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Comprehensive analysis of gene regulatory dynamics, fitness landscape, and population evolution during sexual reproduction

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Abstract

The fitness landscape is a critical concept in biophysics, evolutionary biology, and genetics that depicts fitness in the genotype space and visualizes the relationship between genotype and fitness. However, the fitness landscape is challenging to characterize because the quantitative relationships between genotype and phenotype and their association to fitness has not been comprehensively well described. To address this challenge, we adopted gene regulatory networks to determine gene expression dynamics. We analyzed how phenotype and fitness are shaped by the genotype in two-gene networks. A two-by-two matrix provided the two-gene regulatory network in which a vector with two angle values (Θ) was introduced to characterize the genotype. Mapping from this angle vector to phenotypes allowed for the classification of steady-state expression patterns of genes into seven types. We then studied all possible fitness functions given by the Boolean output from the on/off expression of the two genes. The possible fitness landscapes were obtained as a function of the genetic parameters Θ. Finally, the evolution of the population distribution under sexual reproduction was investigated in the obtained landscape. We found that the distribution was restricted to a convex region within the landscape, resulting in the branching of population distribution, including the speciation process.

1. Introduction

The fitness landscape has been a critical concept in studying evolution in statistical physics and dynamical systems. It describes fitness as the height of the genotype space and enables intuitive visualization of the relationship between the genotype and fitness. It allows for the study of how evolution progresses with genetic change to increase the fitness, and is relevant to studying evolvability, evolutionary pathways, and speciation.

However, it is difficult to obtain suitable fitness landscapes. In a fitness landscape, the genotype is represented by a set of parameters that can be mapped onto the fitness. According to the landscape, how fitness changes depends on the genetic parameters that are prescribed. However, there are two fundamental problems with this approach. It is necessary to determine the genetic parameters that describe landscape axes. Genetic information can be written as the DNA sequence and how continuous parameters are derived from this genetic sequence is not trivial because a slight change in the sequence could significantly change the phenotype. Therefore, the derivation of continuous and measurable genetic parameters that create the fitness landscape must be addressed. Second, fitness is a function of phenotype and is not computed from
Figure 1. The flow chart in this paper and the genotype–phenotype–fitness mapping. First, we analyze the two-node GRN dynamics and its attractor classification, which gives the genotype–phenotype mapping. Next, we introduce the angle vector \( \Theta = (\theta_x, \theta_y) \) of the genotype, which provides convenient parameters to understand the two-node GRN dynamics and classify the attractor. In the second part, we analyze the fitness landscape of the two-node GRN dynamics. We set the simple fitness functions, providing all the 2-input-1-output logic gates. Accordingly, depending on the fitness function, we obtained the fitness landscape on the plane of \( \Theta \). Finally, we carry out numerical evolution of the population under sexual reproduction, which leads to the restriction of the population to a convex region in \( \Theta \) space.

The genotype directly. Usually, the expression of RNAs and proteins is a complex and dynamic process that determines a resultant phenotype. In other words, the relationship between genotype and phenotype depends on complex gene expression dynamics. Fitness, then, is a function of dynamically regulated phenotype.

We adopted gene regulatory networks (GRN) that describe gene expression dynamics to address these questions about the fitness landscape. First, we showed that the degree of activation or inhibition in expression dynamics continuously defines genotype parameters. Second, phenotypes, that is, protein expression levels, are determined by gene expression dynamics; whereas, genomes provide the gene regulatory network. Thus, a complex genotype–phenotype relationship was obtained. Then, fitness was defined by the phenotypes, and thus, represented as a function of the introduced genetic parameters. In fact, the evolution of GRN has been studied extensively with respect to the robustness or the phenotypic plasticity [1–21]. In particular, extensive studies have been carried out using a Boolean gene regulatory network [1], where the expression value can take binary (on or off) values. Furthermore, the Boolean network has also been applied to analyze the actual data in gene expression and networks [22–26].

While there are extensive studies of evolution in the fitness landscape [27–36], the relationship between the global fitness landscape and GRN structures remains uncharacterized. In particular, Heather and Hohenlohe [36] classified GRN dynamics into six cases. However, the global change in gene expression dynamics due to GRN changes and the classification of the fitness landscape need to be further investigated by comprehensively considering all classes of possible GRNs and their relationship to phenotypes and fitness.

Generally, GRNs and their dynamic interactions with many regulatory genes are too complex to analyze. Here, we consider a GRN with two genes, which provides a straightforward and basic system to study comprehensively the dynamics that create a genotype–phenotype relationship. Here, two-node GRNs are represented by \( 2 \times 2 \) matrices. By using on/off expression dynamics, we could evaluate all the possible GRN structures by introducing two-dimensional parameters specifying the matrix.

Still, there can be many types of fitness functions for given expressions; in most theoretical studies, however, a specific fitness function is selected depending on the purpose of the study. Here, we studied all possible fitness functions that depended on the expression of the two genes (i.e. Boolean functions). The possible fitness functions were limited to 16 types, all of which were investigated to obtain the possible fitness landscape. Once the fitness landscape was obtained, the genome distribution of the GRN parameter was computed. The distribution was concentrated on a higher-fitness region; however, the robustness against recombination or sexual reproduction [7, 9, 10, 37, 38] will impose further restrictions on the
are produced from the transcription of genes \( X \) introducing the angle vector (represented by the squares on the line in figure 2). When proteins \( X \) and \( Y \) bind to a gene promoter, transcription is promoted or inhibited. For simplicity, we assumed that a gene is transcribed when the sum of the effects of molecular regulation exceeds a threshold \( \xi \) and \( \theta \), for later fitness function simplicity.

The activation function was given by the sigmoid function: 

\[
\frac{1}{1 + \exp(-a \cdot x)}
\]

where \( a \) is the slope parameter of the sigmoid function. The degree by which gene \( m \) expression is regulated by protein \( N \) transcribed by gene \( n \) is reported by \( n \) as \( J_{mn} \). With the dynamics of the expression level and protein concentration of \( X \) and \( Y \) represented by:

\[
\dot{x} = f[J_{xx}(x - \xi) + J_{xy}(y - \xi)] - x \tag{1}
\]

\[
\dot{y} = f[J_{yx}(x - \xi) + J_{yy}(y - \xi)] - y \tag{2}
\]

We consider the relationship between the fitness landscape and the convex regionalization of epistasis, sexual reproduction, and speciation.

### 2. Dynamics of the two-gene regulatory network and fitness landscape

#### 2.1. Two-gene regulatory network model

Our two-gene regulatory networks model assumed that there were two genes \( X \) and \( Y \). Let \( x \) and \( y \) be the concentrations of proteins transcribed and translated from each gene, respectively, figure 2. Proteins \( X \) and \( Y \) are produced from the transcription of genes \( X \) and \( Y \), respectively, and bind to the promoter regions of \( X \) and \( Y \) (represented by the squares on the line in figure 2). When proteins \( X \) and \( Y \) bind to a gene promoter, they either promote or inhibit transcription of that gene. When protein \( X \) regulates the transcription of gene \( X \), the degree of regulation is represented by \( J_{xx} \). Similarly, the regulation of gene \( Y \) by \( Y \) is represented by \( J_{yy} \), regulation of gene \( Y \) by \( X \) by \( J_{yx} \), and regulation of gene \( Y \) by \( Y \) by \( J_{yy} \). These are collectively denoted by \( J_{ij} \). The value of \( J_{ij} \) can take any real value; thus, when \( J_{ij} \) is positive, transcription is promoted, and when \( J_{ij} \) is negative, it is inhibited.

When a protein binds to a promoter, gene transcription is promoted or inhibited. For simplicity, we assumed that a gene is transcribed when the sum of the effects of molecular regulation exceeds a threshold value. The activation function was given by the sigmoid function \( f[x] = \frac{1}{1 + \exp(-a \cdot x)} \). The degree by which gene \( m \) expression is regulated by protein \( N \) transcribed by gene \( n \) is reported by \( n \) as \( J_{mn} \), with the dynamics of the expression level and protein concentration of \( X \) and \( Y \) represented by:

\[
\dot{x} = f[J_{xx}(x - \xi) + J_{xy}(y - \xi)] - x \tag{1}
\]

\[
\dot{y} = f[J_{yx}(x - \xi) + J_{yy}(y - \xi)] - y \tag{2}
\]

- \( -x \) and \( -y \) on the right-hand side of the second term represent protein \( X \) and \( Y \) degradation, respectively. Here, the expression threshold \( \xi \) was set at 0.5, to make the expression and non-expression states symmetric for later fitness function simplicity. \( \beta \) of the sigmoid function was set to 100 to make the Hill function \( (f[x] = \frac{x^\beta}{1 + x^\beta}) \) sharp.
Figure 3. Nullcline normal vector. First, the nullclines were obtained from \( \dot{x} = \dot{y} = 0 \) in equations (1) and (2). The blue line represents the nullcline for \( x \) and orange that for \( y \). The intersection of these two null lines was a fixed point. The shape of the nullcline was characterized by normal vectors that indicate the directions of the nullclines around \((0.5, 0.5)\) and are denoted as \((J_{xx}, J_{xy})\) or \((J_{yx}, J_{yy})\). Furthermore, the vector field around the fixed point assisted in determining whether the fixed point was a stable fixed point (i.e. when the vector field around the fixed point was directed toward the fixed point from any direction) or an unstable fixed point (i.e. when the vector field around the fixed point is directed away from it). In this figure, \((0, 0)\), \((0.5, 0.5)\), and \((1, 1)\) are nullcline intersections that are fixed points, whereas only \((0, 0)\) and \((1, 1)\) are stable fixed points and \((0.5, 0.5)\) is an unstable fixed point.

2.2. Attractors of gene-expression dynamics
Considering the relationship between genotype and phenotype, we focused on where the expression levels \( x \) and \( y \) converged to a fixed point or cycle. We investigated how phenotype \((x, y)\) was determined depending on the genotype, \( J_{ij}(i, j = x, y) \).

To investigate the fixed point, we first obtained the nullclines of \( x \) and \( y \), respectively, which were curves that satisfied \( \dot{x} = 0 \) or \( \dot{y} = 0 \) in equation (2). The nullclines were represented by the equation:

\[
x = f \left[ J_{xx}(x - \xi) + J_{xy}(y - \xi) \right] \tag{3}
\]

\[
y = f \left[ J_{yx}(x - \xi) + J_{yy}(y - \xi) \right], \tag{4}
\]

which are illustrated in figure 3. Since \( \beta \gg 1 \), \( f \) approaches a step function, so that \( x = f \) is satisfied either at \( x \approx 1 \) or \( x \approx 0 \), as well as \( x \approx \xi = 0.5 \). In this paper, for simplicity, these \( x \approx 1 \) and \( x \approx 0 \) states are written as just \( x = 1 \) and \( x = 0 \) considering \( \beta \gg 1 \).

2.3. Classification of two-gene expression dynamics by attractors
For given gene regulatory networks, phenotypes determined by attractors in gene expression dynamics can be organized into seven classes (figure 4). The attractor types, their numbers, and their configurations are classified based on the value of \( J_{ij} \) as follows:

(a) Equal expression level of \( x, y \) (S, symmetric or synchronized)
\( x = 1, y = 1 \), or \( x = 0, y = 0 \) is the fixed-point attractor depending on the initial conditions. We referred to this as dynamics-type-S.

(b) Different expression level of \( x, y \) (A, antagonistic)
\( x = 1, y = 0 \) or \( x = 1, y = 0 \) is the fixed-point attractor depending on the initial conditions. We referred to this dynamic as type-D.
(c) Same or different expression level of $x, y$ (Q, quad)

All possible four cases with $x = 0$ or 1, $y = 0$ or 1 give the fixed-point attractor depending on the initial conditions and were referred to as dynamics-type-Q.

(d) Intermediate expression level of $x$ (Cx, continuous for $x$)

$x = \alpha, y = 0$ or $x = \alpha, y = 1$ is the fixed-point attractor, where $0 < \alpha < 1$, depending on the initial conditions and were defined as dynamics-type-Cx.

(e) Intermediate expression level of $x$ (Cy, intermediate for $y$)

$x = 0, y = \alpha$ or $x = 1, y = \alpha$ is the fixed-point attractor, where $0 < \alpha < 1$, depending on the initial conditions. We defined this a dynamics-type-Cy.

(f) Half expression level for $x, y$ (H, half)

$x = 0.5$ and $y = 0.5$ is the fixed-point attractors that were observed all initial conditions were defined as dynamics-type-H.

(g) Periodic expression $x, y$ (P, periodic)

When the limit cycle was obtained in all initial conditions, we referred to this as dynamics-type-P.

2.4. Introduction of $\Theta$

We were able to classify steady states that corresponded to phenotypes and introduced a vector that characterized the genotype. Originally, the genotype in the model was represented by a $J_{ij}$ in the $2 \times 2$ real matrix. However, the GRN that adopted a sigmoid function only uses two states, 0, 1, beside 0.5, which reduced the dimension of the genotype. This two-dimensional parameter represented the genotype’s declination angle, $\Theta$.

We then focused on the shape of the nullcline in figure 3. The shape was determined by the direction of the line segment that passed through point (0.5, 0.5), which determined the attractor. Therefore, the change in the dynamics were due to the change in $J_{ij}$ and could be specified in the direction of normal vectors of the $J_{ij}$ row vectors. When examining the direction of this line segment, one of the normal vectors of this line segment (figure 3) pointed in the same direction as the row vector of $J_{ij}$ that is, $(J_{xx}, J_{xy})$ or $(J_{yx}, J_{yy})$. Here, only the direction of the row vectors mattered because the magnitude of the vectors was related to the shape of the nullcline. Hence, the shape of the nullcline was described by the angles of the $J_{ij}$ (Theta) row vectors. In other words, as the angle between a line extended from the origin and the row vector, $(J_{xx}, J_{xy})$ or $(J_{yx}, J_{yy})$, we could take advantage of the symmetry between $x$ and $y$ to define the counterclockwise angle between the positive part of the $x$-axis and $(J_{xx}, J_{xy})$ as $\theta_x$, and the counterclockwise angle between the positive part of the $y$-axis and $(J_{yx}, J_{yy})$ as $\theta_y$. They were defined by

\[
\theta_x = \arg(J_{xx}, J_{xy})
\]

\[
\theta_y = \arg(J_{yx}, J_{yy}).
\]
Here, \( \text{arg}(x, y) \) is the function that returns the argument of the point \((x, y)\), as is defined by

\[
\text{arg}(x, y) = \begin{cases} 
\arctan \frac{y}{x} & \text{if } x > 0 \\
\arctan \frac{y}{x} + \pi & \text{if } x < 0 \text{ and } y \geq 0 \\
\arctan \frac{y}{x} - \pi & \text{if } x < 0 \text{ and } y < 0 \\
\frac{\pi}{2} & \text{if } x = 0 \text{ and } y > 0 \\
-\frac{\pi}{2} & \text{if } x = 0 \text{ and } y < 0 \\
\text{indeterminate} & \text{if } x = y = 0.
\end{cases}
\]  

(7)

The indeterminate case is not considered because we only consider the case where all the \( J_{ij} \) are non-zero.

The dynamics and attractors were determined by using \( \Theta = (\theta_x, \theta_y) \).

2.5. \( \Theta \) torus and attractor classification

Using \( \theta_x \) and \( \theta_y \), we could explicitly classify the attractors of the two-gene regulatory dynamics (figure 4). This section introduces the \( \theta \) torus, which had a finite range of \( \theta_x \) and \( \theta_y \) and specified each attractor type.

First, the behavior of the gene expression dynamics was determined by the direction of the \( J_{ij} \), \( \Theta \) row vector. Here, the phenotype was visualized by introducing the \( \Theta \) torus with \( \theta_x \) on the horizontal axis and \( \theta_y \) on the vertical axis, where \( \theta_x \) and \( \theta_y \) were cyclic with mod 2\( \pi \), as in figure 4. Therefore, \( \Theta \) torus represented the attractor types. Each of the types (S, D, Q, Cy, Cx, H, and P) was characterized by the nullclines shown in figure 4(A). Note that \( \Theta \) determined the directions in the nullcline near point (0.5, 0.5). The attractor determined by the two nullclines could change depending on the relative positions of \( \theta_x \) and \( \theta_y \). Then, S, D, Q, Cy, Cx, H, and P were classified depending on \((\theta_x, \theta_y)\) as presented in figure 4(B). For the derivation of the boundaries for each classification, see supplementary material S1. This diagram shows the relationship from \( \Theta \) to the attractors in \((x, y)\) and the one between genotype and phenotype.

2.6. Defining the fitness function

We obtained the fitness landscape for \( \Theta \) torus by first defining the fitness. Here, the phenotype was determined by the stationary expression levels of \( x \) and \( y \). Here, we consider the case that the fitness takes maximal either at each expression level 0 or 1 (i.e. the fitness depends monotonically on the combination of each expression level, so that the fitness takes maximum at 0 or 1). Hence we focused on the states with values of 0 or 1; therefore, the fitness was described by a combination of the four-state input of \((x, y) = (0, 0), (0, 1), (1, 0), (1, 1)\) and their two-state output. We assumed that binary fitness (fitted or non-fitted) was based on the binary phenotype. Hence, the fitness function was described by a Boolean (logical) function with two binary inputs and one binary output. In this case, \( 2^4 = 16 \) possible fitness degree functions existed, which were reduced to five fitness functions using the symmetry of the model and identifying logical operations. (See supplementary material S2).

As for the fitness function \( w(x, y) \), we introduced a continuous function to satisfy the Boolean function such that \( w(x, y) \) for \( x, y = (0, 1) \) was either 0 or 1, and determined that the simplest form for \( w(x, y) \) had intermediate values between 0 and 1. We characterized four typical fitness functions here. Other cases including NEUTRAL are described in supplementary material S3.

2.6.1. AND: requires expression of both genes

This function used a maximum value of 1 when both \( x \) and \( y \) were set to 1.

\[ w(x, y) = xy. \]  

(8)

This corresponded to the case where \( x \) and \( y \) expression was required for survival (e.g., the formation of complexes by the proteins X and Y).

2.6.2. X ONLY: requires only X, whereas Y expression is neutral

In this function, fitness referred only to \( x \) and thus, if \( x \) was 1, fitness returned a maximum value of 1.

\[ w(x, y) = x. \]  

(9)

This corresponded to the selection pressure affecting only the expression level of \( x \) (e.g., protein X is functionally dominant or protein Y had a neutral function). When the fitness depended only on the expression of \( y \), it presented similarly to that of \( w(x, y) = y \).
2.6.3. XOR: requires the expression of only X or Y
When \((x, y)\) was \((1, 0)\) or \((0, 1)\), the fitness had a maximum value of 1. By contrast, when \((x, y)\) was \((0, 0)\) or \((1, 1)\), the fitness had a minimum value of 0. As the simplest form,

\[
w(x, y) = |x - y|.
\]  

This function required that the expression of only one gene, \(x\) or \(y\), was necessary for survival, but fitness was lost if both genes were expressed (e.g., switching the two pathways by the inputs).

2.6.4. OR: requires the expression of either one of the two genes
If at least one of \(x, y\) is 1, then the fitness had a maximum value of 1.

\[
w(x, y) = 1 - (1 - x)(1 - y) = x + y - xy.
\]  

This corresponded to the case in which the expression of \(x\) or \(y\) was needed for survival (e.g., both X and Y proteins have similar functions).

2.7. Classification of the fitness landscape
We next explored the fitness landscape of genotype spaces that depended on the fitness function represented by \(\Theta\) torus. Here, for a given \(\Theta\), we obtained the attractor of \((x, y)\) for a fixed initial expression \((x_0, y_0) = (0.05, 0.5)\) \((x_0, y_0) = (0.18, 0.18)\) for the XOR. Depending on the fitness function, we defined (AND, X ONLY, XOR, etc) to obtain the fitness landscape for \(\Theta\), as shown in the diagram presented in figure 4(B), which contains information about the complex genotype–phenotype relationship.

We defined four typical landscapes (ONE RECTANGLE, TWO RECTANGLES, L-SHAPE, and ONE BAND in figure 5), as well as additional landscapes that are described in supplementary material S3.

2.7.1. ONE RECTANGLE (seen in AND)
Figure 5(i) presents an example of the ‘rectangle’ landscape, where the region of maximum fitness is in a single rectangle. This landscape is mainly observed in the AND fitness function. The high-fitness region occurs only in the parts of classes S and Q of the AND fitness because the optimal fitness was achieved for only \((x, y) = (1, 1)\). In the \(\Theta\) space for S in figure 5(i) the maximum fitness is determined by the initial expression \((x_0, y_0)\).

2.7.2. TWO RECTANGLES (seen in X ONLY)
This fitness landscape was composed of two rectangular high-fitness regions (figure 5(ii)) where \(x = 1\) and \(y\) values were \(y = 1\) for one region and \(y = 0\) for the other. The fitness maximum was X ONLY when a stable fixed point satisfied \(x = 1\). The dynamics that satisfy this condition were either S or D and the \(\Theta\) space for S and A in figure 5(i) reached the maximum fitness, which was determined by the initial expression \((x_0, y_0) = (0.05, 0.5)\). Q achieved maximum fitness, but all maximum fitness regions were connected.
2.7.3. L-SHAPE (seen in XOR or OR)

The region of maximum fitness was distributed in an L-shape, as shown in figure 5(iii) with the XOR fitness function. The shape of this landscape was an inverted L, which we collectively referred to as an L shape. This landscape was obtained when the fitness function was either an XOR or an OR. When using the XOR or OR fitness functions, the initial condition \((x_0, y_0)\) existed in \(0 \leq x_0 \leq 1\) and \(0 \leq y_0 \leq 1\), except for the unstable fixed-point singularity. We found that the region of L-SHAPE had the maximum fitness for all initial conditions tested. Therefore, the fitness was always maximum in the dynamics-type-A region because the stable fixed points of dynamics-type-A were only \((1, 0)\) and \((0, 1)\), respectively. The L-shaped area was robust against noise in the expression or initial conditions with XOR or OR fitness.

2.7.4. ONE BAND (seen in OR or X ONLY)

The maximum fitness region was extended to the entire range of \(\theta_x, \theta_y\) as a band for the OR fitness function, as shown in figure 5(iv). This band extended over the boundary of the \(\Theta\) torus in a single direction and therefore, the expression level of one \(x, y\) was neutral in this band. In OR, fitness was maximized where \(x = 1\) or \(y = 1\), which was when a stable fixed point was reached and the dynamic types S, D, Q, Cy, and Cx were satisfied. In particular, in dynamics-type-D, the fitness of the OR was always maximally independent of the initial condition because only the stable fixed points were \((1, 0)\) and \((0, 1)\), and there were no other steady states. In S, Q, Cy, and Cx, the maximum fitness regions were determined by the initial expression \((x_0, y_0)\). Depending on the initial expression, this type of landscape was also observed in the X ONLY fitness landscape.

3. Genotype distribution is restricted by evolution with sexual reproduction

Thus far, we have investigated the shape and features of the fitness landscape by comprehensively calculating the correlations between genotype, phenotype, and fitness in a two-gene network. By introducing \(\Theta\) as a genotype parameter, we were able to examine the phenotype and fitness for a given genotype and obtain the global structure of the fitness landscape. Once we have information on the global fitness landscape, we can predict the gene distribution and evolvability of the population during sexual reproduction and mutation. Thus, we investigated how the population distribution of the gene parameter \(\Theta\) changed through evolution, with or without sexual reproduction.

In particular, we focused on how the population distribution was restricted to a convex set of maximum fitness regions by evolution during sexual reproduction (i.e. convex regionalization). We introduced asexual and sexual reproduction into this model and numerically evolved the population distribution in these conditions for each typical fitness landscape.

3.1. Definition of mutation and recombination

Before discussing convex regionalization, we defined the procedure for genetic evolution. We considered two inheritance modes: asexual and sexual reproduction processes. In asexual reproduction, GRN \(J^{\text{new}}\) in the next generation was chosen from a set of \(J^{\text{fit}}\) with high fitness in the population. For mutations, the network adjacency matrix for the next generation, \(J^{\text{new}}\), was changed by adding a random value generated by a normal distribution with mean \(0\) and variance \(\sigma\). Depending on the initial expression, this type of landscape was also observed in the X ONLY fitness landscape.

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Figure 6. Transfer of genetic parameters \((\theta_x, \theta_y)\) to children through recombination. Sexual reproduction involves mixing the row vectors of the adjacency matrix. The vector of children from the parents \((\theta_{fit1}^x, \theta_{fit1}^y)\) and \((\theta_{fit2}^x, \theta_{fit2}^y)\) are randomly chosen from \(\theta_{fit1}^x\) or \(\theta_{fit2}^x\), and \(\theta_{fit1}^y\) or \(\theta_{fit2}^y\). Graphically, two vertices can fall to a child on another diagonal from the parent rectangular axis.

Figure 7. Comparison of the population distributions (blue dots) in two rectangular fitness landscapes with X ONLY \((w(x, y) = x)\) fitness (a) during asexual reproduction (mutation only) or during sexual reproduction, where the population is branched into two cases, (b) and (c), depending on each run of the evolution simulation. The initial expression was chosen as \((x_0, y_0) = (0.05, 0.5)\). The mutation rate was set to 0.01. The generation was 50 and the average fitness was over 0.98 in all these figures.

with mean 0 and variance \(\sigma\) were added to \(J_{\text{new}}\). Thus, sexual reproduction with a slight mutation could create a population of genotypes on the vertices of the rectangle.

3.2. Convex regionalization of sexually reproducing populations from a non-convex fitted area

For each fitness landscape category, we used simulations to examine changes in the population distribution due to sexual reproduction.

3.2.1. Simulation method

We ran simulations for 130 generations with a mutation size \(\sigma\) of 0.01 or 0.1, and a population size of 100. First, we set the initial expression to \((x_0, y_0)\). The initial expression \((x_0, y_0)\) was fixed and did not change throughout the subsequent simulations. Each element of \(J_{ij}\) in the 0th generation was given a uniform random number in the interval \([-1, +1]\). The dynamics of the GRN were calculated using equations (1) and (2) for 100 time steps. The average of each \(x, y\) in the last ten steps was used as the phenotype (expression). Fitness was calculated from the phenotype using the function defined in the previous section.

3.2.2. TWO RECTANGLES fitness landscape

The difference in the distribution between the asexually and sexually reproducing populations in the simulation is shown in figure 7. Here, the brighter (white) color represents higher fitness, whereas blue dots show the genotypes of individuals of the population that existed at the later generation. Two high fitness regions exist separately in TWO RECTANGLES fitness landscape. In the case of asexual reproduction
Figure 8. Comparison of the population distributions (blue dots) in two rectangular fitness landscapes with X ONLY \((w(x, y) = x)\) fitness during (a) asexual reproduction (mutation only) and (b) and (c) sexual reproduction. The population branched into two cases, (b) and (c) either to vertically or horizontally long rectangles, depending on each run of the evolution simulation. The initial expression was chosen as \((x_0, y_0) = (0.18, 0.18)\). The mutation rate was set at 0.1. The generation was 100 and the average fitness was over 0.98 in all these figures.

Figure 9. Partition of L-SHAPE fitness landscape. Regions I, II, and III support the highest fitness, while fitness is lost in region IV. Values with brackets like \((1, 0)\) shows the stable expression levels of \(x\) and \(y\).

(figure 7(a)), the population is distributed in the high fitness regions globally. In the case of sexual one (figures 7(b) and (c)), the population is distributed only in one of the two high fitness regions, depending on the evolutionary courses. These results can be explained as follows. The TWO RECTANGLES fitness landscape had two regions with equal maximum fitness values. If a population was distributed across both, the offspring from the parent of the two rectangles fall onto the non-fitted region and the sexually reproducing population concentrated in one of the two regions was selected. Thus, the population distribution was in a convex region and sustained. In contrast, during asexual reproduction, the population was distributed across the two regions. Therefore, evolution of sexual reproduction could induce speciation. Here, the offspring from the two regions were less fit, resulting in hybrid sterility. Following the definition of speciation by hybrid sterility, it can be concluded that speciation occurred in this case.

3.2.3. L-SHAPE fitness landscape
The difference in population distribution between asexual and sexual reproduction was also observed in the L-SHAPE fitness landscape, as shown in figure 8. During asexual reproduction, the genotype population (blue dots) was spread over the entire L-shaped area of the landscape of high fitness (figure 8(a)); however, through sexual reproduction, the population was biased in one direction of the L-SHAPE either within vertically or horizontally long rectangle region (figures 8(b) and (c)). Here, the L-SHAPE fitness landscape (figure 9) extended vertically and horizontally in two directions, but the offspring from the parent between these two directions (IV of figure 9) were less fit as a result of the genetic change in figure 6.

Hence, the offspring produced from sexual reproduction between the two branches of the L-SHAPE landscape (II and III in figure 9) shrank into a rectangular region, either vertically (I and III in figure 9) or horizontally (I and II in figure 9). Such convex regionalization of the population distribution did not occur during asexual reproduction. This convex regionalization was similar to speciation in the
Bateson–Dobzhansky–Muller model [40–44], which is supposed to be an L-shaped fitness landscape. However, we found that some individuals from the common square area (I in figure 9) of the two edges maintained high fitness and were not reproductively isolated, indicating that complete speciation did not occur. Only some of the two rectangular regions (II and III in figure 9) were not fit, as an offspring could be located in IV in figure 9. However, this convex regionalization was achieved so that the population in the L-SHAPE region was not allowed under sexual reproduction.

4. Discussion

This study analyzed the genotype–phenotype relationship across all two-gene regulatory networks and obtained the fitness landscapes according to possible Boolean fitness functions. Characteristic landscapes including TWO RECTANGLES with the two optimal regions, L-SHAPE and ONE BAND, were obtained. As a result of evolution during sexual reproduction, the population becomes restricted to the convex region, leading in speciation.

To characterize the genetic changes in the gene regulatory matrix, a pair of continuous parameters $\Theta = (\theta_x, \theta_y)$ was introduced and the relationship between genotype and phenotype was characterized to examine how these relationships are associated with fitness and genome distribution. Several studies have discussed how the genotype–phenotype–fitness relationship affects GRN dynamics [32, 35, 36]. However, these analyses were restricted to a specific level. For instance, genotype to phenotype in [36], while a complete analysis of GRN dynamics has not been performed. We conducted a comprehensive analysis of the genotype–phenotype–fitness–distribution relationship. Furthermore, our results improve our understanding of how sexual reproduction changes population distribution or leads to speciation-like events in the fitness landscape, which requires a global landscape structure.

This study demonstrated that sexual reproduction limits populations only in a restricted, convex set in the maximum fitness regions (convex regionalization). The population was restricted to horizontal or vertical rectangular regions in the L-SHAPE landscape. In the TWO RECTANGLES landscape, the population was limited to one of the two separate high-fitness regions, which led to speciation. In the L-SHAPE landscape, complete speciation was not achieved, but convex regionalization led to two distinct population distributions, as in speciation.

When discussing the effects of multiple gene interactions, epistasis is often adopted. Epistasis is defined as a nonlinear change in fitness with multiple mutations. When the fitness change is lower or higher than the addition of changes in multiple mutations, it is called negative or positive epistasis, respectively. Epistasis is applied to local fitness changes, which benefits the study of the effects of relatively small mutations. In contrast, the fitness landscape provides global information on the fitness. This study showed that such information is essential for studying changes in population distribution, robustness of sexual reproduction, and speciation.

The dynamics of gene regulation networks has been extensively studied over decades [45–50], since the pioneering study by Kauffman [1, 2], whereas their comprehensive analysis is still limited even for the two-node network. The method using $\Theta$ can describe all the possible network structures in the limited parameter region. Therefore, from the results of this study, the dynamical characteristics of the random two-node network and their relation to robustness [21] may be unveiled.

Here, we studied the GRN leading to fixed point attractors with on/off states basically by using a high $\beta$ value of the sigmoid function, and accordingly, the fitness is determined by the Boolean function. Of course, such a Boolean model has some limitations in investigating gene expression dynamics. For instance, the intermediate expression level between on/off values cannot be discussed. However, our result included an intermediate expression level (see figure 4) because the model we analyzed used the continuous dynamical system. By adopting the gradual activation function (by using lower $\beta$), one can study the intermediate expression cases seriously, which will be important in the future.

The method and results presented in this study can be used to solve other network related problems. First, the genotype parameter $\Theta$ may be used for evolution in other Boolean network systems such as machine learning and social or ecological networks [51–55]. It can also be applied to a system that interacts with a steep sigmoid function. Here, the threshold $\zeta = 0.5$ was used for simplicity and symmetry, but even if the threshold for each genes is changed, $\Theta$ can be used because the dynamics equivalent to this study were obtained by the transformation of variables, even though the classification of dynamics was more complex. In addition, $\Theta$ can be extended to systems with more than two ($N$) genes. In this case, the $\Theta$ space had $N(N - 1)$ dimensions, which made it more difficult to obtain a global fitness landscape. However, the fixed points and their stability can be evaluated according to the value of $\Theta$. The method of the present study can be applied by maintaining some $\Theta$ values within a certain range. For instance, the present results can be
extended to a system of multiple pairs of corresponding genes. In general, by introducing network modules (network motifs [56]), the application of this method is straightforward.

5. Conclusion

We analyzed how phenotype and fitness are shaped by the genotype in two-gene networks. A two-by-two matrix provided the two-gene regulatory network in which a vector with two angle values ($\Theta$) was introduced to characterize the genotype. Mapping from this angle vector to phenotypes allowed for the classification of steady-state expression patterns of genes into seven types. We then studied all possible fitness functions given by the Boolean output from the on/off expression of the two genes. The possible fitness landscapes were obtained as a function of the genetic parameters $\Theta$. Finally, the evolution of the population distribution under sexual reproduction was investigated in the obtained landscape. We found that the distribution was restricted to a convex region within the landscape, resulting in the branching of population distribution, including the speciation process. Our global analysis of GRNs based on $\Theta$ values and the characterized fitness landscape contributes to the comprehensive understanding of GRN evolution, particularly convex regionalization associated with sexual reproduction and resultant speciation.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Conflicts of interest

The authors declare that they have no conflicts of interest.

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