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Characterizing the D2 Statistic: Word Matches in Biological Sequences

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Characterizing the D2 Statistic: Word Matches in Biological Sequences*

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Abstract

Word matches are often used in sequence comparison methods, either as a measure of sequence similarity or in the first search steps of algorithms such as BLAST or BLAT. The D2 statistic is the number of matches of words of k letters between two sequences. Recent advances have been made in the characterization of this statistic and in the approximation of its distribution. Here, these results are extended to the case of approximate word matches.

We compute the exact value of the variance of the D2 statistic for the case of a uniform letter distribution, and introduce a method to provide accurate approximations of the variance in the remaining cases. This enables the distribution of D2 to be approximated for typical situations arising in biological research. We apply these results to the identification of cis-regulatory modules, and show that this method detects such sequences with a high accuracy.

The ability to approximate the distribution of D2 for both exact and approximate word matches will enable the use of this statistic in a more precise manner for sequence comparison, database searches, and identification of transcription factor binding sites.

KEYWORDS: D2, alignment free sequence comparison, biological sequences

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1 Introduction

Alignment-free sequence comparison methods based on word matches allow sequences to be compared without assuming contiguity of homologous segments. This is of particular interest for the comparison of biological sequences, where deletions, insertions or duplications of segments are common. Several such methods have thus been implemented (see Hazelhurst et al., 2008, for example), and have had various applications, such as the clustering of large EST databases (for example, Christoffels et al., 2001). These applications, however, typically rely on empirical thresholds, rather than on rigorous statistical theory.

One of the statistics for alignment free sequence comparison that has received much attention is the D_2 statistic, which measures the number of words shared between two sequences. The characterisation of this statistic started with the calculation of its mean, and with approximations to the variance (Watterman, 1995). Later, more accurate approximations of the variance allowed asymptotic regimes of D_2 to be derived for non-uniform (Lippert et al., 2002) and uniform (Kantorovitz et al., 2007) letter distributions. More recently, the exact value of the D_2 variance has been computed (Kantorovitz et al., 2007; Forêt et al., 2009). In parallel with this theoretical effort, optimal word sizes for typical biological situations were computed (Forêt et al., 2006), and practical approximations of the distribution of D_2 in these settings were proposed (Forêt et al., 2009).

A more general version of the D_2 statistic is the number of approximate word matches between two sequences. After an initial characterisation of the mean of this statistic, an asymptotic distribution regime was characterised when the logarithm of the sequence size is large compared with the word size (Burden et al., 2008). Here, we further characterise the D_2 statistic in the case of approximate word matches, by computing its variance and proposing approximations of its distribution for typical biologically relevant situations. Finally, we present an application of these results to the identification of regulatory sequences.

2 Results

2.1 Definitions

The statistic $D_2(n_A, n_B, k, t, \eta)$ (D_2 henceforth) is the number of approximate word matches of length k with up to t mismatches between sequences

$A = (A_1 \dots A_{n_A})$ and $B = (B_1 \dots B_{n_B})$ with A_i and B_j belonging to an alphabet \mathcal{A} and distributed according to a letter distribution parameterised by η . As previously (Forêt et al., 2009), for mathematical convenience we will impose periodic boundary conditions, that is, the letter in the first position in a sequence is assumed to follow the last letter of that sequence. Also, only the case of strand symmetric Bernoulli text will be considered, that is, sequences built from alphabets of four iid (independent and identically distributed) letters (A, T, G and C) with the further constraint that the probability ξ_a of letter $a \in \mathcal{A}$ occurring is $\xi_A = \xi_T = \frac{1}{4}(1 + \eta)$ and $\xi_G = \xi_C = \frac{1}{4}(1 - \eta)$, where $0 \leq \eta \leq 1$. Note that the periodic boundary conditions simplify the theoretical calculations considerably, but allow the method to be used for linear as well as circular sequences by appropriate preprocessing (see Section 2.5 for example).

Defining the t neighbourhood match indicator

$$Y_{(i,j)} = \begin{cases} 1 & \text{if } \Delta((A_i, \dots, A_{i+k-1}), (B_j, \dots, B_{j+k-1})) \leq t \\ 0 & \text{otherwise} \end{cases} \quad (1)$$

where $\Delta(w_1, w_2)$ is the number of mismatches between the words w_1 and w_2 , the D_2 statistic is given by

$$D_2 = \sum_{(i,j) \in I} Y_{(i,j)} \quad (2)$$

where the index set is $I = \{(i, j) : 1 \leq i \leq n_A, 1 \leq j \leq n_B\}$.

2.2 D_2 mean

The mean of D_2 was first computed for exact word matches ($t = 0$) and iid letters (Waterman, 1995). This was later extended to the case of letters generated by a Markov model (Kantorovitz et al., 2007). A formula for the mean was also computed for approximate word matches ($t \geq 0$) in the case of Bernoulli symmetric text (Burden et al., 2008) in terms of the perturbed binomial distribution (Melko and Mushegian, 2004). In Appendix A.2 we derive the equivalent formula

$$E[D_2] = \frac{n_A n_B}{4^k} \sum_{l=0}^t \binom{k}{l} (3 - \eta^2)^l (1 + \eta^2)^{k-l}. \quad (3)$$

2.3 D_2 variance

An exact formula for the variance of D_2 was derived in the case of iid letters and exact word matches using periodic boundary conditions in Forêt et al.

(2009). Another study computed the variance for exact word matches using free boundary conditions, in the cases of iid letters and of letters generated by a Markov model (Kantorovitz et al., 2007). Here we extend these results to the case of approximate word matches for iid letters and Bernoulli symmetric text, using periodic boundary conditions. Specific details of this technical derivation are given in Appendix A.3. A brief summary is given below.

To calculate the variance of D_2 for approximate word matches and symmetric Bernoulli text, we follow the method used in Forêt et al. (2009). First we deduce from equation (2) that:

$$\text{Var}(D_2) = \text{Var}\left(\sum_{(i,j) \in I} Y_{(ij)}\right) = \sum_{(i,j) \in I} \sum_{(i',j') \in I} \text{Cov}(Y_{(ij)}, Y_{(i'j')}). \quad (4)$$

We set $u = (i, j)$, $v = (i', j')$ and for fixed u split the sum over v as follows. Let $J_u = \{v = (i', j') : |i' - i| < k \text{ or } |j' - j| < k\}$ be the dependency neighbourhood of Y_u . For $v \notin J_u$, $\text{Cov}(Y_u, Y_v) = 0$. J_u is decomposed into two disjoint sets (Waterman, 1995): an accordion set, $J_u^a = \{v = (i', j') : |i' - i| < k \text{ and } |j' - j| < k\}$ (when two pairs of matching words overlap in both sequences) and a crabgrass set, $J_u^c = J_u \setminus J_u^a$ (when two pairs of matching words overlap in one sequence only). The accordion set is further decomposed into a diagonal part, $J_u^{ad} = \{v = (i', j') : -k < i' - i = j' - j < k\}$ and an off-diagonal part, $J_u^{ao} = J_u^a \setminus J_u^{ad}$.

Table 1 gives a summary of the components of the variance in different settings. The only case that is not analytically characterised is the off-diagonal part of the accordion for approximate word matches and non-uniform letter distribution. In this case, however, numerical tables can be assembled to approximate the entire accordion part of the variance with good accuracy. To see this, note that the accordion part takes the form $n_A n_B \Phi(k, t, \eta)$. When $n_A = n_B = 2k - 1$, the only index set contributing to the variance is the accordion part. Although computing D_2 for approximate word matches requires an algorithm with complexity $o(n_A n_B)$, it is relatively inexpensive to approximate the variance of D_2 by simulation for small n_A and n_B . Tables of the function Φ were thus approximated by simulating a large number of pairs of sequences of length $2k - 1$ for $k \leq 16$ and setting $\Phi(k, t, \eta) = \text{Var}(D_2(2k - 1, 2k - 1, k, t, \eta)) / (2k - 1)^2$ (see below).

2.4 D_2 distribution

It has been shown previously (Forêt et al., 2009) that for exact word matches and in most biologically relevant situations, a distribution chosen *ad-hoc* such

	crabgrass	accordion, diagonal	accordion, off-diagonal
exact matches, uniform distribution ($t = 0, \eta = 0$)	0	Eq. (1) of Kantorovitz et al. (2007)	0
exact matches, non-uniform distribution ($t = 0, \eta \neq 0$)	Eq. (14) of Forêt et al. (2009)	Eq. (17) of Forêt et al. (2009)	Eqs. (20) and (26) of Forêt et al. (2009)
approximate matches, uniform distribution ($t \neq 0, \eta = 0$)	0	Appendix A.4.2	0
approximate matches, non-uniform distribution ($t \neq 0, \eta \neq 0$)	Appendix A.3.1	Appendix A.3.2	

Table 1: Contribution of the index sets of the dependency neighbourhood to the variance of D_2 . See text for definitions.

as the gamma distribution can provide a better estimate of the D_2 distribution than the asymptotic normal distribution. Here we provide approximations for the distribution of D_2 in the case of approximate word matches.

For convenience we have set $n_A = n_B = n$ in our numerical simulations. We have simulated the distribution of D_2 for sequence sizes ranging from small ESTs ($n = 100$) to reasonably large genes ($n = 3200$), for even word sizes k between 2 and 16, for every possible number of mismatches ($0 \leq t < k$), and for both uniform ($\eta = 0$) and non-uniform ($\eta = \frac{1}{3}$) letter distributions. For each combination of parameters, 10^6 pairs of iid sequences were generated. Tables of the accordion contribution function Φ were estimated by generating 10^9 pairs of iid sequences of size $n = 2k - 1$, with k ranging from 2 to 16 with an increment of 2. The Mersenne-Twister random number generator (Matsumoto M. and Nishimura T., 1998) was used, as implemented in the GNU scientific library (<http://www.gnu.org/software/gsl/>). The code was written in ANSI C and is available from the authors' website (http://www.maths.anu.edu.au/cbis/~sf/k_words).

Previously, the gamma distribution was used to approximate the D_2 distribution in the case of exact word matches (Forêt et al., 2009). Here, the beta distribution scaled to the range $[0, n^2]$ is used instead of the gamma distribution. In the range of parameters assessed in our simulations, the gamma and beta distributions are mostly indistinguishable (data not shown). It might be

expected, however, that the beta distribution provides better approximations for very small p-values, as it is bounded within the same domain of definition as D_2 ($[0, n_A n_B]$), whereas the gamma distribution is defined from zero to infinity. Histograms of our numerical simulations the D_2 statistic are compared with the density function of the beta distribution scaled to this interval, that is

$$\frac{1}{n_A n_B} f_B\left(\frac{x}{n_A n_B}; \alpha, \beta\right) \quad (5)$$

where $f_B(x; \alpha, \beta) = \Gamma(\alpha + \beta) / (\Gamma(\alpha)\Gamma(\beta)) x^{\alpha-1} (1-x)^{1-\beta}$ is the canonical density function of the beta distribution. The parameters α and β are set so that the mean and variance of the scaled beta distribution agree with the theoretical values $\mu = E[D_2]$, $\sigma^2 = \text{Var}(D_2)$ derived in the appendix:

$$\alpha = \frac{\mu}{n_A n_B} \left[\frac{\mu(n_A n_B - \mu)}{\sigma^2} - 1 \right], \quad \beta = \frac{n_A n_B - \mu}{n_A n_B} \left[\frac{\mu(n_A n_B - \mu)}{\sigma^2} - 1 \right]. \quad (6)$$

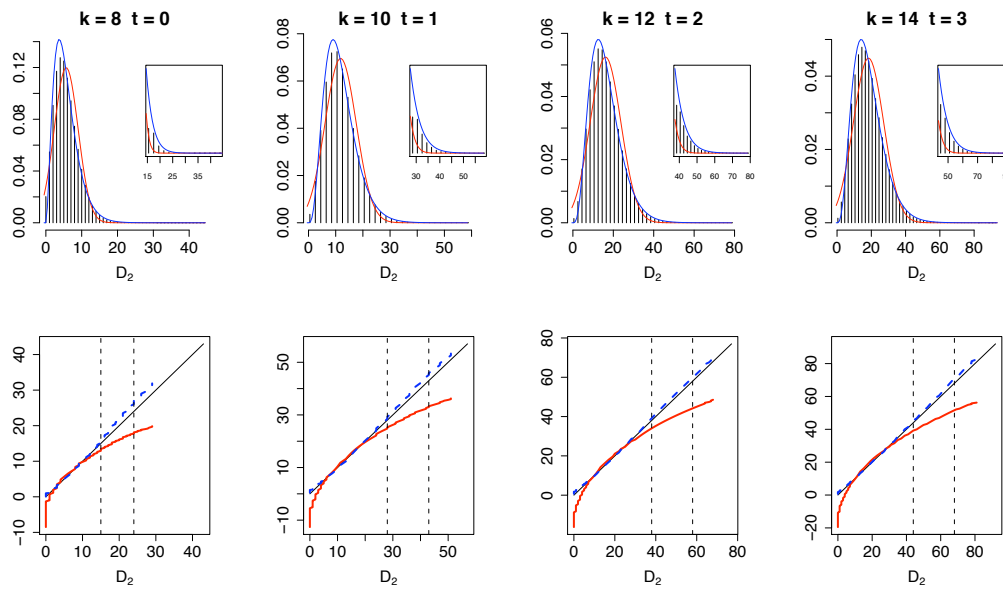
Figure 1 shows the simulated distribution of D_2 for the size typical of a small EST or a read produced by the 454 Titanium technology (sequence size $n = 400$), in the case of non-uniform letter distributions ($\eta = \frac{1}{3}$). The word sizes displayed in this figure are the optimal word sizes corresponding to the associated number of mismatches. We use the optimal word sizes computed previously in Forêt et al. (2006). In brief, a word size and number mismatches combination is optimal when it best captures the relatedness between artificially evolved sequences using the D_2 statistic as a relatedness estimator.

The quantile-quantile plots between the beta and normal distributions, and the simulated D_2 distribution show unambiguously that for these parameters combinations, the beta distribution provides a closer fit to the D_2 distribution than the normal distribution. Similar figures for all the simulations can be found on the authors' website (http://www.maths.anu.edu.au/cbis/~sf/k_words). We observed a few rare situations where the normal distribution outperformed the beta distribution, but these were cases where the number of mismatches was close to the word size, and are of little practical importance.

2.5 Application to the detection of regulatory sequences

We now apply the approximation of the D_2 distribution to a practical biological problem: the identification of sequences containing *cis-regulatory modules* (CRMs).

We use the same dataset as Kantorovitz et al., 2007, which contains seven sets of sequences known to contain CRMs. Within each set, the CRMs are



Non-uniform letter distribution: $n = 400$

Figure 1: *Top row*: Histograms of the simulated distribution of D_2 for sequences of size $n = 400$ and non-uniformly distributed letters. The normal distribution is shown in red and the beta distribution in blue. The insert shows a close up on the far right of the tail larger than the 99th percentile. *Bottom row*: quantile-quantile plots with the simulated D_2 values horizontally, and the normal (continuous red line) and beta (dashed blue line) values vertically. The vertical dashed lines represent the 0.99 and the 0.9999 quantiles.

driving gene expression in one particular tissue or life stage. The sets contain between 9 and 82 sequences. For each of these ‘positive’ sets, a ‘negative’ set was constructed from randomly chosen non-coding sequences of the same species, containing the same number of sequences and with the same sequence sizes as in the positive set.

In Kantorovitz et al., 2007, the authors primarily assessed whether their method can capture an expected effect, namely that sequences known to contain similar (CRMs) are more related to each other than are randomly selected sequences. While they show that the D_2 based approach clearly outperforms other techniques, this approach is of limited practical use.

We chose instead to address a problem more frequently faced by practitioners: given a set of sequences known to contain CRMs, and a query sequence, can the query sequence be classified as containing similar CRMs or not? We

set up the following experiment: each sequence in each positive set was selected as the query sequence and compared both to the remaining positive sequences of this set and to the corresponding negative sequences. In order that our theoretical results for the iid hypothesis null distribution could be applied, each sequence was preprocessed by (1) joining the ends to effect periodic boundary conditions and (2) removing masked tandem repeats present in the data sets and concatenating the pieces either side of the removed portion. The parameters n_A and n_B were taken from the preprocessed sequences and for each pairwise comparison the parameter η estimated from the combined letter frequencies of the two sequences in question. The query sequences were then screened to accept only those for which the smallest p-value of all comparisons was less than 0.01. We used a stringent criterion, namely, a positive query sequence was considered correctly classified if the smallest p-value was obtained with another sequence of the positive set.

Figure 2 shows the results of this experiment. A good sensitivity is achieved in most datasets, with typically 80% or more of the sequences correctly classified for at least one parameter combination using this stringent criterion. The optimal parameters vary from one condition to another. This may reflect different properties of the underlying CRMs, in terms of size, letter composition and level of conservation that they require in order to be functional. The problem of choosing optimal parameters is easily solved by using the above approach, namely by determining a set of positive sequences and using these to estimate appropriate parameters before comparing the query sequence(s) to them.

The percentage of correctly classified negative sequences based on the smallest p-value was typically around 50% (data not shown). This suggests that while this method can successfully identify candidates, further validation of the candidates would be needed.

3 Discussion

In this study we present exact values and approximations of the variance of D_2 for pairs of symmetric Bernoulli texts. These results enable the distribution of D_2 to be approximated with or without mismatches for most situations occurring in biological research.

We illustrate the application of these results by using the D_2 statistic to identify sequences containing regulatory modules. Our results show that this method can be used to identify candidate regulatory sequences for further experimental validation, or in combination with other prediction methods.

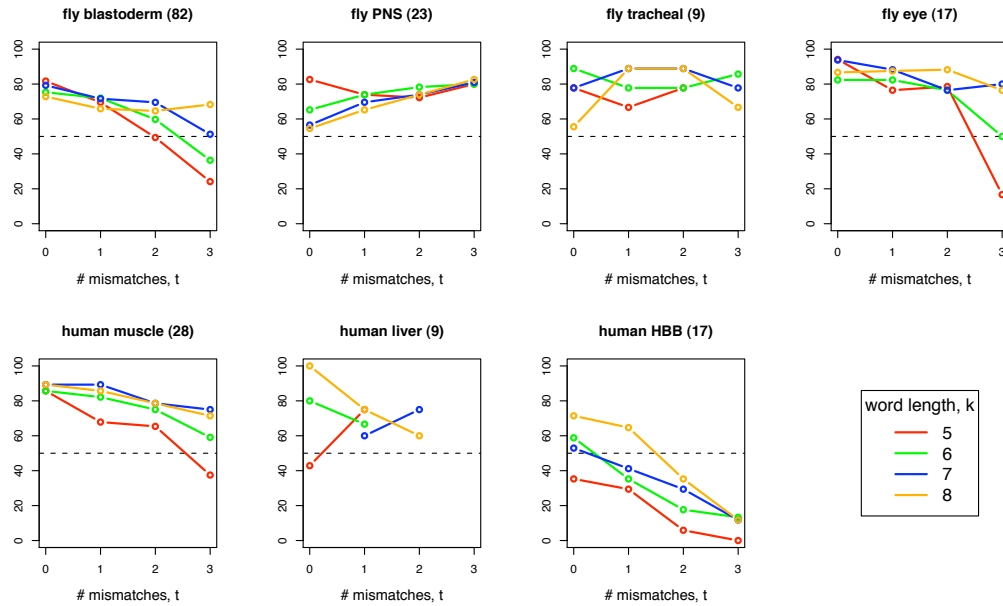


Figure 2: Percentage of times that a sequence containing CRMs is correctly classified: each subplot corresponds to a type of CRM, and the numbers in parentheses are the number of positive control sequences in each set. Percentages are only plotted if at least 4 query sequences survived the screening requirement that the minimum p-value should be less than 0.01.

A remaining theoretical problem is evaluation of the variance and distribution of the D_2 statistic in the case of approximate word matches for strings that are not symmetric Bernoulli texts, such as proteins. This lack of theory could be partially circumvented by using exact word matches for protein searches, but using alphabet reduction to account for most common substitutions. A similar alphabet reduction resulted in increased accuracy in the construction of phylogenetic trees with an alignment free method (Hohl and Ragan, 2007).

Appendix

A Derivation of D_2 mean and variance

Define the statistic D_2 to be the number of k -word matches with up to t mismatches ($t = 0, \dots, k$) between sequences A and B of letters drawn from an alphabet \mathcal{A} . Let the sequence lengths be n_A and n_B respectively, and

assume each sequence to consist of i.i.d. random letters with probability ξ_a of letter $a \in \mathcal{A}$ occurring at any given location, where $\sum_{a \in \mathcal{A}} \xi_a = 1$. Also assume periodic boundary conditions on both sequences, that is, the letter in the first position in sequence A is assumed to follow the letter in the n_A^{th} position, and the letter in the first position in sequence B is assumed to follow the letter in the n_B^{th} position.

In general, we restrict ourselves to the case of strand symmetric Bernoulli texts of nucleotide sequences, that is, i.i.d. sequences for which $\xi_C = \xi_G = \frac{1}{4}(1 - \eta)$, $\xi_A = \xi_T = \frac{1}{4}(1 + \eta)$, where $0 \leq \eta \leq 1$, and write the D_2 statistic as $D_2(n_A, n_B, k, t, \eta)$.

A.1 Preliminaries

We use the following terminology adapted from Burden et al. (2008):

1. For $m = 1, 2, \dots$, define $p_m = \sum_{a \in \mathcal{A}} \xi_a^m$. For strand symmetric Bernoulli texts, $p_2 = (1 + \eta^2)/4$.
2. Define $\Delta(\mathbf{W}_1, \mathbf{W}_2)$ to be a random variable equal to the number of mismatches between the two random k -words \mathbf{W}_1 and \mathbf{W}_2 . When there is no possibility of confusion, we simply write $\Delta(k)$ for the number of mismatches between the two random k -words. One easily checks that $\Delta(k)$ is a binomial random variable:

$$\begin{aligned}
 \Pr(\Delta(k) = l) &= \Pr(\text{Exactly } l \text{ mismatches and } k - l \text{ matches}) \\
 &= \binom{k}{l} (\text{prob. of mismatch})^l (\text{prob. of match})^{k-l} \\
 &= \binom{k}{l} (1 - p_2)^l p_2^{k-l} \\
 &= \binom{k}{l} \frac{1}{4^k} (3 - \eta^2)^l (1 + \eta^2)^{k-l}.
 \end{aligned} \tag{7}$$

3. $Y_{(i,j)} = Y_u$ = the approximate word match indicator, taking the value 1 if the number of mismatches between k -word at i in A and the k -word at j in B is at most t . That is:

$$Y_{(i,j)} = \begin{cases} 1 & \text{if } \Delta((A_1, \dots, A_{i+k-1}), (B_1, \dots, B_{j+k-1})) \leq t \\ 0 & \text{otherwise} \end{cases} \tag{8}$$

Note that $D_2 = \sum_{i=1}^{n_A} \sum_{j=1}^{n_B} Y_{(i,j)}$.

4. $g_t(k, \eta, c)$, $G_t(k, \eta, c)$, probability and cumulative distribution functions of the perturbed binomial distribution (Melko and Mushegian, 2004). Given a fixed k -word with CG-content c ($c = 0, \dots, k$), $g_t(k, \eta, c)$ (resp. $G_t(k, \eta, c)$) is the probability that the number of mismatches between that word and a random k -word will be equal to (resp. at most) t . Specifically:

$$G_t(k, \eta, c) = \sum_{r=0}^t g_r(k, \eta, c) \quad (9)$$

$$g_t(k, \eta, c) = h(k, \eta, c) u_t(k, \eta, c), \quad (10)$$

where $0 \leq c, t \leq k$ are integers, and

$$h(k, \eta, c) = \frac{1}{4^k} (1 - \eta)^c (1 + \eta)^{k-c} \quad (11)$$

$$u_t(k, \eta, c) = \sum_{i=0}^{k-t} \binom{c}{i} \binom{k-c}{k-t-i} v_t(i, \eta, c) \quad (12)$$

$$v_t(i, \eta, c) = \left(\frac{3+\eta}{1-\eta} \right)^{c-i} \left(\frac{3-\eta}{1+\eta} \right)^{t-c+i}. \quad (13)$$

In the above definition, we follow a convention that $\binom{n}{a} = 0$ if $a < 0$ or $a > n$.

5. We set $I = \{(i, j) : 1 \leq i \leq n_A, 1 \leq j \leq n_B\}$. Given $u = (i, j) \in I$, the dependency neighbourhood of u is defined as:

$$J_u = \{v = (i', j') : |i' - i| < k \text{ or } |j' - j| < k\}. \quad (14)$$

Note that for $v \notin J_u$, $\text{Cov}(Y_u, Y_v) = 0$. J_u is divided into two parts, accordion J_u^a and crabgrass J_u^c defined by

$$\begin{aligned} J_u^a &= \{v = (i', j') \in J_u : |i' - i| < k \text{ and } |j' - j| < k\} \\ J_u^c &= J_u \setminus J_u^a. \end{aligned} \quad (15)$$

The accordion set is further decomposed into a diagonal part, J_u^{ad} and an off-diagonal part, J_u^{ao} :

$$J_u^{ad} = \{v = (i', j') : -k < i' - i = j' - j < k\} \quad (16)$$

$$J_u^{ao} = J_u^a \setminus J_u^{ad}. \quad (17)$$

A.2 Mean of D_2

An equivalent and more concise formula for $E[D_2]$ to that given in Burden et al. (2008) is

$$\begin{aligned}
E[D_2(n_A, n_B, k, t, \eta)] &= \sum_{(i,j) \in I} E[Y_{(i,j)}] \\
&= n_A n_B \sum_{l=0}^t \Pr(\Delta(k) = l) \\
&= n_A n_B \sum_{l=0}^t \binom{k}{l} (1-p_2)^l p_2^{k-l} \\
&= \frac{n_A n_B}{4^k} \sum_{l=0}^t \binom{k}{l} (3-\eta^2)^l (1+\eta^2)^{k-l}. \quad (18)
\end{aligned}$$

A.3 Variance of D_2

An exact formula for the variance of $D_2(n_A, n_B, k, 0, \eta)$ (i.e. the case of exact word matches) has previously been given by Forêt et al. (2009). The case of approximate word matches, $0 \leq t \leq k$, is dealt with here. We have

$$\begin{aligned}
\text{Var}(D_2(n_A, n_B, k, t, \eta)) &= \text{Var}\left(\sum_{u \in I} Y_u\right) \\
&= \sum_{u \in I} \sum_{v \in J_u^c} \text{Cov}(Y_u, Y_v) + \sum_{u \in I} \sum_{v \in J_u^a} \text{Cov}(Y_u, Y_v) \\
&= \text{Var}(D_2)|_{\text{crabgrass}} + \text{Var}(D_2)|_{\text{accordion}}. \quad (19)
\end{aligned}$$

Below we give an exact formula for the crabgrass part. A convenient exact formula for the accordion part remains intractable in general, and we give below a practical alternate numerical method for its evaluation. For the case of a uniform letter distribution, $\eta = 0$, we demonstrate below (in section A.4) that only the diagonal part of the accordion contributes to the variance of D_2 , and give an exact formula for this case.

A.3.1 Crabgrass contribution to $\text{Var}(D_2)$

From Eqs. (6) and (7) on page 9 of Burden et al. (2008), the crabgrass contribution is given by

$$\text{Var}(D_2)|_{\text{crabgrass}} = \sum_{u \in I} \sum_{v \in J_u^c} \text{Cov}(Y_u, Y_v)$$

$$= n_A n_B (n_A + n_B - 4k + 2) \sum_{r=-k+1}^{k-1} \text{Var}(f_{|r|}(\mathbf{W})), \quad (20)$$

where, for a given $(k-r)$ -word $\mathbf{w} \in \mathcal{A}^{k-r}$,

$$\begin{aligned} f_r(\mathbf{w}) &= \sum_{l=0}^{\min(r,t)} \Pr(\Delta(r) = l) G_{t-l}(k-r, \eta, c_{\mathbf{w}}) \\ &= \sum_{l=0}^{\min(r,t)} \binom{r}{l} (1-p_2)^l p_2^{r-l} G_{t-l}(k-r, \eta, c_{\mathbf{w}}) \\ &= \sum_{l=0}^{\min(r,t)} \binom{r}{l} \frac{(3-\eta^2)^l (1+\eta^2)^{r-l}}{4^r} G_{t-l}(k-r, \eta, c_{\mathbf{w}}), \end{aligned} \quad (21)$$

where $c_{\mathbf{w}}$ is the GC-content of \mathbf{w} . The variance with respect to the random $(k-r)$ -word \mathbf{W} is calculated using

$$\text{Var}(f_r(\mathbf{W})) = E[f_r(\mathbf{W})^2] - E[f_r(\mathbf{W})]^2. \quad (22)$$

Since the \mathbf{w} -dependence of the function f_r is only via the GC-content of \mathbf{w} , the expectation values are calculated using

$$\begin{aligned} E[\phi(c_{\mathbf{W}})] &= \sum_{c=0}^{k-r} \Pr(c_{\mathbf{W}} = c) \phi(c) \\ &= \sum_{c=0}^{k-r} \binom{k-r}{c} (\xi_C + \xi_G)^c (\xi_A + \xi_T)^{k-r-c} \phi(c) \\ &= \sum_{c=0}^{k-r} \binom{k-r}{c} \frac{1}{2^{k-r}} (1-\eta)^c (1+\eta)^{k-r-c} \phi(c). \end{aligned} \quad (23)$$

A.3.2 Accordion contribution to $\text{Var}(D_2)$

The accordion part is

$$\begin{aligned} \text{Var}(D_2)|_{\text{accordion}} &= \sum_{u \in I} \sum_{v \in J_u^a} \text{Cov}(Y_u, Y_v), \\ &= n_A n_B \Phi(k, t, \eta), \end{aligned} \quad (24)$$

where

$$\Phi(k, t, \eta) = \sum_{r=-k+1}^{k-1} \sum_{s=-k+1}^{k-1} \text{Cov}(Y_u, Y_{u+(r,s)}) \quad (25)$$

is independent of n_A and n_B . For the case $n_A = n_B = 2k - 1$, Eq. (20) implies $\text{Var}(D_2)|_{\text{crabgrass}} = 0$, giving

$$\Phi(k, t, \eta) = \frac{\text{Var}(D_2(2k - 1, 2k - 1, k, t, \eta))}{(2k - 1)^2}, \quad (26)$$

which can be estimated numerically by measuring the variance of D_2 for a large sample of pairs of sequences of length $2k - 1$. Tables of $\Phi(k, t, \eta)$ can be assembled for a range of parameters to provide a practical way of numerically calculating the accordion contribution.

A.4 Var(D_2) for a uniform letter distribution

For the case of a uniform letter distribution, $\xi_a = 1/d$ for all $a \in \mathcal{A}$ where $d = |\mathcal{A}|$ is the alphabet size, we find that the crabgrass and off-diagonal part of the accordion contribution to $\text{Var}(D_2)$ are zero, and that an analytic formula for the remaining, diagonal-accordion, contribution, can easily be found.

A.4.1 Crabgrass contribution, $\eta = 0$

When $\eta = 0$, the perturbed binomial distribution reduces to the ordinary binomial distribution, independent of c (Melko and Mushegian, 2004):

$$g_t(k, 0, c) = \binom{k}{t} \left(\frac{1}{4}\right)^t \left(\frac{3}{4}\right)^{k-t}. \quad (27)$$

Accordingly, the function $f_r(\mathbf{W})$ in Eq. 20 is independent of the random word \mathbf{W} , its variance is zero, and thus $\text{Var}(D_2(n_A, n_B, k, t, 0))|_{\text{crabgrass}} = 0$.

A.4.2 Diagonal-accordion contribution

For arbitrary η we have (see Fig. 3)

$$\begin{aligned} \text{Var}(D_2)|_{\text{diag.accordion}} &= \sum_{u \in I} \sum_{v \in J_u^{ad}} \text{Cov}(Y_u, Y_v) \\ &= n_A n_B \sum_{r=-k+1}^{k-1} \text{Cov}(Y_u, Y_{u+(r,r)}) \\ &= n_A n_B \left[\text{Cov}(Y_u, Y_u) + 2 \sum_{r=1}^{k-1} \text{Cov}(Y_u, Y_{u+(r,r)}) \right]. \end{aligned} \quad (28)$$

The covariance is

$$\text{Cov}(Y_u, Y_{u+(r,r)}) = E[Y_u, Y_{u+(r,r)}] - E[Y_u]^2, \quad (29)$$

where

$$\begin{aligned} E[Y_u, Y_{u+(r,r)}] &= \Pr(Y_u = 1, Y_{u+(r,r)} = 1) \\ &= \sum_{l=0}^{\min(k-r,t)} \Pr(\Delta(k-r) = l) \sum_{s_1=0}^{t-l} \Pr(\Delta(r) = s_1) \sum_{s_2=0}^{t-l} \Pr(\Delta(r) = s_2) \\ &= \sum_{l=0}^{\min(k-r,t)} \binom{k-r}{l} (1-p_2)^l p_2^{k-r-l} \left[\sum_{s=0}^{t-l} \binom{r}{s} (1-p_2)^s p_2^{r-s} \right]^2, \end{aligned} \quad (30)$$

and

$$E[Y_u] = \Pr(Y_u = 1) = \sum_{l=0}^t \Pr(\Delta(k) = l) = \sum_{l=0}^t \binom{k}{l} (1-p_2)^l p_2^{k-l}. \quad (31)$$

The l^{th} term in Eq. (30) accounts for the event that there are up to $t-l$ mismatches between (A_i, \dots, A_{i+r-1}) and (B_j, \dots, B_{j+r-1}) , exactly l mismatches between $(A_{i+r}, \dots, A_{i+k-1})$ and $(B_{j+r}, \dots, B_{j+k-1})$ and up to $t-l$ mismatches between $(A_{i+k}, \dots, A_{i+k+r-1})$ and $(B_{j+k}, \dots, B_{j+k+r-1})$.

For the case of a uniform letter distribution, one simply sets $p_2 = 1/d$ in Eqs. (30) and (31).

A.4.3 Off-diagonal-accordion contribution, $\eta = 0$

The proof that $\text{Var}(D_2)|_{\text{off-diag.accordion}} = 0$ for a uniform letter distribution is non-trivial. First we establish some general results about the distance function $\Delta(\mathbf{W}_1, \mathbf{W}_2)$, equal to the number of mismatches between two random k -words \mathbf{W}_1 and \mathbf{W}_2 .

For a uniform letter distribution, and for two *independent* (i.e. non-overlapping) words \mathbf{W}_1 and \mathbf{W}_2 , we have from Eq. (7)

$$\Pr(\Delta(\mathbf{W}_1, \mathbf{W}_2) = l) = \binom{k}{l} (1-p_2)^l p_2^{k-l} = \binom{k}{l} \frac{(d-1)^l}{d^k}. \quad (32)$$

If one of the words is fixed to be \mathbf{w} , one easily checks that the conditional probability is also binomial:

$$\Pr(\Delta(\mathbf{W}_1, \mathbf{W}_2) = l | \mathbf{W}_2 = \mathbf{w}) = \binom{k}{l} \frac{(d-1)^l}{d^k} = \Pr(\Delta(\mathbf{W}_1, \mathbf{W}_2) = l). \quad (33)$$

Thus $\Delta(\mathbf{W}_1, \mathbf{W}_2)$ and \mathbf{W}_2 are independent random variables.

Now consider the case of three independent random words \mathbf{W}_1 , \mathbf{W}_2 and \mathbf{W}_3 . Then

$$\begin{aligned}
 & \Pr(\Delta(\mathbf{W}_1, \mathbf{W}_2) = l_1, \Delta(\mathbf{W}_2, \mathbf{W}_3) = l_2) \\
 &= \sum_{\mathbf{w} \in \mathcal{A}^k} \Pr(\Delta(\mathbf{W}_1, \mathbf{W}_2) = l_1 | \mathbf{W}_2 = \mathbf{w}) \\
 & \quad \times \Pr(\Delta(\mathbf{W}_2, \mathbf{W}_3) = l_2 | \mathbf{W}_2 = \mathbf{w}) \Pr(\mathbf{W}_2 = \mathbf{w}) \\
 &= \sum_{\mathbf{w} \in \mathcal{A}^k} \Pr(\Delta(\mathbf{W}_1, \mathbf{W}_2) = l_1) \Pr(\Delta(\mathbf{W}_2, \mathbf{W}_3) = l_2) \frac{1}{d^k} \\
 &= \Pr(\Delta(\mathbf{W}_1, \mathbf{W}_2) = l_1) \Pr(\Delta(\mathbf{W}_2, \mathbf{W}_3) = l_2)
 \end{aligned} \tag{34}$$

where we have used the fact that, once \mathbf{W}_2 is fixed, $\Delta(\mathbf{W}_1, \mathbf{W}_2)$ and $\Delta(\mathbf{W}_2, \mathbf{W}_3)$ depend only on \mathbf{W}_1 and \mathbf{W}_3 respectively, and so are effectively independent.

We now generalise Eqs. (33) and (34) to the following proposition P_N , which will be proved by induction:

For given $N \geq 2$, let $\mathbf{W}_1, \dots, \mathbf{W}_{N+1}$ be mutually independent k -words, and define

$$\Delta_i = \Delta(\mathbf{W}_i, \mathbf{W}_j), \quad i = 1, \dots, N. \tag{35}$$

Then for any $\mathbf{w} \in \mathcal{A}^k$,

$$\begin{aligned}
 & \Pr(\Delta_1 = l_1, \dots, \Delta_{N-1} = l_{N-1} | \mathbf{W}_N = \mathbf{w}) \\
 &= \Pr(\Delta_1 = l_1, \dots, \Delta_{N-1} = l_{N-1})
 \end{aligned} \tag{36}$$

and

$$\begin{aligned}
 & \Pr(\Delta_1 = l_1, \dots, \Delta_N = l_N) \\
 &= \Pr(\Delta_1 = l_1, \dots, \Delta_{N-1} = l_{N-1}) \Pr(\Delta_N = l_N).
 \end{aligned} \tag{37}$$

Note that Eq. (36) could equivalently be written as

$$\begin{aligned}
 & \Pr(\Delta_1 = l_1, \dots, \Delta_{N-1} = l_{N-1} | \mathbf{W}_N \in R) \\
 &= \Pr(\Delta_1 = l_1, \dots, \Delta_{N-1} = l_{N-1}),
 \end{aligned} \tag{38}$$

where $R \subset \mathcal{A}^k$ is any restricted set of k -words. Note also that combining Eq. (37) for the propositions P_2 to P_N implies

$$\Pr(\Delta_1 = l_1, \dots, \Delta_N = l_N) = \Pr(\Delta_1 = l_1) \times \dots \times \Pr(\Delta_N = l_N) \tag{39}$$

The proposition P_2 is proved by Eqs.(33) and (34). It remains to prove that P_N implies P_{N+1} . Define $S(\mathbf{w}, l) = \{\mathbf{x} \in \mathcal{A}^k | \Delta(\mathbf{x}, \mathbf{w}) = l\}$. Starting with the left hand side of Eq. (36) with N replaced by $N + 1$, we have

$$\begin{aligned} & \Pr (\Delta_1 = l_1, \dots, \Delta_N = l_N | \mathbf{W}_{N+1} = \mathbf{w}) \\ &= \Pr (\Delta_1 = l_1, \dots, \Delta_N = l_N | \mathbf{W}_{N+1} = \mathbf{w}, \mathbf{W}_N \in S(\mathbf{w}, l_N)) \\ & \quad \times \Pr (\mathbf{W}_N \in S(\mathbf{w}, l_N)) \\ & \quad + \Pr (\Delta_1 = l_1, \dots, \Delta_N = l_N | \mathbf{W}_{N+1} = \mathbf{w}, \mathbf{W}_N \notin S(\mathbf{w}, l_N)) \\ & \quad \times \Pr (\mathbf{W}_N \notin S(\mathbf{w}, l_N)) \\ &= \Pr (\Delta_1 = l_1, \dots, \Delta_{N-1} = l_{N-1} | \mathbf{W}_{N+1} = \mathbf{w}, \mathbf{W}_N \in S(\mathbf{w}, l_N)) \\ & \quad \times \Pr (\mathbf{W}_N \in S(\mathbf{w}, l_N)), \end{aligned}$$

where the second term is zero since “ $\Delta_N = l_N$ ” and “ $\mathbf{W}_N \notin S(\mathbf{w}, l_N)$ ” are mutually exclusive events, and the requirement “ $\Delta_N = l_N$ ” has been dropped from the first term since it is automatically satisfied by the condition “ $\mathbf{W}_{N+1} = \mathbf{w}$ and $\mathbf{W}_N \in S(\mathbf{w}, l_N)$ ”. Then, since $\Delta_1, \dots, \Delta_{N-1}$ are independent of \mathbf{W}_{N+1} , and rewriting the second factor, we have

$$\begin{aligned} & \Pr (\Delta_1 = l_1, \dots, \Delta_N = l_N | \mathbf{W}_{N+1} = \mathbf{w}) \\ &= \Pr (\Delta_1 = l_1, \dots, \Delta_{N-1} = l_{N-1} | \mathbf{W}_N \in S(\mathbf{w}, l_N)) \\ & \quad \times \Pr (\Delta_N = l_N | \mathbf{W}_{N+1} = \mathbf{w}) \\ &= \Pr (\Delta_1 = l_1, \dots, \Delta_{N-1} = l_{N-1}) \Pr (\Delta_N = l_N) \quad \text{by Eqs.(33) and (38)} \\ &= \Pr (\Delta_1 = l_1, \dots, \Delta_N = l_N) \quad \text{by Eq. (37)} \end{aligned} \tag{40}$$

which establishes the first part of proposition P_{N+1} . Starting with the left hand side of Eq. (37) with N replaced $N + 1$,

$$\begin{aligned} & \Pr (\Delta_1 = l_1, \dots, \Delta_{N+1} = l_{N+1}) \\ &= \sum_{\mathbf{w} \in \mathcal{A}^k} \Pr (\Delta_1 = l_1, \dots, \Delta_N = l_N | \mathbf{W}_{N+1} = \mathbf{w}) \\ & \quad \times \Pr (\Delta_{N+1} = l_{N+1} | \mathbf{W}_{N+1} = \mathbf{w}) \Pr (\mathbf{W}_{N+1} = \mathbf{w}) \\ &= \sum_{\mathbf{w} \in \mathcal{A}^k} \Pr (\Delta_1 = l_1, \dots, \Delta_N = l_N) \Pr (\Delta_{N+1} = l_{N+1}) \frac{1}{d^k} \quad \text{by Eq. (40)} \\ &= \Pr (\Delta_1 = l_1, \dots, \Delta_N = l_N) \Pr (\Delta_{N+1} = l_{N+1}), \end{aligned} \tag{41}$$

which establishes the second half of proposition P_{N+1} .¹

¹Aside: For an alternate proof that $\text{Var}(D_2(n_A, n_B, k, t, 0))|_{\text{crabgrass}} = 0$ one can apply the above proposition to the third line of Eq. (5) of Burden et al. (2008).

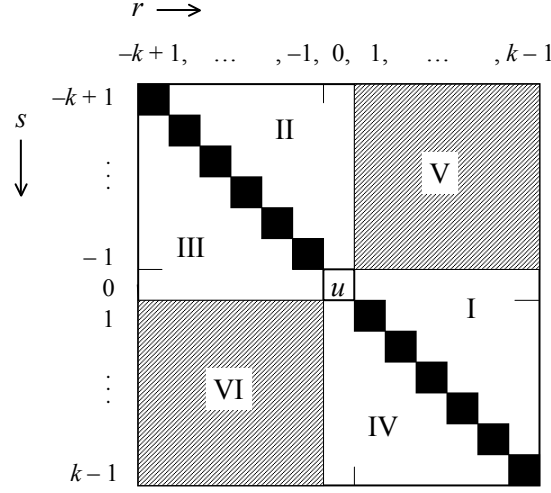


Figure 3: The main diagonal and off-diagonal regions I to VI of J_u^a .

We are now in a position to calculate

$$\text{Var}(D_2)|_{\text{off-diag.accordion}} = \sum_{u \in I} \sum_{v \in J_u^{ao}} \text{Cov}(Y_u, Y_v). \quad (42)$$

Writing $u = (i, j)$, $v = (i + r, j + s) \in J_u^{ao}$, the off-diagonal part J_u^{ao} can be subdivided into six parts illustrated in Fig. 3, namely

- I: $0 \leq s < r \leq k - 1$;
- II: $-k + 1 \leq s < r \leq 0$;
- III: $-k + 1 \leq r < s \leq 0$;
- IV: $0 \leq r < s \leq k - 1$;
- V: $1 \leq r \leq k - 1, -k + 1 \leq s \leq -1$;
- VI: $1 \leq s \leq k - 1, -k + 1 \leq r \leq -1$.

We proceed to prove that $\text{Cov}(Y_u, Y_v)$ vanishes for each of the six cases.

Case I is illustrated in Fig. 4(a). The union of the overlapping words $\mathbf{W}_u^A = (A_i, \dots, A_{i+k-1})$ and $\mathbf{W}_v^A = (A_{i+r}, \dots, A_{i+k+r-1})$ is subdivided into the shaded pieces $\mathbf{W}_0^{A,L} = (A_i, \dots, A_{i+s-1})$ and $\mathbf{W}_0^{A,R} = (A_{i+k+r-s}, \dots, A_{i+k+r-1})$

each of length s , and a set of and a set of single-letter words $\mathbf{W}_\alpha^A = (A_{i+s+\alpha-1})$, $\alpha = 1, \dots, k+r-2s$.

Similarly, the union of the overlapping words $\mathbf{W}_u^B = (B_j, \dots, B_{j+k-1})$ and $\mathbf{W}_v^B = (B_{j+s}, \dots, B_{j+k+s-1})$ is subdivided into the shaded pieces $\mathbf{W}_0^{B,L} = (B_i, \dots, B_{j+s-1})$ and $\mathbf{W}_0^{B,R} = (B_{j+k}, \dots, B_{j+k+s-1})$ each of length s , and a set of and a set of single-letter words $\mathbf{W}_\alpha^B = (B_{j+s+\alpha-1})$, $\alpha = 1, \dots, k-s$.

$$\Delta_0^L = \Delta(\mathbf{W}_0^{A,L}, \mathbf{W}_0^{B,L}), \quad \Delta_0^R = \Delta(\mathbf{W}_0^{B,R}, \mathbf{W}_0^{A,R}) \quad (43)$$

$$\Delta_{\alpha}^L = \Delta(\mathbf{W}_{\alpha}^A, \mathbf{W}_{\alpha}^B), \quad \Delta_{\alpha}^R = \Delta(\mathbf{W}_{\alpha}^B, \mathbf{W}_{\alpha+r-s}^A), \quad \alpha = 1, \dots, k-s. \quad (44)$$

Then

$$\Delta(\mathbf{W}_u^A, \mathbf{W}_u^B) = \sum_{\alpha=0}^{k-s} \Delta_\alpha^L, \quad \Delta(\mathbf{W}_v^A, \mathbf{W}_v^B) = \sum_{\alpha=0}^{k-s} \Delta_\alpha^R. \quad (45)$$

With the indicator variables Y_u and Y_v defined as above, we have

$$\begin{aligned} E(Y_u, Y_v) &= \Pr(Y_u = 1, Y_v = 1) \\ &= \Pr(\Delta(\mathbf{W}_u^A, \mathbf{W}_u^B) \leq t, \Delta(\mathbf{W}_v^A, \mathbf{W}_v^B) \leq t) \\ &= \sum_{\{m_0, \dots, m_{k-s}\} \in I_t} \sum_{\{l_0, \dots, l_{k-s}\} \in I_t} \Pr(\Delta_0^L = m_0, \dots \\ &\quad \dots, \Delta_{k-s}^L = m_{k-s}, \Delta_0^R = l_0, \dots, \Delta_{k-s}^R = l_{k-s}), \end{aligned} \quad (46)$$

where the index set summed over is

$$I_t = \left\{ l_0, \dots, l_{k-s} \mid 0 \leq l_0 \leq s, 0 \leq l_1, \dots, l_{k-s} \leq 1, \sum_{\alpha=0}^{k-s} l_\alpha \leq t \right\}. \quad (47)$$

The set $\{\Delta_1^L, \dots, \Delta_{k-s}^L, \Delta_1^R, \dots, \Delta_{k-s}^R\}$ partitions into a collection of disjoint subsets of the form $\{\Delta_\alpha^L, \Delta_\alpha^R, \Delta_{\alpha+r-s}^L, \Delta_{\alpha+r-s}^R, \Delta_{\alpha+2(r-s)}^L, \dots\}$, $\alpha = 1, \dots, r-s$ (indicated by the zig-zag line in Fig. 4(a)), each of which satisfies the conditions of the proposition P_N for some N . Also, these subsets are independent of one another and of Δ_0^L and Δ_0^R , since they contain random variables which are functions of corresponding disjoint subsets of letters.

Thus we can factor the probability in Eq.(46) and rearrange the sum to obtain

$$\begin{aligned} E(Y_u, Y_v) &= \sum_{\{m_0, \dots, m_{k-s}\} \in I_t} \Pr(\Delta_0^L = m_0) \dots \Pr(\Delta_{k-s}^L = m_{k-s}) \\ &\quad \times \sum_{\{l_0, \dots, l_{k-s}\} \in I_t} \Pr(\Delta_0^R = l_0) \dots \Pr(\Delta_{k-s}^R = l_{k-s}) \\ &= \sum_{\{m_0, \dots, m_{k-s}\} \in I_t} \Pr(\Delta_0^L = m_0, \dots, \Delta_{k-s}^L = m_{k-s}) \\ &\quad \times \sum_{\{l_0, \dots, l_{k-s}\} \in I_t} \Pr(\Delta_0^R = l_0, \dots, \Delta_{k-s}^R = l_{k-s}) \\ &= \Pr(\Delta(\mathbf{W}_u^A, \mathbf{W}_u^B) \leq t) \Pr(\Delta(\mathbf{W}_v^A, \mathbf{W}_v^B) \leq t) \\ &= E(Y_u)E(Y_v). \end{aligned} \quad (48)$$

Thus $\text{Cov}(Y_u, Y_v) = 0$ for v in the Case I part of J_u^{ao} . Cases II, III and IV can be similarly dealt with by reversing the order of both sequences, interchanging the roles of sequences A and B , or both.

Case V is illustrated in Fig. 4(b). This time the union of the overlapping words \mathbf{W}_u^A and \mathbf{W}_v^A is subdivided into the set of single-letter words $\mathbf{W}_\alpha^A = (A_{i+\alpha-1})$, $\alpha = 1, \dots, k+r$, and the union of the overlapping words \mathbf{W}_u^B and \mathbf{W}_v^B is subdivided into the set of single-letter words $\mathbf{W}_\alpha^B = (B_{j-|s|+\alpha-1})$, $\alpha = 1, \dots, k+|s|$. We define

$$\Delta_\alpha^L = \Delta(\mathbf{W}_\alpha^A, \mathbf{W}_{\alpha+|s|}^B), \quad \Delta_\alpha^R = \Delta(\mathbf{W}_\alpha^B, \mathbf{W}_{\alpha+r}^A), \quad \alpha = 1, \dots, k. \quad (49)$$

Then

$$\Delta(\mathbf{W}_u^A, \mathbf{W}_u^B) = \sum_{\alpha=1}^k \Delta_\alpha^L, \quad \Delta(\mathbf{W}_v^A, \mathbf{W}_v^B) = \sum_{\alpha=1}^k \Delta_\alpha^R, \quad (50)$$

and

$$\begin{aligned} E(Y_u, Y_v) &= \Pr(Y_u = 1, Y_v = 1) \\ &= \Pr(\Delta(\mathbf{W}_u^A, \mathbf{W}_u^B) \leq t, \Delta(\mathbf{W}_v^A, \mathbf{W}_v^B) \leq t) \\ &= \sum_{\{m_1, \dots, m_k\} \in I_t} \sum_{\{l_1, \dots, l_k\} \in I_t} \Pr(\Delta_1^L = m_1, \dots, \\ &\quad \dots, \Delta_k^L = m_k, \Delta_1^R = l_1, \dots, \Delta_k^R = l_k), \end{aligned} \quad (51)$$

where the index set is now

$$I_t = \left\{ l_0, \dots, l_k \mid 0 \leq l_1, \dots, l_k \leq 1, \sum_{\alpha=1}^k l_\alpha \leq t \right\}. \quad (52)$$

The set $\{\Delta_1^L, \dots, \Delta_k^L, \Delta_1^R, \dots, \Delta_k^R\}$ partitions into a collection of disjoint subsets of the form $\{\Delta_\alpha^L, \Delta_{\alpha+|s|}^R, \Delta_{\alpha+r+|s|}^L, \dots\}$, $\alpha = 1, \dots, r$, or $\{\Delta_\alpha^R, \Delta_{\alpha+|s|}^L, \Delta_{\alpha+r+|s|}^R, \dots\}$, $\alpha = 1, \dots, |s|$ (indicated by the zig-zag line in Fig. 4(b)), each of which satisfies the conditions of the proposition P_N for some N , and which are mutually independent. Thus we can factor the probability in Eq.(51), rearrange the sum and recombine the probabilities to obtain

$$\begin{aligned} E(Y_u, Y_v) &= \sum_{\{m_1, \dots, m_k\} \in I_t} \Pr(\Delta_1^L = m_1, \dots, \Delta_k^L = m_k) \\ &\quad \times \sum_{\{l_1, \dots, l_k\} \in I_t} \Pr(\Delta_1^R = l_1, \dots, \Delta_k^R = l_k) \\ &= \Pr(\Delta(\mathbf{W}_u^A, \mathbf{W}_u^B) \leq t) \Pr(\Delta(\mathbf{W}_v^A, \mathbf{W}_v^B) \leq t) \\ &= E(Y_u)E(Y_v), \end{aligned} \quad (53)$$

giving $\text{Cov}(Y_u, Y_v) = 0$ for v in the Case V part of J_u^{ao} . Case VI can be similarly dealt with by interchanging the roles of sequences A and B .

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