

LONG-TAIL FEATURE OF DNA WORDS OVER- AND UNDER-REPRESENTATION IN CODING SEQUENCES

A. NOWICKA¹, M. R. DUDEK², S. CEBRAT¹, M. KOWALCZUK¹,
P. MACKIEWICZ¹, M. DUDKIEWICZ¹, D. SZCZEPANIK¹

¹*Institute of Microbiology, University of Wrocław
ul. Przybyszewskiego 63/77, 54-148 Wrocław, Poland*

²*Institute of Physics, Pedagogical University
65-069 Zielona Góra, Poland*

Abstract: We have analyzed DNA sequences of known genes from 16 yeast chromosomes (*Saccharomyces cerevisiae*) in terms of oligonucleotides. We have noticed that the relative abundances of oligonucleotide usage in the genome follow a long-tail Lévy-like distribution. We have observed that long genes often use strongly over-represented and under-represented nucleotides, whereas it was not the case for the short genes (shorter than 300 nucleotides) under consideration. If selection on the extremely over-represented/under-represented oligonucleotides was strong, long genes would be more affected by spontaneous mutations than short ones.

I. INTRODUCTION

Since 1995 more than thirty full genomes have been sequenced and information on the sequences of hundreds of millions of nucleotides has been available for the scientific community. This has opened a new field of research, DNA statistical analysis, where genomic sequences are analyzed both in terms of single nucleotides, oligonucleotides and thousands of nucleotides. In the last case there are studies on the power spectral density and correlation function, especially the question of the existence of statistical long-range base-base correlation. Long-range correlation in DNA was first observed in 1992 by three groups, Li et al. [1, 2], Peng et al. [3] and Voss [4]. This has been a very active topic until now. We will not put a long list of references here but we cite only some of the recent papers by H. E. Stanley et al. [5], Arnéodo et al. [6] and Vieira [7] addressing the problem directly. One can also visit a WWW home page by Li (http://linkage.rockefeller.edu/wli/dna_corr) for the references to this particular topic. We have also contributed to the topic, e.g., in the papers [8-10] we show that replication, which is an asymmetric process, is responsible for introducing strong trends in the third bases of codons and in consequence it causes the long-range base-base correlations.

The examination of the long-range correlation in DNA is strongly connected with the statistical methods applied to texts in natural languages [11-15], where usually one calculates the frequency $f(k)$ of each word in a text ($k = 1, 2, \dots, N$). If the words in the text are arranged in rank order, from most frequent to least frequent, so that $f(1) \geq f(2) \geq f(3) \dots \geq f(N)$ then one observes a power law (Zipf law), $f(k) \propto 1/k^\zeta$, with an exponent ζ and typically $\zeta \sim 1.0$ for natural

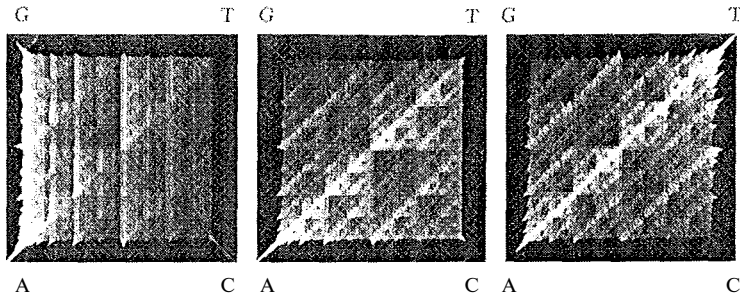


Fig. 1. The chaos game representation of the yeast genes in case of 6-words constructed from the first base position in codons (left), second base position in codons (middle), third base position in codons (right)

languages. This analysis also applies well to studying short-range time correlations in financial signals [14, 15]. In DNA the words are composed from an "alphabet" of four letters A , T , G , C representing the nucleotides *adenine*, *thymine*, *guanine* and *cytosine*. The n -tuplets are termed " n -words". The biological meaning of these «-words depends on the value of n . Typically in the case of coding DNA sequences the words are considered to be 3-tuplets because three nucleotides (codons) code for one amino acid. This triplet structure of DNA coding sequences can be easily detected with the help of the power spectrum because there is a sharp peak at frequency $f = 1/3$ in the spectrum. The connection of the peak with the codon structure has been reported already by Voss [4] in 1992 during discussion of the long-range correlations in DNA. This peak reflects the asymmetry of codons. For example, in the case of the yeast genome (*Saccharomyces cerevisiae*) more than 75% of all genes have more A than T in the first and second positions in codons, more G than C in the first positions of codons, and less G than C in the second positions [18]. Codons for hydrophobic amino acids are rich in T in the second positions whereas codons for hydrophilic amino acids are rich in A in the second positions. In particular, the genes with lower number of A than T in the second positions in codons represent genes coding for transmembrane proteins. Thus, considering 3-tuplets in coding regions to be the words in DNA texts is quite natural, contrary to the words for noncoding regions which are not known. On the other hand, the observation of a much smaller peak around $f \sim 1/11$ in DNA power spectrum [16,17] makes the understanding the DNA words more complex. Namely, this peak might be related to DNA folding structure. The detailed discussion of the meaning of the peak can be found in a paper by Trifonov [19] as well as a discussion of other recognized periodicities in genome sequences, 200- and 400-base periodicities.

The results of Zipf analysis of 40 DNA sequences have been discussed in detail by Mantegna et al. [12], They found that the Zipf exponent ζ for a noncoding region is about 50% larger than that for coding regions and thus noncoding sequences are closer to natural languages with respect to their information content than the coding ones. Notice however that also noncoding regions of DNA can possess a strong signal $f = 1/3$ [20]. The reason is that there can be found both sequences which were coding in past and sequences which may be recognized as genes in future.

Fig. 2. Zipf plots for three DNA subsequences originating from 2772 yeast genes, separately for bases in position (1) in codons, position (2) in codons, and position (3) in codons in the case when the effective frequencies z of 6-words were used

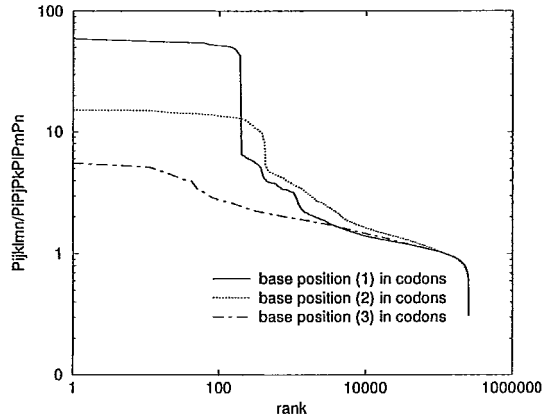
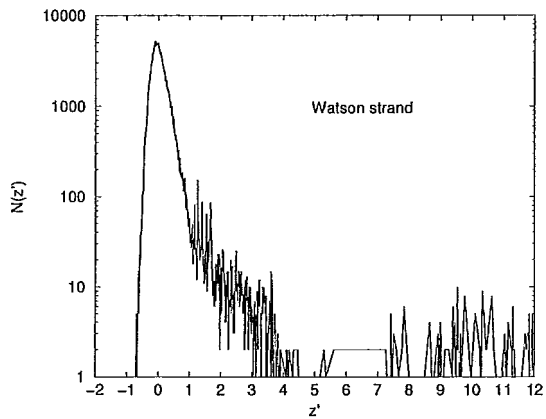


Fig. 3. Distribution of the numbers z' representing 6-words specific for base position (2) in codons of the yeast genes. Here only the genes located at one DNA strand have been considered



The studies of oligonucleotides (n -words) in recent years indicate that they can play the role of a genomic signature. Karlin and Burge, in their paper [21] showed that the relative abundance of dinucleotides (2-words) can discriminate DNA sequences of different organisms. The abundances, particularly for CG and TA can reflect the species-specific replication and repair mechanisms (see also Karlin, Mrázek and Campbell [22]). They analyzed different dinucleotides with the help of effective frequencies:

$$z = \frac{P_{ij}}{P_i P_j} \quad (1)$$

where P_i denoted the frequency of nucleotide i ($i, j = A, T, G, C$) and P_{ij} denoted the frequency of dinucleotide ij under consideration. In particular, they suggested that CG under-representation should be advantageous for organisms which have small genomes and need to replicate rapidly. On the other hand TA under-representation renders DNA more flexible for unwinding. The concept of genomic signature has been extended recently to n -words [23] where the chaos game representation of DNA sequences in the form of fractal images has been used following the

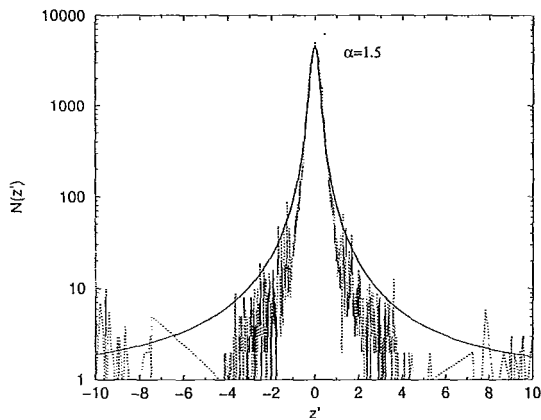


Fig. 4. Distribution of the numbers z' representing 6-words of yeast genes at base position (2) in codons. We associated a value z' with genes of one strand, and a value $-z'$ with genes of the complementary DNA strand. The continuous line represents the Levy distribution with $\alpha = 1.5$

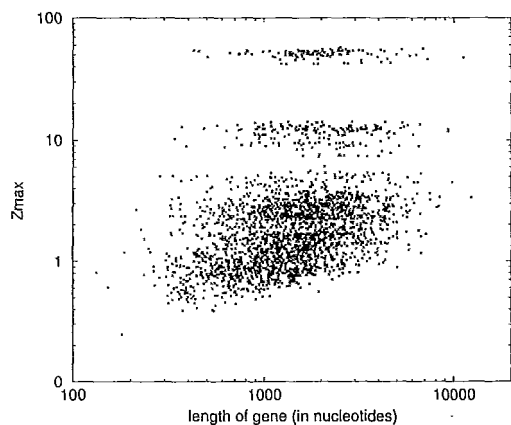


Fig. 5. For each gene length (in nucleotides), the maximum value of z has been recorded. 2722 yeast genes have been analyzed

method developed by Jeffrey [24]. We address this paper because we have introduced a similar concept of DNA representation independently in our paper [25]. In the method, the frequencies of n -words are represented by a complex landscape of "hills" and "valleys" located on a square board. An example of such a chaos game representation of a DNA sequence is presented in Fig. 1 for 6-tuplets constructed from nucleotides located in the first base position in codons, second base position in codons and third base position in codons in the case of the yeast genes. One can observe asymmetric usage of the 6-words. A similar result can be obtained for other values n of length of words under consideration.

In general, one can observe many oligomer repeats in the "hills" of the landscape, especially if one includes noncoding DNA regions. Their number is closely related to the mutation pressure and selection. The statistical properties of short oligonucleotides have been discussed recently by Buldyrev et al. [26]. In particular, they showed that the number of dimeric tandem repeats in coding DNA sequences is exponential, whereas in noncoding sequences it is more often described by a power law.

In the following we restrict ourselves to statistical analysis of 6-tuplets only and to this aim we have considered the relative frequencies of 6-words by a simple generalization of the Eq. 1:

$$z = \frac{P_{ijklmn}}{P_i P_j P_k P_l P_m P_n} \quad (2)$$

where P_{ijklmn} is the frequency of the word $ijklmn$ in the genome under consideration, and P_i, P_j, \dots, P_n are the respective nucleotide frequencies ($i, j, k, l, m, n = A, T, G, C$). Thus, $z = 1$ means that for a chosen 6-word the frequency of its usage in the genome is the same as the expected probability calculated from the nucleotide occurrence. Both under-representation and over-representation of 6-words might introduce the short-range correlation effects. If the words have a biological sense in DNA texts, they will be correlated at least in the region of a gene.

II. DNA WORDS VERSUS MUTATIONS AND SELECTION

The choice of variables z in Eq. 2 to represent effective frequencies of 6-words instead of absolute frequencies P_{ijklmn} guarantees that trivial correlations, the artefacts coming from the nucleotide bias, have been removed. Thus, if the numbers z associated with the respective 6-words represent biased random values only, their Zipf plot should be horizontal. We would like to address the paper by Vandewall and Ausloos [14] who used this argumentation in their analysis of financial data - daily fluctuations of the Apple stock price.

We analyze separately three gene subsequences, obtained by splicing nucleotides from position (1) in codons, position (2) in codons and position (3) in codons. Next, the three resulting nucleotide sequences are partitioned into non-overlapping 6-tuplets. Note that some 6-tuplets can be strongly under-represented. The reason is that 6-tuplets are already gene-specific and in the extreme case it can happen that a 6-tuplet from a gene under consideration does not appear in any other genes. This could introduce a strong correlation effect. Therefore, the values of z in Eq. 2 for a gene under consideration are calculated with the help of the frequencies $P_{ijklmn}, P_i, P_j, \dots, P_n$ in a bank of 6-words representing all genes except the considered one. In Fig. 2 we present the Zipf plots done for 6-tuplets in the case of 2772 yeast genes taken from ftp://genome-ftp.stanford.edu/pub/yeast/genome_seq/all_gcg. The results suggest that we can expect non-trivial correlations between successive 6-words. The reason for the observed step-like structure in Fig. 2 is that some deviations of P_{ijklmn} from the expected value are more frequent than others and, in general, the probability of choosing the next word may depend on several of the preceding words.

Note that the representation of genes by the effective frequencies (Eq. 2) of their 6-tuplets loses some information concerning base arrangement. It is often the case that different 6-tuplets have exactly the same deviation of P_{ijklmn} from the expected value in the genome. Thus a question could arise: is the z representation of genes consistent with the Levy walk analog of a two-dimensional DNA walk in space ($A-T, G-C$), discussed by Abramson, Alemany and Cerdeira [27]? In [27] it has been shown that the mean square displacement of the DNA walker follows the power law $\langle r^2(s) \rangle \sim s^\alpha$, where s denotes the number of steps and $\alpha \sim 1.5$ for yeast chromosomes. Once $1 < \alpha < 2$ this walk corresponds to the Levy walk. We could expect that the

distribution of the effective frequencies of 6-words should keep the memory of the Lévy flights performed in space (*A-T, G-C*). To show this, it will be convenient for us to introduce a new variable z' , which is the effective frequency z defined in Eq. 2 shifted by 1:

$$z' = z - 1. \quad (3)$$

In Fig. 3 we plotted the distribution of numbers z' representing yeast genes solely from one DNA strand. Almost the same distribution we have got for the numbers z' calculated for genes located in the complementary strand. In Fig. 4, we symmetrized the distribution of the numbers z' by introducing the values $-z'$ in the case of 6-tuplets of the complementary DNA strand. The long-tail feature of the distribution of the numbers z' is compared in the figure with a Lévy flight distribution calculated for the exponent $a = 1.5$, a value characteristic for the yeast genome [27]. The property of the large variance of z is consistent with the suggestion of non trivial correlation by Fig. 2. The results are consistent also with other data presented in Fig. 5, where for each gene length the maximum value of z' has been plotted. In the figure we can observe a trend that long genes use more strongly over-represented 6-words than short genes. An analogous situation we have noticed in the case of under-represented 6-words. If selection on the extremely over-represented/under-represented 6-words was strong, long genes would be more affected by spontaneous mutations than short genes. This is suggested also by the results [25] of our Monte Carlo simulations of gene evolution under constant mutation pressure and selection, where we showed that short genes accumulate more mutations per gene length than the long ones [25]. The fact that spontaneous mutation rates per nucleotide are inversely correlated with genome size has been first discussed by Drake et al. [28] and later by Karlin and Burge [21]. Our results might relate this phenomenon to the strong over-representation and under-representation of some n -words representing oligonucleotides.

References

- [1] W. Li, *Int. J. Bifurc. Chaos*, **2**, 137-154 (1992).
- [2] W. Li, K. Kaneko, *Europhys. Lett.*, **17**, 655-660 (1992).
- [3] C. -K. Peng, S. V. Buldyrev, A. L. Goldberger, S. Havlin, F. Sciortino, M. Simons, H.E. Stanley, *Nature*, **356**, 168-170 (1992).
- [4] R. Voss, *Phys. Rev. Lett.*, **68**, 3805-3808 (1992).
- [5] H. E. Stanley, S. V. Buldyrev, A. L. Goldberger, S. Havlin, C. -K. Peng, M. Simons, *Physica*, **A273**, 1-18 (1999).
- [6] A. Arnéodo, Y. d'Aubenton-Carafa, B. Audit, E. Bacry, J. F. Muzy, C. Thermes, *Eur. Phys. J.*, **B1**, 259-263 (1998).
- [7] M. de Sousa Viera, *Phys. Rev.*, **E60**, 5932-5937 (1999).
- [8] S. Cebrat, M. R. Dudek, A. Gierlik, M. Kowalczyk, P. Mackiewicz, *Physica*, **A265**, 78-84 (1999).
- [9] P. Mackiewicz, A. Gierlik, M. Kowalczyk, D. Szczepanik, M. R. Dudek, S. Cebrat, *Physica*, **A273**, 103-115 (1999).
- [10] P. Mackiewicz, A. Gierlik, M. Kowalczyk, M. R. Dudek, S. Cebrat, *Genome Res.*, **9**, 409-416 (1999).
- [11] W. Ebeling, T. Poshel, *Europhys. Lett.*, **26**, 241 (1994).
- [12] R. N. Mantegna, S. V. Buldyrev, A. L. Goldberger, S. Havlin, C. -K. Peng, M. Simons, H. E. Stanley, *Phys. Rev. Lett.*, **23**, 3169-3172 (1994).

- [13] M. Halibard, I. Kanter, *Physica*, **A249**, 525-535 (1998).
- [14] N. Vandewalle, M. Ausloas, *Physica*, **A268**, 240-249 (1999).
- [15] M. Ausloas, K. Ivanova, *Physica*, **A270**, 526-542 (1999).
- [16] S. V. Buldyrev, A. L. Goldberger, S. Havlin, R. N. Mantegna, M. E. Matsu, C. -K. Peng, M. Simons, H. E. Stanley, *Phys. Rev.*, **E51**, 5084 (1995).
- [17] H. Herzel, O. Weiss, E. N. Trifonov, *Bioinformatics*, **15**, 187 (1999).
- [18] S. Cebrat, M. R. Dudek, P. Mackiewicz, M. Kowalczyk, M. Fita, *Microb. Comp. Genom.*, **2**, 259-268 (1997).
- [19] E. N. Trifonov, *Physica*, **A249**, 511-516 (1998).
- [20] A. Gierlik, P. Mackiewicz, M. Kowalczyk, S. Cebrat, M. R. Dudek, *Int. J. Mod. Phys.*, **C10**, 1-9 (1999).
- [21] S. Karlin, Ch. Burge, *Trends Genet.*, **11**, 283-290 (1995).
- [22] S. Karlin, J. Mrazek, A. M. Campbell, *J. Bacteriol.*, **179**, 3899-3913 (1997).
- [23] P. J. Deschavanne, A. Giron, J. Vilain, G. Fagot, B. Fertil, *Mol. Biol. Evol.*, **16**, 1391-1399 (1999).
- [24] H. J. Jeffrey, *Nuci. Acid. Res.*, **18**, 2163-2170 (1990).
- [25] M. Kowalczyk, A. Gierlik, P. Mackiewicz, S. Cebrat, M. R. Dudek, *Physica*, **A273**, 116-131 (1999).
- [26] S. V. Buldyrev, N. V. Dokholyan, S. Havlin, H. E. Stanley, R. H. R. Stanley, *Physica*, **A273**, 19-32 (1999).
- [27] G. Abramson, P. A. Alemany, H. A. Cerdeira, *Phys. Rev.*, **E58**, 914-918 (1998).
- [28] J. W. Drake et al., *Nature*, **221**, 1128-1132 (1969).