Nucleotide spacing distribution analysis for human genome

Andrzej Z. Górski1 · Monika Piwowar2

Received: 15 December 2020 / Accepted: 2 March 2021 / Published online: 15 March 2021
© The Author(s) 2021

Abstract
The distribution of nucleotides spacing in human genome was investigated. An analysis of the frequency of occurrence in the human genome of different sequence lengths flanked by one type of nucleotide was carried out showing that the distribution has no self-similar (fractal) structure. The results nevertheless revealed several characteristic features: (i) the distribution for short-range spacing is quite similar to the purely stochastic sequences; (ii) the distribution for long-range spacing essentially deviates from the random sequence distribution, showing strong long-range correlations; (iii) the differences between (A, T) and (C, G) nucleotides are quite significant; (iv) the spacing distribution displays tiny oscillations.

Introduction
The Human Genome (HG) Project was launched in 1990 and was declared complete in 2003. The reference sequence for the HG was sequenced across all chromosomes. Understanding the coding and explanation of the reading of the genetic information contained in the full genomic sequence in view of the enormity of the data—despite analytical efforts—is still a great challenge (Green et al. 2015). Many studies have proven that the distribution of nucleotides, as well as whole sequences in the human genome is not random as it results from the non-random distribution of coding sequences (genes), CpG regions, as well as regulatory, splice and other functional regions (Denisov et al. 2015) (Majewski and Ott 2002) (Louie et al. 2003) (Piwowar et al. 2006). Fragments that do not encode in human DNA also have their distinctive distribution profile for specific nucleotides (Babarinde and Saitou 2016) (Sotero-Caio et al. 2017). The aim of many investigations has been to pinpoint important structural characteristics of DNA. For example, local irregularities along a DNA strand, compared to surrounding regions, have been associated with biological functionality (Pinkus 1965). On the other hand, it has been established that the regularity of DNA recording is characterized, for example, by fragments of introns. The coding regions in DNA are irregular (Woods et al. 2016). Exon and intron sequences can be identified from trends of the ratio of the 3-nucleotides periodicity to the background noise in the DNA sequences (Zhao et al. 2018). Computation of regularities has been also applied to biological weighted sequences (strings in which a set of letters may occur at each position with respective probabilities of occurrence) to indicate functionally significant fragments of DNA (Iliopoulos 2005). The above facts indicate that the analysis of nucleotide sequences is still a big challenge and any advance in describing DNA might provide a valuable insight. In this paper the (linear) spacing distribution of each of four nucleotides in the Hunan Genome is analyzed.

The motivation for the presented in the paper analysis was to check to what extent the distribution of nucleotides spacing in the human genome is irregular, taking into account our assumptions. We wanted to check where is the point at which the irregularity of the distribution is clearly observed. We start with the investigation of possible self-similar (fractal) patterns and proceed with statistical distribution of the nearest neighbor spacing for all four nucleotides constituting the genome. This type of analysis of data distribution is widely used not only in physics but also in other sciences, ranging from biomedical (Sotero-Caio et al. 2017) to economical (Górski and Skrzat 2006) applications.
**Materials and methods**

The Human Genome (HG) sequence has been taken from the HG Project in the FASTA format (https://www.ncbi.nlm.nih.gov/grc/human/data?asm=GRCh38.p10) (Genome Reference Consortium, Human Reference 2017). It includes the whole HG that is about 3 GB large and contains about 2 billions of nucleotides in chromosome’s fragments. The original text file is converted into numerical files with series of positions of particular nucleotides, A, C, G or T, while the other codes were ignored. The files with concatenated chromosomes are investigated to reveal averaged global properties of Human Genome and they are the starting point for further calculations. It should be stressed that the concatenation has negligible effect on the results because the number of chromosomes as well as the largest spacings are of order $10^2$ while the total length of the HG is of order $10^9$.

**Fractal analysis**

First, the possible generalized fractal dimensions (Mandelbrot 1982) of linear distributions of nucleotides A, C, G, T have been calculated. Such calculations, especially when done with a software that cannot be fully controlled, can give misleading results [see, e.g. (Górski and Skrzat 2006) (Górski 2001) (Górski et al. 2016)]. Hence, the calculation has been done with care, using our own box-counting algorithm code, based on the standard formula for the generalized fractal dimension (Mandelbrot 1982) (Górski 2001).

$$d_q = \frac{1}{1 - q} \lim_{N \to \infty} \frac{\log \sum_i p_i^q(N)}{\log N} = \frac{\log Y(N)}{\log N}$$

(1)

where $N$ is the number of (linear) divisions, parameter $q$ in our case was taken: $q = 0, 1, 2$, for capacity, information and correlation dimensions, respectively; $p_i(N)$ is number of data points found in $i$-th box for a given division $N$. The generalized fractal dimension ($d_q$) is extracted from the plot of log $Y(N)$ vs. log $N$, as a slope of the linear fit.

The resulting standard log–log plot used to extract generalized fractal dimensions for nucleotide A is shown in Fig. 1. Circles, squares and diamonds are for capacity ($d_0$), information ($d_1$) and correlation ($d_2$) dimension, respectively (they strongly overlap). The dotted line has the slope coefficient equal 1, like for homogeneously or randomly distributed data points. The dashed line shows the saturation limit for the ordinate, due to the finite size of the data sample.

Figure 1 implies that the data set has integer (non-fractal) dimension precisely equal to 1.00. Clearly, due to the Hentschel-Procaccia inequality (Hentschel and Procaccia 1983) $d(q) = 1.00$ for all $q < 2$, as the function $d(q)$ is monotonic. Calculations for higher values of $q$ were not performed because for very small $p_i(N)$ in sum in Eq. 1 their high powers are beyond any reasonable compiler accuracy. Hence, one has to conclude that the spacing distribution of nucleotides in Human Genome does not show any trace of direct self-similarity, fractal or multifractal structure.

In this place, it is worth to remind, that within the 2-dimensional Chaos Game Representation (CGR) of DNA sequences (Jeffrey 1990) their fractal structure is well established by many authors (see, e.g. (Moreno et al. 2011)). Self-similarity in those cases is due to the special properties of the CGR transformation, that is a kind of recurrence plot technique (Eckmann et al. 1987). These techniques are useful as randomness tests for random number generators (Jeffrey 1990), as well as stationarity tests for time series (Górski and Skrzat 2006). However, they do not imply self-similarity of the data sample by itself. Hence, it should be stressed, that our calculations...
presented in Fig. 1 are completely different than calculations presented, e.g. in (Moreno et al. 2011) and similar papers. While the cited papers proven the non-stationarity of the data series we have tested its direct fractal properties (of the linear DNA chain). No self-similar structures were found within the linear chain.

Even though the investigated data samples are not self-similar, and they were shown to have high entropy (Schmitt and Herzel 1997)—like random sequences—they are definitely not purely random. This will be shown in the following section. Moreover, even a highly structured data can resemble random series after compression, as the data compression algorithms increase the Shannon entropy.

### Spacing distribution analysis

In this section we analyze the spacing distribution, \( p(s) \), between nucleotides of the same type. Here, spacing \( s \) is defined as the distance between two closest neighbors of the same type. For example, for the nucleotide A and the sequence AA the spacing of nucleotides A is \( s = 1 \). For the sequence AXA, where \( X \) is any nucleotide except A, the spacing is \( s = 2 \), etc. In Figs. 2,3 the circles show (normalized) probabilities, \( p(s) \), of a given spacing in the sample. In addition, we added a dotted line that corresponds to the uniform random distribution of nucleotides,

\[
p_{\text{rand}}(s) = \frac{1}{3s(3/4)^s} \quad \text{where} \quad \sum_{s=1}^{\infty} p(s) = 1
\]  

(2)

---

**Fig. 2** Normalized histogram of spacing distribution, \( p(s) \), for bases A and C. The dotted line corresponds to a purely random distribution. Horizontal axis gives spacing distance and the vertical axis gives probability.

**Fig. 3** Normalized histogram of spacing distribution, \( p(s) \), for bases T and G. The dotted line corresponds to a purely random distribution. Horizontal axis gives spacing distance and the vertical axis gives probability.
Such distribution has no long-range correlations and was given as a reference to show the strength of correlations in our case.

For the Human Genome data the spacing distribution has cutoff for \( s_{\text{max}} \) that is at most of order 103. The total number of occurrences of nucleotide A (and T) is about \( 5.5 \times 10^8 \) and for nucleotide C (and G) about \( 4.1 \times 10^8 \). In Fig. 2 plots are given for nucleotides A and C, while in Fig. 3 for nucleotides T and G. Both pairs of plots are similar, in accordance with the Chargaff’s rule. All probability distributions are normalized to unity to enable comparison of samples with different sizes.

In Figs. 4 and 5 the tails of the histograms are shown up to \( s = 200 \). Here, one can see that for larger spacings (\( s \)) the tail is getting fat and strongly deviates from exponential behavior. Also, one can see a kind of phase transition at \( s_2 \approx 80 \) and the histograms’ bins are more randomly distributed. For \( p(s) \) approaching \( 10^{-9} \) there are only single data points per bin and the statistics becomes less reliable. Hence, though the single events are up to \( s \approx 1000 \) they are not displayed. It should be stressed that fat tails are also common for self-organizing systems in economy, sociology, etc., where long-range correlations (LRC) occur (Górski and Skrzat 2006).

This phenomenological behavior, though as yet not well understood, seems to be important because of its universality. It was observed for very different systems commonly considered as being complex in economy (Górski et al. 2002), sociology, biology (Górski and Skrzat 2006), linguistic (Lestrade 2017) etc. It is interesting to notice that the

**Fig. 4** Normalized histogram of spacing distribution for bases A and C with tail up to \( s = 200 \). Horizontal axis gives spacing distance and the vertical axis gives probability

**Fig. 5** Normalized histogram of spacing distribution for bases T and G with tail up to \( s = 200 \). Horizontal axis gives spacing distance and the vertical axis gives probability
characteristic strong correlations and fat tails do occur for distances \( s \) from about 20 up to about 80. It is also unclear why the first threshold is considerably larger for A and T nucleotides than for C and G nucleotides.

Closer examination of spacing distributions reveals several characteristic features that are listed below:

(i) For small spacing \( (s < s_1 \approx 25) \) probability is higher for their even predecessors. And the difference is slightly higher for \( (C,G) \) nucleotides than for \( (A,T) \) nucleotides. This is a kind of small high frequency oscillations in the distributions.

(ii) The long tails of the distributions are strongly enhanced (‘fat tails’) in comparison with the random distribution. This suggests strong long distance correlations.

(iii) In general, behavior of nucleotide A is similar as for nucleotide T, and the same holds for the \( (C,G) \) pair, though both pairs behave in different way. This can be viewed as another manifestation of the Chargaff’s rule.

(iv) For odd spacing \( (s = 3, 5, 7, \ldots) \) probability is higher than for larger spacing (fatter tails). For even spacings \( (s < s_1) \) it is close to random distribution (exponential decay). Analogous conclusion that the so-called random matches always dominate the distribution for small lengths has also been found recently for eukaryotic genomes (Massip et al. 2015) with similar suggested estimate, \( s_1 \approx 25 \). On the other hand, for larger spacing the distribution shows strong correlations and fat tails.

For large distances, \( s > s_2 \approx 80 \), strong variability around any smooth interpolation was found. Variability of long nucleotide fragments is most likely responsible for structural variation, which is read by molecules interacting with DNA, which are conformationally sensitive.

Existence of long-range correlations within the genome of a living organism has immense importance in understanding the language of DNA sequences. However, the biological meaning of the long-range correlations in DNA is, as yet, not clear. It is still an open and challenging problem. Long-range correlations suggest that to read the functionality of the human genome, one cannot focus solely on the linear reading of individual nucleotides present in the DNA strand. DNA is a three-dimensional object packed in a specific way in a cell nucleus. DNA is read by unraveling specific DNA fragments in the nucleus space. Probably the interaction of unraveled DNA strand fragments in space may explain the described interactions of long-range DNA fragments. The non-random patterns in DNA with long-range correlation can only be confirmation of this fact.

Research reports that there are non-linear chromatin interactions activating, e.g. transcription factors and long distance DNA interaction (Mifsud et al. 2015)(Noonan and McCallion 2010)(Peng et al. 1992). It confirms the computational observations.

Scientific reports also show a number of other pieces of evidence to explain DNA irregularities and long-range correlations. Long-range correlations \( (LRC) \) has been suggested to be related to the duplication of DNA fragments. Some authors claim that \( LRC \) occur only for intron containing DNA sequences, some however, that \( LRC \) does not distinguish between the intron and intronless DNA sequences. There have also been reports that \( LRC \) can be related to the nucleosomal structure and dynamics of the chromatin fiber. Our results are in agreement with conclusions reached by other authors, see, e.g. (Massip et al. 2015) (Messer et al. 2007). Moreover, the \( LRC \) have been shown important to the persistence of resonances of finite segments (Albuquerque et al. 2005).

Attempts are made to analyze the variability of the DNA sequence in terms of structural variation resulting from variation at the sequence level by, e.g. parametric and non-parametric entropy measures. Also, one can speculate, that relatively high entropy of the sequences reported previously (Schmitt and Herzel 1997) (and some similarity to random series) may be an effect of a kind of data compression algorithm. Finally, the A-T and C-G nucleotides have very similar distributions that is in accordance with the Chargaff’s rule. On the other hand, there is clear difference between the two pairs. The C-G nucleotides have significantly higher probability for larger spacing (fatter tails). For \( s = 50 \) the probability for C is about 10 times higher. On the other hand, the tail for C is shorter and its maximum is slightly higher. Such behavior have also been found for genomes of other species (Afreixo et al. 2009).
Declarations

Conflict of interest No competing financial interests exist.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Afreixo V et al (2009) Genome analysis with inter-nucleotide distances. Bioinformatics 25:3064–3070
Albuquerque EL et al (2005) Nucleotide correlations and electronic transport of DNA sequences. Phys Rev E 71:021910
Babarinde IA, Saitou N (2016) Genomic Locations of Conserved Non-coding Sequences and Their Proximal Protein-Coding Genes in Mammalian Expression Dynamics. Mol Biol Ecol 33:1807–1817
Denisov S et al (2015) Correlated Evolution of Nucleotide Positions within Splice Sites in Mammals. PLoS ONE 10:e0144388
Eckmann JP et al (1987) Recurrence plots of dynamical systems Epl 4:973–977
Genome Reference Consortium, Human Reference (2017) Genome Ref. Consortium, Hum. Ref., p p12
Górski AZ (2001) Pseudofractals and the box counting algorithm. J Phys A Math Gen 34:7933–7940
Górski AZ, Skrzat J (2006) Error estimation of the fractal dimension measurements of cranial sutures. J Anat 208:353–359
Górski AZ et al (2002) Financial multifractality and its subtleties: An example of DAX. Phys A Stat Mech its Appl 316:496–510
Górski AZ et al (2016) Accuracy of the box-counting algorithm for noisy fractals. Int J Mod Phys C 27:1650112
Green ED et al (2015) Human Genome Project: Twenty-five years of big biology. Nature 526:29–31
Hentschel HGE, Procaccia I (1983) The infinite number of generalized dimensions of fractals and strange attractors. Phys D Nonlinear Phenom 8:435–444
Iliopoulos CS (2005) Computing the Repetitions in a Biological Weighted Sequence. J Automat Lang Comb 10:687–696
Jeffrey HJ (1990) Chaos game representation of gene structure. Nucleic Acids Res 18:2163
Lestradeg S (2017) Unzipping Zipf's law. PLoS ONE 12:e0185198
Louie E et al (2003) Nucleotide Frequency Variation Across Human Genes. Genome Res 13:2594–2601
Majewski J, Ott J (2002) Distribution and characterization of regulatory elements in the human genome. Genome Res 12:1827–1836
Mandelbrotn, B.B. (1982) The Fractal Geometry of Nature (0716711869, 1982).pdf.
Massip F et al (2015) How evolution of genomes is reflected in exact DNA sequence match statistics. Mol Biol Ecol 32:524–535
Messer PW et al (2007) Effects of Long-Range Correlations in DNA on Sequence Alignment Score Statistics. J Comput Biol 14:655–668
Mifsud B et al (2015) Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C. Nat Genet 47:598–606
Moreno PA et al (2011) The human genome: a multifractal analysis. BMC Genomics 12:506
Noonan JP, McCallion AS (2010) Genomics of long-range regulatory elements. Annu Rev Genomics Hum Genet 11:1–23
Peng CK et al (1992) Long-range correlations in nucleotide sequences. Nature 356:168–170
Pinkus JL et al (1965) The Structures of the Isoisatogens; The Structures of DNA and RNA. J Org Chem. https://doi.org/10.1021/jo01015a037
Piwowar M et al (2006) Tandemly repeated trinucleotides - comparative analysis. Acta Biochim Pol 53:279–287
Schmitt AO, Herzel H (1997) Estimating the Entropy of DNA Sequences. J Theor Biol 188:369–377
Sotero-Caio CG et al (2017) Evolution and Diversity of Transposable Elements in Vertebrate Genomes. Genome Biol Ecol 9:161–177
Travers A (2005) DNA Dynamics: Bubble ‘n’ Flip for DNA Cyclisation? Curr Biol 15:R377–R379
Vologodskii A, Frank-Kamenetskii MD (2018) DNA melting and energetics of the double helix. Phys Life Rev 25:1–21
Vologodskii A, Frank-Kamenetskii D, M. (2013) Strong bending of the DNA double helix. Nucleic Acids Res 41:6785–6792
Woods T et al (2016) Characterizing exons and introns by regularity of nucleotide strings. Biol Direct 11:6
Zhao J et al (2018) Detecting Periodicities in Eukaryotic Genomes by Ramanujan Fourier Transform. J Comput Biol 25:963–975

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.