Evolutionary Ecology Research, 2002, **4**: 317–350

Symbiotic sympatric speciation through interaction-driven phenotype differentiation

Kunihiko Kaneko[1]* and Tetsuya Yomo[2]

[1]*Department of Pure and Applied Sciences, University of Tokyo, Komaba, Meguro-ku, Tokyo 153 and*
[2]*Department of Biotechnology, Faculty of Engineering, Osaka University, 2-1 Suita, Osaka 565, Japan*

ABSTRACT

A mechanism of genetic diversification and reproductive isolation is presented based on the interaction-induced diversification of phenotypes. First, phenotypes of individuals with identical genotypes split into a few groups, according to instability in the developmental dynamics associated with the interaction among individuals. Later, through competition for reproduction and mutational change of genes, the phenotypic differences are fixed to genes, until the groups ('species') are completely separated in terms of genes as well as phenotypes. In addition, we demonstrate that the proposed theory for speciation works also under sexual recombination and provides a basis for the evolution of mating preference. The relevance of the results to natural evolution are discussed, including incomplete penetrance in mutants and the change in flexibility in genotype–phenotype correspondence. Possible experiments are proposed to verify the theory presented.

Keywords: development, hybrid sterility, isologous diversification, mating preference, phenotypic plasticity, sympatric speciation.

INTRODUCTION

Darwin considered why organisms are separated into distinct groups, rather than their character being continuously distributed (Darwin, 1859). According to Maynard-Smith and Szathmary (1995), three hypotheses have been suggested (see also Coyne and Orr, 1998): (1) stable states of living matter, determined by laws of form, are restricted to discrete types; (2) ecological niches are discrete to which organisms are adapted; (3) sexual reproduction leads to a few discrete types. But none of these hypotheses is widely accepted. To date, there is little support for hypothesis 1 and hypothesis 2 is also far from satisfactory. Many physicochemical factors, such as temperature and height, are continuous. Of course, the most important niche is provided by other organisms, which may be separated into discrete groups. However, we cannot assume such discreteness here, since we are addressing why organisms constitute such discrete groups. The third hypothesis is most generally accepted. But several models that have succeeded in demonstrating sympatric speciation (Maynard-Smith, 1966; Felsenstein, 1981; Howard and Berlocher, 1998) have assumed discrete groups in the beginning, for example the genotypes of AA and $A'A'$.

* Author to whom all correspondence should be addressed. e-mail: kaneko@complex.c.u-tokyo.ac.jp

Recently, models have been presented that show the instability of a sexual continuum, without assuming the existence of discrete groups in the beginning. The argument based on the runaway is probably the most persuasive (Lande, 1981; Turner and Burrows, 1995). Proposed mechanisms include disruptive selection and assortative mating (Rosenzweig, 1978; Howard and Berlocher, 1998; Dieckmann and Doebeli, 1999; Kondrashov and Kondrashov, 1999; Kawata and Yoshimura, 2000). These recent studies have succeeded in showing how sympatric speciation can occur through mating preference.

Here, we propose another theory for sympatric speciation. It is based on interaction-induced developmental plasticity and does not require any mating preference in advance, and can even be applied in the genetic diversification of asexual organisms in the same way.[1]

The problem with stable sympatric speciation without mating preference is the lack of a clear mechanism for how two groups, which have just started to separate, co-exist in the presence of mutual interaction. Researchers often tend to search for some mechanisms of how two groups do not mix and survive independently, as is seen in sexual isolation by mating preference. For example, Dieckmann and Doebeli (1999) have shown that two groups are formed and co-exist to avoid the competition among organisms with similar phenotypes, assuming a rather flat fitness landscape. Here, existence of one group does not necessarily 'help' the survival of the other and vice versa.

Of course, if the two groups were in a symbiotic state, co-existence could aid the survival of each. However, since the two groups have very similar genotype at the beginning of the speciation process, it is hard to imagine such a 'symbiotic' mechanism. The problem we address here is as follows: Is there a mechanism for the co-existence of two groups necessary for the survival of each even at the beginning of their separation? In the present paper, we propose such a mechanism and provide a sympatric speciation scenario robust against fluctuations (for an example of sympatric speciation under strong interaction, see Schliewen *et al.*, 1994). The scenario shows the formation of discrete genotypic and phenotypic groups with reproductive isolation, evolving out of a genetic and phenotypic continuum.

Our mechanism is based on isologous diversification recently proposed by Kaneko and Yomo (1997, 1999). Here, two groups with distinct phenotypes appear even with the same genotypes. Based on this theory, through their interaction, the existence of each group mutually eliminates instability in the developmental process that appears when one of the groups is isolated. Hence, the existence of each group is required for the survival of the other, even though every individual has identical, or slightly different, genotypes.

To explain our motivation and the relevance of isologous diversification to evolution, we need to discuss the genotype–phenotype relationship. In the theoretical study of evolution, one generally assumes a mapping from genotype to phenotype. A selection process, fitness – that is, reproducibility of offspring – is applied to the phenotype. With the mutation to genotype, the corresponding phenotype changes and, depending on its fitness, the offspring of mutants change their population. Through this process, the distribution of genes changes. Here, it is generally assumed that the phenotype of an organism is uniquely determined, once its genotype and the environmental condition (including the distribution of other organisms) are known.

However, a phenotype is not necessarily determined uniquely by a genotype; this is known as low penetrance. Furthermore, the isologous diversification mechanism proposed

[1] Note that there are some suggestions that 'species' – that is, discrete types with reproductive isolation – may exist in asexual organisms (Holman, 1987; Roberts and Cohan, 1995).

both theoretically and experimentally also allows differentiation of phenotypes of organisms with identical genotypes. Let us discuss this differentiation of phenotypes from the same genotype in more detail.

First, one of the authors and his colleagues have reported that specific mutants of *Escherichia coli* show at least two distinct types of enzyme activity, although they have identical genes. These different types co-exist in an unstructured environment of a chemostat (Ko *et al.*, 1994), and this co-existence is not due to spatial localization. The co-existence of these types is supported by each other. Indeed, when one type of *E. coli* is removed externally, the remaining type starts to differentiate again to re-establish the co-existence of the two types. It has been demonstrated that the phenotype (enzyme activity) of this *E. coli* is differentiated into two or more groups, according to their interaction with each other in the well stirred chemostat, even though they have identical genes. In addition, even at a molecular level, a mutant gene of xylanase has been shown to produce various levels of enzyme activity (Ko *et al.*, 1994). A mechanism for a single gene to show various levels of molecular function has also been elucidated in physicochemical terms (Kobayashi *et al.*, 1997).

Indeed, some mutant genotypes related to malfunctions show various phenotypes, each of which appears at a low probability (Holmes, 1979). This phenomenon is known as low or incomplete penetrance (Opitz, 1981). Although organisms of low penetrance are a 'headache' in experimental genetics, they exist even in *C. elegans*. At a higher organism level, it is also interesting to note that some cichlids in a Nicaraguan lake show distinct phenotypes corresponding to different ecological niches, even though clear genetic differences have not been observed (Wilson *et al.*, 2000).

Second, a theoretical mechanism for phenotypic diversification has already been proposed as the isologous diversification of 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 units (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 the dynamics. Otherwise, the developmental process will be unstable and, through their interaction, the two types will be 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 question of how the developmental process and evolution are related remains important (Maynard-Smith *et al.*, 1985; Gilbert *et al.*, 1996). The above isologous diversification shows that there can be developmental 'flexibility', in which different phenotypes arise from identical gene sets, as in incomplete penetrance. Note that this flexibility is not the same as 'phenotypic plasticity', in which a single genotype produces alternative phenotypes in alternative environments (Spitze and Sadler, 1996; Callahan *et al.*, 1997; Weinig, 2000). In contrast, in our case, distinct phenotypes are formed in the same environment. Although some of the phenotypic plasticity studied to date may be related to developmental flexibility, we do not use the term 'phenotypic plasticity' here to avoid confusion.

Following the above argument, it is interesting to study the evolutionary process when phenotypes of organisms with identical genes can be different through the developmental process. In this paper, we extend the isologous diversification theory to include genetic evolution by introducing mutation and competition for survival. By studying a simple

model numerically and theoretically, we will show that genetic diversification always occurs whenever interaction-induced phenotypic differentiation occurs. Furthermore, this differentiation is shown to satisfy reproductive isolation and is regarded as speciation. Although the change from phenotypic to genotypic diversity might be viewed as being in the wrong direction, we demonstrate that the evolutionary process in this direction is consistent with the central dogma of molecular biology and natural selection. The extended theory proves that prior diversification in phenotype is sufficient and necessary to establish genetic diversity in a population.

Our theory of genetic diversification from interaction-induced phenotypic differentiation has several consequences for natural evolution. As we show, this genetic separation is maintained by sexual reproduction. Reproductive isolation of genetically distinct groups is confirmed. We also show numerically that the mating preference to stabilize the speciation evolves later. Hence our theory provides a plausible scenario for sympatric speciation. A novel view for the speciation is also provided, including the rather deterministic and fast nature of its process, as well as the origin of mating preference. Furthermore, we explain why mutants show incomplete penetrance more often than wild types. Inhomogeneity in the tempo of evolution is discussed as the change in flexibility in genotype–phenotype correspondence. Finally, we propose a possible experiment to verify our theory.

The paper is organized as follows. In the next section, we introduce a simple toy model that captures the essence of interaction-induced phenotypic diversification, mutation and selection. We then address the essence of the evolutionary process before providing detailed numerical results. Next, we address the robustness of genetic separation under sexual recombination before looking at the evolution of mating preference. Finally, we discuss the relevance of the results to the speciation process (see Kaneko and Yomo, 2000, for a brief report of the present theory).

MODEL

Basic strategy

Since it is necessary to study the correspondence between genotype and phenotype, we need to introduce a developmental process that results in a given initial condition for 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 its general sense. (In this sense, it is not necessarily restricted to multicellular organisms.) To consider this process, we assume that the state of each individual organism is characterized by a set of variables and parameters that govern the dynamics of the variables. For example, we adopt a model where the state of each organism changes dynamically according to some equation (such as a set of discrete-time maps or differential equations).

Here, the phenotype concerned is represented by a set of variables. It can change dynamically with time but stays within some range, which may differ among individuals. Let $(X_t^1(i), X_t^2(i), \ldots, X_t^k(i))$ represent this phenotypic state. This set of variables can be viewed as concentrations of chemicals, rates of metabolic processes or some quantifiers corresponding to a higher function. Although our model study may be most straightforwardly applied to unicellular organisms where the variables refer to the chemical state of a cell, there is no reason in principle not to apply the present theory to multicellular organisms, by using variables that correspond to some states of the organism.

Since genes are nothing but information expressed on DNA, they could in principle be included in the set of variables. However, according to the central dogma of molecular biology (Alberts *et al.*, 1994), 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. The variables corresponding to genes change much more slowly than other variables for biochemicals. In terms of dynamical systems, the set corresponding to genes is represented by parameters that govern the dynamics of phenotypes, since the parameters in an equation are not changed while they control the dynamics of phenotypic variables.

Hence the genotype is given here by a set of parameters. When an individual organism is reproduced, this set of parameters changes slightly by mutation.[2]

Specific model

Based on the above argument and previous studies of interaction-induced differentiation, we choose the following dynamical systems model.

(i) The state of each individual i at time t is given by variables $(X_t^1(i), X_t^2(i), \ldots, X_t^k(i))$ and a set of parameters.

(ii) The temporal change in the variables is given by a set of deterministic equations. They are described by the variables and parameters of the individual and the interaction with other individuals.

(iii) The interaction between the individuals i and j is given in terms of both sets of variables $(X_t^1(i), X_t^2(i), \ldots, X_t^k(i))$ and $(X_t^1(j), X_t^2(j), \ldots, X_t^k(j))$. Here, we choose a very simple form of interaction so that the dynamics of $X_t^l(i)$ are influenced by $X_t^l(j)$ of all the other individuals. A typical example is the interaction through competition for some (nutritional) 'resources' for the change of variable $X_t^l(i)$. We consider global interaction with all other individuals of equal strength. For example, the resources are taken by all individuals and this competition for resources leads to an 'all-to-all' interaction. We do not include any spatially localized interaction, since we focus on sympatric speciation.

(iv) Each individual splits into two when a given condition for growth is satisfied. In the present paper, we introduce some cycles (such as those within metabolic processes) as phenotypic variables. When the accumulated number of the processes goes beyond some threshold, the individual replicates. To introduce competition, individuals are eliminated randomly at some rate and by some death condition (e.g. the elimination of those with a poor ability to convert resources to metabolic processes). With this process, the total number of organisms remains finite and fluctuates within some range.

Note that reproduction occurs through the history of phenotypic variables. In our model, each individual competes for some limited resources and converts them to some factor for growth. If the factor is accumulated beyond some threshold, the individual produces

[2] In reproduction, phenotype variables may also be partly transferred to the offspring. This transfer is subject to a larger error than the mutational variation of parameters. Note, however, our theory of speciation is valid without such transfer, as is shown in the Appendix.

its offspring. The process depends directly on the phenotypic variables, but also indirectly on the genotypic parameters, since the dynamics of phenotypes are, of course, governed by the parameters. For example, the metabolic process may work more efficiently for some parameters and the reproduction may occur much faster. In addition, this change in growth rate with parameters is strongly dependent on the interaction, since the change in resources is strongly influenced by the phenotypes of other individuals.

We have studied several examples with the above requirements (i)–(iv). A simple example is given by a model with the following internal cyclic processes. Assume that there are k cyclic processes. Then, the above set-up of the models (i)–(iv) is given as follows.

First take $X^j_n(i)$ as a state vector of each cell i at time n, which represents the state of the j-th cycle at that instant. Here, we split $X^j_n(i)$ into its integer and fractional parts as

$$X^l_n(i) = R^l_n(i) + x^l_n(i) \tag{1}$$

with $R^l_n(i)$ = the integer part of $[X^l_n(i)]$ and $x^l_n(i) = \mathrm{mod}[X^l_n(i)]$.

The integer part $R^l_n(i)$ is assumed to give the number of the cyclic processes that the individual has passed through since its birth, while the fractional part $x^l_n(i)$ represents the phase of oscillation in the process. As a simple model, we assign a phase of oscillation to each cyclic process and assume that there are mutual influences depending on the phase state of processes (Kaneko, 1998). The l-th process has a flow from other processes, while there is a flow from the process to the other processes. With this set-up and with a choice of some function $F^{l,m}(x)$, the internal dynamics of processes are written as

$$X^l_{n+1}(i) = {}^l_n(i) + \sum_m F^{l,m}(x^m_n(i)) - \sum_m F^{m,l}(x^l_n(i)) + \mathrm{interaction}^l(i) \tag{2}$$

where i denotes each individual unit, l gives the index for the cycle and n is the time step for the process (the interaction term will be determined later). For simplicity in the simulation, we use the discrete-time process. The function $F^{l,m}$ shows a flow from the m-th to l-th process. Considering that only the 'phase' of the cyclic process (i.e. the fractional part $x^l_n(i)$) is relevant to the cycle–cycle interaction, we choose the form $(a^{lm}/2)\sin(2\pi x^m_n(i))$ for $F^{l,m}(x)$. Although only the fractional part is relevant to the dynamics, the integer part $R^j_n(i)$ will be used for the condition for replication.

Second, the interaction between individuals is introduced through competition for resources, with which each cyclic process progresses. The ability to obtain resources generally depends on the internal state of the unit $x^j_n(i)$. Again, we choose our model so that only the phase is relevant to the interaction and take $p \times \sin 2\pi(x^l_n(j))$ as the ability to obtain resources, with p as a fixed parameter. Assuming that all elements (whose number is N_n) compete for resources s^j for each step, we take the following interaction term:

$$\mathrm{interaction}^l(i) = p \times \sin(2\pi x^l_n(i)) + \frac{s^l - p\Sigma_j \sin 2\pi(x^l_n(j))}{N_n} \tag{3}$$

Here, the second term comes from the constraint that $\Sigma_i\, \mathrm{interaction}^l(i) = s^l$, due to the condition that units compete for a given resource s^l at each time step.

Now, summing up all the processes, our internal dynamics are given by

$$X^l_{n+1}(i) = X^l_n(i) + \Sigma_m \frac{a^{lm}(i)}{2} \sin(2\pi x^m_n(i)) - \Sigma_m \frac{a^{ml}(i)}{2} \sin(2\pi x^l_n(i)) + p \times \sin(2\pi x^l{}_n(i)) + \frac{s^l - p\Sigma_j \sin 2\pi(x^l_n(j))}{N_n} \quad (4)$$

Taking into account that the cyclic process corresponds to a metabolic, genetic or other process that is required for the replication, we assume that the unit replicates when the accumulated number of cyclic processes passes some threshold. Thus, the condition is given by

$$\sum_l R^l_n(i) > Thr \quad (5)$$

With division, the parameters (that correspond to genotypes) are transferred with some mutation. In the model, a^{ij} is changed to $a^{ij} + \delta$, with δ as a small random number over $[-\varepsilon, \varepsilon]$. On the other hand, the phenotype state $x^l(i)$ is either not transferred at all or loosely transferred. In the former case, $x^l(i)$ is reset again randomly over [0,1] after each division, while in the latter case $x^l_n(i)$ at the division is taken to be $x^l_{n-1}(i) + \delta'$, with δ' as a random number over $[-\varepsilon_p, \varepsilon_p]$ with $\varepsilon_p > \varepsilon$.[3] Of course, the rotation number $R^l_n(i)$ is reset to zero at each division.

With only the above division process, the number of individuals would increase indefinitely. To include competition, we remove units stochastically with some rate, so that the total population remains around N_{tot}. In the simulations here, we have also imposed a death condition when $R^l_n(i) < -Thr_{\text{death}}$, with $Thr_{\text{death}} = 10$.[4]

In the present model, due to the non-linear nature in the dynamics, x^l_n often includes chaotic or periodic oscillations in time. Then, it is not convenient to use the variables x^l_n for the representation of a phenotype. Instead, it is natural to adopt a quantity that is accumulated (or averaged) since the most recent division of the unit. $R^l(j)$, the integer part of $X^l(j)$, is ideal for this purpose. Here, we represent the phenotype of a unit as a set of variables $R^l(j)$, at each division (therefore, $\Sigma^k_l R^l(j) = Thr$). The value $R^l(j)$ indicates how much the l-th cyclic process is used for reproduction.

Within this setting of the problem, we address the following question: How do the offspring from an identical individual diversify its phenotypes and genotypes and split into two (or more) groups with distinct genotypes and phenotypes?[5] A standard answer for the mechanism of this diversification is that some random changes of genotypes result in different phenotypes and, through the accumulation of such changes, the phenotypes split into distinct groups, each of which is adapted for a different niche. In contrast, our proposal here is that the interaction-induced phenotypic differentiation first occurs for organisms with a single genotype and the differentiation is later fixed to genes, even though only a flow from gene to phenotype exists. In other words, interaction-induced phenotypic diversification is essential to genetic differentiation. We show later that this

[3] In fact, the scenario to be presented holds for both cases.

[4] Even without this additional death condition, the evolution scenario to be presented works.

[5] Inclusion of sexual recombination in our model will be discussed later, when we show that the mechanism presented here holds in such a case.

differentiation also satisfies reproductive isolation. Hence, a theory for sympatric speciation is provided.

PROCESS FOR GENETIC DIVERSIFICATION

We carried out several simulations of the model with $k = 3$, 4 and 5. An example of the speciation process is given in Figs 1 and 2. In Fig. 1, values of $a^{12}(i)$ and $R^2(i)$ are plotted at every division, while (R^2, a^{12}) are plotted as a representation of the genotype–phenotype relationship. Note that the phenotype separates into two groups initially. Indeed, this differentiation of phenotype is induced by interaction, for example through competition for resources.

On the other hand, the plot (R^2, a^{12}) results in a two-dimensional ('phenotype', 'genotype') plane in Fig. 2. In the figure, the colour of the points changes with time. The plot shows that the phenotype differentiates initially and the change is subsequently fixed to the genotypes. Several simulations demonstrate that the phenotypes (rates of use of each cyclic process) differentiate into two groups first (see Fig. 2).[6] Then, the parameter values ('genotype') split into two groups, as shown in Fig. 1. Once started, the splitting process progresses rapidly.

First, we sketch our interaction-induced process of genetic diversification triggered by isologous diversification, obtained from our model simulation and dynamical systems theory. The process of genetic diversification is shown schematically in Fig. 3, where we have plotted the phenotype on the vertical axis; that is, one $R^l(i)$ or some quantity given as a function of $x^l(i)$. The horizontal axis represents a genotype $a^{lm}(i)$, one of the parameters. This plot corresponds to Fig. 2, obtained from the simulation.

The process of genetic diversification is summarized as follows.

Stage 1: 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. This differentiation often appears if non-linearity is involved in the internal dynamics of some phenotypic variables. Then, slight phenotypic differences between individuals are amplified by the internal dynamics (e.g. metabolic reaction dynamics). Through interaction between organisms, the difference in phenotypic dynamics tends to be grouped into two (or more) types, as was first studied in clustering in coupled non-linear dynamics (Kaneko, 1990, 1994) and then discussed in connection with cell differentiation mechanisms (Kaneko and Yomo, 1994, 1997, 1999). Indeed, the orbits of ($x_t^1(i)$, $x_t^2(i)$, . . ., $x_t^k(i)$) lie in a distinct region according to individuals. Note that the difference at this stage is not fixed in either the genotype or the phenotype. The progeny of a reproducing individual may belong to a different type from the parent. In addition, the differentiation is highly interaction-dependent. For example, if a group of one type is removed, then some individuals of the other type change their type to compensate for the missing type.

With this interaction-induced differentiation, the phenotypes $R^l(i)$ split into two groups (Fig. 3a), which will here be called 'upper' and 'lower' groups.

[6] Although one R^i starts much larger than the others, this does not mean that each group is specialized to the 'niche' of a different resource s^i. Indeed, even if $s^2 = s^3 = \ldots = 0$, the present scenario is observed.

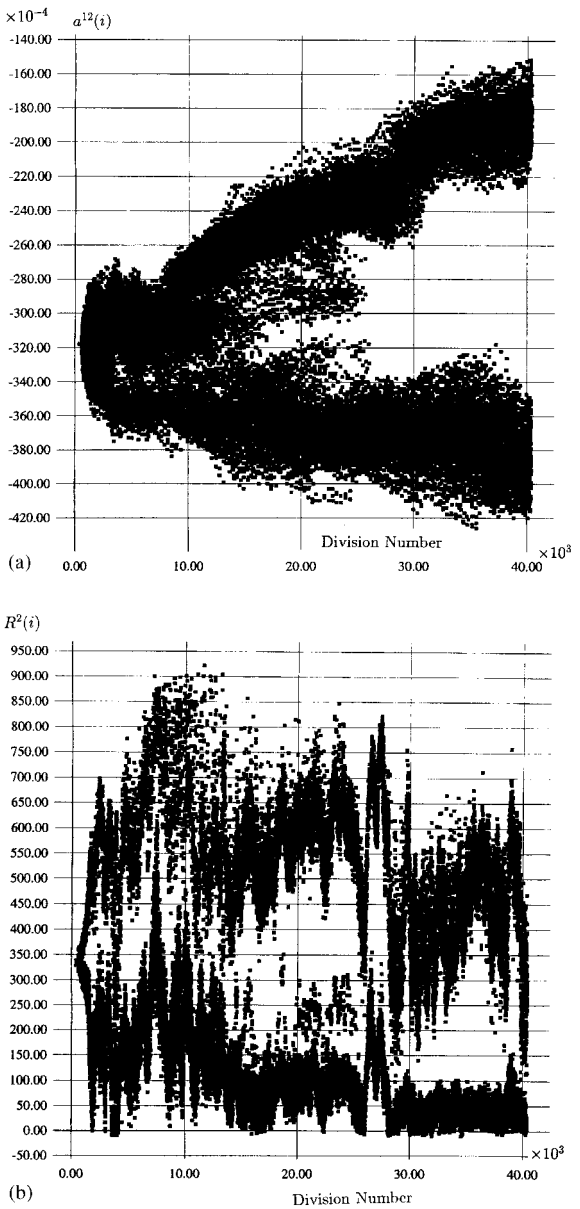


Fig. 1. Evolution of genotype (a) and phenotype (b). The genotypic parameter a^{12} (a) and the phenotype R^2 (b) are plotted at every division (reproduction) event. The ordinate is the parameter a^{12} (a) or R^2 (b) of the individual at each division, while the abscissa shows the number of divisions in the simulation in order. Throughout the paper, the threshold number of cycle processes, *Thr*, is set at 1000 and the mutation rates of parameters are set at 0.001. In the simulation of Fig. 1 and Fig. 2, $p_k = 2/(2\pi)$, $s^1 = s^2 = s^3 = 6$. Initially, the genotype parameters are set at $a^{ij} = -0.2/(2\pi)$, while the variables $x^k(i)$ are assigned randomly over [0,1]. By each division, the variables $x_n^l(i)$ are changed by δ' from those of the mother, where δ' is a random number over [–0.1,0.1] (i.e. $\varepsilon_p = 0.1$). Individuals are eliminated randomly so that the total population is around 500.

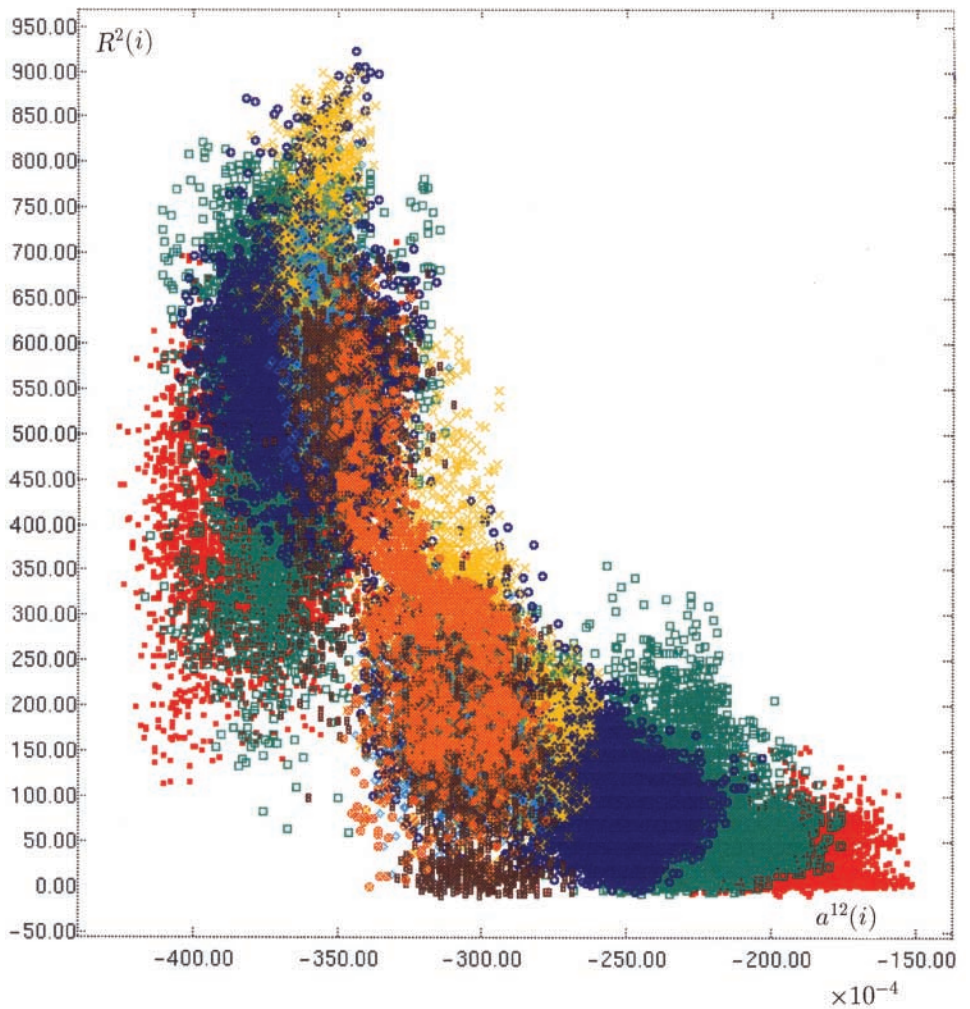


Fig. 2. Evolution of genotype–phenotype relationship: (R^2, a^{12}) plotted for every division of units, for the simulation given in Fig. 1. The first 2000 divisions are plotted in orange, divisions 2001–4000 in brown, 4001–6000 in sky blue, 6001–10,000 in yellow, 10,001–20,000 in dark blue, 20,001–30,000 in green and 30,001–40,000 in red.

Stage 2: amplification of the difference through the genotype–phenotype relationship

The second stage of our speciation is the amplification of difference in both genotypes and phenotypes. This is realized by a kind of positive feedback process between the changes in genotypes and phenotypes.

This process consists of two parts. The first part, essential to the genetic fixation, is genetic separation due to the phenotypic change. This occurs if the parameter dependence of the growth rate is different between the two phenotypes. In other words, there are one

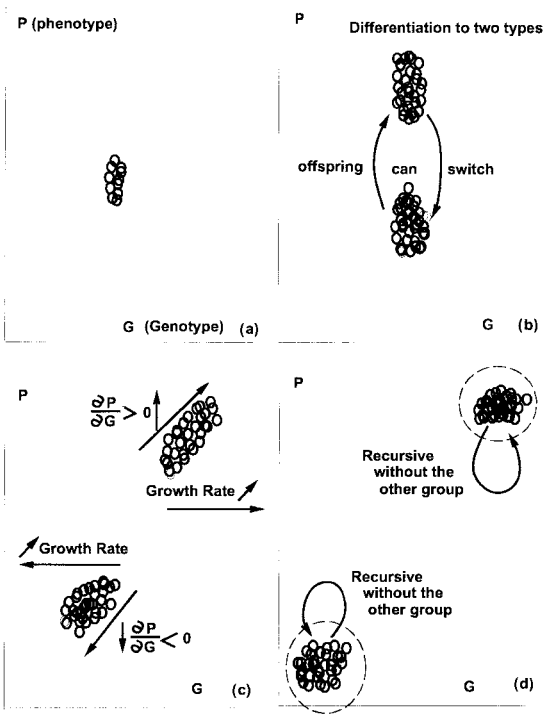


Fig. 3. Schematic representation of the speciation scenario obtained from our simulation and theory. A pair (phenotype, genotype) is plotted successively with time: (a) the stage of interaction-induced phenotypic separation, (b) the stage of genotype–phenotype feedback amplification, (c) the stage of genetic fixation and (d) formation of two groups distinct both in phenotype and genotype.

or several parameters such that the growth rate increases with them for the upper group and decreases with them for the lower group (or the other way around).[7]

Indeed, such parameter dependence is not exceptional. 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 a^{lm} to enhance the cycle will favour the upper group, while a change to reduce it may favour the lower group. Indeed, several numerical results (given, for example, in Figs 1 and 2) support the existence of such parameters. This dependence of growth on genotypes leads to genetic separation of the two groups, as long as the population is limited with competition for survival (see the horizontal shifts in Fig. 3b).

Although the above process is essential to speciation, the genetic separation is often accompanied by the second process, the amplification of phenotypic difference due to the genetic difference. In Fig. 3, as the parameter a^{lm} is increased, the phenotype variable R^j tends

[7] By choosing $-a^{lm}$ as the parameter, the latter case (in which the lower group has a higher reproductive rate with the increase in a^{lm}; as in Fig. 1) can be converted to the former case. Thus, we consider only the former case without losing generality.

to increase and vice versa. This is possible if $\partial R^j/\partial a^{lm}$ is larger for the upper group. In a typical and clear example, as in Fig. 3c, $\partial R^j/\partial a^{lm}$ is positive for the upper group and negative for the lower group (and vice versa for Fig. 2). With this process, the separation of the two groups is amplified both in terms of genotypes and phenotypes.

We note that the existence of such parameters that satisfy the two conditions is not unusual. If there are rather complicated dynamics among phenotypic variables, the number of parameters is rather large and it is expected that parameters that satisfy the above condition exist in general.

With this separation of two groups, each phenotype (and genotype) starts to be preserved by the offspring, in contrast with the first stage. Now, distinct groups with recursive reproduction have been formed. However, up to this stage, the two groups with different phenotypes cannot exist by themselves in isolation. When isolated, offspring with the phenotype of the other group start to appear. The two groups co-exist dependent on each other. The two groups co-evolve, keeping their 'symbiotic' relationship while competing for the same niche for survival.

Note that the metabolic (or developmental) dynamics in each group, when isolated, are unstable and some individuals start to be differentiated to recover the other group. The dynamics, for each phenotype, are stabilized by each other through interaction. Hence, evolution of one group is related to that of the other. To have such stabilization, the population of each group has to be balanced through the interaction. Even under random fluctuation due to finite-size populations or mutation, the population balance of each group is not destroyed. Indeed, the speed of growth of each group is of the same order at this stage, as will be demonstrated later. When the genotype parameter of one group is shifted by mutation and selection, that of the other group is also shifted (in the opposite direction) and the balance of speed of growth is preserved.

Accordingly, our mechanism of genetic diversification is stable. This is why our mechanism works as a stable sympatric speciation, as will be shown later.

Stage 3: genetic fixation and isolation of differentiated groups

Complete fixation of the diversification to genes occurs at this stage. Here, even if one group of units is isolated, the offspring of the phenotype of the other group are no longer produced. Offspring of each group keep their phenotype (and genotype) on their own. This is confirmed by numerically eliminating one group of units.

Each group has one phenotype corresponding to each genotype, even without interaction with the other group. Hence, each group is a distinct reproductive unit at this stage. This stabilization is possible since the flexibility of the phenotypes at the first stage is lost, due to the shift in genotypes (parameters). The initial phenotypic change introduced by the interaction is now fixed to genes. Genetically distinct groups with independent reproduction are formed with this genetic fixation.

To check the third stage of our scenario, it is straightforward to study the further evolutionary process from only one isolated group. To do this, we choose some population of units only of one type after the genetic fixation is completed and then both the genotypes and phenotypes are separated into two groups. Then we start the simulation again. When the groups are chosen from later generations after the genetic fixation process, the offspring keep the same phenotype and genotype. Now, only one of the two groups exists. Here, the other group is no longer necessary to maintain

stability. This recursive production by each group characterizes the third stage of our scenario.

At an earlier generation of the genetic diversification process (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 genotype and phenotype that had existed before the transplant experiment. This is in strong contrast with the third stage.

FURTHER REMARKS ON THE DIFFERENTIATION SCENARIO

Condition for genetic diversification

We now show that in our model interaction-induced phenotypic differentiation is a necessary (and sufficient) condition for the formation of genetically distinct groups.

First, the genetic differentiation always occurs when the phenotype (represented by the rate of each cyclic process, R^k) differentiates into two (or more) groups. After the initial separation into two groups, the fixation into parameters always follows, as long as mutation exists.[8] In this sense, phenotypic differentiation is a sufficient condition for the genetic diversification process, in standard biological circumstances (with reproduction, mutation and a genotype–phenotype relationship).

Second, if the interaction-induced differentiation does not exist initially, there is no later genetic diversification process. In fact, for some resource and coupling parameters, or for some initial distribution of parameters a^{ij}, no phenotypic differentiation occurs. The distribution of phenotype values $R^k(i)$ over all individuals i is concentrated around a single point, rather than forming two disjunct groups. In this case, even if we take a large mutation rate, the distribution only becomes broader, without any split. Thus, no separation process into two groups is observed, even if we wait for many generations of reproduction and death of units. This clearly shows that the interaction-induced differentiation is also a necessary condition for the speciation in our model.

To be specific, in our model, the condition for the genetic diversification to occur is given by the initial parameters. First, the parameter p should be larger than some threshold value. For example, for a model with $s^1 = 2$, $s^2 = 4$, $s^3 = 6$ and with the initial parameters $a^{lm}(i) \approx -0.2/(2\pi)$, the differentiation appears for $p \gtrapprox 1.8$. Second, the resource term per unit ($\Sigma_j s^j/N$) should be smaller than some threshold value. For example, the threshold resource is $s_{\mathrm{thr}} \approx 10$, for $s^1 = s^2 = s^3$, $p = 1.5/(2\pi)$, $N \approx 300$ and the initial parameters $a^{lm}(i) \approx -0.1/(2\pi)$. These two conditions imply a strong interaction when competing for resources. The phenotypes differentiate to several groups if the interaction is strong enough. For example, as the individuals competing for given resources reproduce, the number N increases so that the threshold condition for $\Sigma_j s^j/N$ is satisfied. Then, the interaction-induced phenotypic differentiation always follows.

Next, if the magnitude of the initial parameter a^{lm} is too large for all units, the interaction term is not relevant to the dynamics of each individual. It is mainly determined by its own state, without being affected much by the states of other individuals. Then, the distribution of phenotype R^i does not split initially and no differentiation follows. Hence, the internal

[8] For some parameters or for some events, one of the groups may go extinct at a rather early stage.

dynamics have to support the possibility of diversification. As has been noted in the case of isologous diversification in cell differentiation, some balance between internal dynamics and interaction is required to have interaction-induced differentiation. Indeed, such a condition for internal dynamics (to have phenotype differentiation) implies low penetrance in terms of biology (Opitz, 1981).

Co-evolution of differentiated groups

The rate of reproduction depends on each stage of evolution. In Fig. 4, we plot the distribution of reproduction time for each differentiated group. At the first and second stages of evolution, the rate of reproduction is similar for the two groups. Indeed, at these stages, the reproduction of each group is strongly dependent on that of the other group, and 'fitness' as the speed of reproduction for each group by itself alone cannot be defined. At stage 2, the reproduction of each group is balanced through the interaction, so that one group cannot dominate in the population. This is why the rates of growth of the two groups are almost the same, as shown in Figs 4a and 4b.

Later, at the third stage, the division speeds start to be different (Fig. 4c). Here, the interaction term between the two groups becomes weaker compared with the internal dynamics term. Now 'fitness' as the speed of reproduction can be defined, with the group with the higher reproduction rate becoming dominant in the population; the other group may go extinct due to a difference in the reproduction rates. (In this example, after Fig. 4d, the group with lower reproduction rate goes extinct.) After this extinction, the remaining group exists on its own, since the other group is not required for the existence of this group at the third stage. In other words, 'fitness' as a reproduction rate leads to the selection of one group at the third stage.[9]

In Fig. 5b, we plot the average division time for the two groups, taking another example with different parameters, while the time course of the average parameter value a^{12} is plotted in Fig. 5a for five runs with different random numbers for initial distribution and mutations. As can be clearly seen, the speeds of division for the two groups are balanced, within their fluctuation range. With the formation of the two groups, the population doubles in size; hence the division speed of each group doubles, to allow for a stationary population. Over a few hundred to thousand generations, the growth speeds are balanced, although the genotypes of each group change with mutation. Later in this stage, one of the groups may disappear through the finiteness of the population, when the total population becomes half again. By having two groups, twice the population co-exists in this system, which is an indicator of co-evolution.

On the other hand, if the extinction of one group occurs at the second stage, the later time course is different. Indeed, in a few examples with a small number of populations, one group happens to go extinct at an earlier (i.e. the second) stage, due to the finite-size fluctuation in populations. In this case, the remaining group starts the genetic fixation process again, triggered by phenotypic differentiation. Figure 6 is an example of such a process, where the phenotype R^1 differentiates into two groups after the extinction.

[9] As the value of k is increased, more than one group co-exist (up to our range of simulation time steps). Complex organization of several species will then be possible.

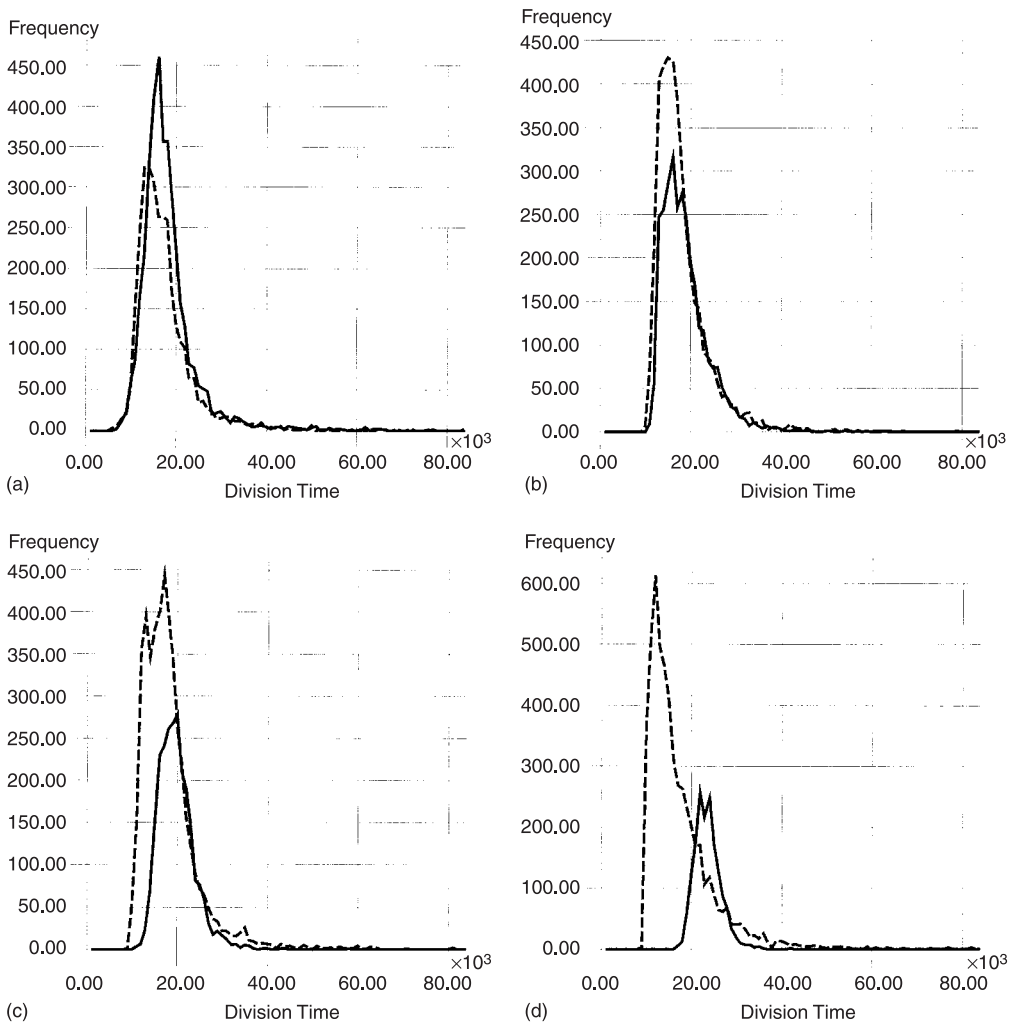


Fig. 4. Histogram of the reproduction time for each of the two differentiated groups. The time steps required for the reproduction are sampled for all division processes over a given duration, to obtain the histogram. The dashed line is a histogram for the 'upper group' given by the condition $a^{12} > -1/2\pi$, while the solid line gives the data for the lower group with $a^{12} < -1/2\pi$. The parameters are set as $p = 1.5/(2\pi)$ and $s_1 = s_2 = s_3 = 8$, and the population N fluctuates around 400–500. Initially the parameter a^{ij} is set at $-1/(2\pi)$. (a) The histogram over division events 3000–10,000; (b) the histogram over division events 10,000–17,000; (c) the histogram over division events 17,000–24,000; (d) the histogram over division events 24,000–31,000. In this example, two groups with $a^{12} \gtrless -1(2\pi)$ are formed around division event 3000 (6–8 generations). A few hundred divisions later after (d), the lower group goes to extinction.

Deterministic nature of evolutionary process

As mentioned, if phenotypic differentiation (stage 1) occurs in our model, then the genetic differentiation of the second stage *always* follows, in spite of the random mutation process. Once the initial parameters of the model are chosen, it has already been determined whether

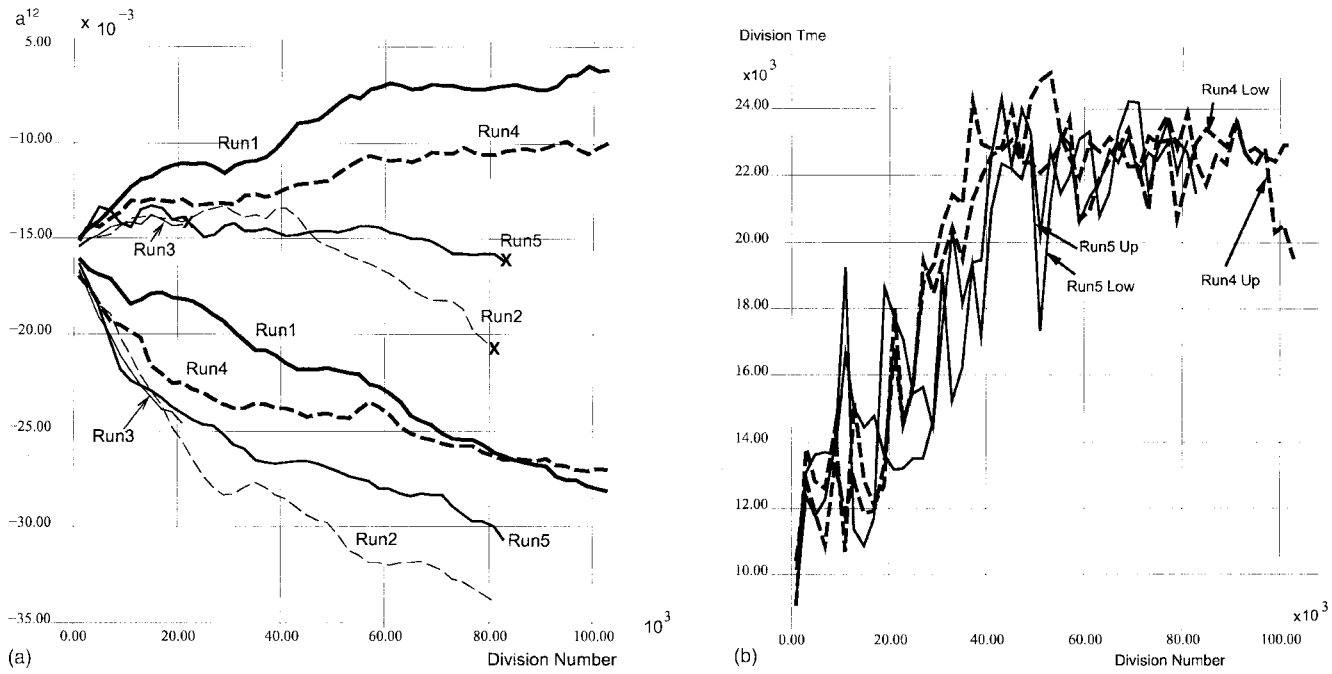


Fig. 5. (a) The evolution of the average parameter for the upper and lower groups. Plotted is the average parameter value a^{12} for the upper and lower groups, where the average is taken over 2000 division events. Five examples of the evolution by a different random number for mutation are overlaid, to demonstrate also that the differentiation process always occurs, although in some runs one of the groups goes extinct later (for runs 2, 3, 5). The parameters are set as $p_k = 1.8/(2\pi)$ and $s^1 = 8$, $s^2 = 7$, $s^3 = 2$. Initially, the genotype parameters are set at $a^{ij} = -0.1/(2\pi)$, while the variables $x^k(i)$ are assigned randomly over [0,1]. Individuals are eliminated randomly so that the total population is around 300. (b) The average division speed for the upper and lower groups, corresponding to Fig. 5a. The average division time is again computed from 2000 division events. Only two examples of the evolution by a different random number sequence for mutation are overlaid, to make the figure clearly visible. As the two groups are formed around 5×10^4 division events, the population size becomes twice that at the beginning, and each division time also becomes approximately twice. Note that the average division speeds of the two groups remain balanced, even if the genetic parameter evolves in time.

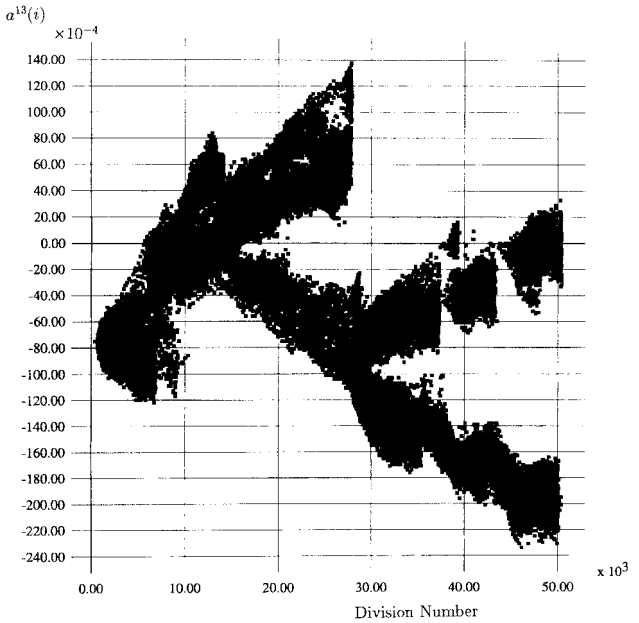


Fig. 6. Speciation after extinction of one group. In this figure, the ordinate is the parameter a^{13} and the abscissa is the division event. $p_k = 1.0/(2\pi)$ and $s^1 = s^2 = s^3 = 3$. $a^{12} = -0.0074$. Initially, the genotype parameters are set at $a^{12} = -0.0074$, $a^{13} = -0.0075$, $a^{21} = -0.0076$, $a^{23} = -0.0084$, $a^{31} = -0.0095$, $a^{12} = -0.0076$, while $x(i)$ is put randomly over [0,1], whose rate of change per division, ε_p, is 0.1. The total number fluctuates around 700.

such differentiation will occur or not. Indeed, the separation process (e.g. the time necessary to have two groups, or the population number ratio between the two groups) changes little between runs that adopt a different random number.[10]

In Fig. 5a, we show the differentiation and evolution process of the two groups by adopting a different random number sequence for mutation. For the dozen or more runs we checked, differentiation and evolution of the two groups always occurred. Here the phenotypes (R^i) taken by the two groups are almost independent of runs. The separation of genotypes – as shown, for example, by the upper and lower values of a^{12} – always occurs, although their mean values can change by runs.[11] Note also that the division speeds are balanced at the same level as shown in Fig. 5b.

[10] If the model parameters are identical by process (i.e. $s^i = s_0$ for all i and the initial parameters a^{ij} take almost identical values for all i, j), the model is symmetric with the change of the process i. Hence, for k groups there can be k possible paths of genetic diversification with equal possibility. For example, when differentiation to two groups with $R^1 > R^2 > R^3$ and $R^3 > R^2 > R^1$ has occurred, differentiation with $R^2 \gtrless R^3 \gtrless R^1$ or $R^3 \gtrless R^1 \gtrless R^2$ can occur with the same probability. In such symmetric cases, the choice of one path among k symmetric paths is stochastic. Still, the time course of genetic diversification is almost the same except for this 'permutation of cyclic process'. In general, as the number of degrees of freedom involved increases, there may be freedom in the choice of each path. Nevertheless, whether genetic diversification occurs or not is determined according to the initial interaction-induced differentiation of phenotype.

[11] In this model, several control parameters a^{ij} are related to the dynamics to change R^i. Although a^{12} change by runs, other parameters also change, leading to two groups with almost the same division speeds and phenotypes independent of runs.

Of course, the existence of mutation is required, but the genetic separation is not mutation driven. The evolution to distinct genetic groups is rather deterministic in nature in this sense.

Note also that the evolution process is rather fast. In the simulations shown in Figs 1 and 2, the total population is around 400 and the separation to genetically distinct groups is completed after the first 20,000–30,000 (total) divisions. This means that the separation is completed around the first 50 generations, when it is started.

The speed of the change of the control parameters a^{ij} depends on the mutation rate. The rate of the parameter change per generation (or division) is found to be proportional to the mutation rate. Although this rate is reduced with the mutation rate, the time required for the two groups to split does not increase so much. The reasons are as follows: First, the phenotype is already separated. Second, since the population distribution in the genotype parameters has sharper double peaks for the case of a lower mutation rate, the separation of the two groups is completed earlier – that is, when the parameter difference between them is smaller. Individuals with intermediate parameter values between the two disappear and genetic separation is completed. In summary, a fast separation process is a characteristic feature of our mechanism.

In the present model, the number of separated groups is limited. Indeed, only two species co-exist in most simulations with $k = 3$ or 4. To see successive diversification processes, a higher number of internal degrees of freedom is required.

Relevance of phenotypic differentiation, rather than genetic change, to genetic diversification

To clarify the importance of interaction-induced phenotypic differentiation, we study the evolution process by initially introducing diverse genotypes. We show that the genetic diversification process is not driven by imposing distributed genotypes initially, even though the mutation of genotypes (parameters) is itself necessary for the genetic separation process.

To examine this point closely, we have made several simulations starting from a population of units with widely distributed parameters (i.e. genotypes). In Fig. 7, the distribution of parameters that was initially broad shrinks to a small range. Initial genetic diversity cannot be fixed and disappears. After a few generations, genes (parameters) are no longer widely distributed but lie only within a narrow interval. Thus, the genotype distribution returns to that seen in the previous sections. Then, if the interaction condition on pp. 329–330 is satisfied, phenotypic differentiation occurs later, as shown in Fig. 7, and genetic separation progresses later as in the previous examples.[12]

The above result (as well as the one in footnote 12) demonstrates that genotypic variation is not sufficient to form genetically separated groups in our model. Even though the variation is assigned initially, this does not help to form the distinct groups. If, and only if, phenotypic differentiation appears can genetic separation proceed.

[12] In some other examples, phenotypic differentiation can appear initially under the presence of genetic distribution. For every parameter value a^{ij}, there are (two) different phenotypes R^l. Initially, this differentiation is not supported by the genotype. In later generations, for each of the two groups with different phenotypes, there is competition for parameters, to have a higher reproduction rate. Then, the parameters start to be differentiated by the phenotypes, as discussed earlier. Finally, (two) different genotypic groups are formed.

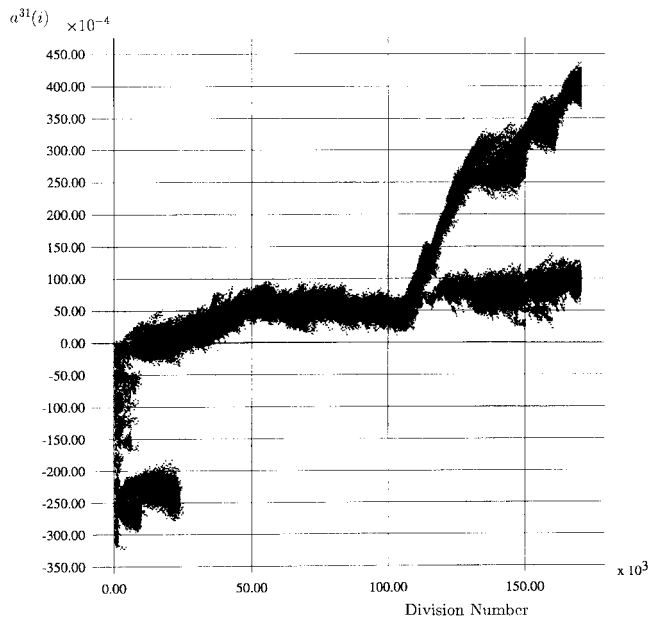


Fig. 7. Speciation process from widely distributed genotypes (parameters). In this figure, the ordinate is the parameter a^{31} and the abscissa is the division event. $p_k = 1.7/(2\pi)$ and $s^1 = 6$, $s^2 = 4$, $s^3 = 2$. Initially, the parameters a^{ij} are distributed as $(-0.1 + \sigma)/(2\pi)$, with σ as a random number over [–0.1, 0.1]. The initial value of $x(i)$ is assigned randomly over [0,1] and its rate of change per division, ε_p, is 0.01. The total number fluctuates around 400.

It is also important to note that the separation into *discrete* groups is necessary to have differentiation into discrete genetic groups. Indeed, when the phenotype is broadly distributed without forming discrete groups, evolution does not proceed to form discrete groups in genotype and phenotype, even though the phenotype variation is large. In fact, we have sometimes observed such a broad, continuous distribution in phenotypes in our model with larger k (say $k = 10$). Then, discrete groups are not formed even after a long time span of mutation to parameters.

Accompanied separation of parameters

Dominance of phenotypic differentiation is also seen in successive changes of parameters. When the interaction-induced differentiation has started, only one or a few phenotypes are separated. In the example in Fig. 1, R^1 and R^2 are differentiated after a few generations, but R^3 is not differentiated. In this case, the parameter a^{12} is most relevant to the differentiation. Indeed, the separation of parameters starts from a^{12}, before a^{13} splits into two groups.

In the example of Fig. 1, a^{12} is relevant to the differentiation. However, the differentiation is later transferred to other parameters, say a^{13}. For a system with a larger number of processes k, some parameters are not relevant to initial differentiation at all. (For example, consider the case of differentiation by R^2 and R^1 for $k \geq 4$. Then, the parameters a^{ij} with $i, j > 2$ do not govern directly the dynamics of x^1 and x^2.) In this case, the separation of such

parameters does not occur initially. Still, such initially irrelevant parameters may differentiate at a later generation. Since the dynamics of each cycle is mutually related, most parameters cannot be completely neutral to the change in phenotypes and can have an effect. Then, following the initial split of relevant parameters, parameters (genotypes) that are initially irrelevant and not responsible for the differentiation also separate later. Note that such a difference in genotypes, even though it has a one-to-one correspondence with a difference in the phenotype, can never be a 'cause' of such differentiation.

As the number of processes k increases, it takes more time for the differentiation to spread over all parameters. In this case, more than one differentiation can occur and several species (i.e. separated groups) co-exist over a long time.

SPECIATION: REPRODUCTIVE ISOLATION UNDER SEXUAL RECOMBINATION

The speciation process is defined both by genetic differentiation and by reproductive isolation (Dobzhansky, 1937; Futsuyma, 1986). Although the evolution through stages 1–3 leads to genetically isolated reproductive units, one might say that the term 'speciation' should not be used unless the process shows isolated reproductive groups under 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 modified our model so that sexual recombination occurs to mix genes. To be specific, the reproduction occurs when two individuals i_1 and i_2 satisfy the threshold condition ($\Sigma_l R_n^l(i_k) > Thr$), 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

$$a^{lm}(j) = a^{lm}(i_1) r_j^{lm} + a^{lm}(i_2)(1 - r_j^{lm}) + \delta \tag{6}$$

with a random number $0 < r_j^{lm} < 1$ to mix the parents' genotypes[13] besides the random mutation term δ (asexual reproduction is not included in the simulation). We have performed several simulations with this model by choosing the same setting as the previous model. The question we address here is as follows: Even if two separated groups start to be formed according to our scenario, the above recombination can form 'hybrid' offspring with intermediate parameter values a^{lm} between the two groups, since two individuals from different groups can mate to produce offspring. Can our speciation scenario work in spite of this drastic disturbance?

In Fig. 8, we plot the evolution of the parameter $a^{12}(i)$ by each reproduction event. As shown in Fig. 8, the two distinct groups are again formed in spite of the above mixing of genotypes by sexual recombination. Of course, the mating between the two groups can produce an individual with the parameters in the middle of the two groups, according to equation (6). Then, why does separation into the two groups remain stable? The reason for the present stability is due to the low reproduction rate of the individual with intermediate parameters between the two groups. When parameters of an individual take intermediate values between those of the two groups, it takes much longer to reach the threshold condition for reproduction whatever phenotype it takes. Reproduction here takes much longer than in the two groups. Before the reproduction condition is satisfied, the individual has a

[13] We have also performed simulations with different methods to mix the genotype, for example by fixing $r_j^{lm} = 1/2$, or by taking a random number depending on each parameter a^{lm}. In all cases, the speciation process to be discussed is observed.

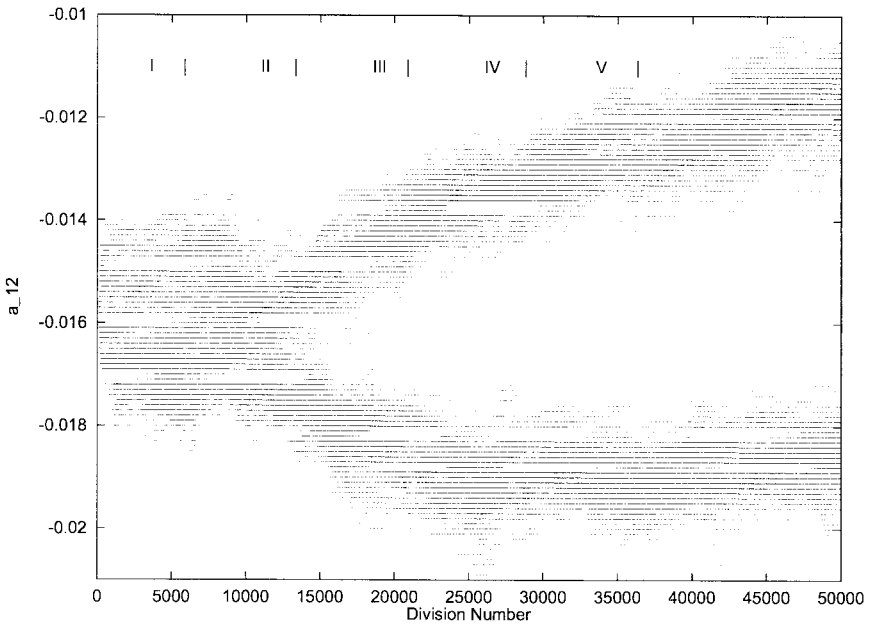


Fig. 8. An example of the speciation process with sexual recombination. The parameter a^{12} of divided units is plotted with the division event. The parameters are $p_k = 1.6/(2\pi)$ and $s^1 = s^2 = s^3 = 2$, with initial parameters $a^{ij} = (-0.1)/(2\pi)$. The total population fluctuates around 350.

higher probability of being removed by death. As the separation process to the two groups progresses further, an individual with intermediate parameter values never reaches the condition for reproduction before it dies, since X_n^i starts to be negative. Hence, the offspring between two distinct groups no longer produce the next offspring.

To demonstrate this post-mating isolation, we have measured the average number of offspring over given parameter (genotype) ranges and over some time span. An example of this average number of offspring is plotted in Fig. 9, as the speciation process progresses. As the two groups are formed with the split of the parameter values, the average number of offspring for an individual having the control 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. Hence it is proper to call stages 1–3 'speciation', since they satisfy genetic differentiation and reproductive isolation (under sexual recombination).[14]

We also performed simulations with other choices of recombination. Note that the mixing of each parameter in (6) is derived by assuming that a genotype constitutes many genes, which are responsible for a given phenotype, and that they are randomly recombined.

[14] The parameter region in which the speciation clearly progresses seems to be narrower than in the previous case without recombination. In particular, if s^j is larger, an individual with intermediate parameter values has some probability for reproduction. Hence, the upper limit of s to have speciation is smaller when the recombination process is considered. Still, within a large enough range of parameters, the speciation process works even under sexual recombination.

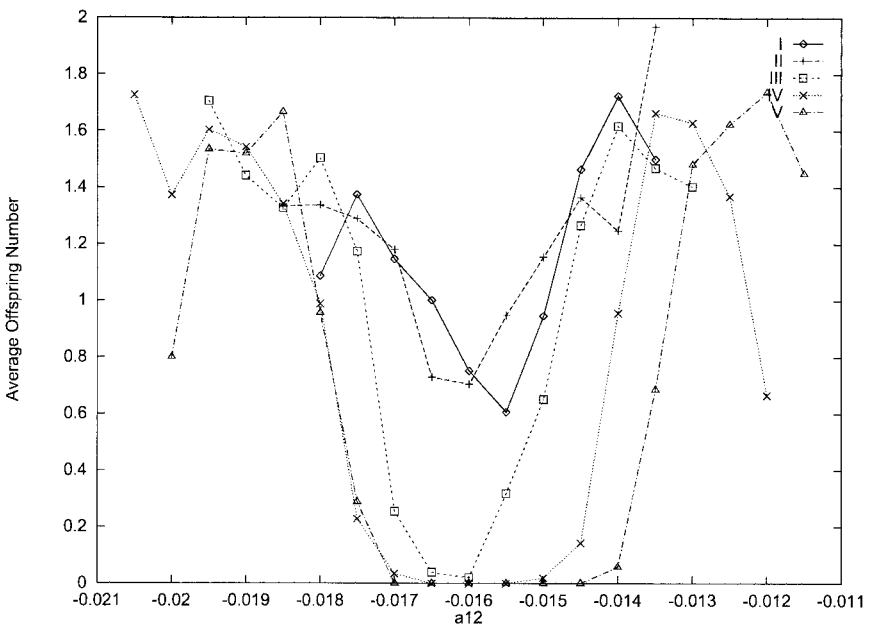


Fig. 9. The average offspring number before death plotted against the parameter (genotype). We measured the number of offspring for each individual during its life span. By taking a bin width of 0.005 for the genotype parameter a^{12}, the average offspring number over a given time span is measured to give a histogram. We have adopted the same model as Fig. 1, and imposed the mating and recombination process, with the parameters $p_k = 1.6/(2\pi)$ and $s^1 = s^2 = s^3 = 2$. The total population fluctuates around 350.

If genes for each parameter are recombined together, the recombination process will be given by

$$a^{lm}(j) = a^{lm}(i_1) + \delta \qquad \text{or} \qquad a^{lm}(i_2) + \delta \tag{7}$$

where the choice of *or* is taken randomly for each l and m.

An example of the simulation result is given in Fig. 10. Here, two groups are formed according to the difference of (R^1, R^2, R^3), and then speciation by the difference of the parameter a^{21} is established. The stable speciation to two groups works well in the present case, although the parameter region for it is a little smaller than in the previous case.

Before closing this section, it should be noted that our mechanism for speciation works in asexual and sexual reproduction in the same way. The phenotype (R^j) separates into two groups first, also in the present case with sexual recombination. Later the change is mapped onto the parameters a^{lm}. The speciation process progresses following stages 1–3 (see pp. 324–329). Indeed, the stability of speciation against sexual recombination is naturally expected, since the co-existence of two distinct phenotype groups is supported by isologous diversification – that is, differentiation to distinct phenotypes under the same genotypes. Even though the genes are mixed, the phenotypes tend to be separated into distinct groups, due to the interaction. Hence the separation into distinct groups is not blurred by the recombination. Here, sexual recombination is just another factor in fixing the

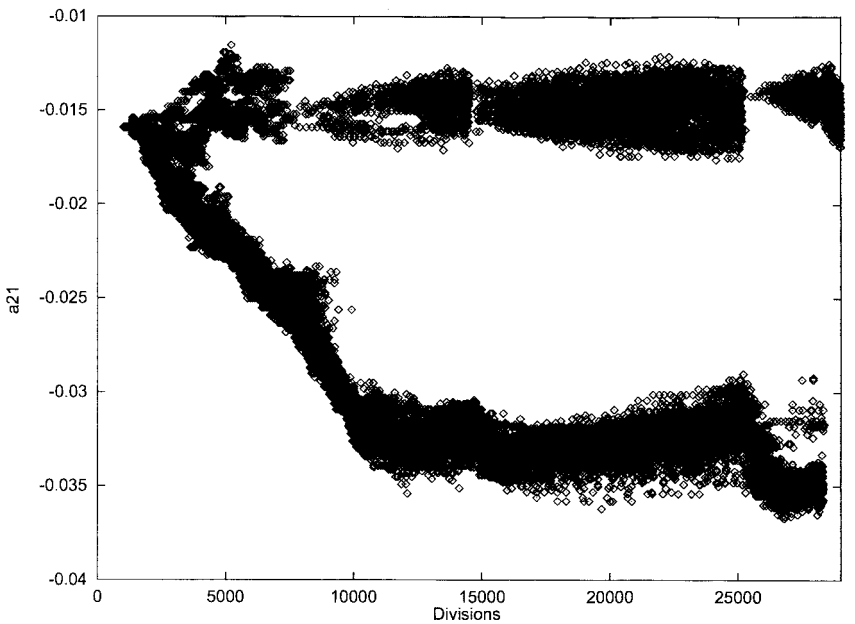


Fig. 10. An example of the speciation process with sexual recombination, using the method of equation (7). The parameter a^{21} of divided units is plotted against the division event. Here two phenotypic groups of (large R^1, small R^2) and (small R^1, large R^2) are formed. The parameters are $p_k = 1.5/(2\pi)$ and $s^1 = 3$, $s^2 = 2$, $s^3 = 1$, with initial parameters $a^{ij} = (-0.1)/(2\pi)$. The total population fluctuates around 150.

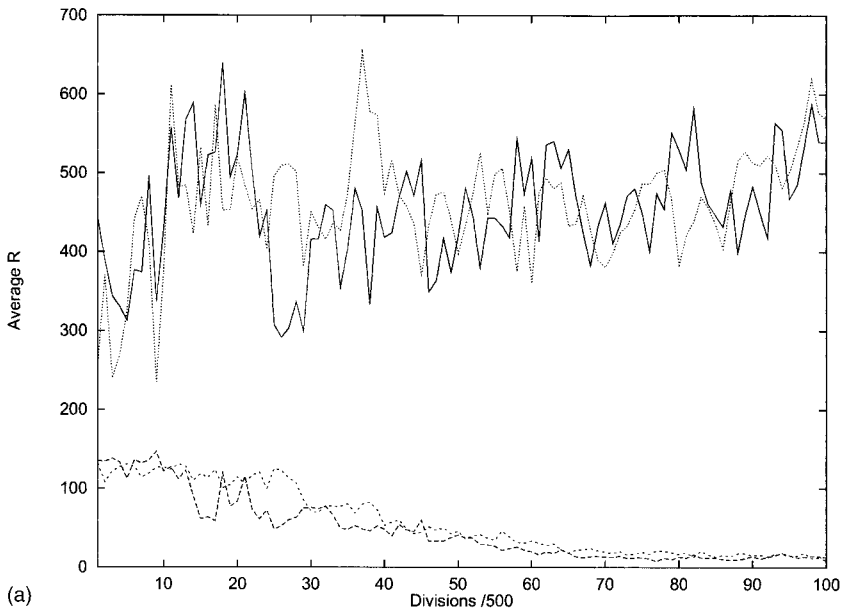
differentiation; it is not the essence of the present process. In short, the speciation process is initiated by phenotype difference, which is later fixed to genotypes either by mutation and/or sexual recombination.

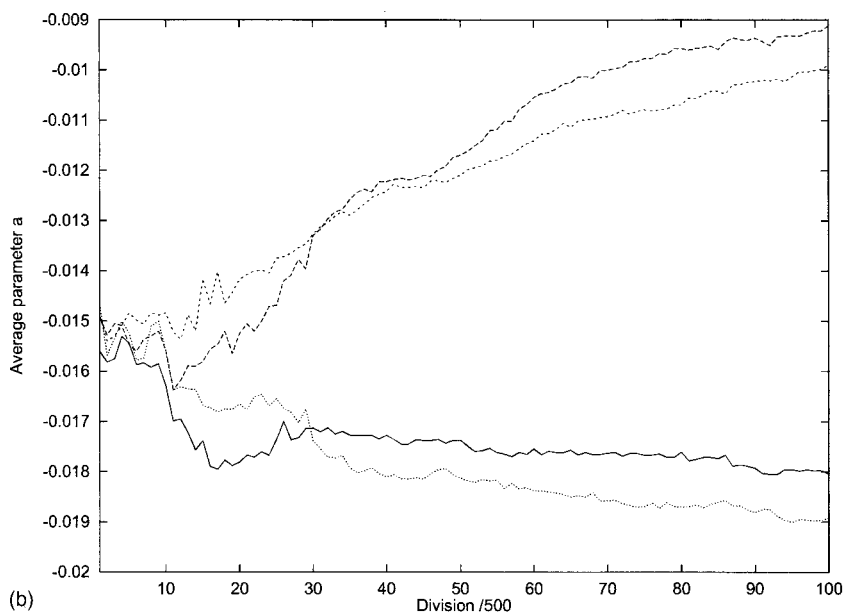
EVOLUTION OF MATING PREFERENCE

So far, we have not assumed any preference in mating choice. Hybrids, although they cannot produce offspring, continue to be born. It is thus natural to expect that some kind of mating preference evolves to reduce the probability to form a sterile hybrid. Here we study the evolution of mating preference, in contrast with most studies for sympatric speciation assuming it in the beginning.

As a simple example, consider the following model for the evolution of mating preference.[15] Each individual i has a set of mating threshold parameters ($\rho^1(i)$, $\rho^2(i)$, ... $\rho^k(i)$), corresponding to the phenotype ($R^1(i)$, $R^2(i)$, ..., $R^k(i)$). First, each individual i_1 that satisfies the threshold condition of $\Sigma_j R^j(i_1) > Thr$ chooses a potential partner i_2 that

[15] We use a one-allele model here. In a two-allele model, it is often believed that the evolution of mating preference is more difficult (Felsenstein, 1981). However, since our mechanism is quite robust against mixing and speciation is already provided by post-mating isolation, it is difficult to consider the possibility that the evolution of mating preference shown here may be destroyed in a two-allele model. Indeed, preliminary numerical studies suggest that the speciation of the present mechanism works well with the two-allele case (Kaneko, in press).





always satisfies the threshold condition, in the same way as in the previous section. Then, instead of random mating, the mating is assumed to occur only if the pair (i_1, i_2) satisfies the condition $R^m(i_2) > \rho^m(i_1)$ and $R^m(i_1) > \rho^m(i_2)$ for all $m = 1, \ldots, k$. If these conditions are not satisfied, individuals i_1 and i_2 wait for the next step to find a partner again. Note that only when both satisfy the mating preference condition does mating occur (in other words, if at least one of them denies the mating, the mating process does not occur).

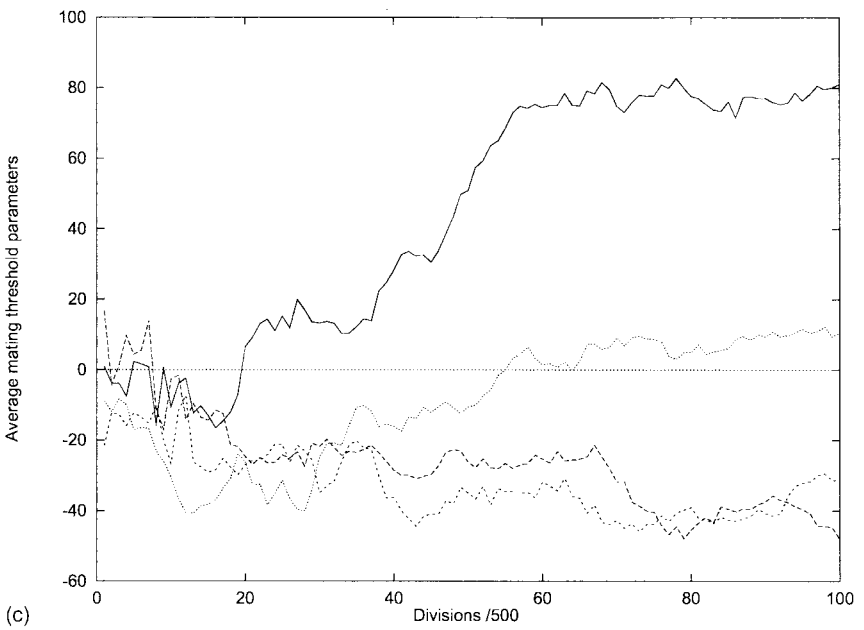


Fig. 11. An example of the speciation process with sexual recombination and the evolution of mating preference, with the model described in the text. Here two groups of distinct phenotype (large R^1, small R^2) and (small R^1, large R^2) are formed after the first few generations, which we call 'up' and 'down' groups. We have measured the average $\overline{R^j}$, $\overline{a^{lm}}$, $\overline{\rho^j}$ for each group per 500 divisions. (The population here is roughly 500, and thus the average is roughly over one generation.) Changes in the average $\overline{R^j}$, $\overline{a^{lm}}$ and $\overline{\rho^j}$ are plotted with divisions (generations). (a) $\overline{R^1}$ (up group; solid line), $\overline{R^1}$ (down group; broken line), $\overline{R^2}$ (up group; thin broken line) and $\overline{R^2}$ (down group; dotted line). (b) $\overline{a^{31}}$ (up group; solid line), $\overline{a^{31}}$ (down group; broken line), $\overline{a^{32}}$ (up group; thin broken line) and $\overline{a^{32}}$ (down group; dotted line). (c) $\overline{\rho^1}$ (up group; solid line), $\overline{\rho^1}$ (down group; broken line), $\overline{\rho^2}$ (up group; thin broken line) and $\overline{\rho^2}$ (down group; dotted line). The parameters are $p_k = 1.6/(2\pi)$ and $s^1 = s^2 = s^3 = 2$, with initial parameters $a^{ij} = (-0.1)/(2\pi)$.

Here the set of $\{\rho^m\}$ is regarded as a set of (genetic) parameters, which changes by mutation and recombination. The mutation is given by addition of a random value over $[-\delta_\rho, \delta_\rho]$. Since R_j is larger than $-Thr_d$ and is typically non-negative, the individual with $\rho^m \leq 0$ (for $m = 1, \ldots, k$) has no mating preference at all. If $\rho^m(i)$ is positive, individual i rejects the mating with the individual whose R^m is less than it. Here, by phenotype differentiation, one group has a large R^m value for some $m = l$ and almost null values for some other $m = l'$. Hence, suffciently large positive $\rho^{l'}$ give a candidate for mating preference. If there is no disadvantage for the mixing of genetic parameters by mating, ρ^m will be negative to increase the chance of mating. (Recall that the individual has to wait for the next step if mating is rejected. Then it will be advantageous to keep ρ negative, taking into account the possibility that ρ may increase by mutation.) Here we start the simulation from $\rho^m = 0$ ($m = 1, \ldots, k$) and study if a mating preference evolves.

In Fig. 11, the evolution of the mating threshold parameters, and the change in phenotype R and some of the parameters a^{lm}, are plotted corresponding to the simulation in the

previous section. Immediately after the formation of two genetically distinct groups, which follows the phenotype separation, one of the mating threshold parameters ($\rho^1(i_1)$) starts to increase for one group. In the example in Fig. 11, one group has a phenotype with (large R^1, small R^2) and the other with (small R^1, large R^2). The former group starts to increase $\rho^1(i_1)$ as shown; $\rho^1(i_1) > R^1(i_2)$ is satisfied for an individual i_2 of the latter group. Now mating between the two groups is no longer allowed, and mating occurs only within each group. Hence a mating preference has evolved and mating to produce a sterile hybrid no longer takes place.

Here the speciation progresses in the order of phenotypic differentiation, genetic differentiation and mating isolation. Although each process progresses rapidly, this order provides an interesting, testable consequence of our theory. Furthermore, two interesting points became apparent after a variety of simulations.

(i) *Two groups do not simultaneously establish the evolution of mating preference*. In several examples, we found that only one group establishes the evolution of mating preference. If one group rejects interspecies mating, a hybrid is no longer formed. Hence, once one group establishes the evolution of mating preference, the other group does not need to have a mating preference by itself. Indeed, in Fig. 11, $\rho^2(i_2)$ of the other group does not show such a clear increase as $\rho^1(i_1)$ of the former group, and it fluctuates around a slightly positive value. See Fig. 12 for other examples, where only one group has a positive threshold parameter for mating again. Hence the symmetry on mating discrimination is broken. When the mutation rate of the mating threshold is larger, however, the other group often establishes the evolution of the mating preference later. This evolution is relevant to avoid 'erroneous' mating between the two groups that can occur due to mutational change of the threshold, if only a single group evolves the preference. In all cases, the mating preference starts to evolve in one group, not simultaneously in the two groups.

(ii) *Co-existence of the two species is stabilized by the evolution of mating preference*. Indeed, in some examples without the evolution of mating preference, after the separation is completed (i.e. at stage 3) one species may go extinct (as in the example of Fig. 5a) after many generations. With the inclusion of sexual reproduction and the evolution of mating preference here, the co-existence of two species is much more stable and extinction is not observed (Fig. 12). Since the loss by forming a sterile hybrid is removed by mating preference, each group has more stable sexual reproduction. Phenotypic differentiation is first fixed to genetic difference and then to mating preference here. This successive fixation makes the speciation more rigid.

As for point (i), one may expect that the evolution of mating preference for both groups is expected if we assume a different mating condition: If either of the two (instead of *both*) satisfies the mating threshold condition, mating occurs. (In other words, mating is prohibited only if both avoid mating.) In this case, it is found that the evolution of mating preference is itself difficult. As shown in Fig. 13, any mating threshold parameter does not increase to, and keep, a positive value, although phenotypic and genetic differentiation have already taken place. This is because suitable mating threshold parameters have to take large positive values simultaneously for both groups to establish the mating preference; such a case should be rare. In fact, none of our simulations performed by changing parameter values or initial conditions showed a clear increase in ρ^m in this case.

To summarize this section, *pre-mating* isolation evolves as a *consequence* of post-mating isolation. Note that the evolution of mating preference *per se* is not necessary for our

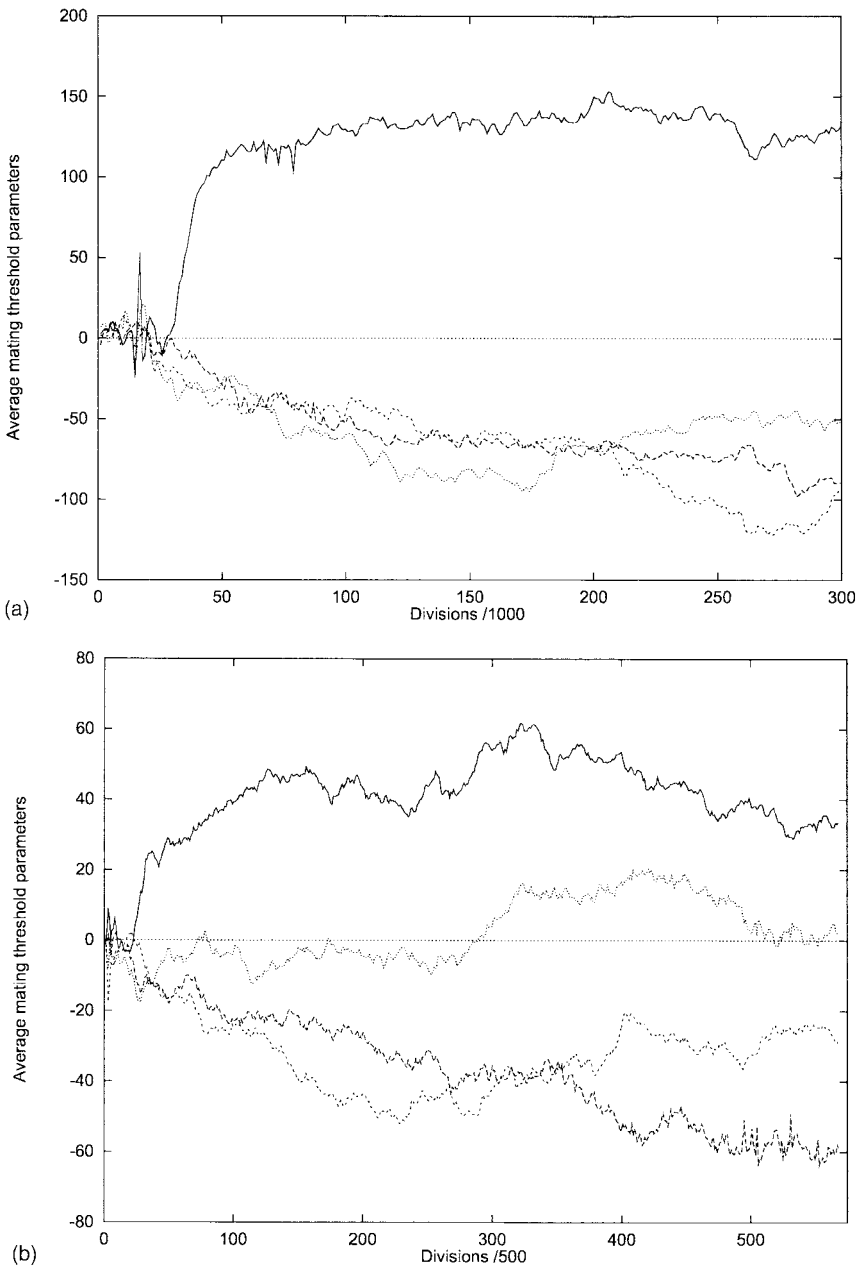


Fig. 12. Examples of the evolution of mating preference. Evolution of the average mating threshold parameter $\overline{\rho^j}$ for each group is plotted. As in Fig. 11, two distinct phenotype groups with (large R^1, small R^2) ('up') and (small R^1, large R^2) ('down') are formed after the first few generations. We have plotted the average ρ^j for each group per 500 divisions. (The population here is roughly 500, and the average is roughly over one generation.) $\overline{\rho^1}$ (up group; solid line), $\overline{\rho^1}$ (down group; broken line), $\overline{\rho^2}$ (up group; thin broken line) and $\overline{\rho^2}$ (down group; dotted line). (a) $p_k = 1.6/(2\pi)$, $s^1 = s^2 = s^3 = 4$. (b) $p_k = 1.8/(2\pi)$, $s^1 = s^2 = s^3 = 2$.

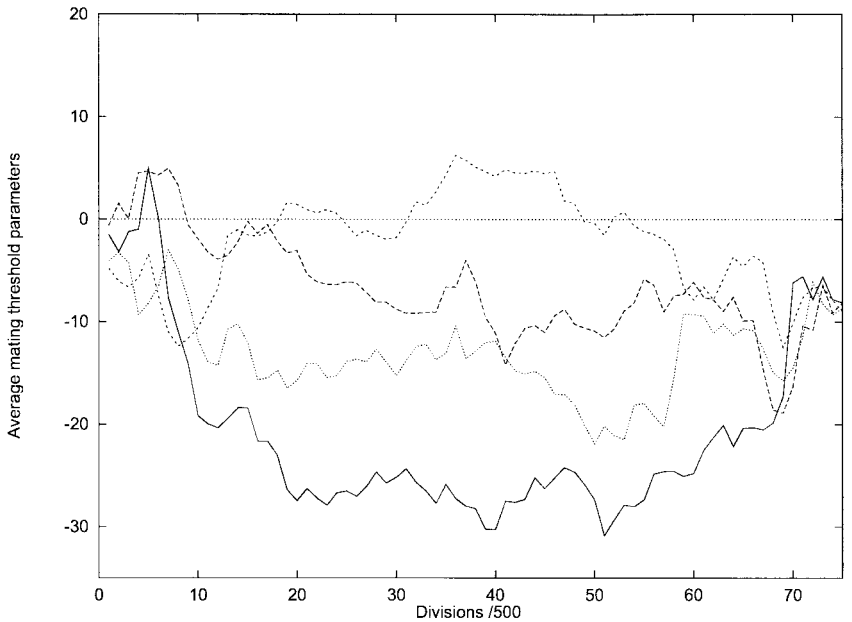


Fig. 13. An example of the evolution of mating preference, using the condition that the mating succeeds if either of the two mating threshold conditions is satisfied (see text). Evolution of the average mating threshold parameter $\overline{\rho^j}$ for each group is plotted. Here, again, 'up' and 'down' groups with (large R^1, small R^2) and (small R^1, large R^2) are formed after the first few generations. We plot the average ρ^j for each group per 500 divisions. (The population here is roughly 500, and the average is roughly given over one generation.) $\overline{\rho^1}$ (up group; solid line), $\overline{\rho^1}$ (down group; broken line), $\overline{\rho^2}$ (up group; thin broken line) and $\overline{\rho^2}$ (down group; dotted line). $p_k = 1.6/(2\pi)$ and $s^1 = s^2 = s^3 = 2$.

speciation theory, but with this evolution of mating preference, the rate of forming a sterile hybrid is reduced, and the speciation of our mechanism is consolidated.

DISCUSSION

According to our results, speciation is initiated by the diversification of phenotypes through interaction among individuals. During the first step of the speciation, a few distinct phenotypes are formed through the interaction among individuals of a single genotype. In other words, each individual changes its surrounding environment through interaction with the others; then, the changed environment induces one of the latent phenotypes. This phenotypic diversification is sufficient and necessary for the speciation presented in this paper. Therefore, the speciation here requires a one-to-many correspondence between genotype and phenotype at the initial stage. The potential of single genotypes to produce various phenotypes works in many biological processes, such as development, brain function, and so forth, where the cells with a single genotype show several functions and structures.

In the process of speciation, the potential 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. In this sense, the process is a kind of genetic takeover, where the phenotypic diversity caused by interaction becomes maintained by genetic diversity. Through the present process of speciation, the potential of single genotypes to produce various phenotypes, which depends on non-linearity to amplify a small difference in phenotypes (Kaneko and Yomo, 1997, 1999), 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 potential 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 potential to produce various phenotypes in the course of evolution. As a reflection on the evolutionary decline of potentiality, one can expect that mutant genotypes tend to have a higher potentiality than the wild-type genotype. As mentioned in the Introduction, a low or incomplete penetrance (Opitz, 1981) is known often to occur in nature, compared with higher penetrance in a wild type. Our result is consistent with these observations, since wild types are in most cases a consequence of evolution (i.e. they are at stage 3), where the one-to-one correspondence is recovered, while mutants have a higher potential to have a loose correspondence (i.e. at stage 1).

Taking our results and experimental facts into account, we predict that organisms emerging as a new species have a high potential to produce a variety of phenotypes, while living fossils, such as *Latimeria chalumnae* and *Limulus*, have stable expression of a few phenotypes.

Of course, there will be occasions when the potentiality is regained, so that the evolution continues. For example, a change in environment may influence the developmental dynamics to regain loose correspondence, or the introduction of novel degrees of freedom, such as the novel element in the reaction network,[16] may provide such looseness. Also, a change to the interaction by a spatial factor may introduce novel instability in dynamics, resulting in the loose correspondence.

According to our theory, sympatric speciation under sexual reproduction starts first from phenotypic differentiation, followed by genetic diversification and, finally, the speciation is fixed by mating preference. This order may be different from most theoretical work. A difficulty in confirming this particular order from the field is that the process from phenotypic differentiation to the last stage is rather fast according to our simulations. Still, it may be possible to find this order in the field, by first searching for phenotypic differentiation of organisms with an identical genotype and an identical environment. 'Phenotypic plasticity' has so far been studied from the standpoint that plasticity is due to environmental change, but it may be interesting to examine the data again from our viewpoint. In this respect, the data for cichlids in a Nicaraguan lake may be promising (Wilson *et al.*, 2000), since

[16] Endosymbiosis may be one such cause.

phenotypic differences corresponding to different ecological niches are observed, even though a clear genetic difference has yet to be identified.

Comparison with previous theories

Since our mechanism depends crucially on the interaction, one might think that it is a variant of frequency-dependent selection. The important difference here is that phenotypes do not have one-to-one correspondence with genotypes, even though the population of organisms is given. Through the 'development process' given by a dynamical system, the phenotype differentiates into two or more types, even if the genotypes are identical or similar. 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. We also stress that the present mechanism works well (or *better*) for 'sympatric' speciation, since the interaction is stronger when individuals are not separated spatially.

Genetic 'takeover' of phenotype change was also proposed by Waddington as genetic assimilation, in possible relationship with the Baldwin effect (Waddington, 1957). Using the idea of epigenetic landscape, he showed that the displacement of phenotypic character is fixed to genes. In our case, phenotypic differentiation is not given by 'epigenetic landscape', but rather the developmental process to form different characters is due to the interaction. Distinct characters are stabilized through the interaction. With this interaction dependence, the two groups are necessary for each other. Accordingly, a robust speciation process is possible.

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. Rather, our theory provides a basis for how the mating preference has evolved. Since the hybrid is inferior in terms of rate of reproduction, the mating preference based on the discrimination in phenotype is shown to evolve then. Indeed, a mechanism to amplify the differentiation by mating preference has been searched for since Dobzhansky (1951). In this sense, our theory also provides a plausible basis for such reinforcement even without any presumption about the inferiority of the hybrid.

Our speciation process often leads to specialization with regard to resources (see, however, footnote 7). Indeed, the co-existence of two or more species after the completion of the speciation is also discussed as resource competition by Tilman (1976, 1981). Although his theory provides an explanation for the co-existence, the speciation process is not discussed, because two individuals with a slight genotype difference can have only a slight difference in resource use, as long as the phenotype is uniquely determined by genotype. In our theory, even if the genotypes of two individuals are the same or only slightly different, their phenotypes need not be similar and can, in fact, be quite different. Accordingly, our theory also provides a basis for resource competition.

Tempo in evolution

Since the present speciation is triggered by interaction and not by mutation, the process is not so much random as deterministic. As shown by our simulations, 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. Our speciation scenario possibly gives an interpretation of punctuated equilibrium (Gould and Eldredge, 1977). Some of the phenotypic explosions in the history of evolution that have been recorded as having occurred within short geologic periods may have followed the deterministic and fast way of interaction-induced speciation.

Adaptive radiation

When exposed to a new environment, a process of successive speciations often follows, called 'adaptive radiation'. By choosing a model with many cyclic processes (e.g. $k = 10$), we can observe successive speciations into several groups from a single genotype. With the increase in population, the phenotypes first split into two groups, each of which is specialized in some processes. They survive being dependent on each other. With evolution, they form distinct genetic groups. With the further increase in population, new instability arises, resulting in further separation into more groups from (each) group, which is later fixed to genotypes. This process can continue successively. Accordingly, we can study adaptive radiation using our model.[17]

Allopatric speciation

It is often believed that allopatric speciation is more common than sympatric speciation. Of course, geographical isolation due to a sudden change in environment may lead to allopatric speciation. Still, some of the data regarded as evidence for allopatric speciation may be interpreted otherwise. After sympatric speciation has taken place and is established, the niches of two groups will be different. Then the two groups may segregate in space according to the difference in niches. After this process is completed, the two species are spatially separated. This might be regarded as a demonstration of allopatric speciation, but in this case a sympatric mechanism is the trigger to the speciation. Such a speciation process will be expected by extending our model to include a spatial factor, which will be reported elsewhere (Kaneko, in press).

Experimental verification

The mechanism of evolution, however, remains anyone's guess. Most important in our scenario, in contrast, is its experimental verifiability. For example, the evolution of *E. coli* is observed in the laboratory, as has been demonstrated by Kashiwagi *et al.* (1998, 2001) and Xu *et al.* (1996). Since the strength of interaction can be controlled by the resources and the population density, one can check whether or not the evolution at the genetic level is accelerated through an interaction-induced phenotypic diversification (Kashiwagi *et al.*, 2001). The time is now ripe to study evolution as an experimental science. Examination of the validity of our speciation scenario provides a first step to such study.

[17] It should again be noted that successive formation of *discrete* groups is necessary. As mentioned earlier, a broad phenotype distribution without discrete groups is often formed for large k, in which case speciation to discrete groups does not follow.

Concluding comments

Darwin asked why organisms are separated into distinct groups, rather than their character being continuously distributed (Darwin, 1859). To summarize the present study, one possible answer to this question is that the phenotypes are first differentiated into distinct groups through developmental dynamics and interaction following the isologous diversification mechanism (Kaneko and Yomo, 1997, 1999), and then the differentiation is fixed genetically through mutation and competition for survival, leading to reproductive isolation.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Masakazu Shimada for useful discussion. This research was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (11CE2006, 11837004).

REFERENCES

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J.D. 1994. *The Molecular Biology of the Cell*. New York: Garland.

Callahan, H.S., Pigliucci, M. and Schlichting, G.D. 1997. Developmental phenotypic plasticity: where ecology and evolution meet molecular biology. *Bioessays*, **19**: 519–525.

Coyne, J.A. and Orr, H.A. 1998. The evolutionary genetics of speciation. *Phil. Trans. R. Soc. Lond. B*, **353**: 287–305.

Darwin, C. 1859. *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. London: John Murray.

Dieckmann, U. and Doebeli, M. 1999. On the origin of species by sympatric speciation. *Nature*, **400**: 354–357.

Dobzhansky, T. 1937, 1951. *Genetics and the Origin of Species*. New York: Columbia University Press.

Felsenstein, J. 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution*, **35**: 124–138.

Furusawa, C. and Kaneko, K. 1998. Emergence of rules in cell society: differentiation, hierarchy, and stability. *Bull. Math. Biol.*, **60**: 659–687.

Futsuyma, D.J. 1986. *Evolutionary Biology*, 2nd edn. Sunderland, MA: Sinauer Associates.

Gilbert, S.F., Opitz, J.M. and Raff, R.A. 1996. Resynthesizing evolutionary and developmental biology. *Develop. Biol.*, **173**: 357–372.

Gould, S.J. and Eldredge, N. 1997. Punctuated equilibria: the tempo and mode of evolution reconsidered. *Paleobiology*, **3**: 115–151.

Holman, E. 1987. Recognizability of sexual and asexual species of Rotifers. *Syst. Zool.*, **36**: 381–386.

Holmes, L.B. 1979. Penetrance and expressivity of limb malformations. *Birth Defects Orig. Artic. Ser.*, **15**: 321–327.

Howard, D.J. and Berlocher, S.H., eds. 1998. *Endless Form: Species and Speciation*. Oxford: Oxford University Press.

Kaneko, K. 1990. Clustering, coding, switching, hierarchical ordering, and control in network of chaotic elements. *Physica*, **41D**: 137–172.

Kaneko, K. 1994. Relevance of clustering to biological networks. *Physica*, **75D**: 55–73.

Kaneko, K. 1998. Coupled maps with growth and death: an approach to cell differentiation. *Physica*, **103D**: 505–527.

Kaneko, K. in press. Symbiotic sympatric speciation: compliance with interaction-driven phenotype differentiation from a single genotype. *Res. Pop. Biol.*

Kaneko, K. and Yomo, T. 1994. Cell division, differentiation, and dynamic clustering. *Physica*, **75D**: 89–102.

Kaneko, K. and Yomo, T. 1997. Isologous diversification: a theory of cell differentiation. *Bull. Math. Biol.*, **59**: 139–196.

Kaneko, K. and Yomo, T. 1999. Isologous diversification for robust development of cell society. *J. Theor. Biol.*, **199**: 243–256.

Kaneko, K. and Yomo, T. 2000. Symbiotic speciation from a single genotype. *Proc. R. Soc. Lond. B*, **267**: 2367–2373.

Kashigawa, A., Kanaya, T., Yomo, T. and Urabe, I. 1998. How small can the difference among competitors be for coexistence to occur? *Res. Pop. Biol.*, **40**: 223.

Kashigawa, A., Noumachi, W., Katsuno, M., Alam, M.T., Urabe, I. and Yomo, T. 2001. Plasticity of fitness and diversification process during an experimental molecular evolution. *J. Mol. Evol.*, **52**: 502–509.

Kawata, M. and Yoshimura, J. 2000. Speciation by sexual selection in hybridizing populations without viability selection. *Evol. Ecol. Res.*, **2**: 897–909.

Ko, E., Yomo, T. and Urabe, I. 1994. Dynamic clustering of bacterial population. *Physica*, **75D**: 81–88.

Kobayashi, C., Suga, Y., Yamamoto, K., Yomo, T., Ogasahara, K., Yutani, K. and Urabe, I. 1997. Thermal conversion from low- to high-activity forms of catalase I from *Bacillus stearothermophilus. J. Biol. Chem.*, **272**: 23011–23016.

Kondrashov, A.S. and Kondrashov, A.F. 1999. Interactions among quantitative traits in the course of sympatric speciation. *Nature*, **400**: 351–354.

Lande, R. 1981. Models of speciation by sexual selection on phylogenetic traits. *Proc. Natl. Acad. Sci. USA*, **78**: 3721–3725.

Maynard-Smith, J. 1966. Sympatric speciation. *Am. Nat.*, **100**: 637–650.

Maynard-Smith, J. and Szathmary, E. 1995. *The Major Transitions in Evolution*. New York: W.H. Freeman.

Maynard-Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D. and Wolpert, L. 1985. Developmental constraints and evolution. *Q. Rev. Biol.*, **60**: 265–287.

Opitz, J.M. 1981. Some comments on penetrance and related subjects. *Am. J. Med. Genet.*, **8**: 265–274.

Roberts, M.S. and Cohan, F.M. 1995. Recombination and migration rates in natural populations of *Bacillus subtilis* and *Bacillus mojavensis*. *Evolution*, **49**: 1081–1094.

Rosenzweig, M.L. 1978. Competitive speciation. *Biol. J. Linn. Soc.*, **10**: 275–289.

Schliewen, U.K., Tautz, D. and Paabo, S. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. *Nature*, **368**: 629–632.

Spitze, K. and Sadler, T.D. 1996. Evolution of a generalist genotype: multivariate analysis of the adaptiveness of phenotypic plasticity. *Am. Nat.*, **148**: 108–123.

Tilman, D. 1976. Ecological competition between algae: experimental confirmation of resource-based competition theory. *Science*, **192**: 463–465.

Tilman, D. 1981. Test of resource competition theory using four species of Lake Michigan algae. *Ecology*, **62**: 802–815.

Turner, G.F. and Burrows, M.T. 1995. A model for sympatric speciation by sexual selection. *Proc. R. Soc. Lond. B*, **260**: 287–292.

Waddington, C.H. 1957. *The Strategy of the Genes*. Bristol: George Allen & Unwin.

Weinig, C. 2000. Plasticity versus canalization: population differences in the timing of shade-avoidance responses. *Evolution*, **54**: 441–451.

Wilson, A.B., Noack-Kunnmann, K. and Meyer, A. 2000. Incipient speciation in sympatric Nicaraguan crater lake cichlid fishes: sexual selection versus ecological diversification. *Proc. R. Soc. Lond. B*, **267**: 2133–2141.

Xu, W.-Z., Kashigawa, A., Yomo, T. and Urabe, I. 1996. Fate of a mutant emerging at the initial stage of evolution. *Res. Pop. Ecol.*, **38**: 231–237.

APPENDIX: EPIGENETIC INHERITANCE EFFECT

In the present model, speciation occurs even if the phenotypic state is not transferred, as long as the genes (parameters) are transferred to offspring. To confirm this, we carried out a simulation where the variable $x_n^k(i)$ is reset at each division to take a value randomly over the interval [0,1]. In Fig. A1, we plot an example of genetic separation that proceeds without any epigenetic inheritance. Since one phenotype group has higher productivity when a parameter (gene) is shifted in one direction, the scenario of the genetic separation process works well, as mentioned in the section on 'Process for genetic diversification'. Hence, our evolution process does not require any epigenetic inheritance; only the interaction-induced phenotypic differentiation is necessary.

In our model, the variables $x_n^m(i)$ are attracted to each stable state rather quickly, from any initial condition. If the transient time for the attraction is longer, it is more important to choose the initial condition so that the variables $x_n^m(i)$ are attracted to one of the final phenotype states within a short time interval. For such fast attraction, it is relevant to adopt the mother's state as the initial condition for the next generation. Hence, the existence of epigenetic inheritance is useful, to some degree, to stabilize the genetic differentiation process. When the initial condition for the phenotype ($x^m(i)$) is transferred with the epigenetic inheritance, each distinct phenotype for R^i is reached within a few time steps and the corresponding phenotype is formed. Thus, the differentiation process occurs smoothly. Starting from a random initial condition for $x_n(i)$ (i.e. without any epigenetic inheritance), it takes more steps to achieve the fast cyclic process corresponding to each type of R^i than starting from a neighbourhood of the mother's $x_n(i)$. Without epigenetic inheritance, one of the differentiated groups may have more difficulty in attaining stable growth. In this sense, the epigenetic inheritance may be relevant to evolution for some case. However, in the present study, we should emphasize that interaction-induced phenotype differentiation does *not* require any epigenetic inheritance and our scenario for genetic evolution works well without epigenetic inheritance.

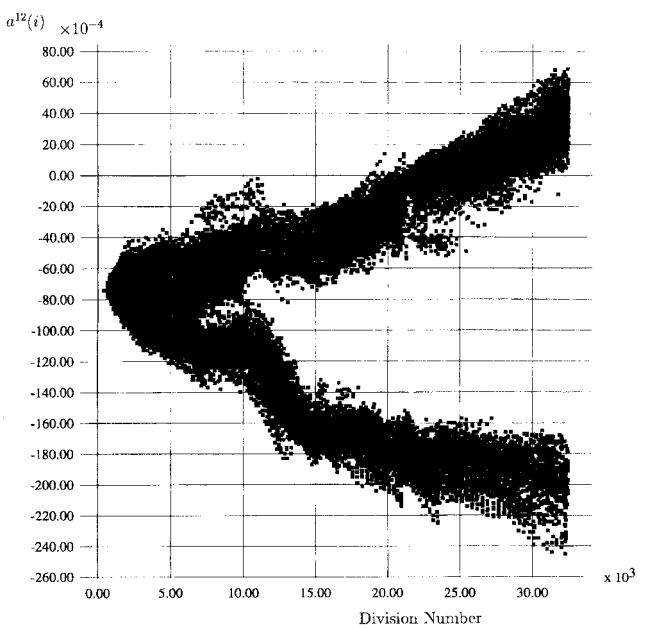


Fig. A1. An example of the speciation process with complete loss of epigenetic inheritance. The parameter a^{12} of divided units is plotted against the division event. The same set of parameters as in Fig. 4 is adopted, while $x^k(i)$ is assigned randomly over [0,1] at every division.