Pseudogenes and bacterial genome decay

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Contrary to the evolutionary idea of junk DNA, many pseudogenes still have function in the genomes of archaea, bacteria, and also eukaryotes, such as humans. As part of the creation model, the genomes of organisms such as bacteria are undergoing processes of decay whereby their genes lose function and are eventually not expressed, and they may eventually be excised from the genome. This type of loss of gene function is called pseudogenization, which is widespread in bacteria, and is characteristic of both pathogenic and non-pathogenic species of bacteria. Pseudogenization occurs via non-synonymous base pair substitution, base pair insertion, frameshift mutation, gene truncation, loss of promoter, disruption by transposon, or failed horizontal gene transfer. Gene loss is a frequent characteristic of adaptation by pathogenic bacteria to different host species. It is also a differential process according to the kind of host species that is colonized by bacterial species. Reflective of this is the way certain genes undergo different patterns of pseudogenization in different baranomes.

Pseudogenes are considered to be dysfunctional genes that are either diminished in expression, are no longer expressed or no longer code for protein. In other cases, still functional genes may be misannotated pseudogenes. According to some creation models, pseudogenization in bacteria can be diagnostic of genome decay.[1] The ENCODE project has revealed near-total functionality of the genome and shown that about one fifth of all pseudogenes are being transcribed in humans.[2] Pseudogenes are quite widespread in bacterial genomes,[3] and even if the proportion of actively transcribed ones is lower than in human genomes, so their possible functionality still warrants an examination. Like the evolutionary model, the creation model predicts both functional and non-functional parts of the genome. Currently it is unclear whether pseudogenes should be classified as functional or non-functional parts of the genome. In this paper we will explore the recent scientific progress on pseudogenes to gain insight into how pseudogenes should fit into the creation model.

Darwinian evolution has been defended by arguing that genomes are composed of pseudogenes and junk DNA that could not possibly be the result of direct creation by God. Creationists state that pseudogenes are the result of degenerative processes beginning after the Fall, and have also correctly predicted that functions for the supposed 'junk' DNA would be discovered. However, two reasons exist for why the creation model also predicts non-functional parts of the genome. After the Fall and consequent advent of death in the world, a biological mechanism for death must have appeared. Genome decay (which leads to non-functional parts of the genome) may be part of this mechanism. The second reason is that God designed kinds to diversify and fill the earth. As the kinds diversified, some of the original genetic information God included in the kinds was no longer needed and therefore may now be absent through processes of gene loss. This can also give rise to non-functional parts of the genome. The goal of this paper is to describe current understanding of pseudogenes in light of the creation model.

Bacteria serve as a good model to study pseudogenization due to several of their characteristics: because of their short generation time and large population size, they can be easily studied. Furthermore, because they are haploid, the effects of genes loss readily become evident.

The process of pseudogenization

Pseudogenes can undergo different kinds of mutations which are more or less deleterious to the function of the genes. They usually contain premature stop codons, meaning that while the full length protein cannot be formed, the truncated mRNA can still function as a regulator of the original gene.[4] Pseudogenization is the process of gene death whereby mutations accumulate within a gene, resulting in that gene losing its original function. During this process, the pseudogene region may still be active and functional, albeit at an ever diminishing expression level. Even if the pseudogene itself is not actively translated, it still can be transcribed, as recent studies have shown that many pseudogenes take part in siRNA-mediated gene silencing.[5,6] In eukaryotes, pseudogenes often form an important subclass of long non-coding RNA genes and are key components of complex regulatory networks of gene expression.[7]

The definition of a pseudogene is not precise since pseudogenization is a process whereby the function of the gene diminishes or is immediately destroyed. Therefore opinions differ as to when a gene can be considered a true pseudogene. One standard is if a gene loses its regulatory region and is no longer transcribed, it is categorized as a

Table 1. Pseudogenes affecting DNA replication and repair in selected bacterial species

Species	Process	Gene name
Vibrio vulnificus CMCP6	Protein synthesis	Elongation factors Tu, G
	DNA replication	DNA gyrase A
	DNA replication	RuvB helicase
	DNA replication	RuvG helicase
	Protein synthesis	Ribosomal protein L5
	Protein synthesis	Ribosomal protein L35
Vibrio vulnificus YJ016	DNA replication	recN
Mycobacterium leprae	DNA repair	dnaQ
Buchnera aphidicola	DNA replication	recA/F
Rickettsia sp.	DNA repair	phrB
		radC
		mutM
		mutT
		alkylated DNA repair genes
Shigella flexneri 2a	DNA replication	dnaA
Blochmannia floridanus	DNA replication	dnaA
Wigglesworthia glossinidia	DNA replication	dnaA
Shigella sp.	DNA metabolism	Lhr
Shigella sp.	DNA repair	ung
Escherichia coli	DNA recombination	recE

pseudogene since it cannot be expressed. Further, if genes are effectively silenced by prophages or transposon disruption these are also categorized as pseudogenes.

In general, pseudogenes may also be eventually removed from the genome because of energetic costs involving continuous translation and transcription of functionless genes. However, this is not true for all genes, since DNA replication is only a minor process compared to protein synthesis, so that there is no real selective pressure against these genes. On a larger scale this coincides with genome size reduction. Microbial pathogens and endosymbionts exist at different stages of pseudogenization accompanying genome reduction.[1]

The mutation accumulation required for pseudogenization can be compared to defect accumulation in an automobile. First, one headlight is broken. Then a window won't roll up. Then a spark plug fails and the engine begins misfiring. Eventually, after the accumulation of minor defects, a major malfunction will prevent the car from being able to be driven. Similarly, pseudogenization often begins with a gradual breakdown of gene functionality until a functional domain, for example such as the active centre of an enzyme, is broken down.

Bacterial pseudogenization differs from that in eukaryotes in a number of ways. Since bacterial cells are haploid, loss of gene function is felt more readily in the phenotype, compared to diploid organisms, where mutations can be masked by functioning gene copies. In eukaryotes, transposons may deactivate only a single copy of a gene, whereas the other copy is free to function. Proviruses must also integrate into the germline in eukaryotes, and most of the genes they are inserted into must also be homozygous in order for their mutations to take effect. Furthermore, the protein coding structure of bacteria is different than in eukaryotes, meaning that available genome space is highly compact with genes lacking introns and packed tightly into operons serving as functional units. Up to 90% of the genome in bacteria is used for coding genes.[8]

In bacteria there are three main ways for pseudogenes to form: disruption by transposons, degradation preceded by duplication, and incomplete (failed) horizontal gene transfer (HGT). The latter process can be more than two times as prevalent as pseudogenization of host genes; therefore we will examine it at a closer level.[9]

Evolutionary predictions for pseudogenes

According to evolution, besides gene loss, pseudogenes could serve as raw material from which new genes are built by random mutations and natural selection, allowing simpler organisms to evolve into more complex ones. However, previous studies have shown that pseudogenes accrue over a line of related species within bacterial groups.[1] Also, entropy tends to break information down, rather than build it up.

Secondly, evolving genes could possibly be in an intermediate stage on a trajectory between random sequences and a new gene. Even if pliable proto-genes were capable of transforming into functionally new genes, they would still have to acquire whole regulatory regions (promoters, enhancers, insulators, etc.) to make them fully functional.

Lastly, pseudogenes also have to stay around long enough for mutations and natural selection to transform them into new genes. However, natural selection cannot act upon a forming pseudogene, since natural selection acts upon a whole gene as a unit. In other words, the forming pseudogene or proto-gene would have to become an entirely new gene

in order for natural selection to kick in and start having any effect on it.

Thus, upwards evolution meets a number of hurdles which are very hard for it to overcome in order to form new genetic information out of nothing in the form of a pseudogene.

Horizontal gene transfer

The most common cause of pseudogenization is the *failed* horizontal transfer of prophages, plasmids, transposons, or other conjugational elements.[10] Failed HGT is 2.3 more likely to occur than the disruption of vertically inherited genes.[9] The reason this rate is so high is that it has been estimated that the concentration of bacteriophages is about ten times as high as that of bacteria, meaning that the rate of HGT is a lot higher compared to plasmid transduction and other forms of DNA uptake from the environment.[11] Thus, failed HGT is a constant threat to bacteria, and eliminating such elements also causes the occasional deletion of useful DNA. A deletion rate at equilibrium thus helps streamline the bacterial genome.

Certain species of pathogenic bacteria paradoxically have a very high ratio of pseudogenes, such as *Rickettsia prowazekii* and *Mycobacterium leprae*. This is because, as intracellular parasites, their exposure to horizontally transferred genetic elements dramatically decreases. *Borrelia burgdorferi* on the other hand harbours linear plasmids, which allows unequal crossing over to increase the number of pseudogenes residing on them. Indeed the coding capacity of these linear plasmids is only 52%, which is very low compared to that of other bacteria.[12]

The nature of mutations occurring during pseudogenization

Many kinds of mutations can accumulate in a gene, thereby deactivating it. These include premature start or stop codons, loss or change of functional domain, loss of the promoter, and frameshift mutations, which cause the truncation of the gene. Such mutations can intensify if the proofreading machinery of the bacterium is disabled, such as mutations in the genes which take part in DnaQ-mediated proofreading in DNA polymerase III in *M. leprae*,[13] or recA/F in *Buchnera*.[14] Other genes include Elongation factor Tu and G, DNA gyrase A, helicases RuvB and RecG, ribosomal proteins L5 and L35, and DNA repair protein RecN in certain *Vibrio* species.[3] Several *Rickettsia* species have lost the following DNA repair proteins: *phrB*, *radC*, *mutM*, *mutT*, and two alkylated DNA repair genes.[15] See table 1 for a sample list of such genes.

Premature stop codons (TGA, TAA, and TAG) may arise in the AT-enriched bacterial genomes. Indeed, pseudogenes occur in higher numbers in bacteria with high AT% values.[16] Bacterial genome decay has been shown to coincide with a non-synonymous to synonymous mutation rate greater than one, with a non-random directionality towards higher AT%. For example, *Rickettsia* species have a mean AT% of 68.3%, and a mean coding capacity of 75%. Even interruption of ORFs is not necessarily detrimental to a gene's 'health'. Thirty-seven genes in the genome of *Rickettsia conorii* have been observed to be split into 105 fragments, of which 59 continue to be expressed, at least on a minimal level.[17]

A summary of different kinds of pseudogene mutations can be seen in table 2.

Table 2. Different classes of mutations during the pseudogenization process

Type of mutation	Frequency	Severity
Non-synonymous base pair substitution	Infrequent	Not severe
Base pair insertions	Infrequent	Disruptive
Frameshift mutation	Frequent	Very disruptive, lethal
Premature stop codon/truncation	Frequent	Very disruptive
Loss of promoter	Infrequent	Lethal
Transposon/prophage disruption	Very frequent	Very disruptive, lethal

Pseudogenization as adaptation to a new host environment

Bacteria can transition to a new host environment involving either an obligate symbiotic or pathogenic lifestyle. The event itself of this transition renders genes necessary for nutrients already provided by the host organism superfluous, as well as accumulation of deleterious mutations due to the decrease of effective population size and relaxation of selection, when the species is restricted to a single individual host organism as its environment.[3]

Massive genomic changes occur during this process, also involving pseudogenization. As an example, the proteomes of *Mycobacterium tuberculosis* and *leprae* have been compared with each other, showing that approximately 1,000 genes have been corrupted in *leprae* as compared to tuberculosis. During further genomic decay, pseudogenes are randomly deleted, rarely being shared between even closely related species, signifying complete loss of function. Despite lack of pressure for genes to remain in the genome, the proportion of pseudogenes is about the same in non-pathogenic and pathogenic bacteria, as well as archaea (3.9%, 3.3%, and 3.6%).[9]

We also studied the ratio of pseudogenes to all genes for 45 bacterial species (11 free-living, 31 pathogenic) taken from the Pseudogene.org database.[18] By checking the number of genes for

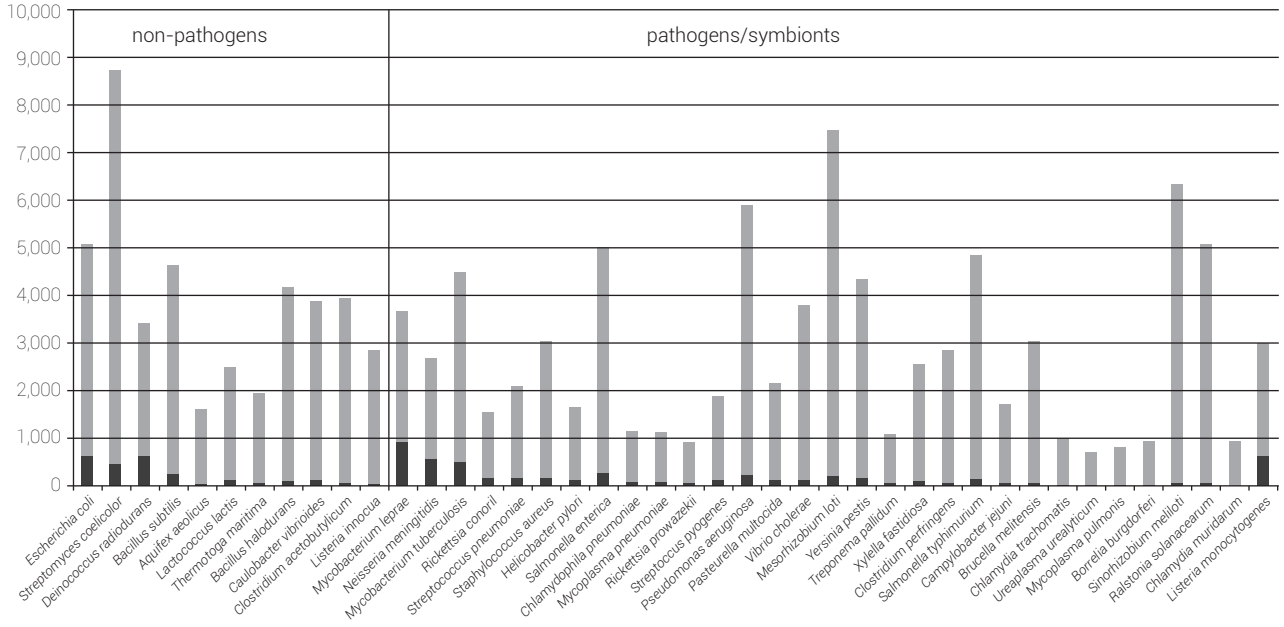

Figure 1. Number of common pseudogenes between pairs of species from the same baramin from the Pseudogene database

each species at NCBI we found that, on average, 3.51% of the genes of non-pathogenic bacteria were pseudogenes (std. dev. 3.09), whereas on average 4.41% of genes of pathogenic bacteria were pseudogenes (std. dev. 5.45). The difference of pseudogene ratios between the two groups proved to be insignificant even at the 10% level, based on a t-test which was performed between the pathogenic and non-pathogenic bacteria. The number of true genes and pseudogenes for each of these 46 species can be seen in figure 1. An important conclusion of this study is that bacterial genomes lose genes through pseudogenization, whether they are pathogenic or not. This means that genomic decay is an inherent process to organisms, irrespective of pathogenic status. This points to devolution and not evolution.

The degree of host specificity also correlates with the number and specific type of pseudogenes accrued in genome decay. *S. Gallinarum* and *S. Pullorum*, which infect birds, have 231 and 212 pseudogenes, respectively. Twelve of 15 metabolic pathways are affected by the same set of pseudogenes in both serovars before their divergence. *S. Dublin*, which is associated, but not exclusively, with cattle has only 95 pseudogenes.[19] This is an example of how bacterial species colonizing the same host undergo a similar trajectory of gene loss through pseudogenization to members of the same baramin which infect/colonize other host species. Examples of pseudogenes in several cultivars of *Salmonella*, mobile genetic elements called Salmonella pathogenicity islands (SPI), are accumulated, which encode type VI secretion systems. Loss of some of these elements as well as the pseudogenization of fimbriae proteins led to host adaptation.

The age distribution of pseudogenes

In a study of several *Salmonella* genomes, Kuo *et al.*[20] studied 378 pseudogenes, of which 364 (96.3%) were shown to have a single deactivating mutation. This indicates that these pseudogenes are very young, whereas pseudogenes with more inactivating mutations are rare. Kuo *et al.* found that this is because the single mutation pseudogenes had a lot more interaction partners. Since they had a relatively higher connectivity, their loss would have been much more detrimental to the organism, and would have been quickly removed, as opposed to multiple mutation pseudogenes, which were shown to have a lot lower connectivity, and thus persisted much longer in the genome. This concept of synergistic epistasis has not been proven and is only a theoretical speculation. This also poses a further problematic question: if the majority of potential pseudogenes are so highly connected (implying that they have necessary function), then how is that so many genes would be lost due to relaxation of selective pressure upon entering a host?

Pseudogenes can arise quite quickly, as seen in the case of five *Shigella* species: [21]*Shigella boydi*, *dysenteriae*, *flexneri 2a*, *flexneri 5*, and *sonnei*. *Shigella dysenteriae* had almost twice the number of pseudogenes (432) as *sonnei* (219). Furthermore, the two very recently diverged *flexneri* strains (2a and 5) have 17 and 14 strain-specific pseudogenes, respectively, as well as 14 and 10 strain-specific truncated pseudogenes, respectively. Bacterial strains have been known to diverge from each other fairly rapidly, implying that pseudogenization is a rapid process and that these pseudogenes are quite young.

Table 3. Number of common pseudogenes between pairs of species from the same baramin from the Pseudogene database

Species 1	Number of pseudogenes	Species 2	Number of pseudogenes	Number of common pseudogenes
B. halodurans	67	*B. subtilis*	214	1
C. muridarum	6	*C. trachomatis*	11	2
C. acetobutyliticum	37	*C. perfringens*	45	1
L. innocua	11	*L. monocytogenes*	9	2
M. leprae	888	*M. tuberculosis*	363	33
M. pneumoniae	47	*M. pulmonis*	9	0
P. abyssi	54	*P. horikoshi*	72	7
R. conorii	142	*R. prowazekii*	40	4
S. enterica	222	*S. typhimurium*	86	49
S. pneumoniae	110	*S. pyogenes*	63	3
S. solfataricus	168	*S. tokodaii*	117	24
T. acidophilum	41	*T. volcanicum*	81	1
T. acidophilum	41	*U. urealyticum*	8	0
T. volcanicum	81	*U. urealyticum*	8	0

We analyzed the common pseudogene content of 25 species of bacteria and archaea from 12 baramins from the Pseudogenes database. There were usually 2–3 species only from each baramin. These interbaraminic species-wise pseudogene content comparisons can be seen in table 3. As we can see, in many of the groups, much of the pseudogene content has diverged. Only *Mycobacterium*, *Pyrococcus*, *Salmonella*, and *Sulfolobus* have a substantial number of pseudogenes in common (3.7–9.1%, 9.7–13.0%, 22.1–57.0%, and 14.3–20.5%, respectively).

The high number of shared pseudogenes between two species may be the result of their colonization of the same host (representing a uniform environment), as in the case of *S. enterica* and *S. typhimurium*, which have 22–57% of their pseudogenes in common. Otherwise, different species living in different environments (such as two wild type species or one wild type species and one pathogen) may undergo different selective pressure, thereby losing different genes. *B. subtilis* and *B. halodurans*, on the other hand, are both free-living soil bacterium species, and have only one pseudogene in common. *C. acetobutylicum* and *C. perfringens* are also examples of a soil-living bacterium and a pathogen which have only one pseudogene in common. Also, *C. muridarum* infects lung tissue in mice, whereas *C. trachomatis* infects the eye and urogenital tract in human (different species, different tissues), and have only two pseudogenes in common.

Pseudogenes in archaea

It has been reported that relative to eukaryotes, deletions occur more frequently than insertions in both archaea and eubacteria. Further, the incidence of deletions is higher in archaea than in eubacteria. For example, the ratio of insertions to deletions was observed to be 0.07 in *Geobacter* and 0.9 in *Wolbachia*.[22] This deletion bias prevents a net gain of genetic information over time. In archaea, the coding fraction of the genome is higher than in eubacteria, with only 0.3% to 8.6% of the genome residing in pseudogenes in 15 species studied by van Passel.[23] Archaeal genes also contain more inactivating mutations (more than three times as many) on average than eubacteria. Also, strand slippage due to mononucleotide repeats is more of a cause of mutagenesis in bacteria than in archaea, due to its association of immune evasion of pathogenic bacteria.[24] Furthermore, truncation as a cause of pseudogenization is more prevalent in archaea than in eubacteria, with up to 30% of all archaeal pseudogenes formed in this manner.

Taken together, archaea and eubacteria are fundamentally different with respect to pseudogene content and possible pseudogenization mechanisms, which implies a separate origin of these two groups of organisms (apobaramins). Table 4 lists these differences in pseudogenization between archaea and bacteria.

Conclusion

Bacterial pseudogenes may indeed have functions, because many of them are shared between several unrelated or distantly related bacterial species. This conservation between species implies function and, as such, proves designed genomes. If they did not have any function, such as the 1,100+ pseudogenes in the genome of *M. leprae*, they would have been quickly removed from the genome. Further, due to their haploid state, the process of pseudogenization in bacteria is distinctly different from that in eukaryotes. The functionality of pseudogenes is more obvious in bacteria, since in eukaryotes, which are diploid, a still fully functioning version of the gene would mask the pseudogenized allele of that gene.

Table 4. Differences and similarities in pseudogenization between archaea and bacteria

Characteristic	Bacteria	Archaea
Pseudogene fraction	Up to 50%	<10%
Average pseudogene to total gene ratio	3.5–3.9% non-pathogenic, 3.3–4.4% pathogenic	3.6%
Insertion to deletion ratio	Higher	Lower
Number of inactivating mutations per gene	Lesser	Greater
Strand slippage due to mononucleotide repeats	Greater	Lesser
Truncation as a cause of pseudogenization	Lesser	Greater

Obviously, the wide extent of species-specific pseudogenes, as well as the observed deletion-bias, poses a problem for evolutionary theory. Pseudogenization is an ongoing process not only in pathogenic bacterial species (where there is no need to retain genes), but in non-pathogenic species as well, since, according to two studies, there is no statistically significant difference in the proportion of pseudogenes to all genes in these two groups. Pseudogenes are also known to accumulate in free-living bacterial species as a part of genome degradation.[3] This may have serious consequences for grand scale evolutionary theories, since the deletion bias renders it impossible to transform smaller prokaryotic genomes into larger eukaryotic. However, it fits the picture that genetic information is gradually degraded, pseudogenized, and eventually lost from the genome.

The creation and distribution of pseudogenes within bacterial baramins fit well with the baranome model of Peer Terborg.[25] According to this model, baranomes are pluripotent, undifferentiated, uncommitted genomes representing a single holobaramin. Speciation and adaptation may now occur due to their genome shuffling, loss of redundancy, and transposable elements activity (coined: variation-inducing genetic elements). The environment determines to what extent the frontloaded genetic programs are being preserved. Thus, the way certain bacteria adapt to a new host environment directly determines which genes become fixed (conserved by selection) and which degrade into pseudogenes (due to lack of selection). As we have seen, 1,456 genes have differentially pseudogenized in five species representing the *Shigella* baranome.[21] In seven *Rickettsia* species 1,252 genes have differentially pseudogenized, with 100–274 genes specifically lost to each species, which are well-known pathogens.[25] A study of *Salmonella enterica* serovars showed that 66 pseudogenes were common to serovars *S. paratyphi A* and *S. typhi*, which contained common inactivating mutations. Twenty of the shared pseudogenes encoded surface proteins, indicative of host interactions and thus environment-induced conservation.[26] A similar study of 19 *S. typhi* strains showed that their last common ancestor had 180 pseudogenes, whereas individual isolates of diverged successors had 10–28 additional pseudogenes.[27] The results presented in this study further demonstrated that in some baramins a large part of their pseudogenes can be held in common, whereas in other baramins, species-specific pseudogenization can be fairly rapid. This may reflect distinct biological mutational mechanisms, as well as distinct environmental constraints.

Overall, as a response to the original problem posed in the introduction about the possible functionality of bacterial pseudogenes, we can say that pseudogenes are themselves a microcosm of genomic decay. A subset of pseudogenes may be functional, but accumulation of inactivating mutations eventually renders them functionless. Their species-specific restriction within baraminic boundaries further supports the baranome theory. All of this supports devolution rather than upwards evolution.

Materials and methods

Bacterial pseudogene information (species, DNA, and protein sequence), among others, was retrieved from the Pseudogene.org database from the following file: pseudogene.org/prokaryotes/All_Prokaryotic_pseudogenes.txt. The number of pseudogenes was tallied for each bacterial species. The number of genes for bacterial species was retrieved from the following NCBI webpage: www.ncbi.nlm.nih.gov/genome. A given bacterial species was classified as pathogenic, non-pathogenic, or symbiotic according to the species description in their NCBI genome description page.

The Linux version of NCBI BLAST was used to identify the pseudogenes of 25 species of bacteria and archaea classified into 12 different baramins (shown in table 3) present in the Pseudogene database and compared against each other. Since we were dealing with pseudogenes which have diverged from each other sequentially, two pseudogenes were counted as a match with each other if they were at least 75% identical throughout at least 50% of their alignment with each other.

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Errata

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Royal Truman, Nylon-eating bacteria—part 4: interpretation according to Coded Information System theory

The chess problem in figure 3 on p. 83 should be as follows: