

## MODEL FOR THE GROWTH OF BACTERIAL GENOMES

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Received 17 June 2002

Analysis of the frequency of occurrence of short oligonucleotides in typical bacterial genomes reveals that they exhibit the statistical characteristics of a DNA sequence of a much shorter length. This peculiar property suggests a model for genome growth in which a genome evolves by random mutation but primarily grows by random segmental self-copying. Computer-generated genome sequence based on this model indeed has statistical properties similar to those of bacterial genomes.

### 1. Genome as a Text of Nucleotides

How much can we learn about a microbial genome's evolutionary history from its present state? When a genome is viewed as a text composed of the four "letters" A (adenine), C (cytosine), G (guanine) and T (thymine), it is essentially a random text. This is so because, as far as we know, genomes are made by a "blind watchmaker."<sup>1</sup> Whatever is not random about a genome is caused by the forces of selection that indirectly, subtly and slightly favor some random patterns over others. This is why it is such a challenge to delineate coding parts of a genome including genes and regulatory sequences from non-coding parts, and especially so when the effort cannot benefit from sequence similarity to other known coding sequences.<sup>2</sup>

As is well known, there is complexity in the randomness of genomes. An example that hints at the complexity of the genome-as-text is the distribution of the frequency of occurrence of oligonucleotides. In what follows, frequency will always mean frequency of occurrence, a  $k$ -mer is an oligonucleotide of length  $k$  and a distribution of frequency of  $k$ -mers will be called a  $k$ -mer distribution. The frequency of short  $k$ -mers has been used in studies of molecular evolution.<sup>3–5</sup> The frequency of a  $k$ -mer is the number of times it is seen through a sliding window of width  $k$  when it traverses once across the genome. If the length of the genome is  $L$ , the act just described is similar to distributing  $L$  objects (we think of the genome as being circular) into  $4^k$  boxes, the total number of different  $k$ -mers. Hence, when  $L$  is much greater than  $4^k$ , the  $k$ -mer distribution for a simple random genome sequence is expected to be a Poisson distribution with the mean and deviation both being  $L/4^k$ . By a simple random genome sequence of a given base composition, we mean

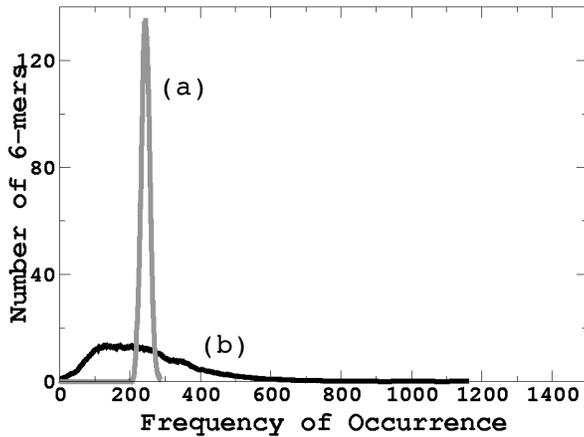


Fig. 1. Distribution of frequency of 6-mers of (a) a simple random sequence 1 Mb long with 50% A+T content, and (b) the genome of *E. coli*, whose A+T content is approximately 50%.

the sequence that would be obtained when any sequence of that base composition is thoroughly scramble.

The 6-mer distribution in a simple random sequence of length one million bases (1 Mb) with unbiased base composition is shown in Fig. 1(a). The mean of 244 and root-mean-deviation of 15.5 characterize the distribution as being Poisson. Figure 1(b) is the distribution obtained from the complete genome of *Escherichia coli*<sup>6</sup> whose base composition is essentially unbiased. (Microbial complete genome sequences are taken from the GenBank.<sup>7</sup> In this work, the frequencies of  $k$ -mers in microbial complete genomes are normalized to correspond to those of a 1 Mb long sequence by multiplying each frequency by a factor equal to  $10^6$  divided by the length of the genome.) While strikingly different from Fig. 1(a), Fig. 1(b) is representative of microbial complete genomes with an unbiased base composition. It has a root-mean-deviation (140) that is nine times that of the simple random sequence. Whereas simple random sequence contains no 6-mers whose frequency is greater than 400 or less than 100, the corresponding numbers of 6-mers in the genome of *E. coli* are about 500 and 510, respectively.

The distribution of a simple random sequence whose A+T content is 70% is shown in Fig. 2(a). (It is a general fact of genomes that the number of A and T bases are almost the same, similarly for C and G contents.) The single narrow peak seen in Fig. 1(a) is now broken into seven smaller peaks whose appearance is caused by the bias in the base composition; the mean frequency of 6-mers with  $m$  A or T's is  $244 \times (7/5)^m (3/5)^{6-m}$ , giving the positions of the seven peaks to be 11.4, 26.6, 62.0, 144, 337, 787, 1837, for  $m = 0$  to 6, respectively (the last peak is off scale in Fig. 2). Figure 2(b) is the distribution obtained from the complete genome of *Methanococcus janaschii* whose A+T content is approximately 70%.<sup>8</sup> The two distributions are again clearly dissimilar in detail.

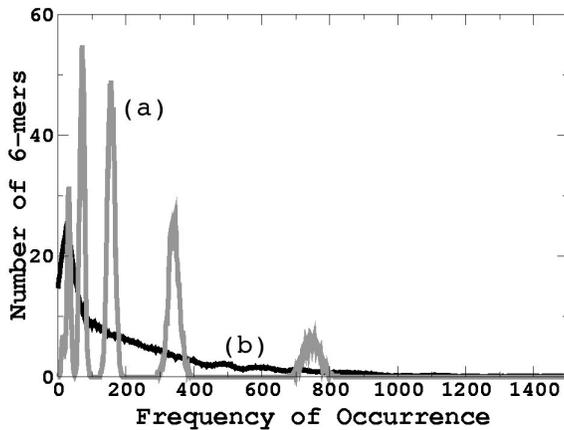


Fig. 2. Distribution of frequency of 6-mers of (a) a simple random sequence 1 Mb long with 70% A+T content, and (b) the genome of *M. janaschii*, whose A+T content is approximately 70%. The positions of the peaks in (a) are explained in the text.

Similar discrepancies between a simple random sequence and complete microbial genomes persist for  $k$ -mer *dist*s with  $k = 4$  to 9. (When  $k = 2$ , the number of types of  $k$ -mers is too small for the present analysis; when  $k = 10$ , the average frequency is too small;  $k = 3$  is a special case because of the existence of codons.) We know of no previous explanation of this discrepancy. Even as one is tempted to attribute the cause of the discrepancy to biological effects, we shall show that it is more likely that the observed distributions have an interesting stochastic origin.

## 2. Microbial Genome Sequences Have Statistics of Shorter Sequences

The ratio of the mean of the 6-mer distribution to its root-mean-deviation suggests that microbial genomes, typically of the order of 1 Mb long, exhibit the statistical property of a much shorter sequence, perhaps as short as 10 kb. In *E. coli*, that ratio is 1.74 (as opposed to a ratio of  $\sqrt{244} = 15.6$  for a simple random sequence of 1 Mb long). Over all the complete microbial genomes, the root-mean-deviation (after the bias in the base composition is corrected for), ranges from 96 to 218 and has an average of 154. This gives an average ratio of mean to root-mean-deviation of 1.58. In terms of a Poisson distribution such a ratio corresponds to a mean of 2.5 and a simple random sequence about 10 kb long, since there are 4096 6-mers. The 6-mer distribution of a 10 kb simple random sequence would have about 3310 of the 6-mers occur one to four times, 3 to 4 of the 6-mers occur nine times and about one 6-mer occur 10 times. It would also have 350 of the 6-mers not occurring altogether. Suppose we now duplicate this simple random sequence 100 times to produce a 1 Mb long sequence and let it undergo a number of single-base mutations, then we may expect the long sequence to have a 6-mer distribution that begins to resemble Fig. 2(b). That is, it should have many 6-mers occurring more than 400

many times, some occurring close to 1000 times, and many occurring fewer than 100 times.

Ohno conjectured that great leaps in evolution had been the result of whole genome duplications.<sup>9</sup> The idea has remained controversial; the present state of gene sequence information on vertebrates makes it difficult to either prove or disprove this hypothesis,<sup>10</sup> and phylogenetic studies of families of mammalian genes indicate that if ancient events of genome duplication did occur, they did not play an important role in structuring the mammalian chromosomes bearing such genes.<sup>11</sup> In any case, even if events of whole genome duplication had occurred, it probably did not occur a very large number of times. On the other hand, there certainly have been a very large number of events of duplications of shorter sequences. Indeed most genomes have repetitive sequences (or repeat sequences) with lengths ranging from 1 base to many kbs whose numbers of copies far exceed those that would be found in a simple random sequence. For example, in the human genome, repeat sequences account for at least 50% and probably much more,<sup>12,13</sup> because most ancient repeats presumably have been rendered unrecognizable as such by degenerative mutation.

### 3. The Model

We propose a minimal model for microbial genome growth that incorporates the duplication of DNA of all lengths, and that exhibits the observed  $k$ -mer distributions of real genomes. The model employs the two types of events that drives genomic changes, mutation and DNA duplication. For simplicity, mutation events are represented by single base replacement (SBR). DNA duplication events are represented by occasional random duplication (RD) of a stretch of oligonucleotide with a characteristic length of  $\sigma$  bases.

In this model, the single-stranded genome evolves and grows from the initial state of a simple random sequence of length  $L_0$  with a given base composition by (base composition preserving) SBR and RD events until its length just exceeds 1 Mb. In an RD event, the length  $l$  of the copied sequence is first randomly chosen (see below), then a site  $p$  sufficiently far from the end of the genome is randomly chosen and the sequence from  $p$  to  $p + l - 1$  is copied and inserted into the genome behind a second randomly chosen site. The model has three parameters: the initial length  $L_0$ , the ratio  $\eta$  of the chances of having an SBR or an RD event and the length scale  $\sigma$ . For the work reported here,  $L_0$  was held fixed at 1000 and only the two parameters  $\eta$  and  $\sigma$  were varied.

At each instance of an RD event, a length  $l$  not greater than the current length  $L_c$  of the (artificial) genome for the duplicated segment is chosen as follows. We construct a function  $G$  such that, given a random number  $y$  between zero and one, the duplicated segment length is  $l = G(\sigma; y)$ . Let  $w(x)$ , the probability per unit length of selecting a segment of length  $x$ , be proportional to  $e^{-x/\sigma}$ . Then from  $\int_0^{L_c} w(x) dx = 1$ , one has  $w(x) = \sigma^{-1} e^{-x/\sigma} (1 - e^{-L_c/\sigma})^{-1}$ . The inverse of  $G$  is given

by  $G^{-1}(l) = y = \int_0^l w(x)dx$  yields

$$l = G(\sigma; y) = -\sigma \ln[1 - y(1 - e^{-L_c/\sigma})]. \quad (1)$$

Note that when  $\sigma \gg L_c$ , the simplification  $l \approx yL_c$  is obtained. When  $\sigma \ll L_c$ ,  $l \approx y\sigma$  when  $y$  is close to zero, otherwise  $1 - y \approx e^{-l/\sigma}$  as long as  $y$  is much greater than  $e^{-L_c/\sigma}$  away from 1. In all cases,  $G(1) = L_c$ . For fixed  $L_c$  the average length of copied segments is  $\bar{l} = \sigma - L_c e^{-L_c/\sigma} / (1 - e^{-L_c/\sigma})$  which approaches  $\sigma$  when  $L_c$  becomes much greater than  $\sigma$ .

Suppose the final genome length  $L$  is much greater than  $L_0$  and  $\sigma$  (this will be the case here), then the total number of RD events will be somewhat greater than  $L/\sigma$  and the total number of SDR events will be somewhat greater than  $\eta L/\sigma$ .

#### 4. Results

Numerous simulation runs show that if the model sequence is to have a 6-mer distribution similar to those of the representative real microbial genomes, the total number of mutations (for a sequence of canonical length 1 Mb) acting on the model sequence needs to be around 40,000. From the discussion in the previous section, this implies the relation  $\sigma \approx 25\eta$  should hold. The best results are obtained when  $\sigma \approx 15,000$ . In Fig. 3 the model genome with an unbiased base composition generated with the parameters  $\eta = 500$  and  $\sigma = 15,000$  is seen to have a 6-mer distribution (gray) surprisingly similar to that of *E. coli* (black). No attempts were made to fine-tune the two parameters to get a “perfect” fit. In Fig. 4 the distributions for the model genome (gray) generated with  $\eta = 600$  and  $\sigma = 15,000$  and for the genome (black) of *M. janaschii* are compared. The peaks caused by the biased base

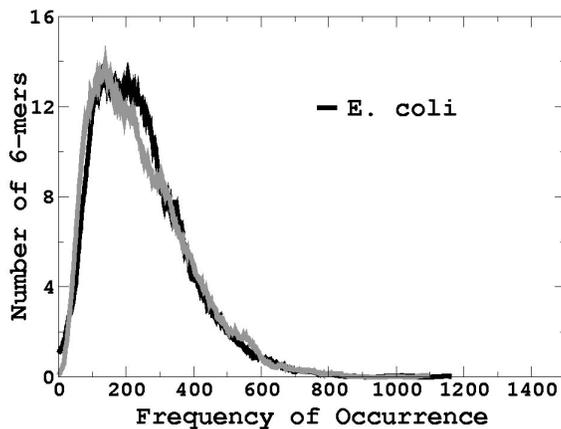


Fig. 3. 6-mer distribution of the genome of *E. coli* (50% A+T content) (black) and a simple random sequence (50% A+T content) including segmental duplication mechanism with  $\eta = 500$  and  $\sigma = 15,000$  (gray).

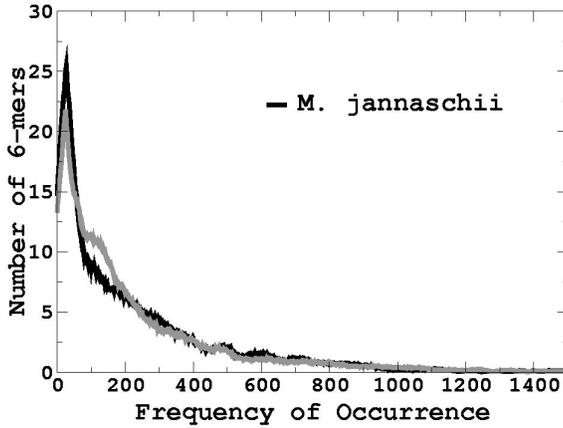


Fig. 4. Same as Fig. 3. Black: *M. jannaschii* (70% A+T content); Gray: model sequence with  $\eta = 600$  and  $\sigma = 15,000$ .

composition that one expects to see in a Poisson distribution (and seen in Fig. 2(a)) are no longer evident in the distributions from the model genomes in Fig. 4, just as they do not appear in the distributions from real genomes.

The 6-mer distributions of microbial genomes are well represented by the two-parameter gamma distribution:

$$D(y) = y^{\alpha-1} \beta^{-\alpha} e^{-y/\beta} / \Gamma(\alpha). \tag{2}$$

The distribution has a mean  $\langle y \rangle = \alpha\beta$  and a mean-square deviation  $\Delta = \alpha^{1/2}\beta$ . In Table 1, the  $n$ th order deviations, defined as  $\Delta^{(n)} = ((y - \langle y \rangle)^n)^{1/n}$ ,  $n$  from 2 to 5, of 6-mer distributions of real genomes are compared with those of: (a) the gamma distribution with the parameters  $\alpha$  and  $\beta$  (in brackets) obtained from the real genome distribution; (b) the 6-mer distribution of a simple random sequence without duplication; (c) the 6-mer distribution of the corresponding sequence given by the minimal model shown in Figs. 3 and 4. The values of  $\Delta^{(n)}$

Table 1. High order deviations  $\Delta^{(n)}$  of 6-mer distributions of microbial genomes and simple random sequences;  $\Delta^{(2)}$  is the root-mean-deviation. See text for description of the deviations and artificial sequences labeled (a), (b) and (c).

Sequence	$\Delta^{(2)}$	$\Delta^{(3)}$	$\Delta^{(4)}$	$\Delta^{(5)}$
<i>E. coli</i> (50% A+T content)	140	147	213	252
(a) ( $\alpha = 3.05, \beta = 80.0$ )	140	146	208	243
(b) Random Sequence	15.6	3.6	20.7	10
(c) ( $\eta = 500, \sigma = 15K$ )	144	148	212	247
<i>M. jannaschii</i> (70% A+T)	320	465	650	810
(a) ( $\alpha = 0.58, \beta = 418$ )	320	439	609	767
(b) Random Sequence	264	369	500	603
(c) ( $\eta = 600, \sigma = 15K$ )	321	462	635	783

in rows (a) show that the 6-mer distributions of the real genomes are well represented by gamma distributions. The values of  $\Delta^{(n)}$  in rows (c) show that the 6-mer distributions from the real and model genomes agree to a very high degree.

## 5. Discussion

We have shown that the full-length microbial genome could have grown stochastically and have the observed  $k$ -mer distribution provided that it grew mostly by random self-copying. We propose that this stochastic process, instead of some unknown biological process, is the main cause for the long genome to exhibit the statistical characteristics of its much shorter ancient self. Because the probability that a random stretch of DNA would be a gene (that codes an RNA or a protein that would fold and function) is infinitesimal, a population of genomes that stumbled upon a self-copying mechanism would have had an enormous evolutionary advantage over another unfortunate population that did not. The preponderance of intra-genomic and inter-genomic homologous genes<sup>14</sup> across all lifeforms are evidence of the importance of this mechanism.<sup>6,8,12,13</sup>

Self-copying growth may not be the only mechanism through which microbial genomes acquire the statistical characteristic of a much shorter sequence. Such a characteristic may well have an as-yet unknown biological rather than stochastic origin. Our model has the virtue of simplicity. The fact that a present-day long genome shares vital characteristic of its theoretical shorter earlier self implies one knows something about its *ancestor*, or the common ancestor of its relatives. Perhaps, by pushing this notion harder and examining the genomes closer, one may gain a deeper understanding of our universal ancestor.<sup>15</sup>

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