Title: It's life Jim, but not as we know it

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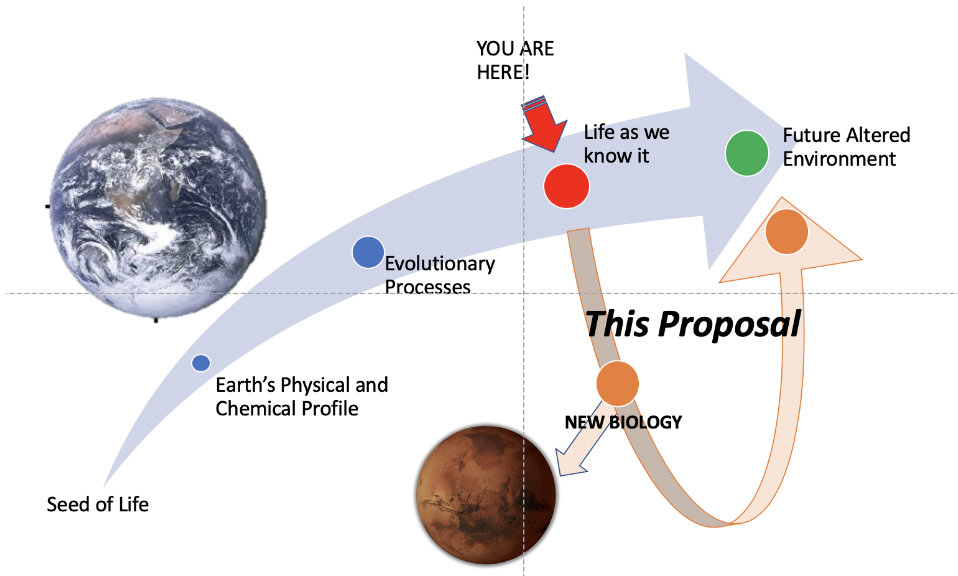


Introduction

Our understanding of life and the potential for variation in biology are based on what we have observed on Earth. Irrespective of if life exists on other planets, the potential "biological space" far exceeds what we have historically experienced (Figure 1). This unexplored space, limited only by the universal physical laws offers the potential for new biology. New technology can now allow us to explore this space offering the opportunity to harness novel mechanisms and understand new concepts potentially expanding the possible biological solutions to current technological and environmental challenges. Moreover, understanding the similarities and differences between this unexplored space and the biology of life on Earth will allow us to establish the rules of life and understand the limits of our existence.

The overarching goal is to understand the influence of specific constraints on the origin and maintenance of life on Earth. These constraints can be classified into three broad groups - (1) those that emerge from the universal laws of physics, (2) those that emerge due to the environment (e.g., elemental composition, the availability of nutrients, pressure, temperature, etc.,) and (3) those that derive from the specific mechanisms and trajectories of evolutionary and selection pressures (Box 1). While the constraints imposed by the laws of physics cannot be altered (hard constraints), new and emerging technologies allow the modification of many environmental conditions and indeed generate others that are not available in nature (soft constraints). Such an approach provides the opportunity to enhance robustness under specific conditions, evolve new functions from precisely defined initial states and perhaps, create new biology that does not exist or has not existed in the natural world. There are many reasons for conceptualizing such an approach that go beyond obtaining a fundamental understanding of the emergence and diversity of life on Earth e.g. the creation of new organisms whose requirements for survival are different from those conditions found currently in nature perhaps by increasing the ability to survive under rapidly altering environmental conditions, lack of specific nutrients, etc.

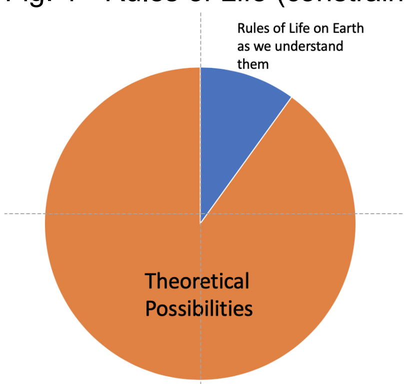
However, the major challenges in implementing these ideas go beyond the obvious experimental difficulties and include identifying all the major constraints and determining the nature of their interactions that have shaped life as we know it.

We are aware that the ideas proposed here will have ethical implications. We argue that these exploratory experiments, though uncontrolled, are already happening in nature due to human-caused changes to the environment. So we feel that it is essential to initiate these discussions on what is, and could be possible, so in the near future, we can work with social scientists, science policy-makers, ethicists, etc. to prepare for these new circumstances. The social and implications of these ideas, although important, are not the focus of this proposal.

Why now?

The rapidly changing environmental and climatic conditions today challenge the long-term survival of much of the diversity of life on Earth that in turn directly or indirectly affect human survival. It is necessary to explore all options to maintain life under more and more extreme conditions. By addressing how environmental changes that are largely mediated by human activity act on life's constraints and thus lead to differences in diversity at the genetic, cellular, and organismal levels, we can anticipate and possibly manipulate the effects of biodiversity loss at the ecosystem scale. In addition, new technologies provide powerful opportunities to enable organisms to escape from prior constraints to exist in new environments. These new technologies can lead to new economies requiring a novel work-force.

Fig. 1 - Rules of Life (constraints) on Earth relative to theoretical possibilities.



Hard Constraints

Physical Forces: Gravitation, electromagnetism, the weak force, and the strong force

Soft Constraints

<u>Earth's Physical-Chemical Profile</u>

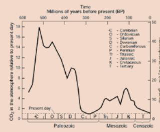
Elemental Profile
- Abundant Carbon
- Abundant Water
- Mineral profiles and Mineral accessibility

Temperature range & distribution
Solar light spectrum
- Frequency Distribution
- Light intensity
- Variation in intensity

24h Day
Seasonal variation
Day-length variation
Atmosphere that protects from radiation
Position of the magnetic poles

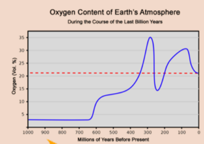
<u>Earth's History</u>

History of atmospheric content
- CO_2 (high, low, high, low)



Historic atmospheric CO_2 levels image from MacKenzie, 1996

- O_2



Historic atmospheric O_2 levels image from Wikipedia

Catastrophic events

<u>Biological History</u>

Starting points for evolution

The process of evolution evolution of life

The feedback of life on Earth's physical properties

Box 1: Life exists in the form as we know it because of not only the hard constraints imposed by the laws of physics but also because of the soft constraints of Earth's physical profile, physical and biological history, and the nature of evolution. Some example constraints for each category. These examples are, of course, not exhaustive.

What can we do with the knowledge about how constraints historically and currently shapes life on Earth?

1. *Better fundamental understanding of life on Earth during the current epoch*

A fundamental understanding of the constraints that shape life, and their connections to each other, will help us address important outstanding questions at various scales of biology. Exploring the existing environmental diversity is one approach that can help us understand how constraints shape the rules of life that we observe. Additionally, technological advances now enable us to manipulate many aspects of life on earth, and certain experimental manipulations are now possible at different biological scales that will help us elucidate the most critical constraints and limitations for life. However, it is extremely challenging to integrate insights about constraints on life across scales (atomic to molecular to organism to ecosystem). Establishing a framework for how to scale across different biological levels would be beneficial.

2. *Prepare for future altered environments here on Earth*

A better knowledge of the constraints on life will enable us to prepare for Earth's future altered environment.

- New environments are being created by human actions; appearance of non-native invasive species with increased globalization, increased urbanization and crop production leading to homogenization of landscapes and decreased biodiversity, presence of persistent plastics in the environment, etc.,. We could identify organisms whose survival is most threatened by these novel conditions to potentially enhance their possibilities of survival.

- There are potential large-scale economic consequences of rapid environmental change. For example, crop plants evolved at (or are adapted to) a certain range of environmental regimes, those we have experienced on Earth for the last few decamillenia. As environmental patterns change rapidly, this has combined effects on crops, pests, and beneficial microbes. Understanding how the environmental constraints limit growth, we could anticipate how crop plants adapt to changing environmental conditions and where the critical tipping points are. When those conditions change, we can anticipate which food systems may be in danger of collapsing.
- Similar predictions can be made at various scales. How might the system react at the cellular, organism, population, community, and ecosystem levels? Human history provides examples of cellular events that have widespread ecological consequences e.g. the 14th century "Black Plague" (Stenseth et al., 2008).

3. *Prepare for future environments away from current constraints on Earth*

An understanding of the constraints that shaped life on Earth and their interactions and limitations will help us design life away from those constraints. For instance, colonization of Mars by humans will be aided by the ability to anticipate which current Earth-originated lifeforms as well as which engineered novel synthetic lifeforms can thrive in an environment with different gravity, atmospheric pressure, day-length, geochemistry, etc.

4. *Use knowledge for synthetic design.*

When understanding the constraints acting on life on Earth, with some creativity, we can redefine bioengineering. We will not be able to alter some of the constraints, but others we can circumvent or ignore the effects of others in certain instances. This will open up a new space for designing novel forms of synthetic life.

5. *Use knowledge for directed bioinspired design*

Understanding the constraints of life will aid in the development of successful bioinspired design defined as using biology to inspire novel technological innovations. The process of bioinspired design can be made more efficient by using evolutionary analysis, but that will require a knowledge of the constraints working in the design space for which you are designing the new technology (Patek, 2014).

How does this Drive Integration of Biology?

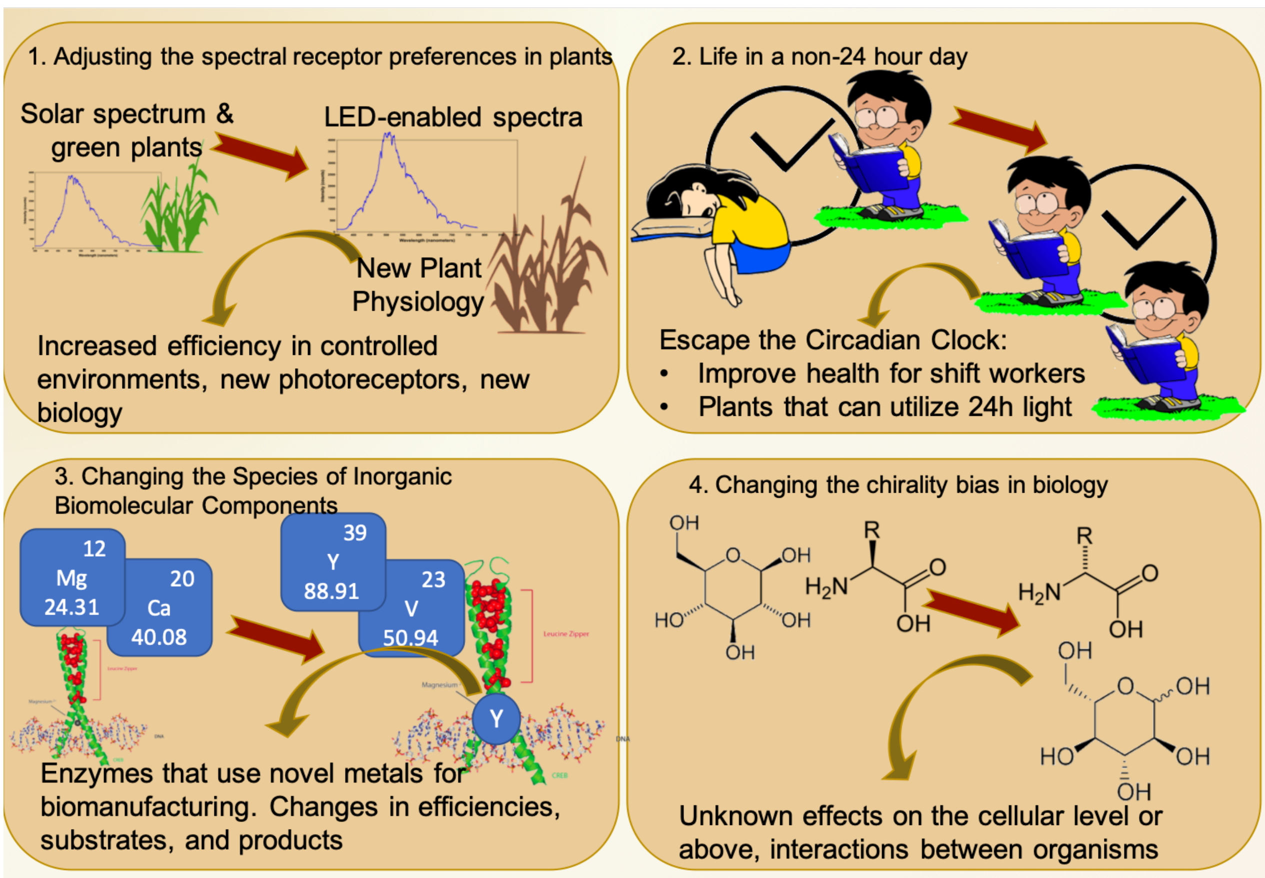
The question of how life might differ if it had not occurred under Earth's conditions is not a new one. It has long been a subject of science fiction, as well as planetary biology and astrobiology. However, for the first time, we have the opportunity to test these questions and apply them to practical and urgent human needs. Directed evolution experiments offer an approach to exploring this space and have been successful in designing new functionality e.g. the ability of enzymes to efficiently utilize novel substrates (Arnold, 2018). But an integrated biological approach offers the opportunity to explore these ideas in a visionary way that goes beyond manipulating individual biomolecules. For example, by combining directed evolution experiments with chemical biology tools optimized to a specific outcome, we can determine how the starting conditions impact the final outcome. Synthetic biology also offers the opportunity to change the starting point and examine how evolution would change if the trajectory started in a different possibility space (e.g. novel nucleotides, changes in chirality, the presence of unnatural amino acids). Thus, through the integration of multiple biological approaches, we can bring together the expertise of researchers in evolution, molecular biochemistry, material science, computational science etc. to both generate a (better understanding) and creatively apply the outcomes of this research in new and exciting ways.

Examples of how we might use knowledge about constraints
(This will be graphically represented in our final draft)

1. Solar -> LED lights
    a. Constraint: solar spectrum
    b. What we observe: green plants
    c. New technology: Light-emitting diodes with a specific spectral profile
    d. Outcome: Red plants + the availability of red light for other uses
        i. New biology- how would plant light sensors be different if they monitored green light
2. 24 hour day
    a. Constraint: Earth has a 24h day
    b. What we observe: most organisms have biological clocks evolved to account for the 24 hour day
    c. New Technology & New environment: tech: Plants that can thrive in 24 hours of light, maximizing growth: facilitating shiftwork for people
    d. Outcome: If we know how this constraint shapes biology we can “escape the clock” - improved health for shift workers and increased yield in crop plants
3. Mineral profile:
    a. Earth has accessible Mg^{2+} (and Ca^{2+} and Fe^{3+})
    b. What we observe: many proteins principally use Mg^{2+} in cells though other metal ions could be more efficient for specific processes (e.g. (Bray et al., 2018)). Organisms also primarily use Ca2+ as a signaling molecule, but other molecules could work. Are these the most efficient, or simply the consequence of what was available and selected as life began in primordial Earth?
    c. New Technology: The elemental composition of specific environments or biology could be changed. For example, in bioreactors, enzymes selected to favor new metal co-factors could have enhanced activity, stability, or substrate specificity. Outcomes: Could enzymes that use different metals be
        i. More efficient
        ii. Produce new products
        iii. Escape current rate limits
        iv. Use new substrates
4. Chirality (Nature utilizes L-amino acids and D-sugars)
    a. Constraint: Unclear

b. What we observe: Molecules have a specific chirality
c. New technology: change chirality
d. Outcomes: Unknown effects on the cellular level or above

5. Folding: In proteins, only observe a fraction of the structurally possible folds is observed.
    a. Constraint: Physics of interactions encoded in a protein sequence (but unknown why we only see a fraction of the folding possibilities).
    b. What we observe: of all the possible protein folds we only observe a fraction in biology
    c. New technology: we can fold proteins in different conditions (e.g., microgravity)
    d. Outcomes:
        i. An understanding of fundamental biology
        ii. New proteins- new enzymes- new technologies- new economies
6. Microbiome vs holobiome
    a. Constraint - presence/absence of the different microbes
    b. What we observe: holobiome has different fitness based on presence/absence of microbiome components
    c. New technologies: we can manipulate the microbiome and make the holobiome better adapted to a different environment
    d. Outcomes: survival of holobiome



Box 2: Examples of potential new biology that could develop from understanding how historical and physical constraints shape life on Earth.

Limitations and Roadblocks

Identifying all possible constraints that influence life for any specific organism can be a difficult problem, and elucidating all of its interactions within the space of the other constraints is likely intractable. Using the extent that their effects can be altered in an experimental setting. The hard constraints, mentioned above, are immutable factors that cannot be altered, including, for example, the laws of physics, and they represent the absolute limits of what will ever be accessible experimentally. Soft constraints may be altered using current and future technologies. These constraints represent the space of variables that may be manipulated by the experimenter. These soft constraints exist on a continuum, from those that are easily manipulated to those that are extremely difficult to alter.

How would one proceed to identify the various constraints on a system? Perhaps a simple place to start would be to start with a simple, mailable manipulatable organism e.g. *E. coli*, that is a fast grower under a variety of precisely defined conditions. The major constraints that influence growth under a set of conditions would then have to be identified through existing insight combined with trial and error. However, this would get to be increasingly more difficult in higher organisms e.g. going from *E. coli* to yeast and from yeast to a multicellular species like *C. elegans.* These studies would have to be driven by large-scale computational models based in part on the available experimental data with experimentally testable outcomes. Extension to colonies and accounting for cell-to-cell variations even in systems as simple as *E. coli* would represent additional levels of complexity and would require the development of novel experimental and theoretical tools.

It is evident that the implications or consequences of changing any organismal characters or processes also be difficult to predict. This necessitates serious ethical reflection before any experimental manipulations of life. This will require careful collaboration between biologist and bioethicists to enable careful consideration of the ramifications of any future experimental manipulations. However, we do not touch on these ethical issues in this paper.

Conclusion

Life on Earth evolved under a set of constraints that if released or altered would have generated a different trajectory and we would perhaps have a different distribution of life with different biology than we have currently. While determining these constraints and indeed the interactions between them may be an intractable problem in general, significant insight may be obtained in working with simpler homogeneous or heterogeneous systems that are experimentally tractable. This will allow new functions to be created, perhaps lifeforms with different biology to be generated. The possible applications are numerous and include the generation of new crops and biotechnologies with their obvious economic effects. However, significant ethical concerns that will certainly arise will need careful consideration.

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Refs in illustrations
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