At face value, this appears to be tautologically true, but misleads since "evolution" is a word with many connotations including change, and development to better, more complex replicators. Let us rephrase. Assume three conditions hold:

(i) A population can replicate for many generations
(ii) the copies are not all identical with the original
(iii) a criterion establishes preferential selection of one lineage

What results? Change, yes. More sophisticated replicators? Not inevitably. Suppose the original state was optimal at some point in time and all subsequent variation degrading. Differential fitness would select the less damaged on average, but the long-term outcome will still be an inferior population. Dennett's criteria do not ensure neo-Darwinian processes result in the complex biological structures we observe.

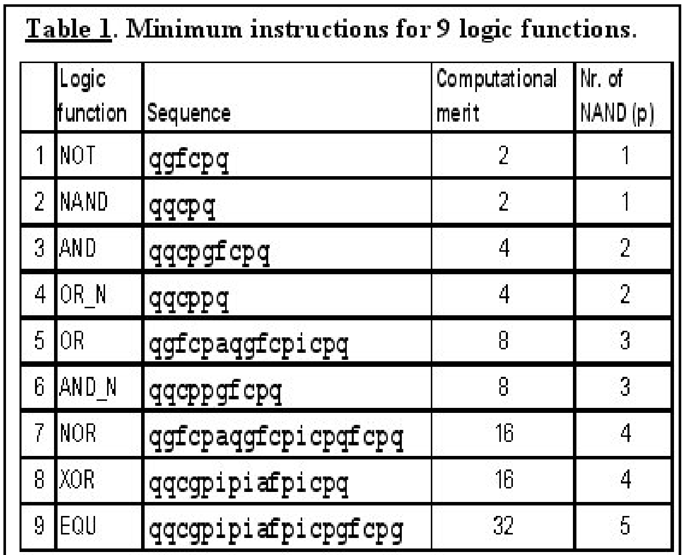
<u>Using Avida to test neo-Darwinian notions</u>. Leading Avida-based researchers believe their digital experiments are analogous to cellular evolution. Adami and colleagues explain[10], ***"Computations can be carried out by evolved programs if they develop sequences of code ("genes") that perform logical, bitwise, computations on random numbers provided in their environment. Such genes evolve spontaneously if the performance is rewarded with bonus CPU cycles (their unit of energy). The complexity of the environment can be controlled by changing the number of logical operations whose performance is rewarded."***

He states elsewhere[5], ***"Because it is these computations which provide the organism with the "energy" (in the form of CPU time) it needs to replicate, we can think of this computational code as the genes that code for the organism's metabolism. To this extent, we are able to observe the emergence of metabolic genes in self-replicating organisms, and thus the evolution of complexity."***

Then to Dennett's list additional criteria must be satisfied if "evolution" is to mean the grand conceptual scheme leading from simple molecules to living multicellular organisms:

(iv) the replicators must not all self-destruct after a few generations
(v) differential fitness must not be accomplished by simplification or net degradation of existing biological functions (e.g., parasites, which do reproduce faster than the progenitor)
(vi) opportunities to attain more complex fitness states must be feasible: the organisms are not trapped in local fitness peaks, and irreducible complexity does not pose statistically prohibitive barriers.

<u>Avida run details.</u> The Avida computer instructions are generally analogous to codons or amino acids. Any of 26 different letters are present at each position of the digital sequence, reminiscent of the 21 (including 'Stop') in the genetic code. A mutation is assumed to be equally likely to change any of the 26 possibilities to any other.



<u>Table 1</u>. Minimum instructions for 9 logic functions.

	Logic function	Sequence	Computational merit	Nr. of NAND (p)
1	NOT	qgfcpq	2	1
2	NAND	qqcpq	2	1
3	AND	qqcpgfcpq	4	2
4	OR_N	qqcppq	4	2
5	OR	qgfcpaqgfcpicpq	8	3
6	AND_N	qqcppgfcpq	8	3
7	NOR	qgfcpaqgfcpicpqfcpq	16	4
8	XOR	qqcgpipiafpicpq	16	4
9	EQU	qqcgpipiafpicpgfcpg	32	5

In a recent paper in Nature<6>,<11>, many combinations of instructions ("codons") in a particular environment can produce **9** different logical functions ("genes" or "operons"), e.g. Table 1<14>. A single initial organism with a genome of **50** instructions reproduces to a final fixed population of 3600 members. Several one letter symbols at the beginning and end of the genome, **15** positions in total, contain all instructions<12>, <13> required for self-replication:

qucavc…utzcarvab [1]

The remaining 35 positions are originally identical and have no use, thus serving as raw material to evolve novel functions. Default settings<6> in the *environment.cfg* file include: point mutations lead to copying errors at a rate of **0.0025** per instruction copied; single-instruction deletions and insertions occur with **0.05** probability each per genome copied (multiple mutations can occur in a single generation).

The organisms compete conceptually for energy, which is provided in quanta called SIPs. One SIP suffices to execute one instruction. "***In Avida, organisms can acquire energy by two mechanisms. First, each organism receives SIPs in proportion to its genome length. Second, an organism can obtain further SIPs by performing one- and two-input logic operations on 32-bit strings***"<6>, p. 140.

Digital "organisms" evolving new logic functions are rewarded in proportion to the complexity of the derived function.

Calibrations necessary to ensure biological realism. Mutations in the symbols of [1] can produce logic functions (*And*, *Nor*…) when tested against numeric strings. The proportion of digital genomes possessing such patterns builds up rapidly<6> for reasons which will be discussed in this two part series. Simple logic functions are statistically easier to obtain, and once significant amounts of these exist, provide the basis for evolution to the more demanding logic functions. The runs reported<6> have not been calibrated against real organisms nor validated with biological experiments. The necessary sensitivity analysis tests were not performed. We conclude that the simulations "work" because of several judicious assumptions and parameter settings which ensure this outcome *a priori*:

(1) Miniscule genomes are assumed with far more superfluous than necessary genetic material

- The ratio of indispensible / total genetic material is initially 15 / 50
- Increasing the genome size with worthless genetic material is compensated with extra metabolic energy<15> instead of penalized, as should be the case in nature.

(2) Extremely high mutational rates are used

- In default settings point mutations occur at a rate of 0.0025 instructions per instruction position per generation; deletions and insertions have a probability of 0.05 per genome copied. This permits generation of logic functions before genome truncation would remove superfluous genetic material.

(3) Critically important functions for "life" are difficult to destroy by mutations

- All functions necessary for an organism to replicate and survive are carried out initially by only 15 instructions. The physical machinery to transcribe, translate and perform metabolic processes are not coded genetically and thus not subject to mutational damage.

(4) Statistically insignificant sequence space distances are assumed between novel, more complex functions

- This is an artefact of logic functions and not protein sequence space.

(5) Dramatic rewards are provided for new logic functions

- New, more complex functions, like EQU, once formed are essentially guaranteed to perpetuate, i.e., fix in the population due to very high relative fitness rewards.

(6) No path for graceful degradation of functions is provided.

- Less effective protein variants which are still functional are more numerous than the more specific ones, and often decrease fitness but minimally. With logic functions no such variants exist, which permits "natural selection" to easily identify degraded functionality with 100% accuracy.

Miniscule genomes with much superfluous genetic material. What kinds of biological organisms might correspond to Avida digital ones? The generation of new genetic information by the neo-Darwinian mechanisms under discussion can only be relevant for very small, asexual, free-living organisms, with short generations times.

In the absence of mutations, Avida lineages as usually generated will never go extinct. In biological terms, the organisms are assumed to possess significant repair mechanisms and to execute all biochemical processess effectively to survive. Clearly a **56** base pair (15 X 3 X 26/21) stretch of nucleic acid cannot represent any real living organism (the extra 35 no-operation codons perform no services). The smallest living organism known is the parasite Mycoplasma genitalium[16] with a genome of ca. 578,000 bp, and a mutational rate of about **5.9X10^{-9}**. Gene knockout studies[17], [18] in a nutured laboratory setting suggests a mutant might limp along for a few generations on perhaps 250 to 400 genes (outside the laboratory 500 to 600 genes is considered more likely[19]). A theoretical lower limit of about (250 genes) X (1000 bp/gene) implies about **250,000** bp. Nevertheless, for computation convenience generous parameter settings could be used, but the results must be followed by extrapolations to real world constraints.

Our analysis must be limited to very small, self-contained asexual organisms able to replicate almost flawlessly: the smallest of bacteria.

Large amounts of additional genetic material must be made available if new logic functions are to be coded for. Indeed, "***The 23 pivotal genomes ranged in length from 49 to 356 instructions; the ancestor had 50 instructions and so there was a strong tendency for increased length to precede the origin of EQU.***" [6], p. 142

In nature, brand new metabolic networks, which the logic functions are analogous to, don't evolve into existence every few mutations. A key but unstated assumption, is that the unneeded genetic material would have to remain present for a very large number of generations. This crucial requirement would not be fulfilled in nature since streamlined variants of very small genomes out-reproduce those with unnecessary coding material, as required for evolutionary experiments.

For example, the bacteriophage Qβ parasite genome[20] uses a single strand of RNA with **4,500 nucleotides**. Sol Spiegelman placed Qβ, a necessary replicase and certain salts in a test tube. Portions of the genome dispensible in this environment were lost, permitting the descendants to reproduce more quickly. After about 70 generations everything superfluous had been eliminated, and a single species with a single RNA strand **550 nucleotides** long remained. Only 70 generations were enough to remove almost 88% superfluous genetic material, which incidently is far more than the 35/50 = 70% initially made available in the major paper<6> under consideration.

In a second experiment<21>, RNA already shortened optimally was allowed to replicate in the presence of a drug that slowed down replication. The drug attached to a specific three nucleotide sequence. After a few generations all members possessed the same new sequence, in which the recognizer address for the drug had mutated to 3 other nucleotides, preventing drug binding and permitting faster reproduction.

Other studies<22>, <23> show that currrently unnecessary DNA added to genomes tends to be removed quickly, sometimes due to the loss of a large number of genes<19>.

For realistic Avida simulations, generation times must be inversely proportional to amount of superfluous genetic material, *ceteris paribus*, at least until an advantageous logic function evolved. The authors permitted deletions, but fail to provide any reward when they occur: generation times would be shortened; and less (biological) energy and material (nucleic and amino acids to produce proteins) would be needed. Some Avida researchers seem to be aware of this fact, as further discussed in Part 2, but a parameter is not provided in the *genesis* file to include this effect during simulations.

The proportion of members possessing a genetic change, given budding or binary fission reproduction and constant population size, can be modelled mathematically<24>, <25>:

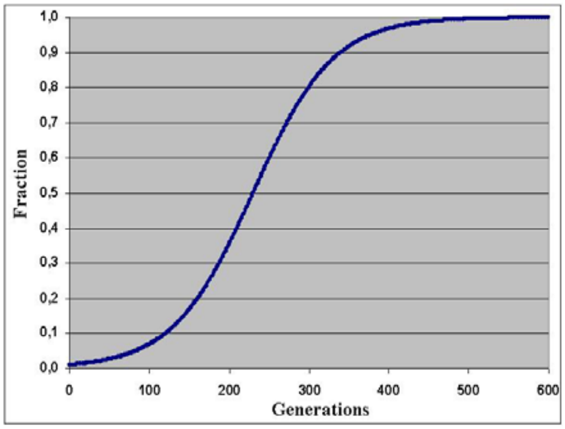
$$x = \frac{x_0 e^{(st)}}{1 + x_0[e^{(st)} - 1]} \qquad [2]$$

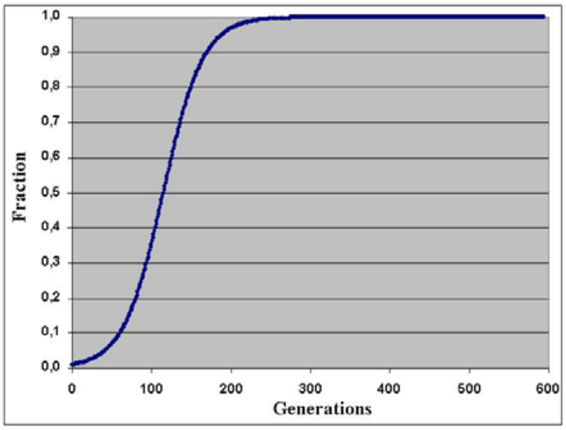
where x is the fraction of a population possessing a mutation; s is the selectivity factor; and the unit of time, t, is the generation interval.

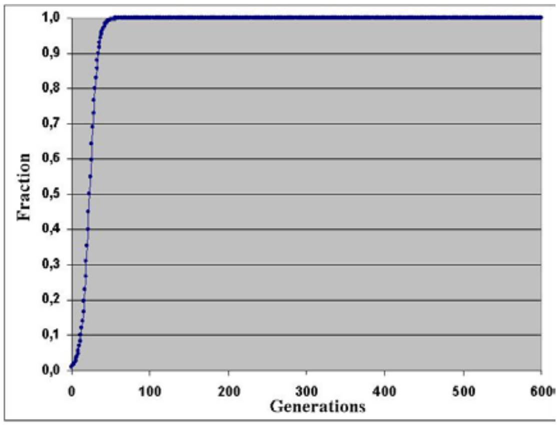
Let's approximate the selectivity coefficient, s, by assuming the offspring with one codon less than the original 50 would replicate in about 49/50 the amount of time (and lineages with an insertion would suffer 51/50 times longer generation times). Since initial sequence [1] does nothing else than replicate, this is reasonable. The favorable selectivity assumption is also consistent with other biological advantages: less energy and material requirements, and less dangerous, interfering polypeptides. About **3.5%** of the 3600 organisms would receive a harmless deletion during just the first generation<27>. Subsequent generations will offer additional opportunities to remove more 'junk'.

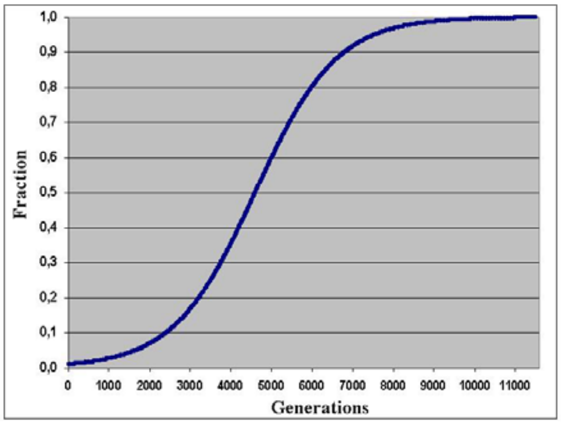
Many experiments could be run with the Avida software and some may indeed undergo slower genome truncation on average, but for very large populations the Central Mean Theorem predicts such deviation will be small. Assume just **1%** of one of the lineages had at some point but one fewer "no-operation instruction". Using equation [2] produces the results shown in Figure 1. On average, only **576** more generations shifts the population from 1% to 99.9% possessing the shorter genome. This result is independent of population size. The population would actually initially have a distribution of members with some having several more and others several fewer unnecessary codons. This broader spread merely strengthens our argument.

1% genomes having shed two codons would on average require **288** generations for 99.9% of the member of any population size to have streamlined their genome, see Figure 2.









Before 1% of the population has obtained a 49 instruction genome, one member on average will undergo yet another harmless deletion (0.01 X 3600 X 0.05 X 34/49 > 1 deletion). This new lineage has a yet greater advantage, leading to a snowball effect. The authors assume mutational additions can also occur. Note that additions after the final instruction to divide would not be incorporated in the next generation. At some point a distribution of genome lengths would be present. Figure 3 illustrates how rapidly the population would shift from 1% to >99.9% members with 10 fewer codons: in just 70 generations.

Of course, the selectivity estimates, s, might be too large. We can use a very modest s = 0.001 for the member having shedded several percent junk coding material. Then on average within 11,512 generations the population would shift from 1% to >99.9% streamlined genome, see Figure 4 (ignoring the mentioned compounding effect, which would shorten the generations considerably). For a proto-bacteria of generation time 10 minutes, this means far less than 80 days, for any population size involved.

This demonstrates that we cannot assume a stable starting population with genomes possessing much unnecessary DNA, and expect natural selection to ignore this.

Extremely high mutational rates

Having demonstrated how quickly small genomes eliminate superfluous DNA, we must consider the mutational rates as typically used in Avida runs<6>. Low values provide much time and many generations for the streamlining process to take place. Such lineages, which reproduce more quickly, would take over the population before opportunities to evolve new functions became available.

High rates of mutation and small sequence distances between logic function are typically assumed, to permit higher development. These also facilitate escape from local fitness traps: "***The presence of deleterious mutations along the line of descent is more surprising. Fifteen of the 18 deleterious mutations reduced fitness by <3% relative to the parent… However, two mutations reduced fitness by >50%.***"<6>, p. 141 This lineage resulted in the dominant genotype.

A primitive, free living (non-parasite) bacteria <1/5 the size of *E. coli* at some point during the evolutionary scenario would have about 1000 coding genes, each averaging 300 codons plus regulatory regions, all critical to life, with little worthless DNA overhead. The genome must code for the translation machinery (ribosomes, DNA polymerase, transcription factors); tools to transcribe DNA to RNA (RNA polymerase); DNA coding for enzymes to permit metabolic reactions; tRNA and accompanying enzymes; and the equipment to duplicate the chromosome during fission. Based on typical (default) parameter settings for Avida runs<6>, p. 143, about 1000 X 300 X 0.0025 = **750** point mutations, plus (2)(0.05)(1000*300)(/50)(21/26) = **485** indels (insertions or deletions) would occur per genome every generation when extrapolated to a ca. 1000 gene organism. The latter would produce many reading frameshifts in every individual virtually every generation.

These model assumptions would lead unavoidably to error catastrophe in biological organisms. Error correction machines must also be coded for to prevent this, but this poses addition questions: how would a miniscule genome survive until these evolved; and these additional genes would also be subject to damage from mutations.

Nevertheless, the authors wrote, "***Hence, in the ancestral genome of length 50, 0.225 mutations are expected, on average, per replication. Various organisms from nature have genomic mutation rates higher or lower than this values.***"<6>, p. 143 However, no free living organisms have genomes on the order of 50 codons with such mutational rates, and **the values need to be scaled to real biological sized free-living organisms to test for reasonableness**.

From the reference cited<28> by the authors, the simplest kinds of single cell free living organisms, such as *E. coli* (genome ca. 4.6X10^6 bp - base pair -) and *S. cerevisiae* (genome ca. 1.2X10^7 bp) have mutation rates on the order or **5 X 10^{-10}** per base pair each generation, or about **0.002** to **0.006** total mutations per genome each generation. Properly scaled, we see that a vastly greater rate is being assumed by the authors for Avida runs. Literature estimates<16>, <28>, <29>, <30>, <31> for various living organisms (which exclude parasite virus) demonstrate that the mutational rates used in the simulations are not realistic.

A much greater than 3600 member population size could tolerate a higher mutational rate with less risk of total extermination, but this exacerbates the preceding objection: fortuitious mutations would require more generations to fix, and thereby provide yet more opportunities to streamline away superfluous DNA.

Functions critical for life are virtually impossible to destroy by mutations

Biologically unrealistic mutation rates to not lead to complete population extinction in Avida runs due to a fact subtle enough to escape the attention of many specialists. Recall from above that the molecular machines needed for translation, transcription, metabolism and so on must be coded for genetically in real, biological free-living organisms. Adami and collegues claim that, "***The Avida system controls populations of self-replicating computer programs in a complex and noisy environment within a computer's memory."*** [32] This is misleading. The only thing mutating are short strings of symbols such as [1] and not any computer programs. The software shell actually or figuratively performing critical functions ("replicate your genome from here to there"; "generate metabolic energy"; etc.) are written in C++ and are neither coded for by the genome (unlike real organisms) nor subject to mutation. The rest of the infrastructure is provided for free in the form of computer technology (stacks, registers, electricity, cables, chips, etc.), unlike real cells in which physical ribosomes, cell walls, ATP and so on must be genetically coded, and thus in the Avida platform cannot be damaged by mutations.

The starting strings such as [1] usually soon increase in size under typical Avida settings (documented experimentally in Part 2). The minimal 15 instruction string, assumed to be necessary to support all life processes autonomously, is but one pattern from among many alternatives able to code for the cellular replication (Repl.) function (Table 2). (ID is the mutant lineage; the first column refers to the position in the genome on which the 1-letter instruction is found)[33].

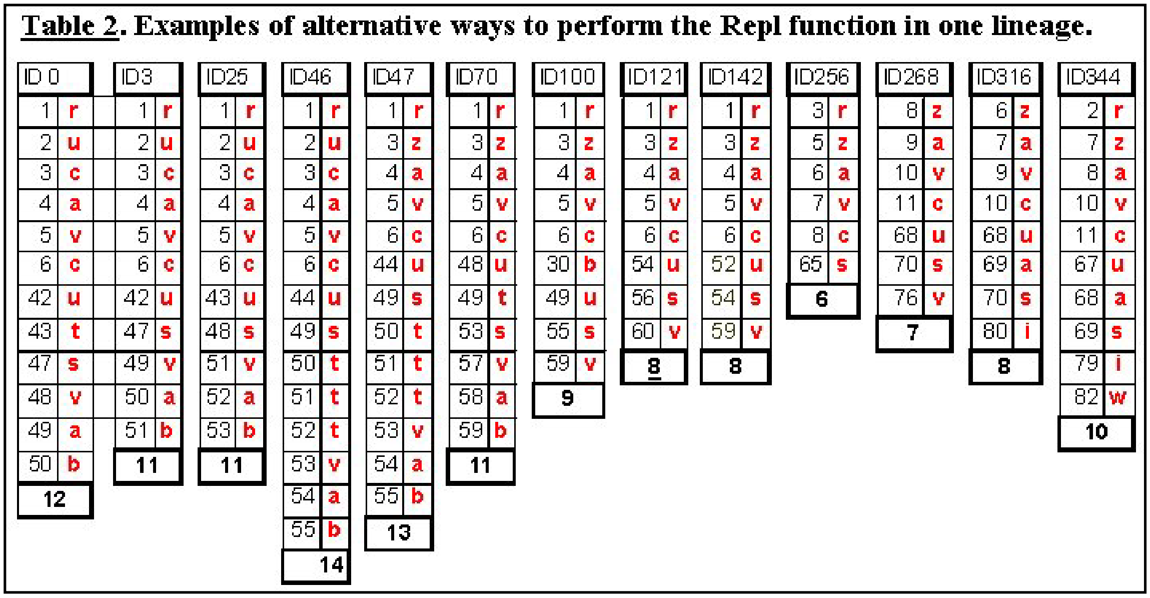


Table 2. Examples of alternative ways to perform the Repl function in one lineage.

ID 0	ID3	ID25	ID46	ID47	ID70	ID100	ID121	ID142	ID256	ID268	ID316	ID344
1 r	1 r	1 r	1 r	1 r	1 r	1 r	1 r	1 r	3 r	8 z	6 z	2 r
2 u	2 u	2 u	2 u	3 z	3 z	3 z	3 z	3 z	5 z	9 a	7 a	7 z
3 c	3 c	3 c	3 c	4 a	4 a	4 a	4 a	4 a	6 a	10 v	9 v	8 a
4 a	4 a	4 a	4 a	5 v	5 v	5 v	5 v	5 v	7 v	11 c	10 c	10 v
5 v	5 v	5 v	5 v	6 c	6 c	6 c	6 c	6 c	8 c	68 u	68 u	11 c
6 c	6 c	6 c	6 c	44 u	48 u	30 b	54 u	52 u	65 s	70 s	69 a	67 u
42 u	42 u	43 u	44 u	49 s	49 t	49 u	56 s	54 s	**6**	76 v	70 s	68 a
43 t	47 s	48 s	49 s	50 t	53 s	55 s	60 v	59 v		**7**	80 i	69 s
47 s	49 v	51 v	50 t	51 t	57 v	59 v	**8**	**8**			**8**	79 i
48 v	50 a	52 a	51 t	52 t	58 a	**9**						82 w
49 a	51 b	53 b	52 t	53 v	59 b							**10**
50 b	**11**	**11**	53 v	54 a	**11**							
12			54 a	55 b								
			55 b	**13**								
			14									

Over 99.9% of the indispensible coding material needed for a minimally sized real organism to survive has been shielded from the possibility of any kind of mutation. These functions are provided by the external hardware and software. For real, compact genomes it is thousands of times more likely[34] that a mutation would be deleterious than that a portion of the critical 15 instruction string would be affected, as implied by the Avida runs[6]. Therefore, such Avida runs ensure *a priori* that a 3600 member population will almost always easily survive the mutational onslaught for endless generations even without the development of new logic

functions. By generously rewarding instruction patterns which produce logic functions, the net effect of Avida type mutations over many runs is virtually guaranteed to ensure increased functional complexity over time (demonstrated in detail in Part 2). This is not some kind of law of nature, but an inevitable result of how the Avida system and runs are designed.

Statistically small sequence space distances assumed

Novel functions are easy to attain, compared to living organisms, and these offer immediate, dramatic selection advantages. For the dominant genome the authors report: "***After the origin of EQU, another 233 steps occurred along the line of descent leading to the final dominant genotype. Of these, 62 were beneficial, 132 neutral and 39 deleterious.***"<6>, p. 141 (underlines added). Within very few attempts many new logic functions developed, since so many useful mutations are available. In one example, "***Forty-five of the steps increased overall fitness, 48 were neutral and 18 were deleterious relative to the immediate parent***."

The logic functions are assumed to be close together in coding sequence space. How close is examined statistically in Part 2. In one report, "***Of the 35 instructions required for EQU, 22 were needed for simpler functions. Three instructions required for EQU were also essential for replications; these were conserved from the ancestral sequence, as were five others.***"<6>, p. 141

We now see why digital organisms manage to derive a series of ever more complex logic functions, culminating in the final EQU one. "***The number of instructions required for EQU ranged from 17 to 43, with a median of 23 instructions***"<6>, p. 142 . EQU was the most complex logic function to be evolved, and there is a large number of available intermediate plateaus leading up to it. When these intermediate stepping stones are removed, none of the organisms managed to evolve EQU<6>, p. 143. "***Some readers might suggest that we 'stacked the deck' by studying the evolution of a complex feature that could be built on simpler functions that were also useful. However, that is precisely what evolutionary theory requires, and indeed, our experiments showed that the complex feature never evolved when simpler functions were not rewarded.***"<6>, p. 143 While this is indeed what current neo-Darwinian Theory requires, the simulation cannot use biologically implausible distances<35> to imply the theory has been validated.

We can contrast this with the estimated proportion of cytochrome c sequences which would be minimally functional: **<10^{-65}** <25>, <26>, <36>. This is a rather small and not too demanding protein. Until a probabilistic barrier of this rough order of magnitude has been bridged, no selection to fine-tune the sequence and fix this gene in a population would be possible.

Let's put things in perspective. Evolutionary theory must explain the origin of hundreds of new, unrelated protein families, based on different folding characteristics. Since increased complexity is under discussion, we are not interested in relatively trivial protein variants which are able to catalyze virtually identical molecules. Professor Sauer at MIT systematically deleted small pieces from viral proteins and inserted altered pieces back into the genes at the sites of

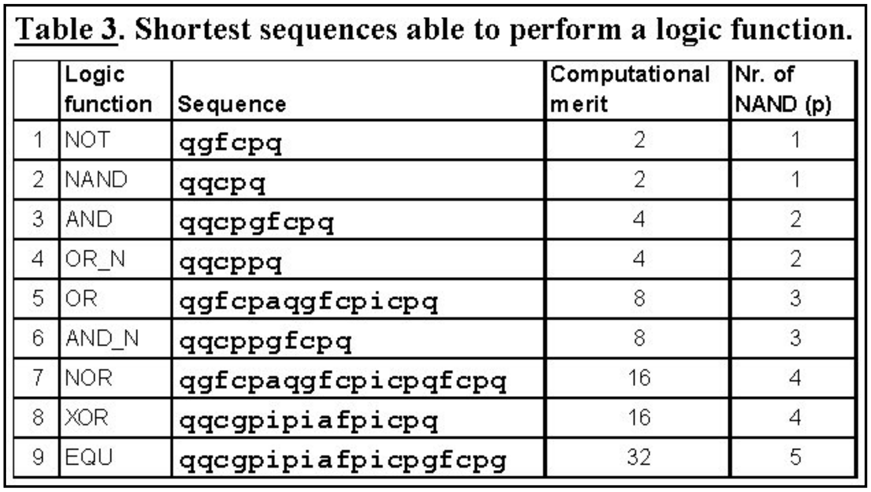


Table 3. Shortest sequences able to perform a logic function.

	Logic function	Sequence	Computational merit	Nr. of NAND (p)
1	NOT	qgfcpq	2	1
2	NAND	qqcpq	2	1
3	AND	qqcpgfcpq	4	2
4	OR_N	qqcppq	4	2
5	OR	qgfcpaqgfcpicpq	8	3
6	AND_N	qqcppgfcpq	8	3
7	NOR	qgfcpaqgfcpicpqfcpq	16	4
8	XOR	qqcgpipiafpicpq	16	4
9	EQU	qqcgpipiafpicpgfcpg	32	5

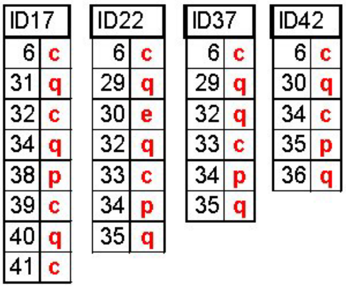
the deletions to determine how much variation at various portions of the sequence can be allowed<37>, <38>. He concluded that the likelihood of finding a folded protein by a random mutational search is about 1 in 10^{65} for the examples studied. That is equivalent to guessing correctly one particular atom out of our whole galaxy.

Clearly the Avida computer logic functions assume much too great a proportion of useful sequence variants, compared to biological random polypeptides<39>, <40> . A new domain, to be stably folded, should contain at least 50 amino acids. However, Table 3<41> illustrates the shortest sequences able to mimic each of the nine novel logic functions that do not depend on the initial content of stacks and registers. Three functions require only six instructions or "codons" although "***nearly every major process in a cell is carried out by assemblies of 10 or more protein molecules***"<42> and not merely a portion of a single protein. As an example, 60 independent polypeptides form the pyruvate dehydrogenase complex, which does not function if one is missing.

From a web site<11> we find a few examples of different sequences from one lineage which produced the logical function NAND, in various manners, within 42 mutations (Table 4). The first column is the position at which the necessary 1-letter instruction is found, ID is the mutation number.

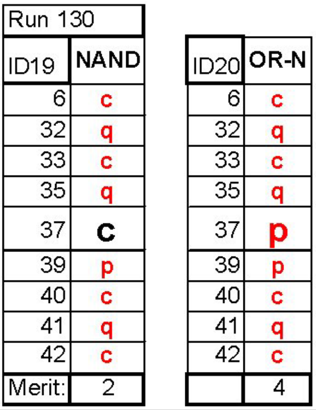
In one lineage<11>, the new and more valuable OR-N function was derived from the NAND function by a single point mutation at position 37, with no intermediate loss of NAND function, see Table 5.

Table 4 Some seqs. which produced NAND. 1st column: position in sequence.



ID17		ID22		ID37		ID42	
6	c	6	c	6	c	6	c
31	q	29	q	29	q	30	q
32	c	30	e	32	q	34	c
34	q	32	q	33	c	35	p
38	p	33	c	34	p	36	q
39	c	34	p	35	q		
40	q	35	q				
41	c						

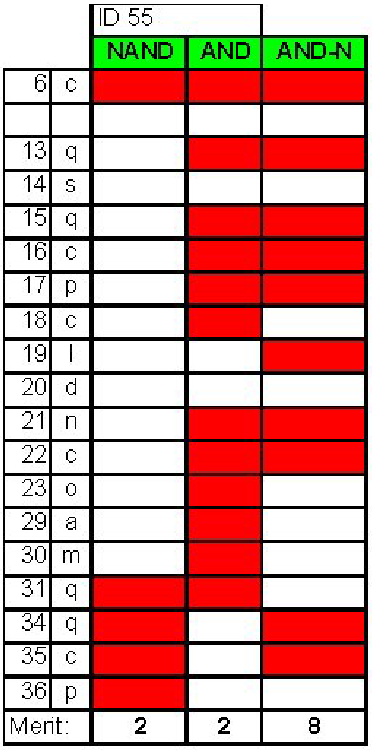
Table 5. Evolving novel function from simpler one "easy", e.g. 1 mutation.



Run 130			
ID19	NAND	ID20	OR-N
6	c	6	c
32	q	32	q
33	c	33	c
35	q	35	q
37	c	37	p
39	p	39	p
40	c	40	c
41	q	41	q
42	c	42	c
Merit:	2		4

In the example in Table 6, NAND and AND functions already work. A new, more complex function, rewarded with a factor of 8, could arise from a single mutation at position 19. (Red boxes indicate the instruction is necessary for the logical function shown in green). No other instructions were necessary.

Table 6. Once NAND and AND exist, 1 mutation was needed at position 19 to generate the valuable AND-N.



		ID 55		
		NAND	AND	AND-N
6	c	🟥	🟥	🟥
13	q		🟥	🟥
14	s			
15	q		🟥	🟥
16	c		🟥	🟥
17	p		🟥	🟥
18	c		🟥	
19	l			🟥
20	d			
21	n		🟥	🟥
22	c		🟥	🟥
23	o		🟥	
29	a		🟥	
30	m		🟥	
31	q	🟥	🟥	
34	q	🟥		🟥
35	c	🟥		🟥
36	p	🟥		
Merit:		2	2	8

Dramatic rewards are provided for new logic functions

Each higher, more complex function type (often separated from simpler ones by a single mutation) are typically rewarded by accelerating the rate of replication of that new lineage by about a factor of two. Since the population was doing very well without the new function, this is unrealistic. A function such as EQU, with a computational merit factor of 32 (!) makes the probability very high that successful mutants will rapidly generate new lineages and fix in the population.

The opportunity to degrade functions gracefully

Avida logical functions either work with 100% effectiveness, and are rewarded richly, or have 0% effectiveness. This has considerable theoretical significance. On average, Avida-type lineages which lose these functions are very likely to be out-reproduced. Alternatively, lineages with intact functions reproduce between about 2 to 32 times faster and on average are very likely to dominate.

In cells, the exact 3-dimensional topology of proteins, location, and their proportion during the lifetime are important. Compared to an optimal solution, there are statistically far more inferior possibilities. We can group these variants into categories, each less effective but more numerous than the preceding. As the population becomes more distant from the optimal, the next degradation step carries decreasing fitness penalties, as long as a necessary function is still performed.

This argument can be extended to a large number of genes, resulting in a snowball effect: multiple degradations over many loci makes additional degradation harder to remove by natural selection.

This fact demonstrates how easy it would be to slide slowly down a fitness slope in real biological organisms, as illustred in Figure 5.

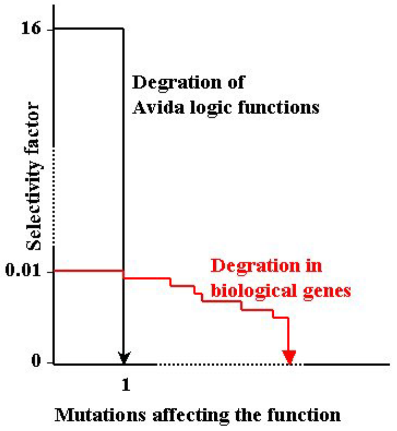


Figure 5. Loss of of an Avida logic functions has a dramatic effect. Biological systems can degrade easily.

Summary.

A very short string of symbols is used in Avida computer runs to represent a genome. Over 99.9% of the indispensible biological functions which must be coded for genetically by a minimally long biological organism (physical metabolic machines, actual replication, real-world transcription and translation, etc.) are not exposed to mutational risk. These are external to the organism and executed by external hardware (stacks, registers, electricity) and software (C++ programs).

This permits very high mutational rates for the 15 symbols (such as 0.0025 point mutations per symbol per generation) for the 3600 member Avida population. Individual deaths are easily replenished by offspring of the survivors.

Having ensured a non-ending supply of replicators, a series of judicious model settings essentially guarantee that development of increased complexity via novel functions must result. The details are examined in Part 2.

With no analysis nor convenient manner to explore under what conditions neo-Darwinian processes might in reality lead to net degradation instead of higher development, the reported studies merely confirm the assumptions. Nevertheless, the Avida platform does provide a rich enough environment to explore many scenarios, and it will be argued in Part 2 that properly designed experiments cast severe doubt as to whether neo-Darwinian processes have led to many novel, complex biological features.

The lineages of small, rapidly replicating asexual organisms with streamlined genomes would quickly out-reproduce their competitors. Without sufficient mutable genetic material the foundation for increasing complexity and development will be denied.

Many details must be considered to permit extrapolation towards biological realities.

(a) The probability of generating coding sequences for real, new biological functions by chance mutations needs to be lowered by many orders of magnitude compared to typical Avida runs<6>, as discussed in more detail in Part 2.

(b) In biological organisms the rest of the over 99.9% critical functions for survival, coded on nucleic acids, would also be at risk of destruction by mutations. There are also multiple external factors which stochastically affect which organisms survive and reproduce. This means that average relative fitness values should be on the order of < 0.01 instead of 2^n, where n=1 … 5. This implies that genetic drift effects will cause the vast majority of fortuitious novel logic functions to disappear before enough build up to fix. This is explored in detail in Part 2.

The simplest functions (***Nor***, ***Nand***) are typically rewarded with an increased reproductive rate of almost 2<43> . Eventually most of the population cannot fail to possess this function. The next more complex logic function is rewarded with an increased rate of almost 2^2 compared to the initial population, and of 2^1 compared to the members possessing only the preceding simpler function.

(c) Minimal genome size studies suggests a minimal genetic string such as [1] needs to be at least 1000 times longer for model testing purposes, and therefore the mutation rate also lowered dramatically.

Factors (a) - (c) are independent and multiplicative. During the vast number of generations needed to produce useful candidates for natural selection to act upon, genome simplification and compaction would be occurring, removing the necessary genetic playing material. We will see in Part 2 that more realistic Avida-type scnenarios, which still do not remotely approach real biological challenges, contradict the picture of easy development of ever increasing biological sophistication.

It is convenient, of course, to work under simulation conditions which are tractable, such as 50,000 updates. However, before any kind of conclusions as to the feasibility of producing a truly new, complex cellular function via neo-Darwinian processes can be claimed, realistic extrapolations are necessary. Our analysis suggests that not much novelty is to be expected during a few billion years of mutations by the mechanism underlying Avida models. Mutations in coding and regulatory portions of genetic material can decrease the effectiveness of genes. The differences in their selectivity, would be very small. The proportion of slightly inferior alternatives is always greater. There are many and very wide downhill paths to degradation, with small penalties unless a critical function is lost. As a population degrades due to random mutation, the relative penalties for additional degradation must be compared to the new lower average fitness, and therefor facilitated.

We conclude that the computer experiments reported using the Avida framework so far have not demonstrated that neo-Darwinian processes could have produced the necessary coding information to produce the hundreds of molecular machines found in natural cells.

Acknowledgements.

I wish to thank Dr. Lenski for some clarifications he kindly provided and Mr. Kaben Nanlohy for helpful technical comments on Avida. Critical exchanges with Dr. Charles D. and Dr. RBH forced more careful rewording. Mr. Salvador Cordova stimulated / provoked much thought. Mr. David Coppedge's suggestions improved an earlier draft.

References

<1> Adami, C. and Brown, C.T., in “Artificial Life IV: Proceedings of the 4th International Workshop on the Synthesis and Simulation of Living Systems”, eds. Brooks, R. A. & Maes, P., MIT Press, Cambridge, MA, (1994) p. 377.

<2> Adami, C., “Introduction to Artificial Life”, Springer, New York, (1998).

<3> Ray, T.S., in “Artificial Life II: Proceedings of the 2nd International Workshop on the Synthesis and Simulation of Living Systems”, eds. Langton, C.G., Taylor, C., Farmer J.D., and Rasmussen S., Addison Wesley, Redwood City, (1992), p. 371.

<4> http://dllab.caltech.edu/avida/v2.0/docs/cpu_tour.html

<5> Adami, C., “Ab Initio Modeling of Ecosystems with Artificial Life”, *Natural Resource Modeling*, **15**, 133-146 (2002).
http://dllab.caltech.edu/pubs/ http://arxiv.org/abs/physics/0209081

<6> Lenski, R.E.; Ofria, C.; Pennock, R.T.; and Adami, C., “The evolutionary origin of complex features”, *Nature*, **423**(8), (2003), 139.
http://myxo.css.msu.edu/papers/nature2003/

<7> http://dllab.caltech.edu/avida/v2.0/docs/environment.html

<8> http://dllab.caltech.edu/avida/v2.0/docs/genesis.html

See also: http://dllab.caltech.edu/avida/v2.0/docs/print_data.html

<9> http://dllab.caltech.edu/avida/v2.0/docs/events.html

<10> Ofria, C.; Adami, C.; and Collier, T.C., “Selective Pressures on Genomes in Molecular Evolution”, *J. Theor. Biol.* 222 (2003), 477-483.
http://arxiv.org/abs/quant-ph/0301075

<11> http://myxo.css.msu.edu/papers/nature2003/case-study/lineage.html

<12> From the Supplementary Information of ref. <6>

<13> http://myxo.css.msu.edu/papers/nature2003/glossary.html

<14> http://dllab.caltech.edu/avida/v2.0/docs/cpu_tour.html

<15> From ref. <6>, (p. 140): “***Digital organisms compete for the energy needed to execute instructions.” “First, each organism received SIPs in proportion to its genome length.***” And p. 143: “***Each digital organism obtained ‘energy’ in the form of SIPs at a relative rate (standardized by the total demand of all organisms in the population) equal to the product of its genome length and computational merit***”.

<16> Drake, J.W., *Annual Reviews of Genetics*, **25**, (1991), 125–146.

<17> (a) Pennisi, E., *Science*, **272**, (1996), 1098.

(b) Mushegian, A. and Koonin, E., *Proc. Nat. Acad. Sci. USA*, **93**, (1996), 10268.
(c) Bult, C. *et al.*, *Science*, **273**, (1996), 1058.

<18> Süßmuth, R., “Die Bakteriengruppe der Mycoplasma“, *Tagesband der 18. Fachtagung für Biologie*, p. 69, 16–18 März 2001. Studiengemeinschaft Wort und Wissen e.V. Rosenbergweg 29, D-72270, Baiersbronn, Germany.

<19> Scherer, S., “Kleinstes Genom einer photosynthetischen Bakterienzelle als Hinweis auf einen genetisch ’komplexe’ Vorfahen?“, *Stud. Int. J.* **11,** (2004), 29-31.

<20> Shapiro, R., *Origins: A Skeptic's Guide to the Creation of Life on Earth*, Bantam Books, New York, 1987, p. 157–160.

<21> Ref. <20>, p. 160.

<22> Petrov, D. and Hartle, D.L., “High rate of DNA loss in the Drosophila melanogaster and Drosophila virilis species groups”, *Moleculer Biology and Evolution*, **15**, (1998), 293.

<23> Mira, A.; Ochman, H.; and Moran, N.A., “Deletion bias and the evolution of bacterial genomes”, *Trends Genet.*, **17**, (2001), 589.

<24> Hoyle, F., *Mathematics of Evolution*, Acorn Enterprises LLC, Memphis (1999), p. 11.

<25> Truman, R. and Heisig, M., “Protein families: chance or design?”, *TJ*, **15**(3), (2001), 115.

To favor the evolutionary viewpoint, we assumed a 10 minute generation time for a bacterial progenitor and enough non-functional DNA to evolve a new gene. The genomes with unneeded DNA would take about 0.1 second longer to replicate per generation. We pointed out that each of 2 growing forks on *E. coli* can replicate less than 1000 base pairs (bps) of DNA per second. Using equation [2] we showed that if 0.01% of the population had the slightly faster generation time, within 3 years over 99.99999% of any population size would no longer carry the superfluous DNA.

Extrapolation from all known viable sequences of cytochrome c suggests that a proportion of about $2X10^{-44}$ of the candidate sequences 110 residues long would have any activity. (A large number of mutations not actually found were also assumed would be acceptable). This conclusion implies that any tolerated mutation would be functional in the presence of all other single mutations which are tolerated, which as a rule is not true of proteins. This estimate is surely many orders of magnitude too great. Other genes show almost no variability throughout nature, for example ubiquitin and histones H3 and H4.

<26> Yockey, H.P., *Information Theory and Molecular Biology*, Cambridge University Press, Cambridge, 1992, p. 250.

<27> 35 / 50 initial positions are ‘no-operation’ instructions, not needed for replication (p. 143), although the on-line data (ref. <11>) shows less than the 15 are absolutely necessary. Single instruction insertions and deletions occur with probability 0.05 each per generation. Some indels might not damage the instructions needed for replication: (0.05)(35/50) = 0.035

<28> Drake, J.W.; Charlesworth, B.; Charleswroth, D.; and Crow, J. F., "Rates of spontaneous mutation", *Genetics*, **148**, (1998), 1667.

<29> Spetner, L., *Not by Chance! Shattering the Modern Theory of Evolution*, The Judaica Press, Inc., 1998, Chapter 4.

<30> Fersht, A.R., *Proceedings of the Royal Society (London)*, **B 212,** (1981), 351.

<31> Grosse, F.; Krauss, G.; Knill-Jones, J.W.; and Fersht, A.R., *Advances in Experimental Medicine and Biology*, **179**, (1984), 535.

<32> Ofria, C.; Adami, C.; and Collier, T.C., "Selective Pressures on Genomes in Molecular Evolution", *J. Theor. Biol.* 222, (2003), 477-483.
http://arxiv.org/abs/quant-ph/0301075

<33> A large number of strings can accomplish the same service as [1]. In cells these instructions must produce physical RNA and proteins which face very demanding folding (3 dimensional) constraints to actually work. The proportion of viable sequence alternatives to [1] is therefor many orders of magnitude lower than implied in the work with digital organisms.

<34> (250000 bp)/(45 bp X 26/21) = 4487 (bp = base pairs)

<35> We could perform a laboratory experiment: insert a superfluous 1000 base pair stretch of DNA into thousands of the smallest bacteria we can engineer. Under optimal laboratory conditions, generation times would be about 20 minutes, providing about 26,000 generations in one year per initial ancestor. Will we find a new genome which exhibits a novel biological functions, or will only streamlined variants be found after a few years?

<37> Meyer, S., "The Message in the Microcosm: DNA and the Death of Materialism", http://www.arn.org/docs/meyer/sm_message.htm

<38> Meyer, S., "The Origin of Life and the Death of Materialism", http://www.arn.org/docs/meyer/sm_origins.htm

<39> Lodish *et al.*, *Molecular Cell Biology*, 4th ed., W. H. Freeman and Company, New York, 2000, p. 60 & 456.

Instead of whole genes, one could examine highly "conserved", i.e., invariant protein domains. An example which comes to mind are the the 260-residue core of protein kinases. Domains must be properly embedded within a larger polypeptide, and according to Lodish *et. al*., 'A structural domain consists of 100–200 residues in various combinations of *a* helices, *b* sheets, and random coils.'

<40> Axe, D. D., "Extreme functional sensitivity to conservative amino acid changes and enzyme exteriors", *J. Mol. Biol.* **301**, (2000), 585.

<41> http://myxo.css.msu.edu/papers/nature2003/logic_programs.html
Supplemental Material.

<42> Alberts, B., “The cell as a collection of protein machines: Preparing the next generation of molecular biologists”, *Cell* **92**, (1998), 291.