Evolutionary implications of a power-law distribution of protein family sizes

Joel S. Bader

(February 2, 2008)

Corresponding Author
Joel S. Bader, CuraGen, 555 Long Wharf Drive, New Haven, CT, 06511.
Tel. (203)401-3330x236; Fax (203)401-3351; Email jsbader@curagen.com
Current-day genomes bear the mark of the evolutionary processes. One of the strongest indications is the sequence homology among families of proteins that perform similar biological functions in different species. The number of proteins in a family can grow over time as genetic information is duplicated through evolution. We explore how evolution directs the size distribution of these families. Theoretical predictions for family sizes are obtained from two models, one in which individual genes duplicate and a second in which the entire genome duplicates. Predictions from these models are compared with the family size distributions for several organisms whose complete genome sequence is known. We find that protein family size distributions in nature follow a power-law distribution. Comparing these results to the model systems, we conclude that genome duplication is the dominant mechanism leading to increased genetic material in the species considered.
I. INTRODUCTION

Current-day genomes are the result of generations of evolution. One of the marks of evolution is the existence of protein families. These families comprise groups of proteins that share sequence similarity and perform similar biological functions [1,2]. The most likely explanation for the similarity in sequence and function is that all the proteins in a family evolved from a single common ancestor.

The size of a family, defined here as the number of proteins in a family for a particular species, evolves over time through processes that increase the physical size of an organism’s genome. Genomes in many major lineages are thought to have undergone ancient doublings one or more times [3]. It is thought that genome doubling can provide an evolutionary advantage by permitting redundant genes to evolve rapidly and perform different biological roles, potentially allowing entire pathways to acquire more specific function [4].

At finer scales, chromosomal regions or individual genes may be duplicated or lost through evolution. Even without physical loss, protein coding regions may suffer loss of function and cease to be expressed, leading to the existence of pseudogenes [5].

Previous studies have detected patterns supporting growth and loss of genetic information. Evolutionary processes consisting of duplication and mutation can introduce long-range, power-law correlations in the sequences of individual genes [6]; reports of such correlations in intron-rich regions sparked considerable interest [7].

In contrast to studies of individual gene sequences, we developed a model to explain the evolution of the physical size of a genome [8]. In our model, a speciation rate allowed genome size to increase or decrease, and an extinction rate removed individual species. The ratio of the speciation and extinction rates yielded scaling laws for the distribution of genome sizes: exponential scaling when the amount of genetic material lost or gained was constant, and power-law scaling leading to a self-similar distribution when the change in genetic material was proportional to the existing size. Closed-form approximations agreed with simulation results and explained observations reported by others [9].

Here we use related models to explore size of gene families. Processes that add and remove genetic material are presented in Sec. II. In the first model, we assume that duplication occurs on the level of individual genes. In the second model, we assume that these events duplicate an entire genome. Closed-form solutions are provided for the size distributions of gene families. Next, in Sec. III, we present results from analysis of gene fam-
ilies in sequenced genomes. These results rely heavily on the clusters of orthologous groups (COGs) database, which identifies gene families that span eight individual unicellular species including eubacteria, archaeabacteria, cyanobacteria, and eukaryots \[1\]. We discuss which evolutionary model is most consistent with our observations in Sec. IV.

II. THEORY

For a single organism, let \( P_n \) be the number of gene families that contain \( n \) genes. The total number of families is \( \sum_n P_n = P_{\text{tot}} \). We describe two models for the increase or decrease of the number of genes in the family.

A. Model I: Gene Duplication

In Model I, we assume that each gene in the family evolves independently. Each gene duplicates with rate \( k_+ \) and each gene is lost with rate \( k_- \). With each generation, the change in the number of families of size \( n \) is

\[
\Delta P_n = (n - 1)k_+P_{n-1} + (n + 1)k_-P_{n+1} - n(k_- + k_+)P_n. \tag{1}
\]

After sufficient time, the distribution reaches equilibrium values. Detailed balance indicates that the number of families increasing from size \( n \) to \( n + 1 \) should equal the number of families decreasing from size \( n + 1 \) to size \( n \),

\[
nk_+ P_n = (n + 1)k_+ P_{n+1}. \tag{2}
\]

The resulting expression for the populations is

\[
P_n/P_{\text{tot}} = (1/n)\alpha^n/[-\ln(1 - \alpha)], \tag{3}
\]

where we have defined \( \alpha \) as \( k_+/k_- \). Alternatively, normalizing by the families with a single member, we have

\[
P_n/P_1 = (1/n)\alpha^{n-1}. \tag{4}
\]

In addition to describing dynamics when each gene is duplicated individually, this model can also represent a system in which large genomic regions are duplicated or lost, provided that only one member of the family is present in the duplicated region. If, for example, a single chromosome is duplicated, this model could apply.

The populations \( P_n/P_1 \) predicted by Model I are shown as black lines in Fig. [\textbf{1}] for three choices of the parameter \( \alpha \): 0.1 (thin black line), 0.3 (medium black line), and 0.9 (thick black line). As the value of \( \alpha \) increases, the distribution of families shifts to larger sizes. The shape
of the distribution changes from a straight line on the log-log plot at small \( n \), characteristic of a power-law distribution, to a curved line at larger \( n \), characteristic of the faster decay of an exponential distribution.

**B. Model II: Genome Duplication**

In Model II, we assume that genome duplication dominates the evolutionary process. Each genome can double in size with probability \( k_+ \) or be reduced by half with probability \( k_- \). Writing the size of a family after \( j \) doublings as \( n = 2^j \), the evolution of \( j \) at each generation is

\[
\Delta P_j = k_+ P_{j-1} + k_- P_{j+1} - (k_+ + k_-)P_j. \tag{5}
\]

Again relying on detailed balance, we find that \( P_j \sim \alpha^j \), with \( \alpha = k_+/k_- \) as before. For normalization, we assume that \( \sum_j P_j = P_{tot} \), yielding

\[
P_j = (1 - \alpha) \alpha^j. \tag{6}
\]

To change variables from \( j \) to \( n \), we make an approximation that the discrete values of \( j \) and \( n \) may be replaced by a continuous distribution. The distribution for \( n \) is then \( P_n = P_{j(n)} d_j(n)/dn \), where \( j(n) = \log_2(n) \), giving

\[
P_n/P_{tot} = \lfloor (1 - \alpha)/ \ln 2 \rfloor n^{(\ln \alpha/\ln 2) - 1}. \tag{7}
\]

Because we used a continuous distribution to derive this result, the normalization is not exact. The power-law form of the distribution, however, is accurate, and simple summation may be used to define the normalization constant.

Alternatively, the distribution may be defined relative to the number of families of size 1, or

\[
P_n/P_1 = n^{(\ln \alpha/\ln 2) - 1}. \tag{8}
\]

Results for \( P_n/P_1 \) are shown as grey lines in Fig. 1 for three values of \( \alpha \): 0.1 (thin grey line), 0.3 (medium grey line), and 0.9 (thick grey line). As these are power-law distributions, they are straight on a log-log plot. The distribution favors larger family sizes as \( \alpha \) increases.

**III. RESULTS**

To investigate the size distributions of gene families in nature, we analyzed the contents of the COG database [1]. This database uses essentially unsupervised sequence-similarity comparisons to group proteins into
families of orthologs and paralogs. The current release includes 8328 proteins from eight sequenced genomes (E. coli, H. influenzae, H. pylori, M. genitalium, M. pneumoniae, Synechocystis, M. jannaschii, and S. cerevisiae) and assigns them to 864 individual families. Only proteins with orthologs in at least three species are included in the database. Using this database, we computed the number of families of size $n$, $P_n$, for each species, then normalized the result by $P_1$ for the same species. The results of this analysis are shown in Fig. 2.

As seen in Fig. 2 all the species show power-law behavior for $P_n/P_1$ as a function of $n$ for families of size 10 or smaller. The linear trend indicates that Model II, duplication of the entire genome, is more likely than Model I, in which individual genes are duplicated.

We explore the linear trend more quantitatively by performing a least-squares fit of the data for each model. The quantity we minimize is the RMS error for the log-transformed data,

$$\text{RMS} = \sqrt{\frac{1}{N} \sum_{n: P_n \geq 2} [\log_{10}(P_n/P_1)_{\text{data}} - \log_{10}(P_n/P_1)_{\text{fit}}]^2}$$  \hspace{1cm} (9)$$

with $(P_n/P_1)_{\text{fit}}$ from Eq. 4 or Eq. 8. As noted in the summation, we considered only family sizes $n$ with $P_n = 2$ or more; the total number of family sizes used is $N$. The results of the fit are detailed in Table I, along with the number of family sizes that contributed to the fit. The model with the smaller RMS for the fit is also indicated.

As seen in Table I Model II (complete genome duplication) provides a consistently better fit to the data than does Model I (individual gene duplication). In particular, when all of the protein families for a given organism are considered, each of the eight organisms shows a better fit with Model II than with Model I.

In Table I the fit values for $\alpha$ are also shown for the functional classes defined in the COG database: information storage and processing, cellular processes, metabolism, and poorly characterized \[1]. These individual classes are also fit better by Model II than by Model I. In E. coli, H. influenzae, H. pylori, M. pneumoniae, and Synechocystis, at least three of the four classes are fit better by Model II; in M. genitalium, there are not enough protein families for adequate predictions of $\alpha$. Only in S. cerevisiae does Model I appear to provide a slightly better fit to the distribution of family sizes for two classes, information storage and processing and cellular processes. One possible explanation for the better performance of Model I for S. cerevisiae is that gene families grow through the duplication of chromosomes, rather than the duplication of individual genes or entire genomes. The distinction between the genome and...
individual chromosomes is not applicable to the other organisms, which have a single chromosome.

A trend evident in Table I is that $\alpha$ for cellular processes (molecular chaperones, outer membrane, cell wall biogenesis, secretion, motility, inorganic ion transport and metabolism) is typically larger than $\alpha$ for information storage and processing (translation, ribosomal structure and biogenesis, transcription, replication, repair, recombination) and for metabolism (energy production and conversion, carbohydrate metabolism and transport, amino acid metabolism and transport, coenzyme metabolism, lipid metabolism). Protein families for cellular processes are therefore biased towards larger sizes, while families for information storage and processing and metabolism are biased toward smaller family sizes. This would imply that, in either model, a duplication of cellular process proteins is more likely to be retained than duplications of other functions. This suggests that cells can tolerate changes to cellular process pathways more readily than to other pathways.

The relative performance of Model I and Model II according to protein family functional class is summarized in Table II. When all classes are considered, Model II clearly provides a better explanation of the observed family sizes. When classes are considered separately, Model II provides a better explanation for three classes (information storage and processing, metabolism, and poorly characterized functions), while Model I provides a better explanation only for cellular processes.

The fits provided by Model I and Model II are shown in Fig. 3 for E. coli and S. cerevisiae. The observed family size distributions are shown as points and the best fits as lines, grey for Model I and black for Model II. The top pair of panels shows the results when all protein families are considered. For families up to size 10, the distributions from both organisms clearly follow the power-law prediction of Model II.

For the separate protein classes, the E. coli family sizes continue to follow the power-law prediction of Model II. As mentioned previously for S. cerevisiae, however, the fit to Model II is not good for the storage and processing and cellular processes classes. The size distribution decays much more rapidly than Model II predicts.

**IV. DISCUSSION**

We have investigated the size distribution of protein families. For a selection of single-celled organisms with sequenced genomes, we find that the number of families with $n$ members follows a power-law distribution as a function of $n$. This behavior suggests that evolution increases protein diversity through duplication of
entire genomes, balanced occasionally by the loss of large amounts of genetic information. It is less likely that protein diversity is increased through the duplication of individual genes, since this process would not lead to a power-law distribution.

The power-law we find is that $P_n/P_1 \propto n^{-\alpha}$, where $P_n$ is the number of families of size $n$. The exponent $\alpha$ varies from 0.2 to 0.6 depending on species. In our theory, this exponent measures the ratio of the rate of genome duplication to the rate of gene loss. The behavior we obtain for all species indicates that the rate of genome duplication, relative to the rate of gene loss, is approximately the same for each species. This points to the ancient origin of the cellular machinery responsible for the duplication of DNA.

Different classes of genes evolve at slightly different rates. Families that perform cellular processes tend to be larger than average. Supplementing these functions might provide a disproportionate selective advantage. Also, the remaining functions (information storage and processing and metabolism) could represent core cellular machinery that is relatively standard and requires less variability.

It would be interesting to verify whether the same protein family size distributions are observed in multicellular plants and animals. One might expect that genome duplication would be supplanted by chromosome duplication, which would shift the family size distribution from a power law to a steeper, almost exponential decay. Some evidence in this direction is already provided with the S. cerevisiae data presented in Sec. III. With the C. elegans sequence reported [10], the D. melanogaster sequence promised within a year [11], and a rough draft of the H. sapiens genome imminent [12], this question might soon be answered.

ACKNOWLEDGMENTS

We wish to thank Piotr Slonimksi for drawing our attention to models of genome evolution and Eugene Koonin for providing technical assistance with the COG database.

* CuraGen Corporation, 555 Long Wharf Drive, New Haven, CT 06511.
[1] R. L. Tatusov, E. V. Koonin, and D. J. Lipman, A genomic perspective on protein families, Science 278:
The COG database is available at [http://www.ncbi.nlm.nih.gov/COG](http://www.ncbi.nlm.nih.gov/COG).

[2] M. A. Huynen and P. Bork, Measuring genome evolution, Proc. Natl. Acad. Sci. USA 95: 5849-5856 (1998). M. Pellegrini, E. M. Marcotte, M. J. Thompson, D. Eisenberg, and T. O. Yeates, Assigning protein functions by comparative genome analysis: protein phylogenetic profiles, Proc. Natl. Acad. Sci. USA 96: 4285-8 (1999).

[3] Wolfe, K. H., and Shields, D. C. 1997. Molecular evidence for an ancient duplication of the entire yeast genome. Nature 387, 108-113. Paterson, A. H., et al. 1996. Toward a unified genetic map of higher plants, transcending the monocot-dicot divergence. Nature Genetics 14, 380-382. Ahn, S. and Tanksley, S. D. 1993. Comparative linkage maps of rice and maize genomes. Proc. Natl. Acad. Sci. USA 90, 7980-7984. Gaut, B. S. and Doebley, J. F. 1997. DNA sequence evidence for the segmental allotetraploid origin of maize. Proc. Natl. Acad. Sci. USA 94, 6809-6814. Moore, G. et al., 1995. Grasses, line up and form a circle. Current Biology 5, 737-739. Atkin, N. B. and Ohno, S. 1967. DNA values of four primitive chordates. Chromosoma 23, 10-13. Hinegardner, R. 1968. Evolution of cellular DNA content in teleost fishes. American Naturalist 102, 517-523. Ohno, S., Wolf, U., and Atkin, N. B. 1968. Evolution from fish to mammals by gene duplication. Hereditas 59, 169-187.

[4] T. R. Gregory and P. D. Herbert, The modulation of DNA content: proximate causes and ultimate consequences, Genome Res. 9: 317–24 (1999). T. Galitski, A. J. Saldanha, C. A. Styles, E. S. Lander, and G. R. Fink, Ploidy regulation of gene expression, Science 285: 251–4 (1999). P. Hieter and T. Griffiths, Polyploidy – More is more or less, Science 285: 210–211 (1999).

[5] W.-H. Li, Molecular Evolution, Sinauer Associates, Inc., Sunderland, MA (1997).

[6] W. Li, Spatial $1/f$ spectra in open dynamical systems, Europhys. Lett. 10: 395–400 (1989). W. Li and K. Kaneko, Long-range correlation and partial $1/f^α$ spectrum in a noncoding DNA sequence, Europhys. Lett. 17: 655–660 (1992).

[7] C.-K. Peng, S. V. Buldyrev, A. L. Goldberger, S. Havlin, F. Sciortino, M. Simons, and H. E. Stanley, Long-range correlations in nucleotide sequences, Nature 356: 168–170 (1992). R. F. Voss, Evolution of long-range fractal correlations and $1/f$ noise in DNA base sequences, Phys. Rev. Lett. 68: 3805–3808 (1992). S. Nee, Uncorrelated DNA walks, Nature 357: 450 (1992). V. V. Prabhuv and J.-M. Claverie, Correlations in intronless DNA, Nature 359: 782 (1992). W. Li and K. Kaneko, DNA correlations, Nature 360: 635–636 (1992). C. A. Chatzidimitriou-Dreismann and D. Larhammar, Long-range correlations in DNA, Nature 361: 212–3 (1993).

[8] J. S. Bader, Evolution of genome size, manuscript in review.

[9] Slonimski, P. P. 1999. Comparison of Complete Genomes:
FIG. 1. The family size distribution $P_n/P_1$ is shown for three values of $\alpha$: 0.1 (thin line), 0.3 (medium line), and 0.9 (thick line). Results are displayed both for Model I (grey lines) and Model II (black lines). Model II, which predicts a power-law distribution, is linear on a log-log plot.

FIG. 2. The size distributions $P_n/P_1$ of protein families are shown for the eight organisms included in the COG database. The linear trend on the log-log plot is evidence for genome duplication being the primary evolutionary mechanism driving the growth of gene families. Lines are provided as a guide to the eye.

FIG. 3. The family size distributions $P_n/P_1$ are shown for protein families in E. coli (left half) and S. cerevisiae (right half). Also shown are predictions of Model I (gene duplications are independent, grey line) and Model II (the entire genome duplicates, black line).

TABLE I. The parameter $\alpha$ as calculated from Model I and Model II is presented, along with the RMS of the fit, for the organisms and functional categories in the COG database.

| Organism / Functional category | Model I $\alpha$ | RMS | Model II $\alpha$ | RMS | $N_{fit}$ | Better Model |
|-------------------------------|-----------------|-----|-----------------|-----|----------|--------------|
| E. coli / All                 | 0.84            | 0.39| 0.50            | 0.16| 17       | II           |
| Information                   | 0.77            | 0.55| 0.47            | 0.39| 6        | II           |
| Cellular processes            | 0.84            | 0.20| 0.66            | 0.12| 7        | II           |
| Metabolism                    | 0.81            | 0.32| 0.53            | 0.17| 10       | II           |
| Poorly characterized           | 0.89            | 0.39| 0.64            | 0.25| 8        | II           |
| H. influenzae / All           | 0.56            | 0.22| 0.31            | 0.09| 8        | II           |
| Information                   | 0.36            | 0.10| 0.25            | 0.12| 4        | I            |
| Cellular processes            | 0.73            | 0.14| 0.54            | 0.03| 4        | II           |
| Metabolism                    | 0.53            | 0.16| 0.34            | 0.04| 5        | II           |
| Poorly characterized           | 0.56            | 0.10| 0.41            | 0.05| 5        | II           |
| H. pylori / All               | 0.54            | 0.32| 0.30            | 0.13| 7        | II           |
| Functional class       | Model I Better | Model II Better | Tie |
|------------------------|----------------|-----------------|-----|
| All classes            | 0              | 8               | 0   |
| Information*           | 2              | 5               | 1   |
| Cellular processes     | 4              | 2               | 2   |
| Metabolism             | 0              | 7               | 1   |
| Poorly characterized   | 1              | 7               | 0   |

*Information storage and processing

$N_{fit}$ is the number of family sizes used in the fit (all sizes with 2 or more families).

Information storage and processing

TABLE II. The number of organisms for which Model I or Model II is a better fit is summarized according to protein functional classes.
E. coli  

S. cerevisiae  

Functional class

All

Information storage and processing

Cellular processes

Metabolism