Mismatch Repair Error Implies Chargaff’s Second Parity Rule

Bo Deng

Abstract: Chargaff’s second parity rule (PR2) holds empirically for most types of DNA that along single strands of DNA the base contents are equal for complimentary bases, \( A = T, G = C \). A Markov chain model is constructed to track the evolution of any single base position on a given single strand of DNA whose organism is equipped with the process of mismatch repair. Under the key assumptions that the mismatch error rates primarily depend the complementarity and the steric effect of the nucleotides and that the mismatch repairing process itself makes strand recognition errors, the model shows that the steady state probabilities for the base position to take on one of the 4 nucleotide bases are equal for complimentary bases. As a result, Chargaff’s second parity rule is the manifestation of the law of large numbers acting on the steady state probabilities. More importantly, because the model pinpoints mismatch repair as a basis of the rule, it is suitable for experimental verification/falsification. At this point, existing empirical survey supports the proposed mismatch-repair separation between PR2 and non-PR2 genomes.

Introduction. Chargaff’s parity rule with \( A = T, G = C \) for the nucleotide composition of DNA ([4]) is mechanistically explained by Watson-Crick’s double helix model of DNA ([16]) because base \( A \) and \( T \) pair only with each other while base \( G \) and \( C \) pair only with each other along DNA’s complementary double strands. Chargaff and colleagues ([5, 6]) discovered a similar rule for single strands of DNA. For distinction, the former is referred to as Chargaff’s Parity Rule (PR) and the latter as Chargaff’s Second Parity Rule (PR2), respectively. Unlike PR, PR2 is not an exact rule but a statistical one. More specifically, according to a recent comprehensive test in [12], PR2 holds for four of the five types of double stranded genomes: the archael chromosomes, the bacterial chromosomes, the eukaryotic chromosomes, most double stranded viral genomes, but it does not apply to the organellar (mitochondria and plastids) genomes nor to single stranded viral genomes or any type of RNA genome. Another study ([1]) also shows that the rule is length-dependent: for those that PR2 does apply, the longer the strand is the smaller the deviation from the rule becomes.

In contrast to PR, the mechanistic basis of PR2 is still an open question (c.f. [10, 1]). Base inversion, base substitution, base transposition are some of the suggested possible causes ([1, 8, 15, 9]). However, these suggested mechanisms fail to make clear and definitive distinction between those that obey the rule and those that do not. Various mathematical models are also proposed to explain the rule. However, they all led to a wrong prediction that single strands of a given length would eventually evolve to exhibit the exact rule after long enough time, which is inconsistent with the length-dependent property of the rule. This defect was noted in [10].

Hence, any new model must make improvement in two fronts: its applicability only to the PR2 genomes and its dependency on the genomic size. The mathematical model we propose here seems
to have accomplished both by limiting its applicability (a) to those organisms only with mismatch repair and (b) to the base evolution of any arbitrary single position along any single strand of the organism’s DNA. The resulting model is a local Markov process for single bases whose steady state probabilities together with their manifestation via the law of large numbers give rise to the length-dependent empirical PR2.

**The Mathematical Model.** Let $m\{B_t \rightarrow B_r\}$ denote the match or mismatch probability of a replicative base $B_r$ to a template base $B_t$. The mathematical model of DNA replication with mismatch repair assumes the following.

**Mismatch Repair Error (MRE) Model:**

(a) DNA replication makes nucleotide mismatch errors.

(b) At the moment of base replication, the mismatch error occurs independent of the nucleotide base position on the template single strand of DNA.

(c) The match probabilities satisfy the following assumptions:

i. The match probabilities between complementary bases are equal with respect to the complementary pairs:

$$m\{A \rightarrow T\} = m\{T \rightarrow A\} \text{ and } m\{G \rightarrow C\} = m\{C \rightarrow G\}$$

ii. The match probability of a base to its complementary or itself is the same as that of the base’s complementary to the complementary or itself:

$$m\{A \rightarrow A, \text{ or } T\} = m\{T \rightarrow A, \text{ or } T\} \text{ and } m\{G \rightarrow G, \text{ or } C\} = m\{C \rightarrow G, \text{ or } C\}$$

iii. The conditional mismatch probability of a pyrimidine to its non-complementary purine equals that of its complementary purine to the purine’s non-complementary pyrimidine:

$$m\{A \rightarrow C|A \neq T\} = m\{T \rightarrow G|T \neq A\}$$

$$m\{G \rightarrow T|G \neq C\} = m\{C \rightarrow A|C \neq G\}$$

(d) Mismatch repair makes strand recognition errors independent of complementary bases, and as a result it make base substitution errors.

Hypothesis (a) is self-evident, which applies to all DNA, with or without mismatch repair. Hypothesis (b) is a localized and symmetrical assumption that each base position is as critical or ordinary as any other base position. It should be taken to be a primary approximation of this aspect of the replication. This means any assumption about global interactions along single strands of DNA, such as the stem-loop hypothesis of [2], or codon position bias asymmetric assumption from [10], can be taken as secondary approximations or corrections for future refinement of the model.
Hypotheses i,ii(c) implies that the mismatch probability of a base to itself is the same as that of the base’s complementary to the complementary:

\[ m\{A \rightarrow A\} = m\{T \rightarrow T\} \text{ and } m\{G \rightarrow G\} = m\{C \rightarrow C\} \]

Alternatively, Hypothesis ii(c) can also be stated in terms of the mismatch conditional probabilities:

\[ m\{A \rightarrow A|A \neq T\} = m\{T \rightarrow T|T \neq A\} \]
\[ m\{G \rightarrow G|G \neq C\} = m\{C \rightarrow C|C \neq G\} \]

In other words, Hypotheses i,ii(c) are made mainly on the complementarity of bases, which would be automatically true if DNA were a binary code in either the AT-pair or the GC-pair.

On the other hand, however, Hypothesis iii(c) is made mainly on the steric characteristics of the pyrimidines and purines, i.e., when an \( A \neq T \) (resp. \( G \neq C \)) mismatch occurs, the conditional mismatch probability between \( A \rightarrow C \) (resp. \( G \rightarrow T \)) mismatch is the same as the \( T \rightarrow G \) (resp. \( C \rightarrow A \)) mismatch. As a result of Hypotheses i,ii,iii(c), the remaining conditional mismatch probabilities are forced to satisfy:

\[ m\{A \rightarrow G|A \neq T\} = m\{T \rightarrow C|T \neq A\} \]
\[ m\{G \rightarrow A|G \neq C\} = m\{C \rightarrow T|C \neq G\} \]  

(1)

More details can be found from the match/mismatch probability diagrams of Fig.1.

What separates our model from all others is Hypothesis (d). Although similar assumptions such as base inversion, inverted transposition, stem-loop substitution, codon position-bias were made for other models, the precise and systematic mechanisms that made such operations possible were either not known well enough or conjectured too broadly for all types of DNA, to which we know by the result of [12] that PR2 does not always apply. As a result, none is incorporated into the current model, nor is any repairing mechanism outside the phase of replication such as excision repair to spontaneous deamination of cytosine. In contrast, it appears that most types of double-stranded chromosomes are equipped with mismatch repair ([13]). More interestingly, mismatch repair in mitochondria of any organism is not known to exist according to [14], even though other types of repair mechanisms may exist ([3]) which do not negate the model under consideration. This distinction between DNA having or not having mismatch repair is consistent with the applicability of PR2 established in [12]. As a result of this hypothesis, our MRE model applies only to DNA types with mismatch repair satisfying these four hypotheses.

**The Result.** From a modeling perspective, Hypotheses (c,d) imply that the MRE model is a Markov process model for any arbitrary position of any single strand of DNA since the transition probability at the position depends only on its current base. The Markov model is illustrated in Fig.[1] referred to as the *mismatch repair error* chart, or MRE chart for short. Take, for example, a nucleotide \( A \) on a template single strand of DNA as shown at the top of the left chart. With a probability \( 0 < 1 - a < 1 \), the process correctly replicates \( A \)'s complementary base \( T \), showing
Figure 1: MRE charts for base A and base G. Parameters $a, d$ denote the mismatch probabilities $m\{A \not\leftrightarrow T\}, m\{G \not\leftrightarrow C\}$, respectively, and $b_i, e_i$ denote the conditional mismatch probabilities. For example, $b_2 = m\{A \rightarrow C|A \not\rightarrow T\} = m\{T \rightarrow G|T \not\rightarrow A\}$ and similarly, $e_2 = m\{G \rightarrow T|G \not\rightarrow C\} = m\{C \rightarrow A|C \not\rightarrow G\}$.

down the left most branch, but incorrectly with $0 < a < 1$ probability. Of that fraction of mismatches, for a fraction of $0 < b_1, b_2$ each the process mismatches it with a G or a C, respectively, with $b_1 + b_2 < 1$. For the remaining $1 - (b_1 + b_2)$ fraction of mismatches, an A is mismatched to the original A. These assumptions are represented by the middle three branches.

For organisms which proof read and repair mismatches, the MRE chart continues one level down to the bottom branches. In such cases, Hypothesis (d) assumes that they do not always distinguish the replicative strand from the template strand 100% of the time, and make strand recognition errors independent of the replicating bases in question. Thus, for $0 < c < 1$ fraction of time, however insignificant or small it may be at this point, the process mistakes the replicative strand for the replicating template, and proceeds to turn the original base A into a base T if the mismatched pair is an A-A pair, with the hyphenated second base position being the template base. Similarly, with a probability $0 < c < 1$ each, a G-A pair is transformed to a G-C pair and a C-A pair to a C-G pair, all changing the template base A to a non-complementary base. With $1 - c$ probability, the process correctly identifies the template strand, and proceeds to keep the original base A in the template strand when the replication is completed. This particular illustration shows the case that the original mismatch error is corrected by a base T, which needs not to be the case. In fact, what is only assumed and important is that with the $1 - c$ probability the original base A is *preserved* along the template strand upon replication. The replicated new base along the complementary strand may or may not be the complementary base of the old base upon the completion of the mismatch repair. That is, the most general depiction of the chart would replace the base T by a place holder, O, for any of the four bases.

Interchanging A and T and interchanging the parameters $b_1, b_2$ in base A’s MRE chart gives rise to base T’s MRE chart if the replicating base at the top starts with a T. Because of Hypotheses (c,d), all the probability distributions for T’s chart are the same as base A’s chart except for the conditional mismatch probabilities of Hypothesis iii(c) and the consequence Eq.(1) of Hypothesis (c), which are accounted for by interchanging $b_1$ and $b_2$. The transition probabilities from base T
are listed on the second row of the transition probability matrix $P$ below.

Similarly, the diagram on the right is base $G$’s MRE chart with probability distributions in $d, e_1, e_2, f$, which may not be respectively the same as $a, b_1, b_2, c$ for $A$ and $T$. In particular, $a$ and $d$ are very likely not to be the same, so are for $b_i$ and $e_i$. According to Hypothesis (d), we have $c = f$. But there will be no changes to the result if we keep $c, f$ distinct. Also, interchanging $G$ and $C$ in $G$’s MRE chart gives rise to $C$’s MRE chart.

The mismatch repair charts allow us to derive the transition probabilities between bases one replicating generation a time. More specifically, take the $A$’s MRE chart for an example. Let $p\{A \rightarrow A\}$ be the probability that a base $A$ from a template strand remains an $A$ upon replication. Similarly, let $p\{A \rightarrow T\}$ be the probability that a base $A$ from the template strand is substituted by a $T$ upon replication, and similar notation applies to $p\{A \rightarrow G\}, p\{A \rightarrow C\}$. Then these probabilities can be tabulated from probability distributions from the MRE chart as follows:

\[
\begin{align*}
p\{A \rightarrow T\} &= a(1 - (b_1 + b_2))c \\
 p\{A \rightarrow G\} &= ab_1 c \\
 p\{A \rightarrow C\} &= ab_2 c \\
 p\{A \rightarrow A\} &= 1 - p\{A \rightarrow T\} - p\{A \rightarrow G\} - p\{A \rightarrow C\} = 1 - ac
\end{align*}
\]

The first expression, for example, is obtained by following from the top of the chart down the direct branches leading to the substituting base $T$ and multiplying all the probabilities along the branches. The same for the transition probabilities from $A$ to $G$ and $C$ respectively. The probability from $A$ to $A$ can either be obtained by the formula above or by summing all probabilities of the $A$-to-$A$ branches (four branches in all) of the chart. Exactly the same tabulation gives the transition probabilities for all other bases.

Using matrix entry notation, we denote

\[
\begin{align*}
p_{11} &= p\{A \rightarrow A\}, & p_{12} &= p\{A \rightarrow T\}, & p_{13} &= p\{A \rightarrow G\}, & p_{14} &= p\{A \rightarrow C\}.
\end{align*}
\]

That is, the row and column numbers, 1, 2, 3, 4, are in correspondence with $A, T, G, C$, respectively. Similar notation extends to other bases as well. As a result we obtain the following transition probability matrix for our MRE model:

\[
P = [p_{ij}] = \begin{bmatrix}
1 - ac & a(1 - (b_1 + b_2))c & ab_1 c & ab_2 c \\
 a(1 - (b_1 + b_2))c & 1 - ac & ab_2 c & ab_1 c \\
d e_1 f & de_2 f & 1 - df & d(1 - (e_1 + e_2))f \\
d e_2 f & de_1 f & d(1 - (e_1 + e_2))f & 1 - df
\end{bmatrix}
\]

What follows are standard textbook properties of transition probability matrices of Markov chains (c.f.[II]).

1. Denote the $n$th iterate of the transition matrix by

\[
P^n = [p_{ij}^{(n)}].
\]
Then we know that it is again a transition probability matrix with its entry \( p_{ij}^{(n)} \) representing the probability of a base \( i \) becoming a base \( j \) after the \( n \)th generation of replication.

2. Because the transition matrix \( P \) has all positive entries (hence is irreducible and ergodic), by the Perron-Frobenius Theorem, the limit \( \lim_{n \to \infty} p_{ij}^{(n)} \) exists and the limit is independent of the initial base \( i \): \( \lim_{n \to \infty} p_{ij}^{(n)} = \pi_j \), satisfying \( 0 < \pi_j < 1 \) and

\[
\pi_1 + \pi_2 + \pi_3 + \pi_4 = 1.
\]

In terms of DNA replication with mismatch repair, probability \( \pi_j \) is the steady state probability of finding base \( j \) at any base position of any single strand of DNA. This means, regardless of the initial base \( i \) at that position, after sufficiently many generations of replication, the probability of finding base \( j \) at the position is \( \pi_j \). We denote the four steady state probabilities by \( \pi_1 = p(A), \pi_2 = p(T), \pi_3 = p(G), \pi_4 = p(C) \).

3. The transition matrix \( P \) has \( \lambda_1 = 1 \) to be the largest eigenvalue in magnitude and it is simple. Moreover, the steady state probability vector \( \pi = [\pi_1, \pi_2, \pi_3, \pi_4] \) is the only left eigenvector not counting scalar multiple (or the transpose \( \pi^t \) is the only right eigenvector of eigenvalue 1 for the transpose matrix \( P^t \)). Because of this property, we can use the eigenvector equation \( \pi P = \pi \) to find \( \pi \) explicitly as

\[
\pi = \frac{1}{2(a(b_1 + b_2)c + d(e_1 + e_2)f)}[d(e_1 + e_2)f, d(e_1 + e_2)f, a(b_1 + b_2)c, a(b_1 + b_2)c],
\]

with equal probabilities \( \pi_1 = \pi_2, \pi_3 = \pi_4 \) for complementary bases. This, to be explained later, is the basis for the empirical law of PR2.

4. By the Perron-Frobenius Theorem, the remaining eigenvalues of \( P \) are less than 1 in magnitude. In this case, they can be explicitly found as:

\[
\lambda_2 = 1 - a(b_1 + b_2)c - d(e_1 + e_2)f,
\]

\[
\lambda_{3,4} = 1 - ac - df + \frac{1}{2}(a(b_1 + b_2)c + d(e_1 + e_2)f) \pm \frac{1}{2} \sqrt{D}, \text{ where}
\]

\[
D = (-2ac + 2df + a(b_1 + b_2)c - d(e_1 + e_2)f)^2 + 4a(b_1 - b_2)cd(e_1 - e_2)f.
\]

It has the property that for any initial probability distribution \( q = [q_1, q_2, q_3, q_4] \) with \( q_1 + q_2 + q_3 + q_4 = 1 \), \( qP^n \) converges to \( \pi \) at a rate no greater than the order of \( |\mu|^n \) with \( \mu = \max_{2 \leq i \leq 4}\{|\lambda_i|\} < 1 \). This gives a temporal estimate for the convergence rate to the steady state probability at the \( n \)th generation of replication.

We now conclude that Hypothesis (b), Property 2, and the law of large numbers imply the empirical PR2. More specifically, let \( F_L\{i\} \) be the length-averaged frequency of base \( i \), called the ensemble frequency, then we must have \( \lim_{L \to \infty} F_L\{i\} = p\{i\} \) for \( i = A, T, G, C \). To see this, we borrow a prototypical explanation from coin tossing: The steady state probabilities of the head and tail of a coin can be approximated by tossing an ensemble of \( L \) many identical coins and counting
Figure 2: Homo sapiens chromosome 22 genomic contig ref|NT_113818.1|Hs22_111537:1-17927 (from Human Genome Project) with length $L = 17,927$ and ensemble frequency $F_L = (0.1993, 0.2007, 0.2917, 0.3083)$. (a) In the $5' \to 3'$ direction, the sequence of ensemble frequencies $F_N$ with length $N = 1, 2, \ldots, L$ is generated. The convergence error sequence, $\sum_{i=A,T,G,C} |F_N(i) - F_L(i)|$, is plotted against $N$ together with its best fit to the curve $a + b/\sqrt{N}$. (b) The same plot except for the opposite $3' \to 5'$ direction. (c) The same plot except (1) the data is averaged over 10 runs and (2) each run is done for a random permutation of the original sequence.

Discussion. It is interesting to note a contrast between mismatch repair and other possible or conjectured processes such as base inversion, transposition, stem-loop substitution, codon position-bias asymmetry. For organisms without mismatch repair, the MRE chart stops at the first or the second branches with the template base well-entrenched in its position. It is not clear what mechanistic operations these processes adopt to systematically substitute the template base. If such processes only subject the template base to a sporadic, disorganized chance substitution as implied when such systematic mechanisms are lacking, it is hard to see how a regular and steady-state-like pattern such as PR2 can arise. It is also important to note that the model starts with a single strand separated from a double stranded mother DNA during replication and ends with the completion of replication. This means that any damage or repair that may take place before or after replication is not relevant to the model at its current formulation. In other words, if such types of damages and repairs are not the results of some systematic, repetitive, and normal cellular functions, but rather disorganized random events, then the lack of regular, steady-state-like patterns in non-PR2
genome is not a contradiction to the model.

On the surface of it, mismatch repair enhances replication fidelity by enforcing PR. However, if the proposed model is correct, it will imply that mismatch repair results in PR2. The question is then: is PR2 also some sort of genomic fidelity that needs to be preserved? If so, what is the evolutionary advantage of having it? A probable answer can be found in the communication theory for DNA replication proposed in [7] and it will be addressed elsewhere.

Past modeling attempts were not successful mathematically because they did not make the critical distinction between the steady state probability at individual base position and the ensemble frequency of whole length along single strands of DNA. To a lesser extent, they failed because they did not limit their scopes to different types of DNA by pinpointing the mechanistic processes which eventually but systematically led to the manifestation of PR2 we observe today. These assessments motivated the model proposed here which should be viewed as a first attempt to address both issues simultaneously. Future refinements of the model should be done by modifying and/or expanding the MRE hypotheses (a,b,c,d) in a manner as concise as we did here.

Non-PR2 genomes can be used as an empirical control group to guard against possible placebo effect of any proposed mechanism for PR2. For example, the hypothesis of base inversion ([1]) can be ruled out to be the cause of PR2 because it applies to both PR2 and non-PR2 genomes. Our proposed mismatch-repair mechanism, on the other hand, has so far withstood this scrutiny by keeping the empirical separation between PR2 and non-PR2 genomes.

Because of its conciseness, the model is suitable for experimental test. More specifically, an experiment can start with many identical double strands of DNA about 6,000 or more bp long (c.f. Fig[2] with an ensemble frequency disobeying PR2. Divide them into two groups. Replicate one group by a replicator with mismatch repair and replicate the other group by a replicator without mismatch repair. After a sufficiently long period of time, calculate the averaged ensemble frequency from each group and compare. The experiment would be supportive of the model if the ensemble frequency of the mismatch repairing replicator is closer to PR2 than that of the null replicator. The model can also be rejected if one can find a non-PR2 genome with mismatch repair.

Acknowledgement: The author would like to thank Melissa Wilson of Penn. State Univ., Dr. Modrich of Duke Univ., and Dr. Lobry of Univ. Claude Bernard, France, for providing him with various useful and important references. Special thanks to Dr. Mitchell of Trinity College of Dublin for his invaluable comments on the manuscript.

References

[1] Albrecht-Buehler, G., Asymptotically increasing compliance of genomes with Chargaff’s second parity rules through inversions and inverted transpositions, *PNAS*, **103**(2006), pp.17828–17833.

[2] Bell, S.J. & D.R. Forsdyke, Accounting units in DNA, *J. Theor. Bio.*, **197**(1999), pp.51–61.
[3] Berneburg M., Y. Kamenisch, and J. Krutmann, Repair of mitochondrial DNA in aging and carcinogenesis, *Photochem Photobiol Sci.*, **5**(2006), pp.190–198.

[4] Chargaff, E. Structure and function of nucleic acids as cell constituents, *Fed. Proc.*, **10**(1951), pp.654–659.

[5] Rudner, R., J.D. Karkas, E. Chargaff, Separation of *B. subtilis* DNA into complementary strands, III. Direct Analysis, *Proc. Natl. Acad. Sci.*, **60**(1968), pp.912–922.

[6] Rudner, R., Karkas, J.D., Chargaff, E. Separation of microbial deoxyribonucleic acids into complementary strands, *Proc. Natl. Acad. Sci.*, **63**(1969), pp.152–159.

[7] Deng, B., Why is the number of DNA bases 4? *Bul. Math. Bio.*, **68**(2006), pp.727-733.

[8] Fickett, J., D. Torney, and D. Wolf, Base compositional structure of genomes, *Genomics*, **13**(1992), pp.1056-1064.

[9] Lobry, J.R. and C. Lobry, Evolution of DNA base composition under no-strand-bias conditions when the substitution rates are not constant. *Mol. Biol. Evol.*, **16**(1999), pp.719–723.

[10] Lobry, J.R., A nice wrong model for the evolution of DNA base frequencies. *Physica A*, **273**(1999), pp.100–103.

[11] Meyn, S.P. and R.L. Tweedie, *Markov Chains and Stochastic Stability*, Springer-Verlag, 1993.

[12] Mitchell, D., R. Bridge, A test of Chargaff’s second rule, *Bioche. Biophy. Res. Comm.* **340**(2006), pp.90–94.

[13] Modrich, P., Strand-specific mismatch repair in mammalian cells, *J. Biol. Chem.*, **272**(1997), pp.24727–24730.

[14] Modrich, P., personal email communication, (2006)

[15] Sueoka, N., Intrastrand parity rules of DNA base composition and usage biases of synonymous codons, *J. Mol. Evol.*, **40**(1995), pp.318–325.

[16] Watson, J.D. and F.H.C. Crick, Molecular structure of nucleic acids, *Nature*, **171**(1953), pp.737–738.