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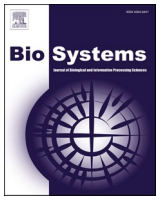

Accurate phenotypic self-replication as a necessary cause for biological evolution.

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ABSTRACT

Since the *Origin of Species*, it has been known that evolution depends on what Darwin called the "strong principle of inheritance." Highly accurate replication of cellular phenotype is a universal phenomenon in all of life since LUCA and is often taken for granted as a constant in evolutionary theory. It is not known how self-replication arose during the origin of life. In this report I use the simple mathematics of evolutionary theory to investigate the dynamics of self-replication accuracy and allelic selection. Results indicate that the degree of self-replication accuracy must be greater than a threshold related to the selection coefficients of the alleles in a population in order for evolution to occur. Accurate replication of cellular phenotype and of the molecules involved in genotype/phenotype linkage is necessary for the origin of evolution and may be considered the fundamental principle of life.

1. Introduction

Perhaps the central issue in the origin of life is the question of how a collection of molecules following the laws of chemistry gave rise to biological evolution by natural selection during the period between the first cell and the arrival of the last universal common ancestor (LUCA) organism. Once biological evolution was possible, the development and refinement of the many complex biochemical machines and systems that we consider essential to all living organisms can be explained. Such systems include those involved in conversion of energy from light and food to useful chemical energy, the complexity of membranes to allow for selective transport of molecules into and out of cells, many metabolic pathways catalyzed by specific and highly efficient enzymes, and, above all, a process for the highly accurate replication of all aspects of cellular phenotype (Szathmáry and Smith 1997).

By definition, evolution is the change in allele frequencies in a population over time (measured in generations) (Millstein, 2022). The key driver of evolutionary change is the difference in "fitness" between alternative alleles of one or more genes. Many discussions of evolutionary mechanisms focus on the selective advantage or disadvantage of particular variations in genes that give rise to differences in phenotype, thanks to changes in protein sequence and function (Brandon 1978). Variations by mutation and natural selection based on environmental factors are not, however, the only two processes required for evolution to work. An improvement in function produced by a mutation can only lead to evolution within a population if the mutation is faithfully copied from one generation to the next (Summers and Litwin 2005; Müller--Wille 2010).

This means that the origin of evolution (biology) in collections of reactive molecules (chemistry) requires at the outset a method to accurately copy phenotypes from a cell to daughter cells during cell division. In modern life that process is accomplished by the enormously complex ribosomal protein translation system that includes the genetic code, transcription of the DNA sequence to mRNA, and the close-to-perfect synthesis of proteins with the correct sequence at the ribosome.

The challenge of understanding how such a complex, elaborate system could have itself evolved before the possibility of natural selection-based evolution as we know it is the subject of intense research (Wills and Carter 2018).

It is possible that some randomly produced polypeptides would be able to catalyze useful reactions in a prebiotic cell and might be candidates for natural selection-based evolution. However, unless such a molecule could be accurately replicated in the following generations, no evolution is possible. In other words, evolution is only possible if such a replication mechanism exists, and if it does, evolution must inevitably follow. In this paper, I will show that the above statement is true.

2. Model and results

The basic mathematics of natural selection has been well known for

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many decades and can be summarized here based on Orr (2009). The theoretical treatment assumes no particular biochemical mechanism for replication, and no specific definition of allele other than as a variant phenotype (see Discussion). We assign P as the identity of an allele with greater fitness (i.e., more likely to improve the survival chances of a protocell) than other alleles. The frequency of the P allele in a population is denoted as p. All other alleles, assumed to have significantly lower fitness, are Q, with a population frequency of q, so that $p + q = 1$.

If the absolute fitness of allele P is W_1, and for purposes of the model, all the other alleles (Q) have the same fitness W_2, ($W_2 < W_1$) then we can calculate the relative fitnesses (w_1 and w_2) of the P and Q alleles as W_1/W_1 and W_2/W_1, respectively. This means the relative fitness of the allele of interest (P) is $w_1 = 1$, and that of Q is $w_2 = 1 - s$, where s is defined as the difference between the two relative fitnesses: w_1-w_2 (Orr 2009).

The average relative fitness of the population $\overline{w}$ is:

$$\overline{w} = p + qw_2 = 1 - qs \tag{1}$$

By definition, the change in the frequency, p, of the P allele, Δp, from one generation (p_{n-1}) to the next (p_n) is $p_n - p_{n-1}$. Evolution takes place when (and only when) $\Delta p > 0$.

$$\text{As shown in Orr (2009)}, p_n = \frac{p_{n-1}}{p_{n-1}w1 + q_{n-1}w2} \tag{2}$$

Combining equations (1) and (2) we get:

$$p_n = \frac{p_{n-1}}{\overline{w}} \tag{3}$$

and Δp from generation n-1 to generation n is given by:

$$\Delta p = \frac{p_{n-1}}{\overline{w}} - p_{n-1} \tag{4}$$

If there is no selective difference in fitness between alleles, then $s = 0$, $\overline{w} = 1$, and

$$\Delta p = \frac{p_{n-1}}{1} - p_{n-1} = 0 \tag{5}$$

meaning no evolution is possible.

This simple mathematics illustrates the role of natural selection in evolution. equations (2)–(5) given above assume that each allele is faithfully reproduced with close to perfect accuracy from one generation to the next. This is a reasonable assumption in all of life from LUCA on since the modern replication system does operate with close to 100% accuracy or $F \approx 1$, where F is the fidelity of phenotypic replication (or fraction of faithful copies) between generations. At $F = 1$, the P allele with $w_1 = 1$ and relative frequency = p will increase as the population grows, while the other alleles will decrease in frequency depending on the value of s.

But at the origin of life such an assumption is not justified since we have no knowledge of more primitive methods for accurate cellular replication (Korfmann et al., 2023; Paul and Joyce 2002). If the assumption of perfect (or near perfect) replication accuracy is not correct, and $F < 1$, then equation (2) must be replaced with

$$p_n = \frac{Fp_{n-1}}{Fp_{n-1}w1 + q_{n-1}w2} \tag{6}$$

where F represents the replication fidelity of the P allele. It can be assumed that the replication fidelity of Q (all other alleles) is ≈ 1 because the probability for any Q mutation to be a non-P allele is ≈ 1.

Equation (6) can be rearranged to give:

$$p_n = \frac{Fp_{n-1}}{\overline{w} - (1-F)p_{n-1}} \tag{7}$$

If we let $Y = (1-F)p_{n-1}$

Then

$$\Delta p = \frac{Fp_{n-1}}{\overline{w} - Y} - p_{n-1}$$

And after rearrangement:

$$\Delta p = \frac{p_{n-1}(F - (\overline{w} - Y))}{\overline{w} - Y} \tag{8}$$

In order to express Δp in terms of s, the selection coefficient, we start with eq. 1

$$\overline{w} = p + qw_2$$

and derive eq. (9) as follows:

$$\overline{w} = p + (1-p)w_2$$

$$\overline{w} = p + w_2 - pw_2$$

$$\overline{w} = p + (1-s) - p(1-s) = p + 1 - s - p + ps$$

$$\overline{w} = 1 - s + ps = 1 + s(p-1) \tag{9}$$

This leads to eq. (10) for the term $\overline{w} - Y$

$$Y = (1-F)p = p - Fp$$

$$\overline{w} - Y = 1 - s + sp - p + Fp \tag{10}$$

We can now determine the minimum value of F required for $\Delta p > 0$, by setting F = 1 - s to give

$$\overline{w} - Y = 1 - s + p[(s - 1 + (1-s)] = 1 - s \tag{11}$$

From eqs. (8) and (11), if F = 1 - s

$$\Delta p = \frac{p_{n-1}\{(1-s) - (1-s)\}}{(1-s)} = 0 \tag{12}$$

We can also reach the same result by deriving the value of p_n assuming s = 1 – F; from eqs. (7) and (9)

$$p_n = \frac{Fp_{n-1}}{1 + (1-F)(p_{n-1} - 1) - (1-F)p_{n-1}} =$$

$$p_n = \frac{Fp_{n-1}}{1 + p_{n-1} - Fp_{n-1} - 1 + F - p_{n-1} + Fp_{n-1}} = \frac{Fp_{n-1}}{F} = p_{n-1} \tag{13}$$

$$\Delta p = p_n - p_{n-1} = 0 \tag{14}$$

We see from eqs. (12) and (13) that the threshold for the value of F to allow for evolution ($\Delta p > 0$) is F = 1 - s. Furthermore, evolution automatically follows if F > 1- s and s > 0, and cannot occur if $F \leq 1 - s$. If $F < 1 - s$, the value of Δp would be negative, which means that the frequency of some other, lower-fitness allele rather than the higher-fitness target P allele would increase. While this can happen in modern life, it would render evolutionary mechanisms inoperable if it occurred at the origin of life.

This analysis shows that a threshold of F = 1 - s for the level of accurate self-replication is necessary for evolution. Since 1 - s = w_2, the average fitness coefficient of the Q alleles, we can say that the level of replication fidelity required for evolution must be higher than the fitness coefficient of the less fit allele. The smaller the fitness differences between two alleles, the larger the value of F must be in order for evolution to take place. If we include the requirement that s > 0, then these two conditions, F > 1 – s and s > 0, are both necessary and sufficient for evolution.

Fixation of the p allele follows a sigmoidal curve as expected for the growth of the frequency of an allele from 0 to 1.0. While the time to fixation and the sigmoid function are highly dependent on the value of s, the effect of F is minor, except as F approaches 1 - s.

The general formula for determining the time for fixation for a new

allele under beneficial selection is given by Kimura and Ohta (1969) as

$$G_t = \frac{2}{s}\, Ln\, 2N_e \tag{15}$$

where G_t is the time in generations from the first appearance of the new allele to fixation, s is the selection coefficient, and N_e is the effective population size. This equation gives a good approximation of fixation time, independently of F.

3. Discussion

In previous work (Garte 2021a, 2021b), I showed that the evolution of high replication fidelity of cellular phenotypes must be a non-continuous process depending on the starting conditions of both the probability of cellular survival and replication fidelity. Cellular growth constants will not exceed 1.0 (the requirement for evolution) unless each of those two parameters surmount thresholds for each value (Garte 2021b). The relevant function is $K \approx Pr(F+1)$, where K is the growth constant, Pr is the probability of survival (related to fitness), and F is the same probability of accurate replication used in this communication (Garte 2021b).

The major implication of the results reported here is that the absence of a system for accurate replication of cellular phenotype makes evolution by natural selection impossible. To operate, evolution requires accurate replication accuracy (the probability of 100% fidelity) greater than the average fitness of the Q alleles ($w_2 = 1 - s$) based on the selected advantage of a new allele P. Evolution by natural selection will automatically occur given a probability of replication fidelity greater than that threshold, and a fitness differential ($s > 0$) between any two alleles. We tend to think that alleles with higher fitness eventually reach fixation, while other alleles disappear from the gene pool. However, this scenario is disrupted if the replication fidelity is below the threshold of 1- s.

Eigen originated the concept of error thresholds and the possibility of error catastrophe (Eigen 1971). Eigen and Schuster (1978a) present a relationship between the threshold for error catastrophe and the "average selective advantage" of a favored allele and "the average quality of symbol copying," which are roughly equivalent to s and F, respectively, in the present work. This relationship takes the form of a function of (s/(1-F)), so that decreases in either s or F or both lead to a catastrophe of errors once past the threshold value. However, Eigen is mostly interested in the way early life was able to overcome the obstacles to self-replication by invoking the hypercycle concept. In Eigen and Schuster (1978b) the authors quote Crick et al. (1976) at the outset: "The origin of protein synthesis is a notoriously difficult problem. We do not mean by this the formation of random polypeptides but the origin of the synthesis of polypeptides directed, however crudely, by a nucleic acid template …." The subject of thresholds in the origin of life is treated exhaustively in a review by Jeancolas et al. (2020).

The error thresholds first discussed by Eigen and further elaborated by Jeancolas et al. are mainly concerned with the problem ("Eigen paradox") of achieving a replicator with sufficient length to overcome the formula for error catastrophe threshold where l (length) < ln (s))/e, e being related to 1-F. In contrast to these studies, the present paper is concerned with the origin and possibility of evolution by natural selection in the face of a threshold for cellular phenotype replication fidelity, without reference to any specific mechanism. The key finding that F must be greater than the threshold 1 - s is a general rule for any form of replication, even those that might not involve polynucleotides, and could even include random processes.

Hofbauer and Sigmund (1998) discuss replication dynamics in the context of evolutionary game theory, including similar scenarios as those presented here, during later stages in the history of life.

While modern life operates with highly accurate self-replication of phenotype, there are exceptions under unusual circumstances. One exception is the phenomenon of hypermutation, a defensive strategy of many single-celled organisms (especially many viruses) facing severe survival challenges due to toxicity or starvation. In this case, replication fidelity is deliberately reduced in order to produce a high mutation rate for a limited time and over a specific genomic region (Pribis et al., 2022). A theoretical treatment of the role of F in this scenario was recently published (Garte 2023).

Others have addressed the question of replication fidelity in the origin of life (Goel and Yčas 1975; Gabora 2006). The development of the modern system for translation of inherited genotype into a highly accurate replication of phenotype is mysterious. It could not have appeared spontaneously, and, as demonstrated here, it could not have evolved by natural selection, since it is required (at least to some extent) for evolution by natural selection to work.

However, these results do not rule out the possibility of evolution in early life even with low replication fidelity. The threshold formula $F = 1 - s$ can also be written as $s = 1 - F$, and since $1 - F$ is the mutation rate, we can say that for evolution to be possible, the selective advantage of a new allele must be greater than the mutation rate. Since mutation rates in modern life (since LUCA) are very low, around 10^{-4} or lower (Kibota and Lynch 1996), even very slightly beneficial alleles will produce an evolutionary change. We can assume that during the origin of life, both mutation rates and selection coefficients were much higher, although details are not known. However, we can conceive of a situation with primordial replication fidelity quite low (say, around 0.1) that would still allow for evolution if some polypeptide or other new molecule conferred an enormous selective advantage to a protocell on the order of, say, 0.95.

The only definitive threshold that cannot be surpassed occurs at $F = 0$; in other words, in the absence of any replication system at all. In that case, the selective advantage of any new biomolecule is irrelevant, and evolution is impossible.

The use of the word "allele" in this report may be confusing since alleles are generally defined as gene variants, whereas this discussion does not presume the existence of genes, DNA, or any other particular genetic mechanism.

In modern life, with the tight linkage between genotype and phenotype, variations in gene allele sequences lead to linked changes in phenotype. But this is not necessarily the case in protolife forms without the modern structures of DNA-based genes, the protein synthesis mechanism, and other elements related to highly accurate phenotypic replication of cells and organisms from one generation to the next. The target of natural selection-based evolution is the phenotype (Mayr 1997). Therefore, a definition of allele is required that applies directly to the phenotype and would therefore be useful in the absence of any specific form of genotype.

One example of an alternative definition of allele is to consider a protobiotic system wherein polypeptides might be constructed by some form of the modern ribozyme peptidyl transferase (Tirumalai et al., 2021). Such polypeptides would consist of amino acids in a random order, without the features of modern proteins allowing for highly efficient and specific catalytic activity as enzymes. On the other hand, it is quite possible that some polypeptides produced in this way might in fact possess a rudimentary catalytic activity due to their size, shape, the reactive nature of many amino acid side chains, or the presence of hydrophobic amino acids acting as surfaces for chemical reactions (Keefe and Szostak 2001).

If a protocell synthesized such a protoenzyme by random chance with some degree of catalytic activity that would, for example, enhance the chemical synthesis of a useful cellular component, then that molecule could be considered a "phenotypic allele" with a positive contribution to the pre-biotic fitness of its cell and contribute to an increase in its overall average fitness. We can refer to this molecule as the P allele. The other non-reactive polypeptides containing other random sequences produced by the ribozyme would have no or much less useful activity and far lower fitness.

In the absence of a mechanism that can produce replication fidelity (F) of sufficient magnitude to allow for a positive change in the frequency (p) of the fitter allele in each succeeding generation, whatever increase in fitness might be given to the original cell in which it was produced will disappear in the following generation. The probability of the ribozyme producing the same (or even a sufficiently similar) protein by chance is vanishingly small (Keefe and Szostak 2001). The loss of any evolutionary pathway is especially true if the selective advantage s of P is minor, in which case the value of F must be closer to 1 than 0 for evolution to occur.

The results presented here suggest that a slow, gradual improvement of replication fidelity by natural selection-based evolution is not a feasible scenario for the development of the near-perfect replication accuracy that has been present in all of life since well before LUCA. The model and results presented here are not meant to have any application to long-term development of life (from first cell to LUCA). This report seeks only to investigate the consequences of having a low fidelity of replication (F) at the earliest stages of modern life, and its effects on evolutionary processes. We know that at some point evolution did begin, and F had to reach the threshold that made evolution possible, but we have no good understanding of how, and how long ago that process occurred.

Furthermore, this report does not address possible alternative ancient evolutionary scenarios that might be independent of natural selection, nor does it imply anything about the details of the replication process. A great deal of research is being done to investigate possible alternative scenarios whereby selection-independent chemical processes could lead to a primitive coding and replication system. Carter and Willis have proposed a number of interesting ideas (Carter and Wills 2021), including the involvement of the dual system of amino acyl tRNA synthetases acting as a primitive selective coding mechanism (Carter and Wills 2018). Many workers have found positive results in the search for self-replicating ribozymes (Lincoln and Joyce 2009; Ichihashi et al., 2013; Le Vay et al., 2019) and the role of peptides in early forms of molecular replication (Jia et al., 2016). However, this research is still in the early stages of providing a convincing solution to the origin of replication (Raggi et al., 2016; Wills and Carter 2018; Szostak, J.W., 2012; Joyce and Szostak, 2018).

Evolution by natural selection is not a separate force of nature, but (as Darwin tried to explain) an automatic result of variation and inheritability in all of life. Therefore, according to its definition, the mathematical support shown here, and the justified assumption that for all of life since LUCA the value of F has been close to 1, the reality of evolution is as proven as any natural phenomenon can be.

This work suggests that high-accuracy self-replication is the central issue in finding mechanisms for the origin of evolution and therefore of life as we know it. Further progress requires research into the origins of the enormously complex and sophisticated system of modern protein synthesis (phenotypic replication), including the genetic code, the ribosomal machinery, the translational system of tRNAs and aaRS enzymes, etc. (Szathmáry and Smith 1997; Vasas et al., 2012; Wolf and Koonin 2007). In the grand scheme of the underlying foundational principle of life, we can conclude that self-replication, rather than evolution, is primary.

CRediT authorship contribution statement

Seymour Garte: Conceptualization, Formal analysis, Methodology, Writing – original draft.

Declaration of competing interest

The author has no competing interests to declare.

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