Symbiotic Sympatric Speciation: Compliance with Interaction-driven Phenotype Differentiation from a Single Genotype

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Abstract

A mechanism of sympatric speciation is presented based on the interaction-induced developmental plasticity of phenotypes. First, phenotypes of individuals with identical genotypes split into a few groups, according to instability in the developmental dynamics that are triggered with the competitive interaction among individuals. Then, through mutational change of genes, the phenotypic differences are fixed to genes, until the groups are completely separated in genes as well as phenotypes. It is also demonstrated that the proposed theory leads to hybrid sterility under sexual recombination, and thus speciation is completed in the sense of reproductive isolation. As a result of this post-mating isolation, the mating preference evolves later. When there are two alleles, the correlation between alleles is formed, to consolidate the speciation. When individuals are located in space, different species are later segregated spatially, implying that the speciation so far regarded to be allopatric may be a result of sympatric speciation. Relationships with previous theories, frequency-dependent selection, reinforcement, Baldwin's effect, phenotypic plasticity, and resource competition are briefly discussed. Relevance of the results to natural evolution are discussed, including punctuated equilibrium, incomplete penetrance in mutants, and the change in flexibility in genotype-phenotype correspondence. Finally, it is discussed how our theory is confirmed both in field and in laboratory (in an experiment with the use of E coli.).

1 Introduction

In spite of progress in the understanding in evolution ever since Darwin(1869), the speciation is not yet fully understood. In the recent book, Maynard-Smith and Szathmary(1995) wrote that *we are not aware of any explicit model demonstrating the instability of a sexual continuum*.

To discuss the problem of speciation, let us start from reviewing basic standpoints in evolution theory, although it might look too elementary here.

- (i) Existence of genotype and phenotype
- (ii) Fitness for reproduction is given as a function of the phenotype and the environment. The "environment" can include interaction with other individuals. In other words, the reproduction rate of an individual is a function of its phenotype, and environment, i.e., F (phenotype, environment).
- (iii) Only the genotype is transferred to the next generation (Weissman doctrine)
- (iv) There is flow only from genotype to phenotype (the central dogma of the molecular biology). For example, through the developmental process the phenotype is determined depending on the genotype. Now, the process is summarized as $Genotype \rightarrow Development \rightarrow Phenotype$.

Here we adopt these standard assumptions. (Although the assumption (iii) may not be valid for some cases known as epigenetic inheritance, we accept the assumption here, since the relevance of epigenetic inheritance to evolution is still controversial, and the theory to be proposed is valid in the presence of epigenetic inheritance, but does not require it.)

In the standard evolutionary genetics, the assumption (iv) is further replaced by a stronger one, i.e., (iv') "phenotype is a single valued function of genotype". If this were always true, we could replace F (phenotype, environment) in (i) by $F(f(\text{genotype}),$ environment) and then we could discuss the evolutionary process in terms of the population dynamics only of genotypes (and environment). This is the basic standpoint in population genetics.

Indeed, this reduction to genes is valid for gradual evolution. It is also supported by the following mathematical argument. The change of genotype is slower in time scale than that of phenotype. As is known, variables with slower time scale act as a control parameter to faster ones, if the time scale separation is large enough (and if the dynamics in the fast time scale do not have such instability that leads to bifurcation).

Still, explanation of the speciation, especially sympatric speciation, is not so easy following this standard evolutionary genetics. If slight genetic change leads to slight phenotype change, then individuals arising from mutation from the same genetic group differ only slightly according to this picture. Then, these individuals compete each other for the same niche. Unless the phenotype in concern is neutral, it is generally difficult that two (or more) groups coexist. Those with a higher fitness would survive. One possible way to get out of this difficulty is to assume that two groups are 'effectively' isolated, so that they do not compete. Some candidates for such isolation are searched. The most well-known example is spatial segregation, known as allopatric speciation. Since we are here interested in sympatric speciation, this solution cannot be adopted here¹ Furthermore, there are direct evidences that sympatric speciation really occurred in the evolution, for example in the speciation of cichlid in some lakes(Schiliewen et al. 1994).

As another candidate for separation mating preference is discussed (Maynard-Smith 1966, Felsenstein 1981, Grant 1981, Doebeli 1996, Howard and Barlocher eds. 1998). Recently, there have appeared some models showing the instability of sexual continuum, without assuming the existence of discrete groups in the beginning. Probably, the argument based on the runaway is most persuasive (Lande 1981, Turner and Burrows 1995,Howard and Barlocher eds. 1998). Even though two groups coexist at the same spatial location, they can be genetically separated if two groups do not mate each other. Hence, the mating preference is proposed as a mechanism for sympatric speciation. However, in this theory, why there is such mating preference itself is not answered. Accordingly, it is not self-contained as a theory.

Another recent proposal is the introduction of (almost) neutral fitness landscape and exclusion of individuals with similar phenotypes (Dieckmann and Doebeli, 1999, Kondrashov and Kondrashov,1999 Kawata and Yoshimura 2001). For example, Dieckmann and Doebeli[1999] have succeeded in showing that two groups are formed and coexist, to avoid the competition among

¹As will be discussed later, sympatric speciation can bring about allopatric one, but not the other way round.

organisms with similar phenotypes, assuming a rather flat fitness-landscape. This provides one explanation and can be relevant to some sympatric speciation. However, it is not so clear how the phenotype that is not so important as a fitness works strongly as a factor for exclusion for a closer value. Furthermore, we are more interested in the differentiation of phenotypes that are functionally different and not neutral.

So far, in these studies, the interaction between individuals lead to competition for their survival. Difficulty in stable sympatric speciation without mating preference lies in the lack of a known clear mechanism how two groups, which have just started to be separated, coexist in the presence of mutual interaction. Of course, if the two groups were in a symbiotic state, the coexistence could help the survival of each. However, the two groups have little difference in genotype in the beginning of speciation process, according to the assumption (iv)'. Then, it would be quite difficult to imagine such a 'symbiotic' mechanism.

Now, the problem we address here is as follows: If we do not assume (iv)' (but by assuming $(i)-(iv)$, is there any mechanism that two groups mutually require each other for the survival in the beginning of the separation of the two groups, In the present paper we propose such mechanism, and provide a sympatric speciation scenario robust against fluctuations.

Note that the above difficulty comes from the assumption that the phenotype is a single-valued function of genotype. Is this single-valued-ness always true? To address this question, we reconsider the G-P relationship. Indeed, there are three reasons that we doubt this single-valued-ness.

First, Yomo and his colleagues have reported that specific mutants of E. coli show (at least) two distinct types of enzyme activity, although they have identical genes(Ko et al., 1994). These different types coexist in an unstructured environment of a chemostat (Ko et al. 1994), and this coexistence is not due to spatial localization. Coexistence of each type is supported by each other. Indeed, when one type of E. coli is removed externally, the remained type starts differentiation again to recover the coexistence of the two types. The experiment demonstrates that the enzyme activity of these E. coli are differentiated into two (or more) groups, due to the interaction with each other, even though they have identical genes. Here spatial factor is not important, since this experiment is carried out in a well stirred chemostat.

Second, some organisms are known to show various phenotype from a single genotype. This phenomenon is often related to malfunctions of a

mutant (Holmes 1979), and is called as low or incomplete penetrance(Opitz 1981).

Third, a theoretical mechanism of phenotypic diversification has already been proposed as the isologous diversification for cell differentiation(Kaneko and Yomo, 1994,1997,1999; Furusawa and Kaneko 1998). The theory states that phenotypic diversity will arise from a single genotype and develop dynamically through intracellular complexity and intercellular connection. When organisms with plastic developmental dynamics interact with each other, the dynamics of each unit can be stabilized by forming distinct groups with differentiated states in the pheno-space. Here the two differentiated groups are necessary to stabilize each of the dynamics. Otherwise, the developmental process is unstable, and through the interaction the two types are formed again, when there is a sufficient number of units. This theoretical mechanism is demonstrated by several models and is shown to be a general consequence of coupled dynamical systems.

The isologous diversification theory shows that there can be developmental 'flexibility', in which different phenotypes arise from identical gene sets, as in the incomplete penetrance aforementioned. Now we have to study how this theory is relevant to evolution. Indeed, the question how developmental process and evolution are related has been addressed over decades (Maynard-Smith et al. 1985). We consider correspondence between genotype and phenotype seriously, by introducing a developmental process with which a given initial condition is lead to some phenotype according to a given genotype. 'Development' here means a dynamic process from an initial state to a matured state through rules associated with genes. (In this sense, it is not necessarily restricted to multicellular organisms.)

2 Model

To consider the evolution with developmental dynamics, it is appropriate to represent phenotype by a set of state variables. For example, each individual i has variables $(X_t^1(i), X_t^2(i), \dots, X_t^k(i))$, which defines the phenotype. This set of variables can be regarded as concentrations of chemicals, rates of metabolic processes, or some quantity corresponding to a higher function characterizing the behavior of the organism. The state is not fixed in time, but develops from the initial state at birth to a matured state when the organism is ready to produce its offspring. The dynamics of the state variables $(X_t^1(i), X_t^2(i),$ \cdots , $X_t^k(i)$ is given by a set of equations with some parameters.

Genes, since they are nothing but information expressed on DNA, could in principle be included in the set of variables. However, according to the central dogma of molecular biology (requisite (iv)), the gene has a special role among such variables. Genes can affect phenotypes, the set of variables, but the phenotypes cannot change the code of genes. During the life cycle, changes in genes are negligible compared with those of the phenotypic variables they control. In terms of dynamical systems, the set corresponding to genes can be represented by parameters $\{g^1(i), g^2(i), \cdots g^m(i)\}\$ that govern the dynamics of phenotypes, since the parameters in an equation are not changed through the developmental process, while the parameters control the dynamics of phenotypic variables. Accordingly, we represent the genotype by a set of parameters. Only when an individual organism is reproduced, this set of parameters changes slightly by mutation. For example, when $\{X_t^{\ell}(j)\}$ represents the concentrations of metabolic chemicals, $\{g^1(i), g^2(i), \cdots g^m(i)\}\$ is the catalytic activity of enzymes that controls the corresponding chemical reaction.

Now, our model is set up as follows:

(1) Dynamical change of states giving a phenotype:

The temporal evolution of the state variables $(X_t^1(i), X_t^2(i), \dots, X_t^k(i))$ is given by a set of deterministic equations, which are described by the state of the individual, and parameters $\{g^1(i), g^2(i), \cdots, g^m(i)\}$ (genotype), and the interaction with other individuals. This temporal evolution of the state consists of internal dynamics and interaction.

(1-1)The internal dynamics (say metabolic process in an organism) are represented by the equation governed only of $(X_t^1(i), X_t^2(i), \dots, X_t^k(i))$ (without dependence on $\{(X_t^{\ell}(j))\mid (j \neq i)\}\$, and are controlled by the parameter sets $\{g^1(i), g^2(i), \cdots g^m(i)\}$.

(1-2) Interaction between the individuals: The interaction is given through the set of variables $(X_t^1(i), X_t^2(i), \dots, X_t^k(i))$. For example, we consider such interaction form that the individuals interact with all others through competition for some 'resources'. The resources are taken by all the individuals, giving competition among all the individuals. Since we are interested in sympatric speciation, we take this extreme all-to-all interaction, by taking a well stirred soup of resources, without including any spatially localized interaction.

(2) Reproduction and Death: Each individual gives offspring (or splits into two) when a given 'maturity condition' for growth is satisfied. This condition is given by a set of variables $(X_t^1(i), X_t^2(i), \dots, X_t^k(i))$. For example, if $(X_t^1(i), X_t^2(i), \dots, X_t^k(i))$ represents cyclic process corresponds to a metabolic, genetic or other process that is required for reproduction, we assume that the unit replicates when the accumulated number of cyclic processes goes beyond some threshold.

(3) Mutation: When each organism reproduces, the set of parameters ${g^{j}(i)}$ changes slightly by mutation, by adding a random number with a small amplitude δ , corresponding to the mutation rate. The values of variables $(X_t^1(i), X_t^2(i), \dots, X_t^k(i))$ are not transferred but are reset to initial conditions. (If one wants to include some factor of epigenetic inheritance one could assumed that some of the values of state variables are transferred. Indeed we have carried out this simulation also, but the results to be discussed are not altered (or confirmed more strongly).

(4) Competition: To introduce competition for survival, death is included both by random removal of organisms at some rate as well as by a given death condition based on their state.

For a specific example, see Appendix.

3 Sympatric Speciation observed

From several simulations satisfying the condition of the model in §2, we have obtained a scenario for a sympatric speciation process(Kaneko and Yomo 2000,2002) The speciation process we observed is schematically shown in Fig.1, where the change of the correspondence between a phenotypic variable $({\rm "P"})$ and a genotypic parameter $({\rm "G"})$ is plotted at every reproduction event. This scenario is summarized as follows.

In the beginning, there is a single species, with one-to-one correspondence between phenotype and genotype. Here, there are little genetic and phenotypic diversity that are continuously distributed.(see Fig.1a). We assume that the isologous diversification starts due to developmental plasticity with interaction, when the number of these organisms increase. Indeed, the existence of such phenotypic differentiation is supported by isologous diversification,, and is supported by several numerical experiments. This gives the following stage-I.

Stage-I: Interaction-induced phenotypic differentiation

When there are many individuals interacting for finite resources, the phenotypic dynamics start to be differentiated even though the genotypes are identical or differ only slightly. Phenotypic variables split into two (or more) types (see Fig.1b). This interaction-induced differentiation is an outcome of the mechanism aforementioned. Slight phenotypic difference between individuals is amplified by the internal dynamics, while through the interaction between organisms, the difference of phenotypic dynamics tends to be clustered into two (or more) types. Here the two distinct phenotype groups (brought about by interaction) are called 'upper' and 'lower' groups, tentatively.

This differentiation is brought about, since the population consisting of individuals taking identical phenotypes is destabilized by the interaction. Such instability is for example, caused by the increase of population or decrease of resources, leading to strong competition. Of course, if the phenotype X_t^j $\frac{\jmath}{t}(i)$ at a matured state is rigidly determined by developmental dynamics, such differentiation does not occur. Only the assumption we make in the present theory is that there exists such developmental plasticity in the internal dynamics, when the interaction is strong. Recall again that this assumption is theoretically supported.

Note that the difference is fixed at this stage neither at the genetic nor phenotypic level. After reproduction, an individual's phenotype can switch to another type.

Stage-II: Amplification of the difference through genotype-phenotype relationship

At the second stage the difference between the two groups is amplified both at the genotypic and at the phenotypic level. This is realized by a kind of positive feedback process between the change of geno- and pheno-types.

First the genetic parameter(s) separate as a result of the phenotypic change. This occurs if the parameter dependence of the growth rate is different between the two phenotypes. Generally, there are one or several parameter(s) g^{ℓ} , such that the growth rate increases with g^{ℓ} for the upper group and decreases for the lower group (or the other way round) (see Fig.1c and Fig.2).

Certainly, such a parameter dependence is not exceptional. As a simple illustration, assume that the use of metabolic processes is different between the two phenotypic groups. If the upper group uses one metabolic cycle

more, then the mutational change of the parameter g^{ℓ} to enhance the cycle is in favor for the upper group, while the change to reduce it may be in favor for the lower group. Indeed all numerical results support the existence of such parameters. This dependence of growth rate on the genotypes leads to the genetic separation of the two groups, as long as there is competition for survival, to keep the population numbers limited.

The genetic separation is often accompanied by a second process, the amplification of the phenotypic difference by the genotypic difference. In the situation of Fig.1c, as a parameter G increases, a phenotype P (i.e., a characteristic quantity for the phenotype) tends to increase for the upper group, and to decrease (or to remain the same) for the lower group.

It should be noted that this second stage is always observed in our model simulation when the phenotypic differentiation at the first stage occurred. As a simple illustration, assume that the use of metabolic processes is different between the two groups. If the upper group uses one metabolic cycle more, then the mutational change of the parameter g^m (e.g., enzyme catalytic activity) to enhance the cycle is in favor for the upper group, while the change to reduce it may be in favor for the lower group. Indeed, all the numerical results carried out so far support that there always exist such parameters. This dependence of growth on genotypes leads to genetic separation of the two groups.

Stage-III Genetic fixation

After the separation of two groups has progressed, each phenotype (and genotype) starts to be preserved by the offspring, in contrast to the situation at the first stage. However, up to the second stage, the two groups with different phenotypes cannot exist in isolation by itself. When isolated, offspring with the phenotype of the other group starts to appear. The two groups coexist depending on each other (see Fig.1d).

Only at this third stage, each group starts to exist by its own. Even if one group of units is isolated, no offspring with the phenotype of the other group appears. Now the two groups exist on their own. Such a fixation of phenotypes is possible through the evolution of genotypes (parameters). In other words, the differentiation is fixed into the genes (parameters). Now each group exists as an independent 'species', separated both genetically and phenotypically. The initial phenotypic change introduced by interaction is fixed to genes, and the 'speciation process' is completed.

At the second stage, the separation is not fixed rigidly. Units selected from

one group at this earlier stage again start to show phenotypic differentiation, followed by genotypic separation, as demonstrated by several simulations. After some generations, one of the differentiated groups recovers the genoand phenotype that had existed before the transplant experiment. This is in strong contrast with the third stage.

4 Remarks on the speciation by interactioninduced phenotypic differentiation

4.1 Dynamic consolidation to genotypes

At the third stage, two groups with distinct genotypes and phenotypes are formed, each of which has one-to-one mapping from genotype to phenotype. This stage now is regarded as speciation (In the next section we will show that this separation satisfies hybrid sterility in sexual reproduction, and is appropriate to be called speciation). When we look at the present process only by observing initial population distribution (in Fig.1a) and the final population distribution (in Fig.1d), without information on the intermediate stages given by Fig. 1b) and 1c), one might think that the genes split into two groups by mutations and as a result, two phenotype groups are formed, since there is only a flow from genotype to phenotype. As we know the intermediate stages, however, we can conclude that this simple picture does not hold here. Here phenotype differentiation drives the genetic separation, in spite of the flow only from genotype to phenotype. Phenotype differentiation is consolidated to genotype, and then the offspring take the same phenotype as their ancestor.

4.2 Robust speciation

Note that the speciation process of ours occurs under strong interaction. At the second stage, these two groups form symbiotic relationship. As a result, the speciation is robust in the following sense. If one group is eliminated externally, or extinct accidentally at the first or second stage, the remaining group forms the other phenotype group again, and then the genetic differentiation is started again. The speciation process here is robust against perturbations.

4.3 Co-evolution of differentiated groups

Note that each of the two groups forms a niche for the other group's survival mutually, and each of the groups is specialized in this created niche. For example, some chemicals secreted out by one group are used as resources for the other, and vice versa.

Hence the evolution of two groups are mutually related. At the first and second stages of the evolution, the speed for reproduction is not so much different between the two groups. Indeed, at these stages, the reproduction of each group is strongly dependent on the other group, and the 'fitness' as a reproduction speed of each group by itself alone cannot be defined. At the stage-II, the reproduction of each group is balanced through the interaction, so that one group cannot dominate in the population (see Fig.2).

4.4 Phenotype differentiation is necessary and sufficient for the sympatric speciation in our theory

sufficient

If phenotypic differentiation at the stage 1 occurs in our model, then the genetic differentiation of the later stages always follows, in spite of the random mutation process included. How long it takes to reach the third stage can depend on the mutation rate, but the speciation process itself does not depend on the mutation rate. However small the mutation rate may be, the speciation (genetic fixation) always occurs.

Once the initial parameters of the model are chosen, it is already determined whether the interaction-induced phenotype differentiation will occur or not. If it occurs, then always the genetic differentiation follows.

necessary

On the other hand, in our setting, if the interaction-induced differentiation does not exist initially, there is no later genetic diversification process. If the initial parameters characterizing nonlinear internal dynamics or the coupling parameters characterizing interaction are small, no phenotypic differentiation occurs. Also, the larger the resource per individual is, the smaller the effective interaction is. Then, phenotypic differentiation does not occur. In these cases, even if we take a large mutation rate, there does not appear differentiation into distinct genetic groups, although the distribution of genes (parameters) is broader. Or, we have also made several simulations starting from a population of units with widely distributed parameters (i.e., genotypes). However, unless the phenotypic separation into distinct groups is formed, the genetic differentiation does not follow. (Fig.4a and b) Only if the phenotype differentiation occurs, the genetic differentiation follows(Fig.4c).

For some other models with many variables and parameters, the phenotypes are often distributed broadly, but continuously without making distinct groups. In this case again, there does not appear distinct genetic groups, through the mutations, although the genotypes are broadly distributed. (see Fig.5).

5 Post-mating Isolation

The speciation process is defined both by genetic differentiation and by reproductive isolation (Dobzhansky 1937). Although the evolution through the stages I-III leads to genetically isolated reproductive units, one might still say that it should not be called 'speciation' unless the process shows isolated reproductive groups under the sexual recombination. In fact, it is not trivial if the present process works with sexual recombination, since the genotypes from parents are mixed by each recombination. To check this problem, we have considered some models so that the sexual recombination occurs to mix genes. To be specific, the reproduction occurs when two individuals i_1 and i_2 satisfy the maturity condition, and then the two genotypes are mixed. As an example we have produced two offspring $j = j_1$ and j_2 , from the individuals i_1 and i_2 as

$$
g^{\ell}(j) = g^{\ell}(i_1)r_j^{\ell} + g^{\ell}(i_2)(1 - r_j^{\ell}) + \delta
$$
\n(1)

with a random number $0 < r_j^{\ell} < 1$ to mix the parents' genotypes (see also appendix §11.2).

In spite of this strong mixing of genotype parameters, the two distinct groups are again formed. Of course, the mating between the two groups can produce an individual with the parameters in the middle of the two groups. When parameters of an individual take intermediate values between those of the two groups, at whatever phenotypes it can take, the reproduction takes much longer time than those of the two groups. Before the reproduction condition is satisfied, the individual has a higher probability to be removed by death. As the separation process to the two groups further progresses, an individual with intermediate parameter values never reaches the condition for the reproduction before it dies.

This post-mating isolation process is demonstrated clearly by measuring the average offspring number of individuals over given parameter (genotype) ranges and over some time span. An example of this average offspring number is plotted in Fig.3, with the progress of the speciation process. As the two groups with distinct values of parameters are formed, the average offspring number of an individual having the parameter between those of the two groups starts to decrease. Soon the number goes to zero, implying that the hybrid between the two groups is sterile.

In this sense, sterility (or low reproduction) of the hybrid appears as a result, without any assumption on mating preference. Now genetic differentiation and reproductive isolation are satisfied. Hence it is proper to call the process through the stages I-III as speciation.

6 Evolution of mating preference

So far we have not assumed any preference in mating choice. Hence, a sterile hybrid continues to be born. Then it is natural to expect that some kind of mating preference evolves to reduce the probability to produce a sterile hybrid. Here we study how mating preference evolves as a result of postmating isolation.

As a simple example, it is straightforward to include loci for mating preference parameters. We assume another set of genetic parameters that controls the mating behavior. For example, each individual i has a set of mating threshold parameters $(\rho^1(i), \rho^2(i), \cdots \rho^k(i))$, corresponding to the phenotype $(X^1(i), X^2(i), \dots, X^k(i))$. If $\rho^{\ell}(i_1) > X^{\ell}(i_2)$ for some ℓ , the individual i_1 denies the mating with i_2 even if i_1 and i_2 satisfy the maturity condition. In simulation with a model with $\{\rho^m(i)\}\$, we choose a pair of individuals that salsify the maturity condition, and check if one does not deny the other. Only if neither denies the mating with the other, the mating occurs to produce offspring, when the genes from parents are mixed in the same way as as in the previous section. If these conditions are not satisfied, the individuals i_1 and i_2 wait for the next step to find a partner again (see also appendix §11.3).

Here the set of $\{\rho^m\}$ is regarded as a set of (genetic) parameters, and

changes by mutation and recombination. The mutation is given by addition of a random value to $\{\rho^m\}$. Initially all of $\{\rho^m\}$ (for $m = 1, \dots, k$) are smaller than the minimal value of $(X^1(i), X^2(i), \dots, X^k(i))$, so that any mating preference doe not exist. If some $\rho^{\ell}(i)$ gets larger than some of $X^{\ell}(i')$, there appears mating preference.

An example of numerical results is given in Fig.5, where the change of phenotype X^m and some of the parameters g^j , are plotted. Here, by the phenotype differentiation, one group (to be called 'up' group) has a large X^m value for some $m = \ell$ and almost null values for some other $m = \ell'$. Hence, sufficiently large positive $\rho^{\ell'}$ gives a candidate for mating preference.

Right after the formation of two genetically distinct groups that follows the phenotype separation, one of the mating threshold parameters $(\rho^1(i_1))$ starts to increase for one group. In the example of the figure, 'up' group has phenotype with (large X^1 , small X^2) and the other ('down') group with (small X¹, large X²). There the 'up' group starts to increase $\rho^1(i_{up})$, and $\rho^1(i_{up}) > X^1(i_{down})$ is satisfied for an individual i_{down} of the 'down' group. Now the mating between the two groups is no more allowed, and the mating occurs only within each group. The mating preference thus evolved prohibits inter-species mating producing sterile hybrid.

Note that the two groups do not simultaneously establish the mating preference. In some case, only one group has positive ρ^{ℓ} , which is enough for the establishment of mating preference, while in some other cases one group has positive ρ^1 , and the other has positive ρ^2 , where the mating preference is more rigidly established.

Although the evolution of mating preference here is a direct consequence of the post-mating isolation, it is interesting to note that the coexistence of the two species is further stabilized with the establishment of mating preference. Without this establishment, there are some cases that one of the species disappears due to the fluctuation after very long time in the simulation. With the establishment, the two species coexist much longer (at least within our time of numerical simulation).

7 Formation of allele-allele correlation

In diploid, there are two alleles, and two alleles do not equally contribute to the phenotype. For example, often only one allele contributes the control of the phenotype. If by recombination, the loci from two alleles are randomly mixed, then the correlation between genotype and phenotype achieved by the mechanism so far discussed might be destroyed. Indeed, this problem was pointed out by Felsenstein (1981) as one difficulty for sympatric speciation.

Of course, this problem is resolved, if genotypes from two alleles establish high correlation. To check if this correlation is generated, we have extended our model to have two alleles, and examined if the two alleles become correlated. Here, we adopted the model studied so far, and added two alleles further (see also appendix §11.4). In mating, the alleles from the parents are randomly shuffled for each locus. In other words, each organism i has two sets of parameters $\{g^{(+)\ell}(i)\}\$ and $\{g^{(-)\ell}(i)\}\$. Each $g^{(+)m}(i)$ is inherited from either $g^{(+)m}$ or $g^{(-)m}$ of one of the parents, and the other $g^{(-)m}(i)$ is inherited from either $g^{(+)}$ of $g^{(-)}$ of the other parents. Here parameters at only one of the alleles work as a control parameter for the developmental dynamics of phenotype.

We have carried out some simulations of this version of our model (Kaneko, unpublished). Here again, the speciation proceeds in the same way, through the stages I,II, and III. Hence our speciation scenario works well in the presence of alleles.

In this model, the genotype-phenotype correspondence achieved at the stage III, could be destroyed if there were no correlation between two alleles. Hence we have plotted the correlation between two alleles by showing two-dimensional pattern $(g^{(+)1}(i), g^{(-)1}(i))$ in Fig.7. Initially there was no correlation, but through temporal evolution, the correlation is established. In other words, the speciation in phenotype is consolidated to genes, and later is consolidated to the correlation between two alleles.

8 Allopatric speciation as a result of sympatric speciation

As already discussed, our speciation proceeds, starting from phenotypic differentiation, then to genetic differentiation, and then to post-mating isolation, and finally to pre-mating isolation (mating preference). This ordering might sound strange from commonly adopted viewpoint, but we have shown that this ordering is a natural and general consequence of a system with developmental dynamics with potential plasticity by interaction.

In a biological system, we often tend to assume **causal relationship** between two factors, from the observation of just correlation of the two factors. For example, when the resident area of two species, which share a common ancestor species, is spatially separated, we often guess that the spatial separation is a cause for the speciation. Indeed, allopatric speciation is often adopted for the explanation of speciation in nature.

However, in many cases, what we observed in field is just correlation between spatial separation and speciation. Which is the cause is not necessarily proved. Rather, spatial segregation can be a result of (sympatric) speciation².

By extending our theory so far, we can show that spatial separation of two species is resulted from the sympatric speciation discussed here. To study this problem, we have extended our model by allocating to each organism a resident position in a two-dimensional space. Each organism can move around the space randomly but slowly, while resources leading to the competitive interaction diffuse throughout space much faster. If the two organisms that satisfy the maturation condition meet in the space (i.e, they are located within a given distance), then they mate each other to produce offspring.

In this model, we have confirmed that the sympatric speciation first occurs through the stages I-III in §3. Later these two differentiated groups start to be spatially segregated, as shown in Fig.8. Now sympatric speciation is shown to be consolidated to spatial segregation (Kaneko, in preparation).

The spatial segregation here is observed when the range of interaction is larger than the typical range of mating. For example, if mobility of resources causing competitive interaction is larger than the mobility of organisms, spatial segregation of symptarically formed species is resulted.

Instead of spatially local mating process, one can assume slight gradient of environmental condition, for example, as gradient in resources. In this case again, sympatric speciation is expected to be later fixed to spatial separation.

To sum up, we have pointed out here the possibility that some of spe-

² Consider, for example, the segregation of resident area in city between rich and poor people. Most of us do not assume that people in 'rich area' are rich because they live there. Rather most think that the spatial separation is a result of differentiation in wealth, but not a cause. In the same way, it is sometimes dangerous to assume allopatric speciation even if the residence of two species are separated.

ciation that are considered to be allopatric can be a result of sympatric speciation of our mechanism. The sympatric speciation is later consolidated to spatial segregation of organisms.

9 Comparison with previous theories

Our theory reviewed here is related with several earlier theories, but is conceptually different. Here we will briefly discuss these points.

9.1 Frequency-dependent selection

Since our mechanism crucially depends on the interaction, one might think that it is a variant of frequency-dependent selection. The important difference here is that phenotype may not be uniquely determined by the genotype, even though the environment (including population of organisms) is given. In the frequency-dependent selection, genetically (and accordingly phenotypically) different groups interact with each other, and the fitness depends on the population of each group (Futsuyma, 1986). At the third stage of our theory, the condition for this frequency-dependent selection is satisfied, and the evolution progresses with the frequency-dependent selection. However, the important point in our theory lies in the earlier stages where a single genotype leads to different phenotypes. Indeed this intrinsic nature of differentiation is the reason why the speciation process here works at any (small) mutation rate and also under sexual recombination, without any other ad hoc assumptions.

9.2 Baldwin's effect

In our theory phenotype change is later consolidated to genotype. Indeed, genetic 'takeover' of phenotype change was also discussed as Baldwin's effect, where the displacement of phenotypic character is fixed to genes. In the discussion of Baldwin's effect, the phenotype character is given by epigenetic landscape (Waddington,1957). In our case, the phenotype differentiation is formed through developmental process to generate different characters due to the interaction. Distinct characters are stabilized each other through the interaction. With this interaction dependence, the two groups are necessary with each other, and robust speciation process is resulted. Hence, the fixation to genotype in our theory is related with Baldwin's effect, but the two are conceptually different.

9.3 Reinforcement

Since the separation of two groups with distinct phenotypes is supported by the interaction, the present speciation mechanism is possible without supposing any mating preference. In fact, the hybrid becomes inferior in the reproduction rate, and the mating preference based on the discrimination in phenotype is shown to evolve. Indeed, a mechanism to amplify the differentiation by mating preference was searched for as reinforcement since Dobzhansky[1951]. Our theory also gives a plausible basis for the evolution of mating preference without assuming ad-hoc reinforcement, or without any presumption on the inferiority in hybrid.

9.4 Phenotypic plasticity

Note that our phenotypic differentiation through development is different from the so called 'phenotypic plasticity', in which a single genotype produces alternative phenotypes in alternative environments(Callahan, Pigliucci and Schlichting 1987; Spitze and Sadler 1996; Weinig 2000). In contrast, in our case, distinct phenotypes from a single genotype are formed under the same environment. In fact, in our model, this phenotypic differentiation is necessary to show the later genetic differentiation. Without this differentiation, even if distinct phenotypes appear for different environments as in 'phenotypic plasticity', genetic differentiation does not follow. In spite of this difference, it is true that both are concerned with flexibility in phenotypes. Some of phenotypic plasticity so far studied may bring about developmental flexibility of ours, under a different environmental condition.

9.5 Resource competition

In our case, competitive interaction is relevant to speciation. Indeed, coexistence of two (or more) species after the completion of the speciation is discussed as the resource competition by Tilman[1976,1981]. Although his theory gives an explanation for the coexistence, the speciation process is not discussed, because two individuals with a slight genotypic difference can have only a slight difference there. In our theory, even if the genotypes of two individuals are the same or only slightly different, their phenotypes can be of quite different types. Accordingly, our theory provides a basis for resource competition also.

10 Relevance of our theory to biological evolution

General conclusion of our theory is that sympatric speciation can generally occur under strong interaction, if the condition for interaction-induced phenotype differentiation is satisfied. We briefly discuss relevance of our theory to biological evolution.

10.1 Tempo in the evolution

Since the present speciation is triggered by interaction, the process is not so much random as deterministic. Once the interaction among individuals brings about phenotypic diversification, speciation always proceeds directionally without waiting for a rare, specific mutation. The evolution in our scenario has a 'deterministic' nature and a fast tempo for speciation, which is different from a typical 'stochastic' view of mutation-driven evolution.

Some of the phenotypic explosions in the history of evolution have been recorded as having occurred within short geologic periods. Following these observations, punctuated equilibrium was proposed [Gould and Eldegridge 1977]. Our speciation scenario possibly gives an interpretation of this punctuated equilibrium. It may have followed the deterministic and fast way of interaction-induced speciation.

10.2 An answer why low penetrance is frequent in mutants

In the process of speciation, the potentiality of a single genotype to produce several phenotypes is consumed and may decline. After the phenotypic diversification of a single genotype, each genotype newly appears by mutation and takes one of the diversified phenotypes in the population. Thus, the one-to-many correspondence between the original genotype and phenotypes is consumed. Through the present process of speciation, the potentiality of single genotypes to produce various phenotypes decreases unless the new genotypes introduce another positive feedback process to amplify the small difference.

As a result, one may see single genotypes expressing only one (or a small number of) phenotypes in nature. Since most organisms at the present time have gone through several speciation processes, they may have reduced their potentiality to produce various phenotypes. According to our theory, if the organisms have a high potentiality, they will undergo a speciation process before long and the potentiality will decrease. In other words, natural organisms tend to lose the potentiality to produce various phenotypes in the course of evolution. As a reflection on the evolutionary decline of the potentiality, one can expect that mutant genotypes tend to have a higher potentiality than the wild-type genotype. As mentioned in $\S1$, the low or incomplete penetrance(Opitz 1981) is known to often occur in mutants, compared with higher penetrance in a wild type. Our result is consistent with these observation, since wild types are in most cases, a consequence of evolution, where the one-to-one correspondence is recovered, while the mutants can have higher potentiality to have a loose correspondence.

10.3 Relevance of developmental plasticity to speciation

Relationship between development and evolution has been discussed extensively. Our theory states the relevance of developmental plasticity to speciation. Taking our results and experimental facts into account, one can predict that organisms emerging as a new species have a high potentiality to produce a variety in phenotypes. It is interesting to discuss why insects, for example, have higher potentiality to speciation from this viewpoint. Also examining if living fossils, such as Latimeria chalumnae, Limulus and so forth, have a stable expression of a small number of phenotypes.

In our speciation theory, plasticity is declined through the evolution. Of course, there should be some occasions when the potentiality is regained, so that the evolution continues. For example, change of environment may

influence the developmental dynamics to regain loose correspondence, or introduction of novel degrees of freedom or genes may provide such looseness. Endosymbiosis can be one of such causes. Also, change of the interaction through spatial factor may introduce novel instability in dynamics, resulting in the loose correspondence.

10.4 Unified theory for speciation in sexual and asexual (and unicellular) organisms

One important point in our theory is that the speciation in asexual and sexual organisms are explained within the same theory. Of course, the standard definition of species using hybrid sterility is applied only for sexual organisms. However, it is true that the asexual organisms, or even bacteria, exhibit discrete geno- and pheno-types. It is suggested that 'species', i.e., discrete types with reproductive isolation, may exist in asexual organisms (Roberts and Cohan 1995, Holman 1987). There are also discussions that the potentiality of speciation in asexual organisms is not lower than the sexual organisms. In this sense, the present theory sheds a new light to the problem of speciation in asexual organisms as well.

10.5 Reversing the order

According to our theory, sympatric speciation under sexual reproduction starts first from phenotypic differentiation, and then genetic diversification takes place, leading to hybrid sterility, and finally the speciation is fixed by mating preference. This order may be different from studies most commonly adopted. Hence, our theory will be verified by confirming this chronic order in the field. One difficulty here, however, lies in that the process from phenotypic differentiation to the last stage is rather fast according to our theory. Still, it may be possible to find this order in the field, by first searching for phenotypic differentiation of organisms with identical genotype and under the identical environment. In this respect, the data of cichlid of Nicaraguan lake may be promising (Wilson, Noack-Kunnmann, and Meyer 2000), since phenotypic difference corresponding to different ecological niche is observed even though clear genetic difference is not observed yet.

10.6 Experimental verification

Discussion on the mechanism of evolution using past data, however, often remains anyone's guess. Most important in our scenario, in contrast, is its experimental verifiability, since the process of speciation is rather fast. For example, the evolution of E . coli is observed in the laboratory, as has been demonstrated by Kashiwagi et al.(1998, 2001) and W.-Z. Xu et al.(1996). As mentioned in $\S1$, phenotypic differentiation of E. coli is experimentally observed when their introduction is strong. Since the strength of interaction can be controlled by the resources and the population density, one can check whether or not the evolution in genetic level is accelerated through interaction-induced phenotypic diversification (Kashiwagi et al., 2001). Examination of the validity of our speciation scenario will give a first step to such study.

10.7 Summary: Dynamic Consolidation

To sum up, we have shown that developmental plasticity induced by interaction leads to phenotypic differentiation, which is consolidated to genes. Thus, distinct species with distinct geno- and pheno types are formed. This leads to hybrid sterility, and later mating preference evolves. Further later, this differentiation can be fixed to correlation in alleles or to spatial segregation. How the original differentiation in phenotypes can be understood as symmetry breaking from a homogeneous state, in the term of physics, while successive consolidation of the broken symmetry to different properties observed at later stages is more important for biological evolution. This dynamic process of consolidation is a key issue in development and evolution (see also (Newman, 2002)).

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11 Appendix: An example of our Model

11.1 A coupled map model

To be specific we consider the following model.

We study a simple abstract model of evolution with an internal dynamical process for development. In the model, each individual i has several (metabolic or other) cyclic processes, and the state of the j -th process at time *n* is given by $X_n^j(i)$. With *k* such processes, the state of an individual is given by the set $(X_n^1(i), X_n^2(i), \dots, X_n^k(i))$, which defines the phenotype. This set of variables can be regarded as concentrations of chemicals, rates of metabolic processes, or some quantity corresponding to a higher function. The state changes temporally according to a set of deterministic equations with some parameters. To be specific, our toy model consists of the following dynamics:

(1) Dynamics of the state: Here, we split $X_n^j(i)$ into its integer part $R_n^{\ell}(i)$ and the fractional part $x_n^{\ell}(i) = mod[X_n^{\ell}(i)]$. The integer part $R_n^j(i)$ is assumed to give the number of times the cyclic process has occurred since the individual's birth, while the fractional part $x_n^{\ell}(i)$ gives the phase of oscillation in the process. The dynamics of the variables $X_n^j(i)$ consist of a mutual influence of cyclic processes and interaction with other organisms. As a simple example, the former is assumed to be given by $\sum_{m} \frac{a^{\ell, m}}{2}$ $\frac{2\pi}{2}sin(2\pi x_n^m(i))$, while the latter is given by the competition for resources among the N_n organisms existing at the moment, given by $I^{\ell}(i) = p sin(2\pi x_n^{\ell}(i)) + \frac{s^{\ell} - \sum_j p sin 2\pi(x_n^{\ell}(j))}{N_n}$ $\frac{N_n}{N_n}$. (The second term comes from the constraint $\sum_i I^{\ell}(i) = s^{\ell}$, i.e., the condition that N individuals compete for a given resource s^{ℓ} at each time step. The first term represents the ability to secure the resource, depending on the state.) Our toy model is given by

$$
X_{n+1}^{\ell}(i) = X_n^{\ell}(i) + \sum_m \frac{a^{\ell m}(i)}{2} sin(2\pi x_n^m(i)) - \sum_m \frac{a^{m\ell}(i)}{2} sin(2\pi x_n^{\ell}(i)) + \frac{1}{2} sin(2\pi x_n^{\ell}(i)) + \frac{s^{\ell} - \sum_j p sin 2\pi(x_n^{\ell}(i))}{N_n}.
$$

(2) Growth and Death: Each individual splits into two when a given condition for growth is satisfied. Taking into account that the cyclic process corresponds to a metabolic, genetic or other process that is required for reproduction, we assume that the unit replicates when the accumulated number of cyclic processes goes beyond some threshold. Thus, the condition is given by $\sum_{\ell} X_n^{\ell}(i) \geq Thr$ (the maturity condition). The state $X_n^{\ell}(i)$ is

reset to a random value between 0 and 1, when the corresponding individual splits. To introduce competition, individuals are eliminated by a given death condition, as well as by random removal with a given rate. As for the former condition, an individual with $X_n^{\ell}(i) < -10$ (i.e., with a reverse process) is removed.

(3) Genetic parameter and mutation: Following the discussion in the text, genes are represented as parameters in the model, since the control parameters affect the dynamics of phenotypic variables, but no direct reverse process exists, as dictated by the central dogma of molecular biology. Here, genotypes are given by a set of parameters $a^{m\ell}(i)$, representing the relationship between the two cyclic processes ℓ and m $(1 \leq \ell, m \leq k)$. This set of parameters changes slightly through mutation when offspring is reproduced. With each division, the parameters $a^{m\ell}$ are changed to $a^{m\ell} + \delta$ with δ , a random number over $[-\epsilon, \epsilon]$, with small ϵ , corresponding to the mutation rate.

In the present model, due to the nonlinear nature of the dynamics, x_n^{ℓ} often oscillates in time chaotically or periodically. Hence it is natural to use $X^{\ell}(j)$ including its integer part, as a representation of the phenotype, since its integer part represents the number of cyclic process used for reproduction.

An alternative model using catalytic reactikon network

We have also carried out some simulations of a model with reaction network, where $X_t^m(i)$ represents the mth chemical concentration of an individual i. Each individual gets resources depending on its internal state. Through the above catalytic reaction process, some products are synthesized from the resources. When they are beyond a given threshold, they split to two, as given in the model for isologous diversification (Kaneko and Yomo, 1994,1997,1999, Furusawa and Kaneko, 1998). With the increase of the number of individuals, they compete for resources, while they are removed randomly to include competition. Since genes code the catalytic activity of enzymes, the rate of each reaction in the catalytic network is controlled by a gene. Hence, as a genetic parameter g^{ℓ} , the parameter for each reaction rate is adopted. Through the mutation to this reaction rate, the speciation process discussed throughout the paper is also observed (Takagi, Kaneko, Yomo 2000).

11.2 Sexual Reproduction

To include sexual recombination, we have extended our model so that organisms satisfying the threshold condition mate to reproduce two offspring.

When they mate, the offspring have parameter values that are intermediate of those of the parents. Here, the offspring $j = j_1$ and j_2 are produced from the individuals i_1 and i_2 as

$$
a^{\ell m}(j) = a^{\ell m}(i_1)r_j + a^{\ell m}(i_2)(1 - r_j) + \delta
$$
\n(2)

with a random number $0 < r_j^{\ell} < 1$ to mix the parents' genotypes.

11.3 Mating Preference

Here the set of $\{\rho^m\}$ is introduced as a set of (genetic) parameters, and changes by mutation and recombination. The mutation is given by addition of a random value over $[-\delta_{\rho}, \delta_{\rho}]$. Initially $\rho^m \leq 0$ (for $m = 1, \dots, k$ is set smaller than the minimal value of $(X^1(i), X^2(i), \dots, X^k(i))$, so that any mating preference does not exist. If $\rho^{m}(i)$ gets larger than some of $X^{m}(i')$ there appears mating preference.

11.4 Model with two alleles and random shuffling by mating

Here we assume that each individual has two sets of parameters $\{a^{\ell m}(j)\},\$ given by $a^{(+)\ell m}(j)$ and $a^{(-)\ell m}(j)$. In mating, the alleles from the parents are randomly shuffled for each locus $(\ell.m)$. Each $a^{(+)\ell m}(i)$ is inherited from either $a^{(+)\ell m(p_1)}$ or $a^{(-)\ell m(p_1)}$ of one of the parents p_1 , and the other $a^{(-)\ell m}(i)(p_2)$ is inherited from either $a^{(+)\ell m(p_2)}$ or $a^{(-)\ell m(p_2)}$ of the other parents p_2 . For the dynamics for X, only $\{a^{(+)\ell m}(i)\}\$ is used. The other parameter $a^{(-)\ell m}$ is not used, but can be used as $\{a^{(+)\ell m}(i_{offspring})\}$ of the offspring after the shuffling.

11.5 Spatial Model

To an individual i in the model of $\S11.2$ (with sexual reproduction), spatial position $(x_t(i), y_t(i))$ is assigned. The individual shows Brownian motion in the 2-dimensional space, by adding random number over $[-\delta_t, \delta_t]$ to $(x_t(i), y_t(i))$ per each step. They move within a square of a given suze with a periodic boundary condition. If two individuals i and j that satisfy the maturity condition $(\sum_m X_t^m(i) > Thr)$ are within a given distance d,

they can reproduce two offspring, which are located between $(x_t(i), y_t(i))$ and $(x_t(j), y_t(j))$.

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Figure 1: Schematic representation of the speciation scenario obtained from our simulation and theory. A pair (phenotype, genotype) is plotted successively with time: (a) the stage of interaction-induced phenotypic separation (b) the stage of genotype-phenotype feedback amplification and (c) the stage of genetic fixation. (Reproduced from (Kaneko and Yomo 2002)).

Figure 2: The evolution of the genotypic parameter. The parameter $q =$ $a^{12}(i)$ is plotted as a dot at every division (reproduction) event, with the abscissa as the division number. The average time necessary for division (reproduction) is plotted for the upper and lower groups, where the average is taken over 2000 division events (6th - 8th generation). As the two groups are formed around the 2000th division event, the population size becomes twice the initial, and each division time is also approximately doubled. Note that the two average division speeds of the two groups remain of the same order, even when the genetic parameter evolves in time. (Reproduced from (Kaneko and Yomo (2000)).

Figure 3: Schematic representation of the evolution starting from large genetic variance. (a) initial genotypic and phenotypic distribution. (b) without the phenotypic differentiation, no speciation follows. (c) if phenotypic differentiation occurs, then the speciation follows later (d).

Figure 4: Schematic representation of the evolution when phenotype is distributed without clear differentiation to discrete state. (a) initial distribution of phenotypes and genotypes. (b) after the evolution, genes are also distributed broadly, but no speciation follows.

Figure 5: The average offspring number before death is plotted as a function of the parameter (genotype), for simulations with sexual recombination. As an extension to include sexual recombination, we have also studied a model in which two organisms satisfying the above threshold condition mate to reproduce two offspring. When they mate, the offspring have parameter values that are randomly weighted average of those of the parents, as given in the text. We have measured the number of offspring for each individual during its lifespan. By taking a bin width 0.005 for the genotype parameter $g = a^{12}$, the average offspring number over a given time span is measured to give a histogram. The histogram over the first 7500 divisions (about 20 generations) is plotted by the solid line (I), and the histogram for later divisions is overlaid with a different line, as given by II (over 7500-15000 divisions), III (1.5-2.25 $\times 10^4$), IV(2.25-3 $\times 10^4$), and V(3.75-4.5 $\times 10^4$). As shown, a hybrid offspring will be sterile after some generations. Here we have used the model of Appendix $(\S11.1,2)$ and the initial condition as in Fig.1 and imposed recombination, with the parameters $p_k = 1.5/(2\pi)$ and $s^1 =$ $s^2 = s^3 = 2$. In the run, the population fluctuates around 340. (Reproduced from (Kaneko and Yomo 2000)).

Figure 6: An example of the speciation process with sexual recombination and the evolution of mating preference, with the model described in the Appendix (§11.1-3). Here two groups of distinct phenotype (large X^1 , small X^2) and (small X^1 , large X^2) are formed at the first few generation, which we call 'up' and 'down' groups. We have measured the average \overline{X} ^{*i*} at reproduction events, $a^{\ell m}$, ρ^j for each group per 500 divisions. (The population here is roughly 500, and thus the average is roughly over one generation). Change of the average R^j , $a^{\ell m}$, and ρ^j are plotted with divisions (generations). $\delta_\rho = 20$. (a) X^1 (up group ; solid line), X^1 (down group; broken line), (b) a^{12} (up group ;solid line), $\overline{a^{12}}$ (down group; broken³lne), (c) $\overline{\rho^1}$ (up group ;solid line), $\overline{\rho^1}$ (down group; broken line), ρ^2 (up group ; broken line), ρ^2 (down group; thin broken line), (Adapted from (Kaneko and Yomo (2002)).

Figure 7: Correlation in alleles. Using the model in the Appendix $(\S 11.1-4)$, the plot of $(a^{(+)12}(i), a^{(-)12}(i))$ is plotted at every reproduction event, with $p = 1.6$, $s_1 = s_2 = s_3 = 4$, $\delta_{\rho} = 20$. Initially genotype parameters a^{ij} is set at $-0.01/(2\pi)$. Two alternate groups with distinct phenotypes are plotted with alternate colors. (a) Plots at the division from 20000 th to 36000th. Here phenotypic differentiation already occurred but the genetic separation is not completed. (b) Plots at the division at a much later stage (from 50000 th to 63000 th) where genetic separation already occurred.

Figure 8: Spatial separation of two species, observed in the model of the Appendix (§11.5) . Model in spatially local mating. Each unit moves the position with the Brownian motion, given as the random number over $[-\delta_f, \delta_f] = [-0.0025, 0.0025]$, while units are located within 2-dimensional square of the size 5x5, with periodic boundary condition. Mating is possible if two units satisfying maturing condition are located within the distance 0.25. The parameters are set as $p = 1.6$, $s_1 = s_2 = s_3 = 2$, while initial genotype parameters a^{ij} is set at $-0.01/(2\pi)$. Position of the units at every division is plotted, at each division event from 45000 to 50000. Two alternate groups with distinct pheno- and geno- types are plotted with alternate colors.

