Statistical analysis of gene and intergenic DNA sequences

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Abstract

Much of the on-going statistical analysis of DNA sequences is focused on the estimation of characteristics of coding and non-coding regions that would possibly allow discrimination of these regions. In the current approach, we concentrate specifically on genes and intergenic regions. To estimate the level and type of correlation in these regions we apply various statistical methods inspired from nonlinear time series analysis, namely the probability distribution of tuplets, the Mutual Information and the Identical Neighbour Fit. The methods are suitably modified to work on symbolic sequences and they are first tested for validity on sequences obtained from well-known simple deterministic and stochastic models. Then they are applied to the DNA sequence of chromosome 1 of \textit{arabidopsis thaliana}. The results suggest that correlations do exist in the DNA sequence but they are weak and that intergenic sequences tend to be more correlated than gene sequences. The use of statistical tests with surrogate data establish these findings in a rigorous statistical manner.

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1. Introduction

The goal of the ongoing genome projects is to detect and extract the genetic information from the DNA sequences. In addition to any biological approaches, that constitute major contributions towards this goal, statistical analysis of DNA sequences has its own merit and has met tremendous interest in the recent years. In particular, much...
effort is focused on the estimation of statistical properties of coding and non-coding regions as well as on the discrimination of these regions. Starting from the pioneer work in Ref. [1] on the so-called “DNA walk” where long-range power law scaling was found on non-coding regions, a number of correlation measures were used, such as power spectrum (e.g. see Ref. [2]), detrended fluctuation analysis [3], redundancy and entropy [4,5], and mutual information [6,7]. The results from the application of the different measures on DNA sequences, as well as from analyses making use of several measures together (e.g. see Ref. [8]), agree in that there is a different structure of the symbolic sequence in the coding and non-coding regions and that the non-coding symbolic sequences are more correlated than the coding ones (see the review in Ref. [9] and references therein). These findings are certainly useful, but they are based on the prior identification of the coding and non-coding regions.

Coding regions are organized in clusters called genes, which contain also non-coding regions, called introns, in-between the coding regions, called exons. Thus intergenic regions have pure non-coding character, while genes are of mixed character since they contain both introns (non-coding) and exons (coding). We choose to pursue the analysis at the lower level of knowledge of the DNA sequence, namely when only the genes are identified and not the exons and introns in each gene. This analysis is more involved because the mechanism underlying the gene expressions is corrupted by the introns and the objective here is to investigate whether the statistical properties for genes and intergenic regions can be distinguished.

The statistical aspects we focus on are, the distribution of homologous nucleotides (successive identical symbols in the DNA sequence) [10–13], the general correlation between two nucleotides being apart by a varying displacement (quantified by the mutual information of the symbols being apart) [14], and the prediction of a nucleotide at a certain position given the other nucleotides in the sequence (by creating a new algorithm making use of a local prediction technique modified for symbolic time series). To facilitate the correct interpretation of the results of this analysis, we make the same statistical analysis on artificial symbolic sequences for which we know the generating mechanism. We use data from a purely stochastic system, a linear stochastic system and a simple chaotic system. The results of the analysis on genes and intergenic regions combined also with the results on the artificial systems give some insight onto the level of structure and randomness in the two types of DNA sequences. In order to assess rigorously the level of randomness or correlations in the two types of DNA sequences we apply also statistical tests using the method of surrogate data [15,16].

The paper is organized as follows. In Section 2, the DNA symbolic sequences are described and the artificial and DNA data sets used in the statistical analysis are presented. In Section 3, the statistical methods are described and in Section 4 the results of the analysis are presented. Finally, in Section 5 the results are discussed, the main conclusions are drawn and open problems are introduced.

2. DNA and artificial symbolic sequences

The primary structure of DNA consists basically of a nitrogenous base of four nucleotides, the two purines, adenine (A) and guanine (G), and the two pyrimidines,
cytosine (C) and thymine (T). Thus the DNA sequence can be simply considered as a symbolic sequence on the four symbols A,C,G,T. The genes are specific subsequences of nucleotides encoding information required to construct proteins. In primary organisms it is found that the genes cover a large proportion of the whole DNA. In higher organisms, such as the human, genes are more sparsely distributed and the coding sequences constitute a very small proportion of the genome. Thus, in terms of data availability, it is difficult to pursue a statistical analysis on the two distinct types of the DNA sequence (the genes and intergenic regions) on higher organisms. In our analysis we use a large segment of the Chromosome 1 of Arabidopsis Thaliana (a plant), which we denote CAT1. The genes in CAT1 have been identified and the proportion of genes is about 50%, relatively high for higher eucaryotes. The complete sequence of CAT1 has 29,640,317 bases, but we use a smaller segment (from base 3760 to base 1,000,232) to make two sequences, one joining together the genes (of total length 574,395) and another joining together the intergenic regions (of total length 425,837). The median size for genes and for intergenic regions is 1608 and 1170, respectively, and the first and third quartiles are (1045, 2542) and (687, 2030), respectively.

In order to interpret the results of the statistical analysis we use also artificial symbolic sequences on four symbols from well-known systems. The simulated symbolic sequences are designed to possess the probability distribution of the four symbols of the DNA symbolic sequence. This means that to each of the gene and intergenic sequence corresponds a different symbolic sequence derived from the same system. The systems we consider here generate numeric time series and we transform them to symbolic. We choose: a purely stochastic system (a), a linear stochastic system with strong and weak correlations (b1 and b2) and a chaotic system (c). The respective toy models are: (a) a purely white noise (uniform in [0,1]); (b1) a first-order autoregressive model, AR(1), with coefficient $\phi=0.9$ to assign for strong autocorrelations and (b2) $\phi=0.4$ to assign for weak autocorrelations; (c) the logistic map $s_{i+1} = 4s_i(1-s_i)$ at chaotic regime.

The transformation of the simulated numeric time series to symbolic time series is done with respect to the probability distribution of the A,C,G,T symbols of the DNA sequence. For this we select three breakpoints in the range of the data of the numeric time series, so that the relative frequency in each of the four bins (formed by the minimum data point, the three breakpoints and the maximum data point) is equal to the relative frequency of an assigned symbol of the DNA sequence. Let $\{x_i\}_{i=1}^n$ be the DNA symbolic sequence, where $x_i \in \{A,C,G,T\}$, and $\{y_i\}_{i=1}^n$ be the artificial symbolic sequence derived from a numeric time series ($y_i \in \{A,C,G,T\}$). Then by construction of $\{y_i\}_{i=1}^n$ we have

$$p_x(a) = p_y(a), \quad \text{or} \quad p(x=a) = p(y=a) \ \forall a = A, C, G, T,$$

where $p_x$ is the probability mass function of $x$ and $p_x(a) = p(x=a)$ is the probability that $x = a$. Note that to each of the two types of DNA sequences corresponds a different symbolic sequence derived from the same artificial numeric time series by a classification mechanism that is defined by the base probabilities of the corresponding DNA sequence.
3. Methods

A full statistical description of a symbolic sequence would require the estimation of the $w$-joint probability function $p_x(x_i, x_{i-1}, \ldots, x_{i-w+1})$ for sufficiently large window $w$. For limited size sequences $\{x_i\}_1^n$, reliable estimations can be achieved only for very small $w$. Therefore, we rely instead on statistical measures related to certain aspects of $p_x(x_i, x_{i-1}, \ldots, x_{i-w+1})$. In the following, we present the methods of our statistical analysis including a hypothesis test that makes use of surrogate data.

3.1. Probability distribution of tuples of symbols

As a first statistical approach towards the investigation of correlations in symbolic sequences we consider the probability distribution of the size of clusters of an identical symbol. Note that if the symbolic sequence $\{x\}$ is completely independent then for a cluster of size $m$ it should be

$$p_x(a^m) = p_x(aa\ldots a) = p_x(a)^m, \quad \text{where } a = A, C, G, T,$$

where $p_x(a^m)$ is the probability that symbol $a$ occurs $m$ times sequentially and $a^m$ is called the $m$-tuple of $a$. The probability $p_x(a^m)$ is estimated by the relative frequency of the occurrence of the $m$-tuples of $a$ in the symbolic sequence. For a tuple of length $w$, $p_x(a^w)$ is actually the evaluation of $p_x(x_i, x_{i-1}, \ldots, x_{i-w+1})$ in the case $x_i = x_{i-1} = \cdots = x_{i-w+1} = a$.

3.2. Mutual information

The mutual information $I(x; y)$ of two variables $x$ and $y$ measures the general correlation between $x$ and $y$ and it is defined as [17,18]

$$I(x; y) = \sum_{a,b} p_{xy}(a,b) \log \frac{p_{xy}(a,b)}{p_x(a)p_y(b)},$$

where $a$ and $b$ in the double sum are the possible values $x$ and $y$ can take. For time series or spatial sequences the mutual information regards the variables that are apart by a lag or displacement $\tau$, namely $x_i$ and $x_{i-\tau}$, and the mutual information is then denoted $I(\tau)$. For the symbolic sequences considered here there are four distinct base probabilities, i.e., $p_x(a) = p_{x,-\tau}(a)$ for $a = A, C, G, T$, and 16 joint probabilities for each $\tau$, i.e., $p_{x,x,-\tau}(a,b)$ for $a, b = A, C, G, T$. The base and joint probabilities are again estimated by the relative frequencies computed on the symbolic sequence. Note that setting $\tau = w - 1$, $I(w - 1)$ is derived from $p_x(x_i, x_{i-w+1})$, which is the projection of the $w$-joint probability function $p_x(x_i, x_{i-1}, \ldots, x_{i-w+1})$ onto the first and last component of the window $(x_i, x_{i-1}, \ldots, x_{i-w+1})$.

3.3. Identical neighbor fit

The identical neighbor fit is actually a modification of the method of nearest neighbor prediction used for nonlinear prediction and modeling of numeric time series [19].
the context of symbolic sequences, the prediction problem is to estimate a symbol \( T \) positions forward when we know the symbols up to the current position \( i \). However, for DNA sequences we consider the modeling or fitting problem rather than the prediction problem since we know all symbols. For each position \( i \) in the symbolic sequence, we want to estimate the probability of correct identification of the symbol in position \( i + T \) using all other symbols in the sequence, i.e., symbols in positions \( 1, \ldots, i, i + T + 1, \ldots, n \), where \( n \) is the length of the sequence. The level of fit as defined by this probability constitutes another measure of the degree of correlations in the symbolic sequence.

Similarly to the state space reconstruction of numeric time series, we assign for each symbol at position \( i \) in the symbolic sequence, the segment \( [i-m+1, i] \) of size \( m \) comprised of the \( m \) last symbols, i.e., the symbols in positions \( i-m+1, \ldots, i \). The parameter \( m \) acts here as the embedding dimension for numeric time series. Thus for each symbol \( x_i \), \( i = m, m+1, \ldots, n-T \) we assign the symbolic vector \( x_i = [x_{i-m+1}, \ldots, x_i] \). If the working position for the fit is \( i \), the target vector is \( x_i \), and we want to estimate the symbol \( x_{i+T} \). To do this, we search across the sequence \( \{x_m, x_{m+1}, \ldots, x_{i-1}, x_{i+T}, \ldots, x_{n-T}\} \) (we exclude vectors that have as components any of \( x_i, \ldots, x_{i+T} \)) to find symbolic vectors that are identical to \( x_i \), which we call identical neighbors (similarly to the nearest neighbors for numeric time series [19]). Let us suppose that we found \( K \) identical neighbors of \( x_i \) in positions \( i_1, \ldots, i_K \) and the respective symbols \( T \) steps forward are \( x_{i_1+T}, \ldots, x_{i_K+T} \). Since we know the actual symbol \( x_{i+T} \) we define the prediction error based on the \( k \)th identical neighbor as

\[
e_{ik}(T) = \begin{cases} 0 & \text{if } x_{i+T} = x_{i_k+T}, \\ 1 & \text{if } x_{i+T} \neq x_{i_k+T}. \end{cases}
\]

The error in the prediction of \( x_{i+T} \) using all \( K \) identical neighbors of \( x_i \) is the proportion of false identical neighbor predictions defined as

\[
E_i(T) = \frac{1}{K} \sum_{k=1}^{K} e_{ik}(T).
\]

If \( E_i = 0 \) the prediction of \( x_{i+T} \) is perfect while if \( E_i = 1 \) the prediction fails completely.

Finally, we average the individual prediction errors over all symbolic vectors (\( i = m, m+1, \ldots, n-T \)) to get a measure of the mean identical neighbor error (MINE) for the whole sequence, defined as

\[
\text{MINE}(T) = \frac{1}{n-m-T+1} \sum_{i=m}^{n-T} E_i(T). \tag{2}
\]

One can also define the weighted MINE (WMINE) from the weighted average with respect to the number \( K_i \) of identical neighbors found at each individual prediction \( i \),

\[
\text{WMINE}(T) = \frac{\sum_{i=m}^{n-T} K_i E_i(T)}{\sum_{i=m}^{n-T} K_i}. \tag{3}
\]

For a symbolic sequence \( \{x_i\}_1^n \), the maximum prediction error level depends on the base probability distribution \( p_x(a) \) for each symbol \( a \) and it is reached when the
symbolic sequence is purely random. It is straightforward to find that the maximum error is
\[ \text{max MINE}(T) = \text{max WMINE}(T) = 1 - \sum_a p_x(a)^2, \tag{4} \]
where the sum is over the symbols of the sequence. For a random sequence of four equally probable symbols \((p_x(a) = 0.25\) for every \(a\)) we get \(\text{max MINE}(T) = 0.75\).

The measure of identical neighbor fit is also related to the joint probability function \(p_x(x_i, x_{i-1}, \ldots, x_{i-w+1})\) through the conditional probability function \(p_x(x_{j+T}|x_j, x_{j-1}, \ldots, x_{j-m+1})\) where \(w = m + T\) and \(i = j + T\). The estimation of this conditional probability is actually the problem of individual \(T\) step ahead prediction for a position \(j\) (actually, the false identical neighbor prediction \(E_j(T)\) estimates the complementary conditional probability). In the computation of the total prediction error MINE, we evaluate the conditional probability on a subset of the set of all possible values \(\{x_j, x_{j-1}, \ldots, x_{j-m+1}\}\) (those found in the sequence), which constitutes a very small fraction when \(m\) gets large. For \(m = 1\), the conditional probability reads \(p_x(x_{j+T}|x_j)\) and MIME measures essentially the same characteristic as the mutual information for lag \(T\), \(I(T)\). Thus, in terms of information processing, the identical neighbor fit can be seen as an extension of mutual information to more than two variables, i.e., a measure similar to Shannon-like entropy [4].

3.4. Hypothesis test with surrogate data

The working null hypothesis \(H_0\) for the DNA sequence is that the examined symbolic sequence is completely random, i.e., there are no correlations in the sequence. We generate an ensemble of \(M\) surrogate symbolic sequences that represent \(H_0\). Each surrogate sequence is simply generated by shuffling the original sequence. Note that in this way, the original base probabilities are preserved in the surrogate sequence and the sequence is otherwise random.

As discriminating statistic \(q\) for the test we consider any of the statistics from the statistical methods presented above. \(H_0\) is rejected if the statistic \(q_0\) on the original symbolic sequence does not fall within the empirical distribution of the statistic \(q\) under \(H_0\), which is formed by the statistics \(q_1, \ldots, q_M\) computed on the \(M\) surrogate symbolic sequences. A formal test decision can be made using a parametric approach or a non-parametric approach (e.g. see Ref. [16]).

4. Results

The statistical analysis with the tools presented in Section 3 extents in two directions: (a) investigation in a rigorous statistical manner whether the gene sequence and the intergenic sequence contain significant correlations and assessment of the degree of departure from the level of no correlation, and (b) comparison of the two types of DNA sequences to each other and to some other symbolic sequences with known dynamical properties.

We choose to present the results for each of the three statistical methods separately.
4.1. Results from the probability distribution of symbol clusters

It is believed that the correlations observed in DNA sequences are mainly due to non-trivial clustering of homologous nucleotides, which are actually the successive repetitions of a single symbol in the DNA symbolic sequence. Moreover, it is found that the density of clusters of identical symbols in non-coding DNA regions is atypically high suggesting long range correlations while the density of the same clusters in coding DNA regions is lower and consistent to short range correlated symbolic sequences [12,13].

In a similar way, we study the density of clusters of homologous symbols on genes and intergenic sequences. A gene is a mixture of coding and non-coding parts and according to the above findings it is expected to contain some form of long range correlation due to the non-coding parts in it. However, the correlation in the genes should be less than the correlation in the intergenic sequences which consist only of non-coding DNA. This reasoning is only partially confirmed by the results from the probability distribution of symbol clusters. Fig. 1 shows the probability distribution of \( m \)-tuples of the symbols A, C, G and T for a gene sequence of 285,000 bases and an intergenic sequence of 210,000 bases, both from CAT1. Superimposed are also the graphs of the same probability function evaluated for each of the \( M = 40 \) surrogate sequences as well as the analytic probability mass function under the assumption of statistical independence.

For both gene and intergenic CAT1 sequences, the probability function of tuples of the symbols A and T (in Figs. 1a, b, j and k) is distinctly higher from the analytic probability function under the assumption of complete randomness. This is confirmed by the surrogate data as their probability distribution of tuples is always concentrated along the theoretical probability function. Obviously, we can reject \( H_0 \) that the original DNA sequence (gene and intergenic regions) has no correlations at high confidence levels taken as statistics the relative frequency of any \( m \)-tuple of A or T. For symbols C and G the distribution of the tuples for the DNA sequences does not differ significantly from random apart from the case of symbol C and intergenic sequence, where remarkably large tuples of C occur with non-zero probability (see Fig. 1e).

From the results in Fig. 1 it cannot be universally concluded that the probability distribution of the tuples of the symbols deviate from randomness more for the intergenic sequence than for the gene sequence. Actually, it seems to hold only for symbol A, as shown in Fig. 1c. This is expected because long repetitions of A have been observed in higher organisms. The opposite effect is observed for large tuples of T (see Fig. 1l).

We turn now to compare the two types of DNA sequences to the four artificial symbolic sequences. The tuple distributions are shown in Fig. 2.

The tuple distribution for the random sequence is the same as the analytic tuple distribution under the assumption of zero correlation and validates that the use of sequences of 30,000 bases gives sufficient estimation of the probability distribution of the tuples.

The symbolic sequences from the chaotic logistic map, which is a system of zero linear correlation but strong nonlinear correlation, have a special order of the symbols.
Fig. 1. (a) The graph of the sample probability function of tuples of symbol A evaluated for a gene CAT1 sequence of 285,000 bases and 40 surrogate sequences of it. Superimposed is also the analytic probability function assuming the sequence is random, as shown in the legend. (b) The same as in (a) but for an intergenic CAT1 sequence of 210,000 bases. (c) The graphs of the probability functions of tuples of the symbol A for the gene sequence in (a) and the intergenic sequence in (b). The same results as in (a)–(c) are shown for symbol C in panels (d)–(f), for symbol G in panels (g)–(i) and for symbol T in panels (j)–(l).

The logistic symbolic sequence based on the base probabilities of the gene sequence does not contain a C followed by C or a T followed by T (i.e., a point of the numerical time series in a region that is assigned to C or T does not map in the same region). The same holds for symbols C and G of the logistic symbolic sequence based on the base
Fig. 2. (a) The graph of the sample probability function of the tuples of symbol A evaluated for a gene CATI sequence and for four artificial symbolic sequences with the same base probability as the gene sequence (specified in the legend). All sequences consist of 30,000 bases. The analytic probability function assuming independent sequence is also superimposed shown with a light gray curve. (b) The same as in (a) but for an intergenic CATI sequence. The same results as in (a) and (b) are shown for symbol C in panels (c) and (d), for symbol G in panels (e) and (f), and for symbol T in panels (g) and (h).
probabilities of the intergenic sequence. Thus the results from the tuple distribution for symbols C, G and T cannot be clearly interpreted. For symbol A, the tuple distribution is similar to the weakly correlated AR(1) for both types of DNA.

The strongly correlated AR(1) system has always by far the highest probability across all tuples and symbols as expected. Apparently, the DNA sequences (gene and intergenic) do not contain strong correlations that would be pronounced as frequent homologous symbol clusters of any size. It seems that even a weakly correlated AR(1) model (for $\phi = 0.4$) contains more homologous symbol clusters of different size than a gene or intergenic sequence. The estimated probability of tuples tends to be higher for the weakly correlated AR(1) model than for either of the two DNA sequences. However, for large tuples the probability decreases slower for the intergenic sequence and for very large tuples it is even larger than the respective probability of the weakly correlated AR(1) symbolic sequence, as expected for distributions with long tails. This does not hold for the gene sequence and this difference suggests that there are stronger long range correlations in the intergenic sequence than in the gene sequence.

4.2. Results from the mutual information

It has been reported in earlier works that the mutual information function has a significantly different functional form in coding and non-coding DNA. However, there are contradicting reports whether the exons or introns have larger mutual information (e.g. see Refs. [7,8]). Here, we study the mutual information of the gene and intergenic CAT1 sequences together with surrogate symbolic sequences of zero correlations and the results are shown in Fig. 3.

![Fig. 3](image-url)

Fig. 3. (a) The graph of the mutual information $I(\tau)$ for displacements $\tau = 1, \ldots, 10$ computed on a gene sequence of 100,000 bases of CAT1 and $M = 40$ shuffled surrogates as shown in the legend. The mutual information for a random sequence is zero for any $\tau$ (this is the curve of “random-analytic” in the legend). (b) The same as in (a) but for an intergenic CAT1 sequence.
The simulations with surrogate data simply confirm that the estimation of mutual information from a sequence of 100,000 is very accurate. Thus the small values of $I(\tau)$ computed on both types of DNA sequences are indeed significant and they cannot be attributed to deviations from zero due to insufficient statistics. So, the immediate conclusion from Fig. 3 is the existence of correlations in both the gene and intergenic sequence (a formal parametric test with surrogate data would give rejection of the null hypothesis of zero correlations at very high confidence levels). Further, it does not appear that the intergenic sequence has larger correlations than the gene sequence. To the contrary, $I(1)$ is much larger for gene sequence suggesting that the general correlations (linear and nonlinear) of adjacent symbols is larger in the genes than in the intergenic sequences. This is indeed expected because in coding regions symbols up to the order of 3 code for aminoacids. Moreover, from Fig. 3a it can be seen that there are more significant correlations in multiples of 3 (gene sequence) while there are no such features in Fig. 3b (intergenic sequence).

Next, we compare $I(\tau)$ from gene and intergenic sequences to $I(\tau)$ from the artificial symbolic sequences. The results are shown in Fig. 4.

The random sequence sets the level of the essentially zero $I(\tau)$ (the plateau is at about $10^{-6}$). Similarly to the tuple distribution, the strongly correlated AR(1) obtains very high values of $I(\tau)$ and is clearly distinguished from all other systems. The $I(\tau)$ of both gene and intergenic sequences is somehow smaller than the $I(\tau)$ for the respective weakly correlated AR(1) sequence and larger than the $I(\tau)$ for the logistic symbolic sequence. So, in terms of mutual information, the DNA sequences would be classified in-between the weakly correlated AR(1) (for $\phi = 0.4$) sequences and the logistic symbolic sequences.

An interesting feature that can be observed from the comparison of the two DNA sequences to surrogate data (in Fig. 3) and to other correlated symbolic sequences (in Fig. 4), is that the $I(\tau)$ of both DNA sequences does not tend to vanish as $\tau$ increases.
Fig. 5. (a) The percentage of target points for which at least one identical neighbor was found given as a function of the segment length $m$ and computed on a gene CAT1 sequence of 20,000 bases and its 40 surrogate symbolic sequences. (b) The same as in (a) but for the intergenic CAT1 sequence.

$(I(\tau) > 10^{-4})$, which suggest that some correlation persists even between symbols that are not spatially close.

4.3. Results from the identical neighbor fit

While mutual information involves only two symbols (displaced by $\tau$ spatial units), the identical neighbor fit involves neighborhoods of same symbols and relates to correlations spanned over segments of symbols. Neighbor fitting and in general nonlinear modeling has not been used much in DNA sequence analysis. In Ref. [20], a similar technique to the identical neighbor fit was applied to coding (exons) and non-coding regions and it was found that coding regions behave as random chains while non-coding regions have deterministic structure. Our analysis is again different in that the DNA sequences are genes and intergenic regions.

The $T$ positions ahead identical neighbor fit for a target position $i$ relies on the existence of neighbor segments (of some length $m$) in the sequence that are identical to the current segment. Obviously, for very large $m$ there might be no identical neighbors, depending also on the length of the sequence. So, the existence of identical neighbors for large $m$ may signify a special deterministic structure or correlation in the sequence. In Fig. 5 we show for the DNA sequences and their surrogates the proportion of target positions over all possible positions in the sequence for which at least one identical neighbor was found (and prediction could be made). We observe that for large segments, say $m > 8$, the percentage of existing identical neighbors decreases to zero, but the decrease is slower for the DNA sequences (gene and intergenic regions) than for their counterpart surrogate sequences. This indicates that there is some form of organization of the symbols in the DNA sequence, so that particular combinations of $m$ symbols occur more often than if the chain of symbols were completely random.

Next, we investigate whether the prediction based on identical neighbors is better for the DNA sequences than for their surrogates. As shown in Fig. 6, the differences are
indeed significant, they increase with \( m \) and persist even when predicting multi-steps ahead. In particular, for large \( m \) the fit error measure MIME falls dramatically for the DNA sequences, whereas for the surrogates, MIME is the same on average for all \( m \) (as expected) but has larger variance due to the decrease of identical neighbor statistics with \( m \). Note that the level of MIME of the surrogates is different for the gene and intergenic sequence because it is determined by the base probabilities which are different for the two DNA sequences (see (4)). From Fig. 6, we cannot confidently draw a different signature for the two DNA sequences in terms of identical neighbor prediction, though it seems that the MIME of the intergenic sequence deviates more from the MIME of the respective surrogates for larger \( m \) than it does for the gene sequence. The results with the weighted MINE (WMINE) were similar.

It should be stressed that the significant differences shown in Fig. 6 for \( T = 1 \) and \( T = 5 \) were also observed for a range of prediction steps \( T \) up to 50 (not shown here) when \( m \) is small (at the level of 4). This indicates that given the occurrence of a particular small sequence of 3–5 nucleotides the probability of observing a specific
Finally, we compare using the identical neighbor fit the DNA sequences to the artificial symbolic sequences. We show in Fig. 7 the results of WMINE(\(T\)) for \(T = 1,\ldots,10\) and \(m = 5\) on all the sequences. The maximum WMIME (and MIME) for a random sequence (see (4)) is essentially the same as the WMIME for the random symbolic sequence. The one step ahead prediction (\(T = 1\)) for the DNA sequences is worse than all but the random sequence. When the prediction is made for many steps ahead, say \(T > 4\), the WMIME for the logistic sequence and the weakly correlated AR(1) converges to the maximum WMIME (that correspond to the MIME of a random sequence) while the WMIME for the DNA sequences does not change much and remains at a lower than the maximum WMIME level. It should be noted that the WMIME for the strongly correlated AR(1) is much smaller.

5. Discussion

In the current study we use three statistical methods to establish differences and similarities between genes and intergenic regions in Chromosome 1 of Arabidopsis Thaliana as well as artificially generated symbolic sequences from well-known systems.

Our analysis could discriminate the correlation structure of the different symbolic sequences. The following results were obtained with the three measures of tuple probability distribution, mutual information and identical neighbor fit: (a) Both genes and intergenic DNA sequences have significant correlations as compared to the respective random surrogate data (sequences having no correlation structure). (b) The intergenic regions tend to be more correlated than genes for larger scales of displacement (addressed by \(\tau\) for mutual information and by \(m\) for tuple probability distribution...
and identical neighbor fit). (c) Compared to simulated systems, the correlations of both gene and intergenic DNA sequences are close to those of an AR(1) model with autocorrelation function $0.4^\tau$, (note that the numerical time series of the system is suitably discretized to four symbols, so that the probability distribution of the symbols is identical to this of the respective DNA sequence). (d) The resemblance of DNA correlation to the correlation of AR(1) holds only for small scales of displacement (according to the measure, $\tau$ or $m$ smaller than 4), while for larger displacements the DNA retains significant correlation.

Certainly, the parallel studies of the DNA sequence of *Arabidopsis Thaliana* and of the different simulated systems was not to find the best suited system for this natural sequence in terms of correlation structure, but rather to compare the correlation signature of DNA sequence to this of other symbolic sequences derived from well known systems. In future efforts, we intend to investigate further whether known deterministic or stochastic systems can give rise to symbolic sequences that imitate the correlation structure of the DNA sequences.

The DNA sequence of genes and intergenic regions used in this analysis is a concatenation of individual genes and intergenic segments, both not exceeding a couple of thousand bases on average. Thus long-range correlations cannot be properly estimated, moderate-range correlations (at the level of hundreds) are underestimated and in general the estimated correlations are reduced due to the frequent discontinuity in both series. Therefore, we found useful to use the surrogate data as a reference of complete lack of correlations and assess the departure from this level. Moreover, in our analysis we concentrated basically on medium scale correlations (e.g. windows of about 10 bases).

In other studies concerning DNA statistical analysis [10–13], long range correlations are found in non-coding DNA while coding DNA presents more short range correlated features. In our work the existence of correlations is partly masked since: (a) gene regions are mixed containing coding and non-coding parts and (b) the DNA sequences used in the analysis consist of pieces of limited sizes put together.

Regarding the particular DNA sequence used in the analysis, it should be stressed that although *arabidopsis thaliana* belongs to the general category of higher eucaryotes, it belongs to the sub-category of dicot and contains high percentage of coding DNA (approximately 50%). In this respect, it resembles more to the lower organisms in which the coding is prevailing. This is why we have found in several cases strong levels of resemblance between genes and intergenic regions. For example, in Fig. 1j there seem to be strong large size T-clusters within the gene regions which can be attributed to the introns of the corresponding gene.

Other plant sequences, such as the rice genome (now nearly completely sequenced), which is a monocot and has characteristics closer to higher organisms, need to be investigated and compared with the results on *arabidopsis thaliana*. Also, the same analysis need to be carried out for different classes of organisms (animals, lower eucaryotes, etc.), so that similarities and differences between various categories of organisms could be assessed.

While all methods discriminate significantly the DNA sequences (genes and intergenic regions) from the respective random surrogate data it remains still to be
investigated whether this discrimination holds for small data sizes at the level of 1000 bases. Some preliminary results showed that the identical neighbor fit has better power at discriminating DNA segments of 1000 bases from surrogate data for small $m$ and $T$ but more systematic analysis is required on this.

References