protein, they may facilitate subtle, but new beneficial functions (Firnberg and Ostermeier 2013).

Biological constraints can also vary in terms of their scope. Constraints vary in scope to the extent they are present across a range of clades. Many of the components of the naturally evolved system of heritable information are conceptualized as universal in scope. The molecular structure of the sugar-phosphate backbones of DNA and RNA, of nucleotides, and amino acids, the full set of codon-tRNA-amino acid assignments, etc. are the same across all of life since (at least) the last universal common ancestor. By contrast, most other biological constraints are only present in some lineages.

The next feature highlights an ambiguity to many biological constraint concepts with regards to its relationship to selection. Sometimes authors can have in mind constraint$_F$ – where a set of structures or processes that structure, limit, or bias form (Amundson 1994). In contrast, one could have in mind constraint$_A$ – a set of structures or processes that structure, limit, or bias adaptation. While biological constraints have occasionally been characterized in opposition to adaptation, the two can interact. Some types of constraint$_F$ may allow variations that are selectively neutral, making the structure or process that structures, limits or biases form independent of constraint$_A$. In this case, the constraint$_F$ is unrelated to constraint$_A$. However, a constraint$_F$ may result in a constraint$_A$ when a constraint on form allows variations that have adaptive value. Thus, biological constraints can range in the degree to which they are independent of selection. On one end of the spectrum, a biological constraint is entirely independent of selection and on the other, the two are intertwined. The standard set of codon-tRNA-amino acid assignments in modern living systems is traditionally theorized by Crick to lie at the extreme end of the spectrum where constraint$_F$ results in constraint$_A$ on the basis that any change in assignments is thought to be lethal (Crick 1968).

Related to the feature of lethality, biological constraints are often said to be generatively entrenched (Wimsatt 2007, 1999, 1986). Generatively entrenched structures or processes are conceptualized as supporting a number of other "downstream" causes. Thus, a change in a generatively entrenched structure will produce changes in the factors upon which they depend. The genetic code is a paradigm example of a generatively entrenched structure of life. For example, the genetic code is theorized to be "frozen," due to numerous, complex processes depend on the precise and faithful translation of nucleic acids into amino acid sequences. Because so many essential life processes depend on a universal genetic code, Crick theorized that any change to the code is likely to be lethal if not strongly selected against (Crick 1968).

This section demonstrates how some of the components of the universal system of heritable information can be conceptualized as biological constraints. Biological constraints are structures and processes that both limit and facilitate evolvability, they can vary in the degree to which variation is lethal and generatively entrenched. Things like the sugar-phosphate backbone of RNA and the genetic code limit and facilitate the evolvability of different aspects of the universal system of heritable information and are generatively entrenched to a very high degree. In what follows, I illustrate how the universal biological constraints face epistemic challenges that nonuniversal constraints don't.

4. The Challenge of Testing (Universal) Biological Constraints

Merely possessing features characteristic to a biological constraint doesn't settle many of the hypotheses that scientists have about the origin and evolution of a constraint. In this section, I illustrate how phylogenetic analysis is essential strategy for testing hypotheses about biological constraints in evolutionary-developmental biology (or evo-devo). Unfortunately, this type of analysis is not available for testing hypotheses about universal biological constraints. However, in Section 5, I show how an analogous strategy is made possible by the alternative biochemistries synthetic biologists develop.

Are the structures of the universal system of heritable information themselves constrained by other structures or processes of life? What role did randomness and chance play in the expansion of the set of codon-tRNA-amino acid assignments? How evolvable is the genetic code? I argue that progress on these questions can be helped by drawing an analogy between the study of universal biological constraints and the study of biological constraints in evolutionary developmental biology (or evo-devo). Evo-devo tackles similar questions by conducting phylogenetic analysis – an analysis unavailable to the study of universal biological constraints.

The above questions concern the universal system of heritable information as a biological constraint, but analogous questions can be asked about developmental constraints in the field of evo-devo. Developmental constraints are a type of biological constraint; however, they limit and facilitate the evolvability of development in a lineage (Schwenk and Wagner 2003). Just like the universal system of heritable information, a structure or process might possess all the features of a biological constraint, yet this is not sufficient evidence to settle various hypotheses about the

structure or process. The rarity or nonoccurrence of some types of variation is compatible with several hypotheses. Some types of variation might not occur in a lineage largely due to chance. Selection might be so strong against some types of variation that it never occurs. By contrast, the regular occurrence of some types of variation is also compatible with various hypotheses. Selection for a structure or process might be so strong that the type of variation is all that is observed. A structure or process might not itself be a biological constraint, but a downstream effect of something else that is and the former simply tags along. This possibility is especially relevant to biological systems with deeply entrenched, integrated components.

Evo-devo derives support for biological constraint hypotheses by establishing several, independent lines of evidence (Schwenk 1995; Alberch 1989). Of the distinct lines of evidence, phylogenetic analysis is an essential methodology for testing constraint hypotheses. An important method for testing biological constraint hypotheses in most cases involves showing the range of variation in a trait is a subset of the range of variation one would expect were selection alone explanatory (Gould 1989). This approach requires a principled method for setting expectations about the range of phenotypic variation that would result from selection alone. How do scientists set their expectations? A major step involves tracing a trait's evolutionary history by mapping out its phylogenetic distribution (Richardson and Chipman 2003; Schwenk and Wagner 2001). Measurement of a trait's expected range of variation can be gleaned from the range of variation displayed by the set of clades within a monophyletic group. Sister clades that consistently display a narrower range of variation deviate from what's expected. This alone is still not sufficient evidence in support of a biological constraint. What's needed is evidence that the sister clade is subject to similar selection pressures as the others. For example, the number of digit bones of hands and feet varies widely in non-mammalian tetrapods, but it does not vary in most mammals (with the exception of some members of Cetacea). Additionally, it is theorized that most mammals are subject to the same environmental conditions as non-mammalian tetrapods. The stable phalanx number in most mammals deviates from what is expected given the phylogenetic distribution and hypothesized selection pressures. Phalanx number in most mammals is theorized to be the result of a biological constraint.

Of course, even with a compelling phylogenetic analysis, a biological constraint hypothesis can still be subject to some uncertainty. It may be that the selection pressures in fact differ between sister clades. In this case, the hypothesis that a biological constraint best explains the range of variation in a trait is more dubious. Finally, it may also be that the trait hypothesized to be constrained is not in fact constrained but is instead a downstream effect of a different trait that is.

While evo-devo employs phylogenetic analysis to test biological constraint hypotheses, it should be clear by now how such a method is not currently available to scientists interested in testing similar hypotheses about the universal system of heritable information. Phylogenetic comparison is largely unavailable when theorizing about the elements composing the universal system of heritable information. On what basis should scientists set their expectations for the range of variation possible when it comes to the universal features of life's heritable system? In the

following section, I argue that semifactual models can play an analogous function to the role related clades play in evo-devo. Semifactual models help provide a way to estimate the possible range of variation that might be relevant to the subcomponents of the universal system of heritable information. Importantly, clarifying this role also sheds light on further epistemic demands that this research should meet, but rarely does.

5. Alternative Biochemistries of Life: Semifactual Models for Expected Variation

Following Knuuttila and Koskinen (2021), I argue for thinking about alternative biochemistries of life as semifactual models. Knuuttila and Koskinen extend Goodman's (1947, 1954) account of semifactual statements to the material models developed by synthetic biology. While counterfactual statements address "what if things had been different questions," semifactuals address "even if things were different" questions. Knuuttila and Koskinen interpret semifactuals as conditional statements where (1) the antecedent of a conditional is false and the consequent is true, and (2) the antecedent represents one way (out of several possibilities) of instantiating the consequent. On Knuutila and Koskinen's account, semifactual models demonstrate the potential multiple realizability of biological systems (also see Ijäs and Koskinen 2021; Koskinen 2018). Successful construction of alternative biochemistries of life demonstrates how a higher-level function of the actual world (such as storing, transcribing, and translating heritable information) can be invariant despite variations in the underlying material make makeup as with the cases of nonstandard nucleobases, alternative backbone structures, unnatural amino acids, etc. Knuuttila and Koskinen argue that achieving knowledge of what is possible, semifactuals can help us learn something new about the actual world – such as whether high-level functions of the biological world can be multiply realized in nearby, but different possible worlds. The aim of this paper is to extend this thesis further by showing how semifactual models can aid in testing hypotheses about universal biological constraints.

One lesson alternative biochemistries of life can provide is what range of variation is very unlikely to occur. An illustrative example of this lesson comes from the study of alternative backbone structures for information bearing molecules. Synthetic biologists interpret the alternative backbone structures they study as models of alternative information-bearing molecules prior to the origin of naturally occurring RNA structure. The naturally occurring system of heritable information contains RNA molecules, which are long chains of nucleotides linked together by regular anionic phosphoric acid groups bound to a sugar molecule (i.e., ribose). Is the sugar-phosphate backbone of RNA a biological constraint on life? What variations on the backbone of information-bearing molecules are possible? For a backbone structure to support an information-bearing molecule like RNA, one thing it must be capable of supporting is complimentary base-pair binding between nucleotides. After many failed efforts to synthesize neutral backbone structures that support complimentary binding, some synthetic biologists now argue that alternative backbone structures must carry a charge (either positive or negative) (Frierand Altmann 1997; Nielsen 2004; Benner 2002). Without a charge, information bearing molecules become a tangled mess unable to support the flow of heritable information. If this is

true, then biologists should set their expectations about the possible range of variation in the backbone structure of RNA and proto-RNA molecules to the set of molecules with a repeating charge along the backbone. The range of possible variation represented by semifactual models shows that the actual variation observed in the backbone structure of DNA and RNA is not a subset of what would be expected – at least when it comes to the feature of molecular charge. The repeated charge along the backbone of DNA and RNA may not qualify as a *biological* constraint on these grounds.[2] Crucial to the function of RNA's sugar-phosphate backbone is its ability to rule out extremely rigid conformations. A further question is whether the backbone of RNA deviates from what one would expect when examining semifactual models.

Another lesson alternative biochemistries of life can provide is what range of variation is possible, at least in principle. Consider the work in synthetic biology to expand the genetic code by developing alternative codon-tRNA-amino acid assignments with orthogonal tRNA and orthogonal amino acyl-tRNA synthetases. This work typically involves assigning an artificial amino acid to one of the three "stop" codons (Liu and Schultz 2010). Orthogonal tRNA associates a specific nonstandard amino acid with a "stop" codon with the same degree of specificity as naturally occurring tRNAs. The successful expansion of the genetic code is a test of the hypothesis that any change to the genetic code is likely to be lethal in living systems that make use of a large number of proteins.[3] Semifactual models of expanded genetic codes serve as a proof of principle that the genetic code may be (both currently and in the past) more evolvable than Crick had originally theorized (Koonin 2017; Plutynski 2005; Kendig 2015; Elliott 2021).[4] If semifactual models indicate that, indeed, a wider range of variation in the genetic code is possible, then the universal genetic code's invariance supports the hypothesis that it may be a biological constraint.

Importantly, semifactual models may serve an analogous role related clades play in phylogenetic analysis, this alone is not sufficient for estimating the possible range of expected variation in a trait. Phylogenetic reasoning in evo-devo involves (i) tracing the distribution of variation in related clades, and (ii) showing that related clades are subject to the same selection pressures. Scientists engaging in this kind of reasoning with semifactual models spend much effort satisfying (i). Insufficient effort is paid to satisfying (ii) as well. Synthetic biologists would do

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[2] It may be that the repeated charge of the backbone structure constitutes a chemical constraint instead.

[3] Crick's theory is commonly interpreted as saying that changes to the genetic code are unlikely to be viable in modern living systems – especially in eukaryotic organisms whose genomes are relatively large.

Expansion of the genetic code has, indeed, been successful to some degree in a variety of eukaryotes.

[4] Evidence for the evolvability of the genetic code is also supported by nonstandard genetic codes in wild microbial populations as well (Ivanova et al. 2014).

well to expand their modeling efforts to account for analogous environmental conditions in which, say, the sugar-phosphate backbone of RNA originated.

6. Conclusion: The Scope of the Life Sciences

The work of synthetic biologists illustrates that the focus of biology extends significantly beyond what is actual or biologically normal – contrary to traditional views in philosophy of biology (Hull 1978; Waters 2007; Weber 2013). Alternative biochemistries of life serve as semifactual models for how aspects of the universal system of heritable information might have been different (Knuuttila and Koskinen 2021). I've argued that alternative biochemistries of life are epistemically important for helping scientists test hypotheses about universal biological constraints. Testing hypotheses about biological constraints ordinarily involves tracing the distribution of variation in a trait in related clades to arrive at an estimate of an expected range of variation (Gould 1989). However, such a method is not naturally or actually available for universal biological constraints. I argue for treating alternative biochemistries of life as semifactual models that serve an analogous role as related clades do in estimating the range of possible variation for components of the universal system of heritable information. Clarifying this role not only illustrates the epistemic value synthetic biology has for more traditional areas of biological inquiry, but it also sheds light on further research efforts that synthetic biologists should pursue that would lend greater rigor to theorizing about universal biological constraints.

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