Disparity mutagenesis model possesses the ability to realize both stable and rapid evolution in response to changing environments without altering mutation rates.

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Abstract

It has been shown that the disparity model, in which mutations are introduced exclusively in the lagging strand, has a great advantage in the promotion of evolution, compared with the conventional parity mutagenesis model. To understand much more about the characteristic of the disparity model, a novel 2-D genetic algorithm (GA) which reflected gene interactions was developed. The GA consisting of the lattice model showed powerful abilities to evolve. Even under conditions of high mutation rates with an extra genetic load, the GA could quickly evolve and finally attain a long stable state with high fitness scores.

Arbitrary interruption of the stable state of evolution by various lengths of environmental stress could produce new individuals with different genotypes within relatively short periods and the mutants finally occupy the population; that brought back the picture of “punctuated equilibrium”. Interestingly, this phenomenon could be reproduced with certainty without changing mutation rates at all.
As long as the fidelity difference between the lagging and leading strand was kept high enough, the robustness of the disparity model was very high. The acceleration or slowdown of evolution can be unambiguously introduced only by environmental changes, and the seesawing mutation rate is not the necessary condition for changing the speed of evolution.

Keywords: Biological sciences, Evolution, Genetics, Mathematical biosciences

1. Introduction

The disparity model of evolution which was deduced from the basic concept of the lagging-strand-biased-mutagenesis (disparity mutagenesis) was proposed by one of the present authors, M.F., and his colleague, H. Doi, in 1992 [1, 2]. The disparity mutagenesis is deterministically described as follows. When a parental linear DNA replicates, one daughter DNA synthesized by the leading strand with high fidelity has zero mutations, but the other by the lagging strand with low fidelity is inevitably mutated. Consequently, there is more variance in the number of mutations in certain offspring, and some offspring always have no mutation. This unbalanced mutagenesis promotes evolution [2]. Indeed, it was estimated that the fidelity of the leading strand was about one hundredfold higher than that of the lagging strand in *Escherichia coli* [3], and that the disparity mutagenesis caused by error-prone pol α was discovered in humans and yeasts [4]. The most remarkable feature of our disparity mutagenesis model (disparity model) is that it works effectively when average mutation rates are well over the so-called “error threshold” [1, 2, 5]. Interestingly, such extreme conditions in mutation rate are not unusual for the living world. It has been reported that the mutation rate of humans and chimpanzees is 68 mutations per diploid genome per generation [6], and that of laboratory mice 28 [7], both of which are thought to be well over the error threshold values [8, 9].

The authenticity of our disparity model of evolution has been verified theoretically and experimentally. It was shown that co-existence of normal and error-prone RNA polymerase gave rise to the increase or the disappearance of error threshold in the Eigen-Schuster’s quasi-species [10], and that in the evolution games, the disparity model well overwhelmed the conventional “parity mutagenesis model”, in which the fidelity difference between the lagging and leading strand were out of consideration [11, 12]. Moreover, using living microorganisms such as *E. coli*, yeasts and malaria parasites, we reported that the “disparity mutator” performing lagging-strand-biased-mutagenesis significantly promoted adaptive evolution [5]. For instance, a disparity mutator of *E. coli* could make colonies in the presence of saturated concentrations of different kinds of antibiotics tested [13], and yeast mutators could proliferate and survive under high temperature conditions (38–41 °C) by virtue of serial mutations, *hot* 1 and (an) other unidentified gene(s) [14]. In short, it has been
proven that when there is a high enough fidelity difference between the leading and lagging strand, the disparity models and disparity-mutator organisms could continue to promote evolution without accompanying error catastrophe even under extraordinarily high mutation rates.

As the genome of living things, however, is so sophisticated and complicated there are methodological limitations for examining the mechanism occurring in the cell body. Thus, we decided to use computer simulations to estimate what actually happens in a mutator’s cell body. We have used so far a very simple GA which consists of a double-stranded structure with a linear arrangement of bits, and each bit corresponds to an individual gene [11, 12].

In the present study, we have further improved the GA in regard to the following three points to make it more realistic. First, as already reported, we used a DNA-type GA consisting of double strands which replicated semi-conservatively using the leading and lagging strand. In the present study, ploidy and sexuality were consciously ignored since we would like to highlight the effect of disparity mutagenesis on evolution. Second, we used a genome having 50 genes and added non-coding redundant regions with variable lengths. Third, it might be true in living things that every gene in a genome somehow interacts with others directly or indirectly. Therefore, we tried to contrive ways to reflect such a complicated gene interaction on the GA.

The present study reconfirmed the results of our former experiments, in that the disparity model could not become extinct even when average mutation rates were over the error threshold, and at the same time, evolution was actually accelerated [5, 11, 12]. The evidence most worth noting in the present study is as follows. 1) The disparity model could redirect and redeploy the process of evolution even under drastic changes in mutation rates and/or environments. 2) Punctuated-equilibria-like phenomena could be introduced only by environmental changes. Namely, the rise in mutation rates is not a necessary condition to introduce a sudden acceleration of evolution, as generally believed.

In the light of new evidence obtained from the present study, the following three points are also discussed: 1) the threshold of the fidelity difference between the leading and lagging strand in terms of error catastrophe, 2) genomic redundancy and evolution, 3) the relationship between genetic diversity and evolution.

2. Methods

2.1. Modeling of Disparity and Parity Mutagenesis

The GA used in the present study consists of a double-stranded-structure like DNA which replicates semi-conservatively by using the lagging and leading strand. The
replication origin is located at the upper terminal. This DNA construction corresponds to a replicore in living things.

2.1.1. Disparity model

As shown in Fig. 1 (right) as a typical example, mutations are introduced exclusively in the lagging strand. The characteristics of this DNA’s pedigree are: 1) Genotypes which existed in the past generations can be observed in the present generation; i.e., the diversity of genotypes is enlarged accompanied by the guarantee of genotypic “principal”. 2) Even when mutation rates exceed the so-called “error threshold”, the population can be prevented from extinction because of the error-less leading strand [5, 9].

![Disparity model diagram]

**Fig. 1.** Deterministic illustrations of the alternative models for the mutagenesis accompanying DNA replications. In the parity model, one mutation per replication is introduced in both daughter strands (Fig. 1 (left)). In the disparity model, two mutations per replication are introduced exclusively in the lagging strand (Fig. 1 (right)). A thin arrow indicates a leading strand and a dotted thin arrow a lagging strand. A short bar crossing strands indicates a misbase. Each number beside a bar indicates a base substitution at a different site. The ori indicates the replication origin. The pedigree by the disparity model implies several interesting features: 1) the ancestor with zero mutations and any genotype that once appeared in the past has been precisely guaranteed forever; 2) the threshold of mutation rates is increased (actually, the threshold disappears in this model); 3) even if circumstances drastically changed, the fittest individual will be selected as a new ancestor and will start again to produce a new pedigree as well.
2.1.2. Parity model

In the parity mode, mutations are introduced evenly into the two daughter DNAs independently of the lagging and leading strand (Fig. 1(left)). In other words, this is a commonly-believed model. When mutation rates exceed the error threshold (> 1 mutation per genome per replication), the population becomes extinct before long due to accumulated deleterious mutations.

2.2. Concept of Model Genome

The genome consists of a double-stranded structure which has N(50) genes in number. There are interactions among each gene. When these gene interactions are interrupted, the cell immediately results in death. In order to reflect these situations, we devised a kind of “information lattice”, where a given gene occupied a specific lattice point (Fig. 2). The vertical scale indicates the position of genes and the horizontal scale represents the coordinate to be occupied by each gene. Because of each gene having a single function, a given gene can occupy only a single position along the horizontal line. As stated later, the position of a gene can horizontally migrate due to newly-introduced mutations. When the distance

![Fig. 2. The “information lattice” which represents gene interactions in a DNA-type genetic algorithm. The vertical scale indicates the position of a gene. The horizontal scale indicates the coordinate to be occupied by each gene and each gene occupies a single position. When the distance between two vertically adjacent genes separates more than “1” coordinate, the cell has to die due to the disconnection of gene interaction (e.g., see the left enlarged circle, where the middle two genes are connected by a dotted line indicating the disconnection).](image)
between two vertically adjacent genes separates more than “1” coordinate, the cell has to die due to the disconnection of gene interaction, which can be regarded as a sort of an inner selection pressure (Fig. 2).

Genes used in the present study were classified into the following two categories depending on whether or not they contain coding regions.

1) Functional gene (FC gene): Gene endows three peaks for fitness score (FS). The biological meaning of the FS peaks is as follows. An amino-acid-replacement occurring at these three particular regions in a given gene product (protein) can only give rise to the increase or decrease of FS; in other words, the replacements occurring at other regions than these three particular regions in the protein are neutral (for details, see the legends of Fig. 3). Concerning each FC gene, these three regions with FS peaks are assigned symbolic names; A, B and C in a left-to-right fashion, respectively. For instance, when the \((i + 17)^{\text{th}}\) gene has a place of B peak, the gene is represented as \(i^{+17}\text{B}\). When the \((i + 7)^{\text{th}}\) gene has

Fig. 3. Fitness scores (FS) in individual functional genes (FC genes). The FC gene has three discrete fitness peaks, each of which consists of three FS values from “1” to “6”. There are five FC genes, \(i + 2, i + 7, i + 12, i + 17\) and \(i + 22\). For detailed explanations, see the text.
no $FS (FS = 0)$, the gene is represented as $i^{+7}$ - (Fig. 3). As shown in Fig. 3, the $(i + 17)^{th}$ gene has three $FS$ peaks; A with numbers $(1, 2, 1)$, B with $(3, 6, 3)$ and C with $(2, 4, 2)$, respectively. Each number in parentheses indicates the $FS$ value. When the $(i + 17)^{th}$ gene occupies the coordinate 10 on C peak, the $FS$ value of the $(i + 17)^{th}$ gene is evaluated as “4”.

2) Junk gene (JK gene): a JK gene has no $FS$ peak. Thus, $FS = 0$ in any position on the horizontal axis. JK genes are comparable approximately to “junk DNA”.

In the present simulation, we used a genomic DNA having 50 FC genes in number. For JK genes, we used 0, 100, 200, 300 and 450 in number, respectively. JK genes are placed evenly between FC genes. Fig. 4 shows an example of the DNA construction including JK genes, in which the DNA consists of 50 FC genes and 200 JK genes; hereafter, this gene construction is represented as 50FC-200JK.

2.3. Basic Rule of Simulations

a) Mutation rates

1) Disparity model: The average mutation rate introduced in the lagging strand was represented as $Pa$. The number of mutations inserted into each lagging strand per replication, $Nm$, was obtained by a random number. They were distributed normally so that $0 \leq Nm \leq 2 Pa$, according to the following distribution equation,

$$P(Nm) = \frac{1}{\sqrt{2\pi}\sigma^2} \exp\left(-\frac{(Nm - Pa)^2}{2\sigma^2}\right)$$

where $\sigma$ was the standard deviation and set to 1.5 in this work.

Within the compass of $Pa = 2$–$7$, the average mutation rates in the leading strand ($Pe$) were changed in the range of 0–0.35. In the case of the leading strand, one gene was selected randomly and a mutation was inserted at a probability of $Pe$. The effects of mutation rates on evolution were examined.

2) Parity model: In the parity model $Pe = Pa$. The average mutation rates were changed in the range of 0.07–0.71. This is because when the rate exceeded 0.71, the population became extinct in the very early stage of evolution.

b) Detailed conditions for simulations

1) The initial state of the simulation: The initial population consisted of 100 cells (or genomes) and the initial coordinates for $FS$ in all individual cells were set as “0” position on the horizontal axis. The starting 100 individuals had exactly the same genotype. The actual value of $FS$ in the starting individual was about 30.

2) Replication and generation number: Synchronized replications were carried out up to >200,000–400,000$^{th}$ generation.
3) Mutation and FS: Both FC and JK genes obeyed the same rule for the horizontal migration by a single mutation. The distance of migration is defined as 1, 2, or 3 coordinate(s) on the lattice per single mutation, respectively (Fig. 2). The probabilities of the occurrence of migration are defined as 1/2 for 1-coordinate-migration, 1/3 for 2-coordinate-migration and 1/6 for 3-coordinate-migration per mutation. The case in which plural mutations are introduced into the same gene during a single replication process was not ignored. Additionally, the rate of deleterious mutations is significantly high in nature. To reflect this situation, when a mutation causes

![Diagram](http://dx.doi.org/10.1016/j.heliyon.2016.e00141)

**Fig. 4.** Two examples of graphic illustrations for whole genotypes of an entire population at the 200,000th generation. (a) $P_t = 2$, $P_e = 0.07$ and 50FC-200JK. (b) $P_e = 0.28$, other parameters are same as in (a). The numerical number in each black frame indicates the number of genes that occupy a given FC coordinate. For instance, GN = 183 in the upper circle indicates the 183rd of FC gene. Between GN = 178 and 183 there are four JK genes with zero $FS$. When fitness peaks are located outside of the coordinate (-10-10), the black frames of fitness score are omitted from the graphs. For detailed explanations for coloring and numbering, etc., see the text.
an advantageous or a deleterious effect on the FC gene, the probability of 0.8 of the occurrence of deleterious effect was selected. In the case of JK genes, as the direction of migration on the lattice has no influence on \( FS \), the probability to migrate on the lattice to the left or the right was set as 0.5, respectively.

4) Selection pressure: As stated above, the number of individuals at the start point was 100. When the population size became more than 2,050 after a replication, a kind of truncation selection pressure was applied in order to adjust the population size to about 2,000. The probability of survival of cells with high \( FS \) was higher than that of cells with low \( FS \). The death or survival of the \( j \)th cell was determined by the \( FS \) of the \( j \)th cell, \( F_s(j) \), and a random number, \( R_n \)

\[
R_n \leq \Theta \quad \text{ (alive) } \tag{2}
\]
\[
R_n > \Theta \quad \text{ (dead) } \tag{3}
\]

and

\[
\Theta = \exp \left( -f_a \frac{F_{\text{max}} - F_s(j)}{F_{\text{max}} - F_{\text{min}}} \right) \tag{4}
\]

where \( \Theta \) is defined by the highest and lowest cell fitness scores, \( F_{\text{max}} \) and \( F_{\text{min}} \), of surviving daughter cells before selection pressure was applied.

The fitting parameter \( f_a \) was applied to keep the total number of cells between 1,950 and 2,050. However, in the case of a very homogeneous population with a very narrow deviation of \( FS \), randomly selected cells were truncated from the population to adjust the population size without using the equation (4).

5) Graphic illustrations of genotypes of an entire population: Fig. 4(a) shows an example. When \( P_a = 2 \), \( P_e = 0.07 \) and 50FC-200JK, genotypes of the entire population at the 200,000th generation are represented on the lattice. Fitness peaks of each FC gene are enclosed by a black frame. Four JK genes are located between adjacent two FC genes.

For example, there are 2,022 cells in the population in Fig. 4(a). Concerning FC gene 183 (GN = 183 in the enlarged circle in Fig. 4(a)), 2,017 cells out of 2,022 cells occupied the coordinate of \( FS = 4 \), four cells occupied \( FS = 2 \) and the remaining one cell occupied \( FS = 0 \), respectively. Thus, this population was very pure for FC gene 183. The coloring represents the density fluctuation of a gene on a given coordinate. A red frame indicates that the great majority of a given gene in the population occupied the same coordinate, blue ones are for a medium number, and colorless ones with a number are for a smaller number. Colorless ones with no number mean that no gene exists. Therefore, graphs mainly consisting of red and dark blue boxes indicate that the population is in a stable and nearly pure state (Fig. 4(a)). The average \( FS \) was calculated as follows: the total \( FS \) value of 50 FC genes was calculated to the individual genome. Then, the total value of \( FS \) of total
2,022 cells was calculated and divided by 2,022. The average $FS$ in the population of Fig. 4(a) is 138.8.

Fig. 4(b) shows the results of a simulation using a bit more severe condition where $Pe = 0.28$. Other parameters besides $Pe$ were not changed from those of Fig. 4(a). As expected, the average $FS$ of the entire population at the 200,000th generation became low (average $FS = 82.9$) and the color of the graph appeared blue, meaning that the genetic diversity of this population increased. The $FS$ became lower compared to the former simulation. This is because the lower fidelity of the leading strand ($Pe$) cannot precisely keep the parental $FS$.

3. Results and discussion

3.1. Disparity Model

3.1.1. The effect of the fidelity difference between the lagging and leading strand on $FS$

The main conditions to win an evolutionary race would be the quick increase of $FS$ at the early stage of evolution and the acquisition of higher $FS$ at the final stable phase. When $Pe$ is adequately small including 0 and $Pa = 2–4$, quick increases of $FS$ were obtained at the early stage of simulation. When $Pa = 5–7$, however, the increase rates of $FS$ became low. Fig. 5(a) and Fig. 6(a) show the results of simulations in the cases of $Pa = 3$ and 5, respectively. The $FS$ of parents is guaranteed with overwhelming probability by the leading strand with low mutation rates (cf. Fig. 1(right)). In fact, when $Pe < 0.03$ and $Pa = 3$, the initial $FS$ of 30 increased to 80–90 by the 23,000th generation, further followed by a gradual increase up to the 29,000th generation. After this step, the $FS$ gradually increased in a staircase pattern and finally gained the highest $FS$ around 140 (Fig. 5(a)).

Before climbing, $FS$ slightly decreased due to looking randomly for other fitness peaks by using the lagging strand with low fidelities. When $Pe > 0.03$, the final $FS$ became lower with increasing mutation rates. When $Pa = 5$, it took much more time to attain a stable state of evolution. As shown in Fig. 6(a), all populations tested seem to have not yet attained their final stages of evolution.

In conclusion, the fidelity of the leading strand is the crucial factor; i.e., the lower the $Pe$, the better for evolution. Adequately, relatively high $Pa$ (2–4) is another necessary condition to attain success of evolution in the disparity model, because too low $Pa$ with low $Pe$ would give rise to a similar result as the parity model.

3.1.2. Changes in genetic diversity in the process of evolution in the disparity model

In order to understand much more about the process of evolution, time-dependent changes of individual genotypes in the course of evolution should be examined.
Therefore, the relationship between time-dependent changes in FS and genetic diversities in a given population were examined. The Shannon-Wiener index number (H') was calculated at a regular interval of 100 generations. H' can be expressed by the following formula, eq. (5), which is regarded as a kind of index number for entropy in the field of thermodynamics or information theory.

\[ H' = -\sum_{i=1}^{R} p_i \ln p_i \]  

(5)

\( p_i \) indicates the probability that a single cell has i-type genotype.

Larger values of \( H' \) indicate that the genetic diversity of a population is larger. To put it the other way around, a too well-stabilized population has to have a very low \( H' \).

When \( Pe \) was very small (0.03) and \( Pa = 3 \) in the disparity model, \( H' \) strongly fluctuated up until the 30,000–40,000 generations (Fig. 5(b)). At the early stages of
evolution, it was estimated that populations should be rich in diversity in order to attain the rapid increase of FS as a population. After several tens of thousands of generations, FS became stabilized and was followed by small $H'$ values. For instance, when $Pa = 3$, $H'$ was approximately 0.1 in the range of $Pe = 0.030 – 0.090$ (Fig. 5(b)). When $Pa = 4$, $H'$ was less than 0.1 in the range of $Pe = 0.012 – 0.024$ at the later stage (data not shown). The $H'$ value 0.1 means that genetic diversity is extremely low and more than 99% of the individuals comprising the population share nearly the same genotype. As stated above, after this step, the FS gradually increased in a staircase pattern and finally gained the highest FS around 140. Exactly corresponding to each position of the increased FS with a staircase pattern, a sharp peak of $H'$ could be observed where the average FS of the population slightly decreased. (compare Fig. 5 (a) and (b), or compare Fig. 6(a) and (b)). Before and after each $H'$ peak, it was ascertained that the genetic constituents were changed.

**Fig. 6.** The effect of the fidelity difference between the lagging and leading strand on evolution in the disparity model; $Pe$ dependency on evolution when $Pa = 5$. See the explanations of Fig. 5 for (a) and (b). $Pe$ values: black line ($Pe = 0.0$), red (0.0036), orange (0.0072), green (0.0180), blue (0.0144), purple (0.15) and dotted black (0.0198).
For instance, when $Pa = 3$ and $Pe = 0.03$ (a red line in Fig. 5(a)), a small gap of $FS$ (a red arrowhead in Fig. 5(b)) was observed near the 97,000th generation, where a very small sharp $H'$ peak appeared (a red line with an arrowhead in Fig. 5(b)). The $FS$ plateau before the $H'$ peak had been occupied by a single genotype: [BBABB-BA-A-CC–CCCB–BCAB-B–CB-B-BAABB-BBBAC–B]. At the 96,900th generation, however, two cells obtained a single new mutation at the 20th FC gene: [BBABB-BA-A-CC–CCCB–BCAB-B–CB-B-BAABB-BBBBAC–B–B]. At the 97,000th generation the number of cells having this new mutation increased to 41, and at the 97,100th generation to 1,959, in number which was more than 97% of the entire population. There was a very small drop of fitness ($−0.1$ in $FS$) at the point corresponding to the sharp peak of $H'$ (Fig. 5(a)). This drop of $FS$ may reflect situations of a transient period. A pioneer individual which could firstly occupy a new higher mountain in the fitness landscape started to search around itself by using the lagging strand with low fidelity, this action might bring about a loss of $FS$. On the one hand, it continued to reproduce its copies having the same genotype by using the leading strand with high fidelity. This transient situation continued until a great majority of members of the population had the new genotype. In the present example it took about 200 generations. This climbing-stairs phenomenon in the process of evolution could be observed at $Pe = 0.03$, 0.06 (not shown) and 0.09, as shown in Fig. 5(a) and (b). As stated later, this phenomenon could be reproduced in a more clear-cut form when the process of evolution was disturbed in arbitrary manners.

When $Pa = 3$ and $Pe = 0.105–0.135$, the $FS$ became low and $H'$ drastically fluctuated between 2 and 7; indicating that the $FS$ could not attain a long-lasting plateau, but different genotypes coexisted in a population all the time as far as simulated (Fig. 5(a), Fig. 6(a)). When $Pe = 0.135$, the variety of genotypes in a population became consistently over 100 in number and the proportion of the most dominant genotype was less than 10% of the entire population. This chaotic situation could be close to a “melting down” of genetic information, but the population could still continue to exist, indicating that the effect of the disparity mutagenesis has a very high robustness. Of course, there were limitations to perform the disparity effect. When $Pe$ was up to 0.150, $H'$ attained the highest value, and the population became quickly distinct due to the destruction of gene interactions (Fig. 5 (a) and Fig. 6(a)). When $Pa = 7$, $FS$ gradually increased without extinction of population as long as $Pe$ was kept low (data not shown).

### 3.2. Parity Model

The evolutionary process of the parity model was completely different from that of the disparity one. In the parity model, mutation rate of the lagging strand and that of the leading strand are identical: $Pe = Pa$. 
Generally speaking, in the case of parity mutagenesis, the degrees of the increase of $FS$ at the early stage of evolution were smaller than those of the disparity mutagenesis. This indicates that the disparity model has an advantage in the evolutionary race. A decisive disadvantage for parity mutagenesis would be due to the existence of its low error threshold. When the total mutation rate is over 1 mutation per genome per generation, the population theoretically becomes extinct eventually by the accumulation of deleterious mutations [8]. In the present simulation conditions, the limit of survival for the parity mutagenesis model was $Pe = Pa = 0.72$; i.e., the total mutation rate was 1.44 per genome per generation, which was just over the theoretical threshold value 1 (Fig. 7(a)). Within a very limited range of mutation rates ($Pe = Pa = 0.28$–0.63), the model could obtain the highest value of $FS$ around 140, which was comparable to that of the disparity mutagenesis model. Additionally, stair-like increases of $FS$ were also observed (Fig. 7(a)). Concerning $H'$, only around $Pe = Pa = 0.63$, the parity model could be

![Fig. 7](http://dx.doi.org/10.1016/j.heliyon.2016.e00141)

**Fig. 7.** The effect of the fidelity of DNA polymerases on evolution in the parity model. $Pe = Pa$ (0.07–0.72). (a) The relationship between generation and fitness score. (b) The relationship between generation and genetic diversity (Shannon-Wiener $H'$). Black line ($Pe = Pa = 0.07$), red (0.21), orange (0.35), green (0.49), blue (0.063), purple (0.70) and dotted black (0.72).
less than 0.1 (Fig. 7(b)). When other mutation rates were used, $H' > 0.5$, where populations were not stable throughout simulations.

In summary, we could not find any striking advantageous point for the parity model in terms of pursuing evolution, when compared with the disparity model.

3.3. Effects of redundant JK Genes on Evolution

Irrespective of the disparity or parity mutagenesis model, when the area occupied by JK genes in a genome was larger, $FS$ became higher and $H'$ lower at a given $Pa$ and $Pe$. That is, populations adapted well to environments and stabilized more with enlarged JK gene regions (Fig. 8(a) and (b)). The JK gene appears to act as a kind of buffering agent to keep the direct interaction between two adjacent FC genes. When the two FC genes move to opposite sides separately on the lattice by

![Fig. 8. The effects of JK genes and Pe values on evolution in the disparity model.](image)

(a) The relationship between $Pe$ values and $FS$ (fitness score). (b) The relationship between $Pe$ values and genetic diversity ($\log H'$). The average data from the 100,000–200,000th generations at $Pa = 3$ were plotted. ○, 50FC–0JK; ▲, 50FC–100JK; □, 50FC–200JK; ◇, 50FC–300JK; ●, 50FC–450JK. For detailed explanations of results, see the text.
mutations, intervening JK genes act as a bridge by which the two FC genes can keep their connection. The disconnection of the bridge brings about death. We could see occasionally that two widely separated adjacent FC genes were connected by four JK genes, most of which become “red” as shown in Fig. 4(a). Although there is no direct relation between JK genes and natural “junk DNA”, it can be stated at least that both JK genes and junk DNA might have a common activity by which harmful effects of mutations are diminished.

Fig. 8 indicates the dependency property of the JK gene on FS and H' in the disparity model with Pa = 3. When Pe is low (0.0–0.06), irrespective of the size of JK gene regions except for JK = 0, steady high FS values were maintained and low H' values were kept at the stable state of evolution. Namely, the population was occupied by individuals having stable and nearly homogeneous genotypes (Fig. 8(a)), evolution being held in equilibrium. When Pe became larger (0.06–0.15), FS became lower. On the contrary, H' became higher and a huge variety of genotypes appeared. Therefore, these populations seemed to be not advantageous in promoting evolution (Fig. 8(b)).

In the parity mutagenesis model, the condition satisfying both conditions of high FS and low H' at the same time could be observed only in a very narrow range of mutation rates, i.e., around Pe = Pa = 0.63. When Pe = Pa was 0.07–0.6, FS was high, but H' was so high that a stable major genotype could not exist (Fig. 9(a) and (b)). Thus, concerning the parity world, it appears to support the hypothesis that evolving living things can exist in narrow regions in terms of mutation rates, which is called the “edge of chaos” [15].

When Pe = Pa > 0.7, FS became remarkably low and H' abruptly increased. This might act as a major handicap for pursuing evolution. Furthermore, it can be predicted that in the parity model it might be difficult to overcome drastic environmental changes.

3.4. Punctuation of Equilibrium

3.4.1. The disparity model has the potential capacity to realize the punctuation of equilibrium.

The concept of punctuated equilibrium was proposed by N. Eldredge and S. Gould in order to explain the gaps in the fossil record; i.e., evolution does not go along progressively but rather abruptly after a long stationary phase [16]. According to the punctuated equilibria theory, species quickly change at the early stage of its differentiation followed by several hundred millions years of equilibrated state without prominent morphological changes. Irrespective of repeated discussions, however, the punctuated equilibrium theory has many problems continuing up to the present time [17].
Here, let us identify the problems concerning the punctuation of equilibrium. First, why is such a long equilibrated state able to exist in the process of evolution? Three factors have been considered: 1) gene flow which provides genetically equalized population, 2) sexual isolation which gives rise to genetic homeostasis, and 3) developmental constraint and chasing of habitat [17]. The disparity mutagenesis might provide another plausible answer to this problem.

A long equilibrium state of evolution can be explained by the disparity model. Independently of total mutation rates, the high fidelity of the leading strand guarantees a very low speed of evolution as long as the environment does not change. When environment drastically changes, the most suitable mutated genotypes might be selected from the stock of mutated genotypes. Using these conditions, the leading strand fidelity can explain why, to a certain extent, a long stable state of evolution can exist.

Fig. 9. The effects of JK genes and Pe values on evolution in the parity model. (a) The relationship between Pe (=Pa) values and FS (fitness score). (b) The relationship between Pe (=Pa) values and genetic diversity (log H'). The average data from the 100,000–200,000th generations were plotted. ○, 50FC–0JK; ▲, 50FC–100JK; □, 50FC–200JK; ●, 50FC–300JK, ●, 50FC–450JK. For detailed explanations of results, see the text.

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selected mutants, a search for the new fitness landscape might start. This situation appears as the acceleration of evolution with a sudden increase of mutation rates as occurring to an observer. In fact, in the disparity model, this jump of evolution occurs even if the total mutation rate is not changed. In the disparity world, the central players of the evolutionary drama are the high fidelity of the leading strand, the fidelity difference between both DNA strands, and natural selection [5].

In the following chapter, it is shown that drastic changes of selection pressure induce clear-cut punctuated equilibrium-like phenomena in the disparity model.

### 3.4.2. Effect of drastic changes of selection pressure on evolution in the disparity model

The process of evolution was experimentally disturbed by introducing a drastic environmental change. At the 200,000th generation in the above-stated

![Graph](image)

**Fig. 10.** The effect of the interruption of evolutionary process for 10,000 generations by a drastic environmental change in the disparity model. $P_e = 0.0, P_a = 3$. The process of evolution was intentionally interrupted by the drastic environmental change. The duration of the interruption is shown as the distance between two horizontal arrowheads. (a) The relationships between generation and $FS$ (fitness score), and (b) those between generation and genetic diversity (Shannon-Wiener $H'$) are shown.
simulations, the fitness landscape of genes in Fig. 3 was totally changed at once with different durations. This manipulation corresponds to applying a different selection pressure. Then, the changed fitness landscape was restored again to the original fitness landscape and this was followed by simulations for additional 200,000 generations. The mutation rate was kept constant throughout simulations. This arbitrary insertion of environmental stress would mimic a drastic environmental change which induced a mass extinction of life on earth, such as that occurring at the end of the Cretaceous.

The shapes of changes in $FS$ are shown in Fig. 10. In proportion to the length of the exposure time to the environmental stress, the degree of genotypic change became larger at the last stable stage of evolution. Namely, when the duration of stress was 5,000 generations, no genotypic change occurred (data not shown). However, when the duration was much more prolonged, new genotypes appeared and were fixed in the population. The stress for 6,000 generations produced a new genotype with two
mutations, that for 10,000 generations produced one with four mutations (Fig. 10) and that for 50,000 generations produced one with seven mutations (Fig. 11), respectively. Some of these mutations were common in these three populations. This is because the start population at the onset of environmental stress was genetically almost homogeneous. While the environmental stress was exerted, the population had to search much higher $FS$-hills of the new landscape by using the lagging strands with low fidelity. Thus, this resulted in the loss of $FS$ and the increase of $H'$. At the end of the simulation experiments, however, these three stable populations acquired nearly equal or higher $FS$ compared with that of the original population just before adding the environmental stress (Fig. 10 and Fig. 11).

In the next place, the simulation conditions were changed. The selection pressure and the mutation rate changed at the same time at the 200,000th generation.

![Graph showing fitness score and Shannon-Wiener H']

**Fig. 12.** The effect of the interruption of evolutionary process for 10,000 generations by a drastic environmental change in the parity model. $Pe = Pa = 0.5$. For the detailed explanations, see the legends of Fig. 10.
followed by continued reproduction without restoring the mutation rate and selection pressure until the end. The disparity model adapted well in a similar way and it seemed to be beyond the previous $FS$ value when the simulation continued for much longer (data not shown). These results strongly indicate that the disparity model is a considerably tough player in the evolutionary race and its genetic machinery has a high robustness.

As stated above in this section, in the disparity model the artificially-produced “punctuated equilibria” gave rise to the redirection and redeployment of the process of evolution. Irrespective of starting with exactly the same stable population, the interruption of the evolutionary process by the environmental stress produced different populations that consisted of different genotypes. It appears that this behavior of population divergence would seem to reflect a situation of the very early stage of species differentiation in nature. Here, it should be noticed that the

![Figure 13](image_url)

**Fig. 13.** The effect of the interruption of evolutionary process for 50,000 generations by a drastic environmental change in the parity model. $Pe = Pa = 0.5$. For the detailed explanations, see the legends of Fig. 10.
present “punctuated equilibria” were not induced by increasing mutation rates but only by a given term of drastic environmental changes. The increase of mutation rates is not the necessary condition for the realization of punctuated equilibria as R. Dawkins predicted [18]. Therefore, it can be stated that the realization of “punctuated equilibria” and performance of divergence are due to the intrinsic characteristics of the disparity model.

On the other hand, in the parity model, we could also observe the similar phenomena of punctuation with only a very restricted range of mutation rates ($Pe = Pa = 0.3–0.6$) (Fig. 12 and Fig. 13). $H'$ was high, however, and the speed of evolution was significantly low because of the low error threshold. (Fig. 7). The speed of evolution was high when $Pe = Pa = 0.4–0.6$, which was comparable to that of the disparity model.

Broadly speaking, sexuality and ploidy might act as positive factors for the promotion of evolution by virtue of increasing genetic diversity. The present fact that the striking behaviors presented by the disparity model and the disparity-mutator microorganisms, both of which were carried out with the mono-ploidy and asexually, should be positively evaluated. Although living things have plural replication origins on a chromosome, the effects of the disparity mutagenesis presented here might be expected as already stated elsewhere [9].

Implications of the disparity mutagenesis in the evolution of real organisms remain to be examined.

4. Conclusions

1) The disparity-mutagenesis model is highly tolerant to mutation rates. The necessary and sufficient conditions for the disparity model to perform evolution are to maintain high total mutation rates and high fidelity of the leading strand. It is noticeable that the disparity model can keep a stable state of evolution even when the total mutation rates are high.

2) The disparity model can display the so-called “punctuated equilibrium” by manipulating a single parameter – selection pressure; i.e., rising mutation rates are not necessary. The parity model can display a similar behavior but only within a very limited and low range of mutation rates. However, the genetic stability is too low to provide a stable population.

3) The disparity model hypothesizes an internal driving force that is responsive to environmental change. In other words, the disparity model includes a mechanism that enables the realization of the experimental acceleration of evolution. A simple genetic manipulation, by which the rate of the lagging-strand-biased-mutagenesis is increased, can bring about the acceleration of
evolution in any living thing with a double-stranded DNA as a genome (refer to [5]).

Declarations

Author contribution statement

Ichiro Fujihara: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mitsuru Furusawa: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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