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Pre-template Metabolic Replicators: Genotype-Phenotype Decoupling as a Route to Evolvability

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Abstract

The RNA World is widely heralded as the leading candidate for a template first vision of the origin of life, yet doubts as to the plausibility of the natural formation of RNA with catalytic function have led to revived interest in the metabolism first paradigm. Recent studies of the evolvability of reflexively autocatalytic sets of polymers have also revealed the nature of limited heredity in compartmentalised reaction networks. In the algorithmic sense, the lack of a meaningful distinction between data storage and functional expression results in a protocell heredity-fitness dichotomy which gives an intrinsic selective advantage to those protocells with limited heredity. An idealised model is used explore a minimal set of dynamical requirements necessary to weaken the dichotomy. This is achieved by explicitly modelling a protocell as an outer compartment 'phenotype', heritable only indirectly, in which competitive exponential growth may occur without compromising the heritability of previously discounted non-competitive growth autocatalysts in an inner sub-compartment, 'genotype'. Results show that heritable variation can be achieved under simulations of natural selection in populations of such metabolic replicators.

Introduction

Evolution by natural selection as proposed by Charles Darwin represents the founding piece and current cornerstone of the modern synthesis - the most complete description of how populations of organsims accumulate adaptation. However, because the mechanisms and units of heredity, variation and selection are considered to be static, the rigidity of the framework that gives it its explanatory power also prevent it from addressing the problem of just how nature came to adopt the Darwinian machine (Calvin (1997)) in its current form. A more complete model of the evolutionary process must then include a description of how the above mechanisms evolved (Pigliucci (2008)). Such evolution of evolvability is essential for the major transitions in evolution (Smith and Szathmary (1997)) and seems an especially relevant issue to consider in any candidate model for the origin of life. This problem is in part that of describing the emergence of a reproduction, variation and heredity dynamic (Maynard Smith (1986)). This criterion though does not truly embody the algorithmic nature of the minimal machine believed to be requisite to the higher order evolution of evolvability and open-ended evolution. A key requisite considered here is the physical decoupling of the data storage device and the processing machinery responsible for functional expression and self-reference (Walker and Davies (2013); Ruiz-Mirazo et al. (2008)).

This paper is intended to summarise an ongoing investigation into the dynamical requisites of a purely metabolic replicator that could simultaneously permit non-trivial heredity, micro-mutation and the resulting phenotypes to be selectable/heritable. That is respectively, a parent replicator with non-trivial heredity must be able to transmit its phenotype to its progeny reliably, these phenotypes must be able to undergo small variation while they must also be selectable in a population. Together, this amounts to heritable variation. Using a minimal set of assumptions necessary, it is demonstrated that a duality between directly inherited autocatalytic molecules (genotype) and indirectly inherited molecules (phenotype) permits such heritable variation. In dynamical terms this corresponds to non-competitive autocatalytic growth in a protocell replicator's inner compartment and faster competitive growth in a disposable outer compartment. Algorithmically, this can be interpreted as the decoupling of the pseudo-instructional data storage and functional expression roles, reminiscent of aspects of gene-protein duality.

The first part of this paper summarises the spectrum of models of the origin of life. Then, an abstracted model of a metabolic replicator is introduced, designed to embody the key dynamical aspects of, and problems with, current models of compartmentalised metabolic reaction networks (protocells). In the model we present here, protocell fissioning dynamics are modified to distinguish fissionable material from non-fissionable (transmittable/non-transmittable to daughter protocells). This is designed to weaken the dichotomy, both allowing a kind of combinatorial heredity permitting micro-mutation, while also increasing the intrinsic fitness of these protocells that makes such variation heritable.

Template and Metabolism based Heredity

The two schools in origin of life research are broadly represented by the template first and metabolism first paradigms. The divergence is caused by the observation that both templating and metabolic function play distinct but intricately coupled and essential roles in all contemporary life. Linked genomes play the role of data storage that ultimately codes for protein enzymes, which elegantly guide biological metabolism, including the replication of the genome. In the current biological context then, each seems requisite to the other, giving rise to the chicken and egg paradox. The RNA World is the dominant candidate for a template first vision, in which naturally forming RNA strands contain sequential nucleotide data and also act as catalyst for their own base pairing replication (Gilbert (1986)). If they are able to multiply with heritable variation, then they may be able to evolve meaningfully under natural selection. Significant doubts have been cast as to whether any such polymer with both capacities, RNA or otherwise, could have formed naturally in the pre-biotic world (Bernhardt et al. (2012)). This kind of system relying on sequence based heredity stands in contrast to models of compartmentalised metabolic reaction networks that rely on attractor based heredity (Szathmáry (2006)). This is the distinction in the units of heredity between sequential instructional data that is internally inert until copied, and information contained as concentration profiles whose replication and hence heredity is the result of autocatalysis. Such models typically assume compartmentalisation of a repertoire of well mixed chemically autocatalytic units. These units are either autocatalytic at the level of the particle or some reflexively autocatalytic food generated set (RAF sets). These units would be potentially heritable as the numbers for each distinct unit type increase through autocatalysis until an enclosing compartment fissions into two. Multiplication and heredity at the compartment level results from the fissioning into daughter compartments, transmitting concentration profiles. Variation is desired as a consequence of the stochastic gain/loss of units during the growth and fissioning processes.

Alexander Oparin was the first to advocate the idea that spatially localised coacervates comprising varied chemical mixtures could have metabolised from the environment and become subject to natural selection. The first attempt to investigate the potential for heritability in metabolic models was taken by Farmer & Kauffman (Farmer et al. (1986); Kauffman (1986)). They studied artificial chemistries of protein polymers equipped with a capacity to catalyse ligation and cleavage reactions. Their aim was to investigate the conditions under which RAF sets of such polymers would form. They were motivated by the idea that when compartmentalised, the possible exponential growth of autocatalysts could both describe their own self-replication as units of heredity, and also confer exponential growth in compartment mass and hence exponential increase in compartment numbers. Selection would conceptually operate at the compartment level and those that increase in mass fastest would fission and multiply at a higher rate (have a higher Darwinian fitness). Compartments containing different RAF sets could then compete under natural selection. Others took this idea further, investigating the potential for heritible variation in these compartmentalised reaction networks when undergoing growth and fissioning cycles (Bagley and Farmer (1990); Bagley et al. (1992)). The idea was that if nature provided some form of spatial structure, such as naturally forming lipid micelles, vesicles, micro-spheres or coacervates, they could enclose the reaction network, preventing it from freely mixing within the environment. Other models have since used the same principles of imposed spatial segregation and autocatalysis, such as the GARD model of a Lipid World (Segré et al. (2000)) and Fernando & Rowe's model of chemical avalanches in reaction networks enclosed in liposomes (Fernando and Rowe (2007)). It seems that if such entities were in fact able to multiply with heritable variation, a set of models exist that assume a much lower level of early world chemical complexity than that of RNA like templating molecules. However there are in fact serious problems with limited heredity.

More recently, Vasas et al. (2012) have investigated the evolvability of the Farmer & Kaufmann type metabolic polymer networks. The essential results of the models were that only a single autocatalytic unit could typically be stably inherited by any single compartment and that heritable variation was in principle possible because stochastic processes could allow loss and gain of a compartment's complete repertoire of each unit. As Vasas et al. put it, this constitutes only one heritable bit of information. This does not seem to allow a meaningful concept of heritable micro-mutation, even if each unit as a set, had more than one attractor. The underlying problem is that the exponential increase in protocell numbers necessary for natural selection to act strongly at that level is caused by the necessarily independent and exponential growth of the chemically distinct units contained within. In general, this results in intra-compartment competition between units, which because of their exponential growth form inevitably results in only the fastest growing surviving within a compartment lineage; hence one heritable bit of information.

Model Aims and Methodology

This is not an investigation into the structure of reaction networks. The aim of this model is to identify a minimal set of physical and chemical requisites to heritable variation in a metabolic model of compartmentalised autocatalytic sets. As such, we focus on symbolic representations which we believe captures the relevant properties and problems. The ultimate aim would be to realise the model results in vitro, but here we aim only to use a theoretical tool to highlight the problems and identify a solution within the space of our

model. The purpose of doing this is to provide conceptual tools that might help elucidate the crux of the problem.

We wish to represent two extreme RAF set growth dynamics possible in artificial chemistries. One is that of unconstrained exponential/competitive growth (if no resource constraint). This is designed to approximate the growth form associated with autocatalytic reactions that is explored in most metabolic attractor based models. The other is that of some form of strongly self-regulating/non-competitive growth. The motivation for only including these growth types is that when compartmentalised they respectively represent the growth form that confers rapid exponential increase in compartment numbers (high intrinsic protocell fitness f), and the growth form that might best allow non-trivial heredity - non-trivial because an ensemble of distinct self-regulating units do not necessarily increase in number competitively. As such, in principle, they are capable of collectively and stably transmitting concentration profiles through a lineage as well as allowing additional such units to be lost or gained through stochastic processes, ie. allow multi-bit heredity. Summarising this point - protocells containing units that only grow competitively gives highly fit protocells, but poor heredity and those with units that grow non-competitively should give protocells with very low fitness but much better heredity. This represents an extreme tension between fitness and hereditary potential.

Non-competitive growth has been largely neglected in the literature on the evolvability of compartmentalised reaction networks for good reason. The protocell fissioning dynamics of current models in which the dichotomy exists uses a protocell which phase separates internal and external material in the environment only. In such cases if it is the protocell that selection operates on, the fittest compartment is the one that grows and hence multiplies fastest, not the one that has good hereditary.

What we aim to do is identify the minimal modifications to the protocell growth and fissioning dynamics that would break the tension between heredity and intrinsic fitness. For this purpose, we assume a two tier protocell system comprising an outer compartment which also houses an inner one. The inner compartment has much the same role as in other models - when a protocell (size of inner + outer) reaches a determined size, its contents are fissioned into two daughter inner compartments. However, during protocell growth, material is also allowed to leak from the inner into the outer compartment. Material in the outer is not directly transmitted to daughter protocells upon fissioning, but assumed washed back into the environment. The idea is that if an inner compartment can host non-competive units, when they leak to the outer compartment, they might either exhibit a competitive growth form due to different spatial constraints, or trigger competitive growth in the richer chemical environment of the outer compartment. Details of this dynamic are described in the following section, but the purpose of this modification is to reduce the large difference in intrinsic fitness between protocells whose inner compartment contains only non-competitve units, and those that contain competitive units by allowing both growth types to co-exist in different parts of a protocell with different function. The inner compartment could act as reliable data storage, while the outer does the bulk of the metabolising, providing the protocell level competitive growth.

Model

In our model, protocell growth and fissioning dynamics are similar to other models in which compartmentalisation of a chemical reaction network is imposed; the dynamics of a contained reaction network is run until the total particle numbers in the inner and outer compartment reaches F particles, then the inner compartment contents are stochastically fissioned into two daughter protocell inner compartments and next generation begun. The key difference is in the protocell structure. We assume a two tier structure in which only an inner compartment is fissionable into daughter compartments while the outer is lost to the environment during fissioning. During the growth phase, material is allowed to leak from the inner compartment to the outer where the chemical dynamics continue, as shown in figure 1.

We do not model a large reaction network, but work with an idealisation of only six types of autocatalytic unit, sacrificing rigour for clarity of principle and operation. We define three of them to grow in number exponentially at different kinetic rates in the inner compartment. These we call type A units. The other three are assigned the same rate constants, but growth forms designed to represent concentration dependant self-regulation in the inner compartment. These we call type B units. When in the outer compartment, both A and B types grow exponentially. The reason that we use three of each is that later we will test protocells containing each of the two types for heritable variation, and three is the minimum number necessary. An example of such a candidate self-regulating reaction is one in which a reactant catalyses its own production from some food set, but also catalyses the production of another particle that either inhibits the first reaction, destroys the unit or otherwise removes it from the system in such a way that both its growth and associated products grow non-competitively in mass. This type of reaction is reasonably well understood in chemistry and within the possibilities of a polymer chemistry such as that of Farmer & Kaufmann. Multi-particle examples are the Brusselator or Oregonator (Nicolis and Prigogine (1977)). It is not necessary for our purposes to describe in detail the many ways a reaction network could realise self-regulation in a many particle system, only to recognise the existence of reactions with either attractors or stable limit cycle solutions for the autocatalytic unit (or for many particle RAF set if autocatalytic unit is not a single particle autocatalyst). We model a single autocatalytic unit species and describe its

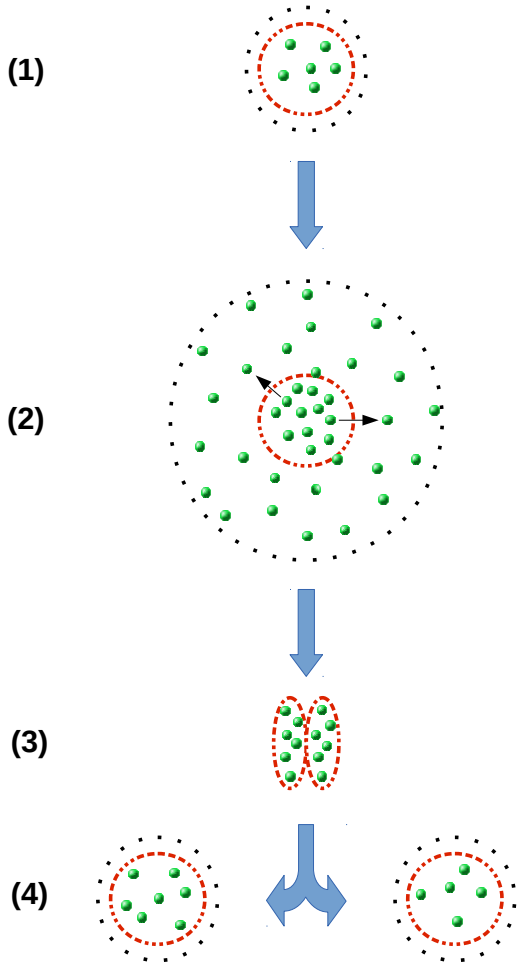

Figure 1: (1) Post-fissioning daughter protocell inner compartment. (2) Growth phase describes by chemical kinetics with uni-directional material leakage to the outer compartment with low volume density. (3) External agitation causes disruption to protocell when contained particle number reaches F. Outer material is washed away and inner compartment fissions each particle into one of two daughters with probability 0.5. (4) Two new daughter protocells are created and the process repeats from (1).

growth form as largely logistic. This was chosen to embody extreme non-competitive growth.

Chemical Kinetics and Protocell Growth Dynamics

The rate equations for $i = 1, ...N$ autocatalytic units with number x_i inside the inner compartment and y_i outside are used to model the growth of each type in a given protocell. In the following rate equations A type units are represented by species $1, 2, 3$ and B type units by $4, 5, 6$. Equations are numerically integrated and deterministic with integer valued particle numbers found by rounding the integrated equations at the end of a growth cycle.

Inner compartment kinetics:

$$\dot{x}_i = k_i \frac{x_i}{\left(\sum_{j=1}^{N} x_j\right)^p} - D x_i \qquad i = 1, 2, 3, \tag{1}$$

$$\dot{x}_i = k_i \frac{x_i}{\left(\sum_{j=1}^{N} x_j\right)^p} \left(1 - \frac{x_i}{C_i(V_I)}\right) - D x_i \qquad i = 4, 5, 6. \tag{2}$$

Outer compartment kinetics:

$$\dot{y}_i = k_i y_i + D x_i \qquad i = 1, 2, 3 \tag{3}$$

$$\dot{y}_i = k_i y_i \left(1 - \frac{y_i}{C_i(V_O)}\right) + D x_i \qquad i = 4, 5, 6, \tag{4}$$

where $C_i(V) = c_i V / V_I$ and c_i is the carrying capacity for unit species i in volume V when $p = D = 0$. V_I is an assumed volume of the inner compartments and V is the volume of either the inner (V_I) or outer (V_O) compartment. Together with the assumption that $V_O/V_I \gg 1$, we treat the factor of the second term in equation [4], $y_i/C_i(V_O)$, as vanishingly small for the y_i ranges of the simulation. D is an approximation of a diffusion coefficient in the case of negligible outer compartment concentrations - we assume that concentrations in the outer compartment are low enough to ignore the actual internal-external concentration difference dependence of diffusion rates. It would also be desirable to represent some form of resource constraint on autocatalytic growth in the inner compartment that is most isolated from the wider environment and theoretical source of food species. Kaufmann, Farmer, Fernando & Rowe, Segre et al., all model resource constraint implicitly by assigning a rate at which food species leak into protocells. We have chosen to neglect detailed kinetics of such reaction dependencies, but would like to describe a monotonic reduction in the per particle resource as particle numbers increase. The ad hoc means by which we do this is to include a $1/(\sum_{j=1}^{N} x_j)^p$ factor for internal compartment unit growth. The effect of this is to apply an effective cost to growth in the inner compartment, modulated by p.

When placed in an inner compartment, the self-regulating B types will grow in a logistic like manner up to an effective carrying capacity (though not always $C_i(V)$ due to the resource constraint and D term). With the $V_O/V_I \gg 1$ assumption, they will diffuse into the outer compartment and begin true exponential growth. A type units will grow at up to exponential rates inside and truly exponentially outside.

The rate equations basically do two things. First, they distinguish growth forms between the inner and outer compartments. As mentioned, this is to apply a cost to all inner

compartment growth. The second, is to distinguish self-regulating growth of type Bs from type As, but only in the inner compartment. The $V_O/V_I \gg 1$ assumption gives them similar exponential/competitive growth in the outer compartment.

The reason that existing models do not properly consider autocatalysts that inhibit or remove themselves from the system, to be good candidates for units of heredity is because of the heredity-fitness tension mentioned in the previous section. That is, if a protocell were to contain only on phase seperated region, it would take far too long to reach a physical size at which it would fission, ie. it would be incredibly unfit. What allowing a non-fissionable outer compartment to do is be the medium in which self-regulators are allowed to change their growth form to competitive/exponential so that they can impart higher fitness upon their host compartment. As the growth form in the inner compartment is still self-regulating and the outer is not fissionable, this will not damage heritability of inner compartment concentration profiles so long as the protocell as a whole is still fit.

Assumptions

Many assumptions of the model are idealised representations of assumptions and results of existing models, such as the existence multiple autocatalytic sets in some chemistry, treatment of low particle number systems as well mixed, chemical dis-equalibrium caused by influx of energy rich food set and removal of material from the system, growth of compartment membrane material, and the compartment particle number dependence of the compartment fissioning parameter. The novel assumptions, also in idealised form are the following:

- There exist RAF sets that chemically self-regulate in a concentration dependant manner, which we approximate as single unit particulate autocatalysts.
- The spatial structuring in an environment is not described solely by a single phase separated protocell, but two phase separated regions, an inner and an outer, in such a way that a protocell's collective particle number contributes to compartment fissioning rates (intrinsic fitness f), but only the inner is fissionable.
- Outer and inner compartments have fixed volumes and $V_O/V_I \gg 1$.
- A $1/(\sum_{j=1}^{N} x_j)^p$ factor in the inner compartment kinetics describes a common resource constraint.

Recent work by Vasas et al. (2012) found that the number of exponentially growing autocatalytic cores (irreducible RAF sets (Hordijk et al. (2012))) was far exceeded by the number that were kinetically incapable of growing exponentially. It is in part from this result that we are motivated to assume the existence of self-regulators in this idealised artificial chemistry. The assumptions with regard to a protocells's structural features are more ad hoc; notably the ability of the outer compartment to contribute to the physical instability of the inner compartment while being non-fissionable and at very low concentration. It is possible that multilamellar liposomes can provide some of this functionality, but this has not yet been explored.

We proceed with these as assumptions in this model while keeping the many alternative possibilities for future work, such as the ability of outer compartment autocatalysts to ignite other reaction pathways, which would not require the $V_O/V_I \gg 1$ assumption.

Hypotheses

- Combinations of self-regulating autocatalytic units can be faithfully transmitted down a lineage and protocells can undergo micro-mutation caused by stochastic loss or addition of distinct unit species.
- A two tier protocell in which outer compartment material is distinguished from fissionable inner compartment material will allow near unity intrinsic fitness f_B/f_A ratios of protocells characterised by self-regulating/non-competitive B type and non-self-regulating exponential A type units. This together with faithful transmission of concentration profiles between generations will permit heritable variation under simulations of natural selection.

Reducing the Intrinsic Fitness Disadvantage of Self-Regulators

If self-regulating units can be transmitted in combination, in order to demonstrate heritable variation, narrower intrinsic fitness differences between protocells containing only type Bs versus type As must be achieved. Otherwise the potential increased heredity allowed by self-regulators will not be heritable as artificial selective advantages assigned will not be great enough to overcome the intrinsic fitness differences. The first task was to identify which combinations of D, k_i, N_{max} and p would best reduce the intrinsic fitness differences of the protocells characterised by each type. The caveat was that after the growth phase, near complete regrowth of the B types in inner compartments would be necessary if combinations of distinct B types are to be heritable. If B types do not reach their effective carrying capacity in each growth cycle, then they will start to compete with one another within inner compartments within a lineage. This can be seen even in the logistic growth approximation for unit types 4,5,6; for particle numbers significantly below carrying capacity, growth is dominated by the first term in the kinetic equations, which potentially gives exponential growth (though will typically be of a parabolic/algebraic form due to the resource constraint assumption).

In all simulations in figure 2, the model was run through 100 growth and reproduction cycles to obtain a mean for

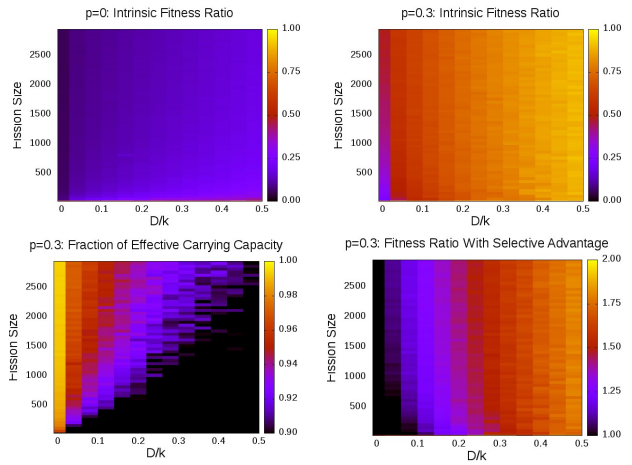

Figure 2: Selection of results from search of parameter space. The upper two plots show the ratio of the intrinsic fitnesses of B and A type compartments $f_B/f_A = T_A/T_B$ for $p = 0$ and $p = 0.3$. T_A and T_B are the mean times taken for protocellss containing A units 1,2 and those containing B units 4,5 to reach fission parameter F. Fitness is defined as their reciprocals. The lower right plot shows the same ratio when a selective advantage is given to the B types by multiplying the corresponding k_i rates by two. The lower left plot shows the total number of B types in an inner compartment prior to fissioning as a fraction of the maximum they can kinetically achieve - their effective carrying capacity.

the relevant observable. B and A type protocells were each seeded with two distinct B units ($i = 4, 5$) or two distinct A units ($i = 1, 2$). Two were used in each so that results would be relevant to later tests of heritable variation. The aim was to identify intrinsic fitness differences and measure the ability of B type protocells to achieve their effective carrying capacity IF a daughter protocell received its parent's full repertoire of unit types upon fissioning. For this reason, if a unit was removed entirely due to stochastic fissioning processes, it would be replaced with a single particle of that unit type.

The upper two plots in figure 2 show the disparity in the intrinsic fitness for $p = 0$ and $p = 0.3$. When $p = 0$ the intrinsic fitness differences are so great for all D that complete regrowth is not particularly relevant because the necessary increase in fitness in any simulation of natural selection would be so great, they would be entirely unreasonable. When a cost to growth in the inner compartment is added by setting $p = 0.3$, as D is increased the bulk of the growth starts to occur in the outer compartment where all unit types ($i = 1, 2, .., 6$) grow exponentially and the intrinsic fitness differences are drastically reduced (at fission size the ratio of total particles in the inner to those in the outer is 0.041 for A type protocells and 0.006 for the B type). The test for whether these fitness differences are great enough is whether increasing the fitness differences by multiplying the logistic type k_i values by a constant (in this case $k'_i = 2k_i$), will bring the ratio f_B/f_A to something greater than unity (changing the reaction rate constants k_i will be how we implement artificial selective advantages in simulations of natural selection for a target profile later). The lower right plot shows that this is the case. So long as this fitness is heritable, this compartment type will be able to compete effectively in a population. The lower left plot is an indication of how heritable such fitness differences might be for compartments of B types. It shows the contained number of particles in the given inner compartment prior to fissioning as a fraction of its maximum effective carrying capacity (separate simulation not shown). If this fraction falls below ≈ 0.9, then the logistic growth starts to become exponential during growth phases, which means competition in the inner compartment for different values of k_i. The results show that low D around $D = 0.2k_0$, $p = 0.3$ and $F = 2500$ will provide the necessary theoretically possible fitness differences, while the effective carrying capacity fraction is high enough to indicate that the inheritance of these differences should be possible. See appendix for parameters.

Natural Selection and Micro-mutation - Heritable Variation

A crude test of heritability is to simulate natural selection acting on sets of protocells in a population. The following tests will allow us to evaluate at a high level whether previously imposed fitness ratios f_B/f_A approaching unity can be achieved in a given selection regime and whether the previously enforced transmission of an inner compartment's repertoire will follow from the earlier requirement that an effective carrying capacity is achieved during growth.

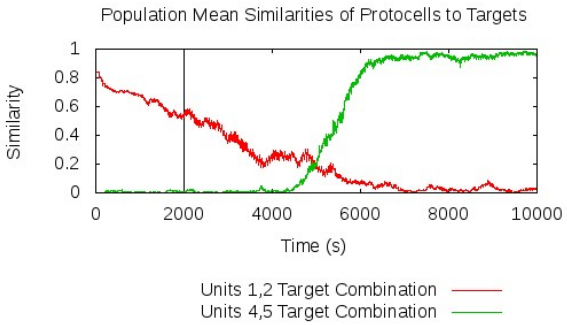

Figure 3: Mean similarity to 1,2 and 3,4 target in two simulations.

Two simulations were run. Stochastic addition and removal of a unit upon fissioning (other than the stochastic sampling process upon fissioning) was not allowed, but occasional migration of small randomly formed protocells con-

taining two particles was. In both, we seeded a population of $N_{compartments} = 200$ protocells, each with a mixture of ten of the exponential units 1 and 2. After 2000 simulated seconds, selection on A type units 1 and 2 was initiated. The second simulation differs only in that selection was initiated for B type units 4 and 5.

Selection was implemented at each iteration of numerical integration by first assessing the similarity of a given protocell's total concentration profile with a target profile. The similarity measure used was the euclidean inner product between the normalised target and concentration vectors. Boltzmann scaling/selection was then used to evaluate a k_i' that was used to replace the normal k_i in the rate equations for all growth in that compartment for that integration step. Specifically, $k_i' = k_i(1 + \exp(\frac{\hat{\mathbf{T}} \cdot \hat{\mathbf{C}} - 1}{S}))$, where $\hat{\mathbf{T}}$ and $\hat{\mathbf{C}}$ are the normalised target vector and normalised concentration vector. Temporarily increasing the rates of reactions is a method used by others in simulations of natural selection for a target (Vasas et al. (2012)). Implementation here differs in that boltzmann scaling is used and the maximum artificial increase is 100% of k_i. See appendix for parameters and target vectors. When a protocell reaches F and fissioned into two daughters, one replaces its parent and the other replaces a randomly chosen protocell in the population.

Figure 3 shows the average similarity of protocells in the population to each target. Even though the populations were seeded favourably for the 1,2 target, the population similarity drops as migrant compartments containing species 3, the species with the highest k_i, invade the population and those already containing 1 and 2 fixate with only unit 2. Within protocell competition results in unit 3, with highest k_i, fixating within protocells and those protocells fixating in the population (as protocells containing only unit type 3 have the highest intrinsic fitness). Neither the targeted 1,2 or fitness is heritable. Invasion of the population by B type 4,5 protocells is possible however because their repertoire and fitness are heritable and so selection is able to induce greater exponential increase in their number until they come to dominance in the population.

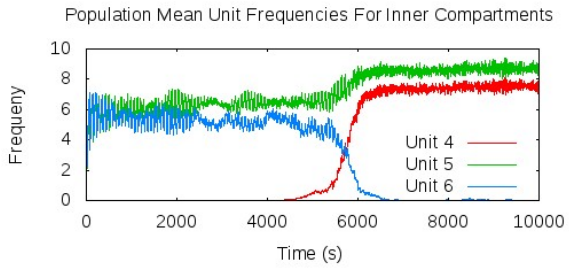

Figure 4: Population seeded with protocells containing unit types 1 and 2. No migration, but unbiased stochastic addition and removal of unit types 1,2 and 3 allowed.

These two simulations of natural selection confirm that there is heritability but do not confirm heritable variation, without which this model would not be demonstrating micro-mutation/heritable variation, but another means of canalisation. To test this, the previous simulation was rerun, being seeded only with unit types 5 and 6. Selection was continuous on unit types 4 and 5. Migration was disallowed as it was heritable micro-mutation/variation we were testing for rather than solely heritability under natural selection. Independent stochastic addition (P_{add} - probability of adding single particle at fission event) and removal (P_{remove} - probability of removing each particle in protocell at each fission event) of single particles of a random unit type of type 4,5 or 6 was introduced.

Figure 4 shows the integration step mean inner compartment particle frequencies. At approximately $t = 5000$, the stochastic fissioning process admits a single particle of unit type 4 to a single protocell which also stochastically and independently loses all particles of unit type 6. Selection is then able to bring this compartment type/genotype to fixation.

Conclusions

The hypotheses that self-regulating/non-competitive autocatalytic units could permit faithful transmission of concentration profiles down a lineage while allowing micro-mutation and that a two tier protocell system would then permit heritable variation has been confirmed. This was achieved by identifying a two tier protocell system in which self-regulating autocatalysts were able to take the role of units of heredity, being directly transmitted to daughter protocells upon fissioning, while the bulk of the protocell's growth mass, and hence intrinsic protocell fitness, was achieved in an outer phenotype like region of the compartment where unconstrained exponential growth could contribute to fitness without harming heredity. The key physically meaningful parameters that influenced evolvability were diffusion rates D from the inner to the outer compartment, cost to inner compartment growth controlled by p and fissioning size F.

The solution to the weakening of the heredity-fitness dichotomy in this model has also coincided with an algorithmically meaningful distinction between data storage in the inner compartment and functional phenotype in the outer compartment. This was not an objective of the model, but a seemingly necessary consequence of minimal conditions for multi-bit heritable variation.

The limited heredity problem exists for both sequence/template and the attractor/metabolism based heredity mediums examined here. Ultimately, there is little experimental evidence to confirm that either RNA like molecules or RAF sets of protein polymers existed in the early world with the necessary high specificity catalytic/enzymatic function, and the problem of limited

heredity in attractor based mediums is even more severe. The model presented here represents one possible dynamical setting in which this problem might be alleviated. Additionally, the resultant 'phenotype', only indirectly heritable, represents the emergence of an algorithmically meaningful distinction between data storage medium and expression machinery. Given the increase in heredity, such a mechanism might also permit adaptations in the form of self-interactions, such as regulation of expression functions. While this possibility has not been explored within the scope of this simplified model, it is a unique feature with relevance to the evolution of evolvability. The evolution of development field has already demonstrated the use of self-regulation to achieve heritable adaptation in the units of phenotypic variation (Watson et al. (2014)).

Future work may include a more detailed treatment of the chemical and physical dynamics with examination of the feasibility of such a naturally occurring two-tier protocells and self-regulating autocatalytic units in the pre-biotic world. Of particular conceptual interest though is the potential of interaction between the inner and outer compartment networks, which might lead to the evolution of a protocell's ability to evolve. Additionally, while we believe we have presented the outline of a minimal dynamic potentially able to support open-ended evolution, we have not addressed the question of whether this system could itself have been the product of an evolutionary process. It is difficult to imagine the evolution of complex adaptations without good heredity, but the spatial structuring we assume here may not have required this to be an product of single-bit heredity (Powers et al. (2007)).

Appendix

Parameters and effective values used for simulations:

k_1	0.1	p	0.3
k_2	0.12	F	2500
k_3	0.14	D	0.02
k_4	0.1	V_I	1
k_5	0.12	V_O	∞
k_6	0.14	c_i	15 $\forall i$
P_{add}	0.0001	$P_{migrate}$	0.005
P_{remove}	0.04	$N_{compartments}$	200
S	0.1		
$T_{1,2}$	$\frac{1}{2^{1/2}}(1,1,0,0,0,0)$		
$T_{4,5}$	$\frac{1}{2^{1/2}}(0,0,0,1,1,0)$		

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